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A SURVEY ON THE LEVEL OF AWARENESS OF SAFETY PRACTICES IN SCIENCE LABORATORIES AMONG SCIENCE LABORATORY TECHNOLOGY STUDENTS IN YABA COLLEGE OF TECHNOLOGY

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ABSTRACT

The laboratory has a central and distinctive role in the acquisition of hands-on knowledge through the practice of science experiments. The importance of safety measure in every laboratory activity cannot be over emphasized. Safety awareness must be an integral part of every laboratory course. Proper implementation of safety practices in a laboratory is the key to preventing an emergency in the laboratory. A poor awareness of laboratory safety practices may be due to non or improper implementation of safety practices in laboratories. The main aim of the study is to evaluate the level of awareness of safety practices in science laboratories among science laboratory technology students in Yaba College of Technology. There are adapted questionnaires on safety practices and checklist on available safety equipment in science laboratories administered to 102 students. The data collected was analyzed using Statistical Package for Social Sciences (SPPS) version 20. Research questions were analyzed using frequency and percentage. The findings of the study revealed that the required and approved safety equipment were available in the laboratories to ensure safety practices while others were not. The study also suggests that the overall level of awareness of students on laboratory safety practices is moderately good but the safety culture and safety compliance can be further improved to international standard.

Keywords: Safety awareness, safety practices, laboratory, safety culture, safety compliance

INTRODUCTION

The laboratory has been given a central and distinctive role in science education, and science educators have suggested that rich benefits in learning science come as a result of using laboratory activities (Hofstein and Lunetta, 2003). In the Department of Science Laboratory Technology, Yaba College of Technology, there are four basic types of science laboratories, namely; physics laboratory, chemistry laboratory and biology laboratory. The ability of any country to produce well-qualified scientists with a solid background in science requires the implementation of a science curriculum that fosters scientific enquiry and engages students in practical experience meeting the needs of the society throughout all levels of education (Wrightson *et al*., 2008). Laboratory has become a place to explore concepts, develop learning methods, and develop a scientific attitude and skills. However, academic laboratories are associated with the potential exposure to hazardous materials or unhealthy situations and the risk of occurrence of accidents. Therefore, there is a need for researchers, students and laboratory personnel to be aware of the hazards of the materials and processes that they and others in the laboratory are working with and are prepared to take rapid and appropriate measures to protect themselves and their co-workers, especially in the case of unexpected events. Ensuring a safe laboratory environment is the combined responsibility of laboratory personnel, the researchers, students and the management of the institution, though the primary responsibility lies with the individual performing the work. Hayble (1998) presented that to prevent continuous exposure to laboratory hazards and thereby endangering the lives of the scientists and laboratory users, laboratory safety must be regarded as a positive undertaken by every laboratory user.
Laboratory safety must be an integral part of every science curriculum. This means that

safety awareness must be integrated into each laboratory course including research with increasingly broader scope at more advanced levels. The creation of a culture of laboratory safety requires a broad commitment from all levels of the educational institution. Laboratory safety practices include appropriate facilities and equipment, adequate training, personal protective equipment, chemical management, standard operating procedures, waste handling, signage, proper laboratory practices and safe working conditions.

The importance of safety measure in laboratories cannot be over emphasized. Many academic institutions lack a clear, unified vision about what a culture of safety entails. According to Leggett (2012), the accident rate in academic chemical laboratories is about 10-50 times higher than in industrial laboratories. Meyer (2012) presented that academic laboratories are more dangerous than those in industry due to the fact that the approach to safety in academic laboratories is more relaxed. Schröder *et al.* (2016) supported this view by pointing out that academic laboratories do not have a well-established culture of safety.

There have been several high-profile accidents in academic laboratories around the world, resulting in significant injuries and fatalities. Contributing factors to this accident can be identified at multiple levels: the individual, the laboratory, the department, the institution and the discipline itself (Baudendistel, 2009). Ménard and Trant (2020) explained that the contributing factors to laboratory accidents can be conceptualized as occurring at multiple levels: risks associated with the materials or equipment being used, risks related to the skills, knowledge and choices of the research personnel doing the study, characteristics or qualities of the principal investigator and the research laboratory in which the research is occurring and risk factors arising from the departmental or institutional level.

In the study conducted by Edward *et al.* (2001) to discover the types of laboratory accidents in Texas public schools, it was discovered that the major factor associated with laboratory accidents is the class size. It was revealed that as the number of students in science classes' increases, the likelihood of accidents occurring also increases. In the work of Wu, Liu and Lu (2007) on the investigation of the influence of organizational and individual factors on safety climate in university and college laboratories, multivariate analysis of variance revealed that organizational category of ownership, the presence of a safety manager and safety committee, gender, age, title, accident experience, and safety training significantly affected the climate. Among them, accident experience and safety training affected the climate with practical significance.

Good laboratory practices can reduce tremendously the rate of accidents in the laboratories. The current study is aimed to examine the level of awareness of safety practices in science laboratories among students using a case study. The following questions guided this study:

To what extent are safety equipment available in the science laboratories?

To what extent do students implement safety practices in the laboratories?

MATERIALS AND METHODS

Description of study area

Geographically, Yaba College of Technology, Yaba, Lagos, Nigeria State is located in the southwestern part of Nigeria. It is geographically 3.3744^0 Longitude and 6.5187^0 Latitude. Yaba College of Technology has eight schools and thirty-four academic departments with a total of seventy accredited programme, across National Diploma (ND), Higher National Diploma (HND) and Post-HND levels. The College also offers certificate courses.

Population

The population of the study comprised 102 students in the final year national diploma (ND) of Science Laboratory Technology. All demographic information of the respondents was considered irrelevant to the scope of the study and were not collected. Participation was completely voluntary.

Instrument for Data Collection

The data was obtained from primary sources through self-administrated questionnaire. There are questionnaires on safety practices in science laboratories and checklist on available safety materials and equipment in science laboratories. The response pattern for the checklist is: Available (A) and Not Available (NA). The checklist was used to assess the availability of safety equipment/materials in

the science laboratory. After reviewing literature, the scale prepared by Ali *et al*. (2018) was adopted as a proper scale for this present study. The questionnaire consists four components, namely (1) work procedure, (2) safety equipment, (3) handling experiments, and (5) emergency response plan. The response pattern for the questionnaire was structured 5-point scale of Strongly Agreed (SA) = 5, Agreed (A) = 4, Moderate (M) = 3, Disagreed (D) = 2 and Strongly Disagreed (SD) = 1 respectively to elicit information on students' awareness of laboratory safety practices. The reliability of the instrument was determined using Cronbach Alpha, the correlation coefficient was 0.78. Research questions were answered using frequency and percentage.

RESULTS

Table 1. Percentage of available safety equipment and materials in science laboratory

S/N	ITEMS	Frequency	Percentage (%)
1	First Aid kits	80	66.7
2	Fire extinguishers	110	91.7
3	Hand gloves	90	75
4	Displayed safety rules and regulations	70	58.3
5	Fire blanket	0	0.0
6	Sand buckets	70	58.3
7	laboratory coat	120	100.0
8	Safety goggles	65	54.2
9	Fume cupboard/chamber	55	45.8
10	Safety showers	0	0.0

No of respondents = 120

Table 2. Students' awareness on laboratory work procedure

S/N	**ITEM**	**Strong agree/agree (%)**	**Moderate (%)**	**Strong disagree/disagree (%)**
1	The science laboratory safety and guidelines poster is displayed in a place that is easy to see and read.	55.2	24.1	20.7
2	The teacher explains science laboratory safety rules at the beginning of the laboratory session.	65.8	30.0	4.2
3	I adhere to all of the established school science laboratory safety guidelines.	58.5	33.2	8.4
4	I clean the school science laboratory after conducting an experiment.	22.9	50.0	27.1

No of respondents = 120

Table 3. Students' awareness on laboratory safety equipment

S/N.	ITEMS	Strong agree/agree (%)	Moderate (%)	Strong disagree/disagree (%)
1	Wearing of laboratory coats	75.0	25.0	0.0
2	Always handling samples with hand gloves	45.5	54	20.5
3	Wearing of safety goggles as required in the experiment	35.5	38.7	45.8
4	Working fume cupboards are prepared in my school science laboratory	15.8	35.7	68.5

No of respondents = 120

Table 4. Students' awareness on handling of experiments in laboratory

S/N	ITEM	Strong agree/agree (%)	Moderate (%)	Strong disagree/disagree (%)
1	Taking precautions to avoid spills	63.7	45.0	11.3
2	The school science laboratory has guidelines for waste disposal	58.5	35.7	25.8
3	Always checking the expiry date of chemicals and reagents before using them	25.0	45.7	49.3
4	The labelled reagent/chemical bottles contain clear and easily understandable safety information	60.9	45.9	13.2

No of respondents = 120

Table 5. Students' awareness on emergency response plan.

S/N	ITEM	Strong agree/agree (%)	Moderate (%)	Strong disagree/disagree (%)
1	An eye wash station is prepared in the school science laboratory	20.5	34	65.5
2	The locations of the alarm and the exit is clear marked in case of emergency	30	24.5	45.5
3	Poster to operate fire distinguisher is displayed in the laboratory	35.5	16	48.5
4	An emergency aid kit is prepared in my school science laboratory	70.5	14.2	15.3

No of respondents = 120

DISCUSSION

The available item is based on the bench mark of 50% and above. Therefore, from table 1 above, it can be seen that some of the safety equipment were not available, these include fire blanket, fume cupboard and safety showers. The indication is that more safety equipment should be provided in the science laboratories.

Table 2 above indicates that majority students (65.8%) agreed that teachers had explained science laboratory safety rules at

the beginning of the laboratory. Laboratory work procedure plays a vital role in protecting students and those around them from injury, as well as to avoid damage to equipment, and to protect the environment. Students should be sensitized upon entering a science laboratory what can and cannot be done by students when they are in the science laboratory. 55.2% agreed that the laboratory safety and guidelines posters were displayed in a place that was easy to see and read. It could be seen from the results that students' attitude toward cleaning of the laboratory after conducting an experiment was negative. The level of awareness of students on adhering to all of the established school science laboratory safety guidelines should be improved.

Table 3 clearly shows that there was a lack of willingness among the students in handling sample with hand gloves, wearing of safety googles, and usage of fume cupboards. This could be as result of non-availability or inadequate functional safety equipment. Ali *et al.* (2018) pointed out that the failure to prepare adequate safety equipment will place the users of science laboratories at great risk. Their finding concurred with Noorden (2011) and Artdej (2012) that negligence in the usage of safety equipment can cause accidents, or sometimes death. However, the majority (75%) of students had a high level of safety compliance in wearing of laboratory coats.

The findings from this study as shown in table 4 suggests that the majority (63.7%) of students gave positive feedbacks towards taking precautions to avoid spills. 58.5% of students agreed that the school science laboratory has guidelines for waste disposal. 60.9% agreed that the labelled reagent and chemical bottles contain clear and easily understandable safety information. There is a negative feedback towards checking the expiry date of chemicals and reagents before using them.

Emergency response plan is essential in a laboratory to avoid fatalities and reduce injuries during an emergency. Table 5 clearly indicates that students still unfamiliar of the infrastructure and system of the emergency response plan available in their own science laboratories. However, 70.5% agreed that emergency aid kits were installed in their science laboratories. The results suggest that students need to be equipped with emergency training to know the emergency equipment available in their laboratories and how to respond if any incident occurs.

In conclusion, this study examined the level of awareness of safety practices in science laboratories among students. There is a need for improvements to the laboratory safety practices and safety compliance among the students to prevent the occurrence of any serious injury or fatality. In line with the findings of the study, government and institutions should provide funds for purchasing laboratory safety equipment and provide laboratory safety training for lecturers, laboratory personnel and students to equip them with the competences needed to ensure adequate safety in science laboratories. Lecturers and laboratory personnel should promote students' awareness of good laboratory practices.

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REFERENCES

Ali, N.L., Ta, G.C., Zakaria, S.Z.S., Halim, S.A., Mokhtar, M.B., Ern, L.K., Alam, L. (2018). Assessing Awareness on Laboratory Safety: A Case Study in Pahang, Malaysia. Jurnal Pendidikan Malaysia, 43(2)(2018): 73-80.

Artdej, R. (2012). Investigating undergraduate students' scientific understanding of laboratory safety. Procedia Social and Behavioral Sciences 46: 5058-5062.

Baudendistel, B. (2009). Investigation Report University of California, Los Angeles, Case No. S1110-003-09. Department of Industrial Relations, Division of Occupational Safety and Health, Los Angeles.

Edward, J.F., Ali, C.P., James, W.C., Philip, S. (2001). An analysis of laboratory safety in Texas. Texas Univ., Austin. Charles A. Dana Center.

Hayble T.S.M. (1998). Laboratory Resources Utilization, Funding and Management of Public Institution in Nigeria. African Journal of Education. 1998:1(1).

Hofstein, A. & Lunetta, V.N. (2003). The laboratory in science education: Foundation for the twenty-first century. Science Education 88(1): 28-54.

Leggett, D.J. (2012). Lab-HIRA: Hazard identification and risk analysis for the chemical research laboratory part 2. Risk Analysis of Laboratory Operations 19(5): 25-36.

Menard, A.D. and Trant, J.F. (2020). A review and critique of academic lab safety research. Nat. Chem. 12, 17–25 . https://doi.org/10.1038/s41557-019-0375-x.

Meyer A. T. (2012). "How About Safety and Risk Management in Research and Education?" Procedia Engineering 42: 854–864. doi: 10.1016/j.proeng.2012.07.478.

Schröder, I., Huang, D. Y. Q., Ellis, O., Gibson, J. H. & Wayne, N. L. (2016). Laboratory safety attitudes and practices: A comparison of academic, government, and industry researchers. J. Chem. Health Saf. 23, 12–23.

Van Noorden, R. A. (2011). Death in the lab - Fatality adds further momentum to calls for a shake-up in academic safety culture. Nature, 472 (7343), 270−271.

Wrightson, I., S. J. Cooper, M. Crookes, C. L. Grundy, N. King, J. Larner, P. Lewis, D. H. Lohmann, C. Maxwell, D. Perry, M. Sanderson and S. Lipworth (2008). Environment, Health and Safety Committee Note on: Health and Safety in theTeaching of Practical Chemistry in Schools. Version 2. London, UK, Royal Society of Chemistry.

Wu, T.C., Liu, C.W. & Lu, M.C. (2007). Safety climate in university and college laboratories: Impact of organizational and individual factors. Journal of Chemical Health and Safety, Volume 38, Issue 1, Pages 91-102 .

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MEASUREMENT OF INDOOR IONISING RADIATION LEVELS WITHIN THE X-RAY BUILDINGS OF FOUR GENERAL HOSPITALS IN LAGOS STATE

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ABSTRACT

Radiation levels associated with radiological procedures in hospitals have come under increasing scrutiny, requiring quantitative monitoring and accurate measurements. In this work, the radiation dose levels within and around the x-ray rooms of four General Hospitals in Lagos, South -West Nigeria were determined using two calibrated handheld radiation survey meters (PRM-9000), preset to take minimum, maximum and mean dose rates measurements for five minutes. The mean dose rates for both background radiation as well as when the x-ray machine is in use were recorded inside five or six different locations within the x-ray buildings of all the hospitals. Background dose rates in the sampled locations range between 0.077 - 0.140µSv/hr, 0.093 – 0.123 µSv/hr, 0.082 -0.116 µSv/hr and 0.092 -0.118 µSv/hr in Gbagada, Lagos Island, Isolo and Igando General Hospitals respectively. The mean annual effective dose (mSv) ranged between 0.53 – 1.96, 0.28 -0.41, 0.24 -0.31, 0.23 – 0.31, 0.23 - 0.28 and 0.24 -0.37 inside the x-ray room, the x-ray room entrance, patients' waiting room, reception, dark room and main entrance respectively in all the hospitals, when the x-ray machine is in use. These values were within the radiation safe limit to the public and radiation health workers thereby putting no radiological risk to them.

Keywords: X-Rays, Exposure, Radio-diagnosis, Effective Dose, General Hospital

INTRODUCTION

The use of radiation in medicine is the largest, and a growing, man-made source of exposure to ionizing radiation (Ribeiro *et al*., 2020). In particular, X-rays have maintained a key role in diagnosis of diseases, injury and in X-ray therapy, thus contributing largely to the effective dose of both patients and personnel. The latest United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) estimates suggest that there are about 4 billion X-ray examinations per year, worldwide (UNSCEAR, 2008). Ionizing radiation emanating during usage of radioactive facilities in hospitals and medical research institutes have been of great concern because of the detrimental effects of high exposure. Exposure to radiographic examination (computerized tomography, fluoroscopic procedures, dental diagnosis, and routine exposure to x-rays), radioisotope procedures and radiation therapy have contributed to increase in background radiation and radiation levels of patients and many occupational workers.

In Nigeria, several X-ray machines which are regularly employed for radio-diagnosis are housed in brick buildings or else in cement-plastered buildings. The effectiveness of these building materials in shielding energy radiations is not too certain. There exists the possibility of leakages of harmful radiations to the environment whenever the machine is in use, the extent of exposure being a function of the intensity and energy of the emitted radiations and the frequency of usage of the machine (Hassan et. al, 2012; Sharma, 2015). This can be a serious problem to both health workers and the public around that area.Hence there is a need for the

measurements of radiation around within such vicinities, especially when the machine is in use. Due to the radiological risks involved, it is usually recommended that dose to patient from x-ray be kept as low as reasonably achievable (ALARA) with adequate image quality (IAEA, 1996).

In this study, the indoor and outdoor radiation dose owing to the use of x-ray machine in selected general hospitals in Lagos state were measured in order to determine radiation dose being received by both patients and health personnel following the continual exposure to emission from the x-ray machines.

2.0 METHODOLOGY

The study was carried out at the department of Radiology of General hospitals at Gbagada, Isolo, Igando and Lagos Island in Lagos state. The specific locations where measurements were carried out in each hospital included the x-ray room, x-ray room entrance, patients' waiting room, reception, dark room, and the main entrance to the x-ray building.

Two calibrated handheld radiation survey meters (PRM-9000), preset to take minimum, maximum and mean dose rates measurements for five minutes were used for 5days in the sample locations in each of the hospitals between 8.00am and 9.30am when it is expected that there would be a high demand and usage of the x-ray machine. The PRM-9000 is suitable for regulatory inspections, and for the detection, measurement and monitoring of broad spectrum, low energy radionuclides, including Naturally Occurring Radioactive Material (NORM). They were calibrated electronically using a pulse generator "relative" to CS -137, with a maximum error that did not exceed 5%.

The background radiations in the designated points within each hospital, including the x-ray room were first measured when the machine was OFF; afterwards the measurements were repeated when the machine was ON.

3.0 RESULTS AND DISCUSSION

The measured mean dose rates for each of five days at the various locations within the x-ray buildings of the four general hospitals have been tabulated in table 1.

Background dose rates in the sampled locations range between 0.077 -0.140µSv/hr, 0.093 – 0.123 µSv/hr, 0.082 -0.116 µSv/hr and 0.092 - 0.118 µSv/hr in Gbagada, Lagos Island, Isolo and Igando General Hospitals respectively. The background values are lower inside the x-ray room of all but Isolo General Hospital, compared to at least one of the other samples locations outside the x-ray rooms. In particular, all the sampled locations in Gbagada and Igando General Hospitals have background dose rates that were higher outside the x-ray room than inside it. At Lagos Island General Hospital, it is only at the reception that background dose rate is slightly higher than inside the x-ray room. This shows in general that within these three hospitals, possible extraneous background radiations from natural or artificial sources outside the x-ray room are attenuated before getting inside the x-ray room. The highest mean background dose rates of 0.140, 0.123, 0.116 and 0.118 µSv/hr obtained respectively within Gbagada, Lagos Island, Isolo and Igando Island General Hospitals compare favourably with those reported for the Radiology Department FMC Asaba Delta State, Nigeria (Abubakar et al., 2017), General Hospital, Abuja (James et al., 2015) and Braithwaite memorial specialist Hospital, Port Harcourt (Okoye and Avwiri, 2013).

Table 1: Mean Dose Rate (μSv/hr) when machine is ON and OFF

S/N	Gbagada		Lagos Island		Isolo		Igando	
	Machine (OFF)	Machine (ON)	Machine (OFF)	Machine (ON	Machine (OFF)	Machine (ON)	Machine (OFF)	Machine (ON)
X-ray Room	0.077	0.532	0.114	0.729	0.116	0.198	0.092	0.378
Entrance of X-ray Room	0.093	0.153	0.106	0.103	0.086	0.144	0.094	0.122
Patient Waiting Room	0.093	0.091	0.100	0.103	0.094	0.088	0.102	0.114
Reception	0.088	0.085	0.123	0.114	N/A	N/A	N/A	N/A
Dark Room	0.085	0.085	0.103	0.104	0.11	0.096	0.096	0.106
Main Entrance	0.140	0.1386	0.0926	0.104	0.082	0.088	0.118	0.116

Figures 1-4 show comparisons between the background dose rates (when the x-ray machine is OFF) and the dose rate values when the machine is ON. At Gbagada General Hospital, the dose rate when the machine is ON is higher than the background dose rates in the x-ray room and at its entrance. The same is observed at Isolo and Igando General Hospitals. This is expected since the x-ray machine is the major source of and contributor to the radiation level within that environment at that instance. The proximity of the entrance of the x-ray room to where the x-ray machine is installed and the fact that the door demarcating the two points is being opened frequently would make the dose rate comparison at the entrance not too different from what is observed inside the x-ray room. At Lagos Island General Hospital, the background dose rate is approximately same as the dose rate when the x-ray machine is in use. This shows a very good containment of the radiation being emitted from the x-ray machine.

3.1 Mean annual effective dose

The mean annual effective dose was calculated following the hourly dose rates measured at the various locations and assuming that a worker spends averagely 8 hours in the hospital each day.

Annual effective dose =
Hourly dose rate X 8hours X 336*

….(1) (Olubosede *et al*., 2012)

(*336 days =48 weeks)

The result is depicted in figure 5

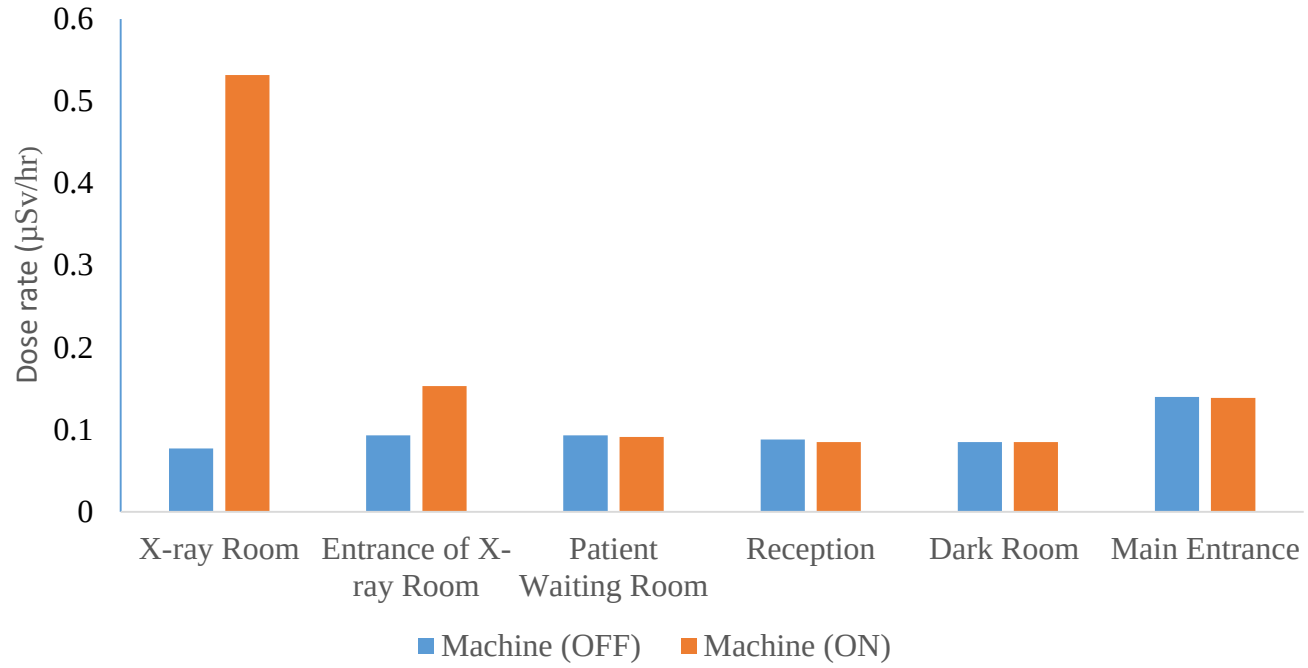

Figure 1: Dose rate measurements at Gbagada General Hospital

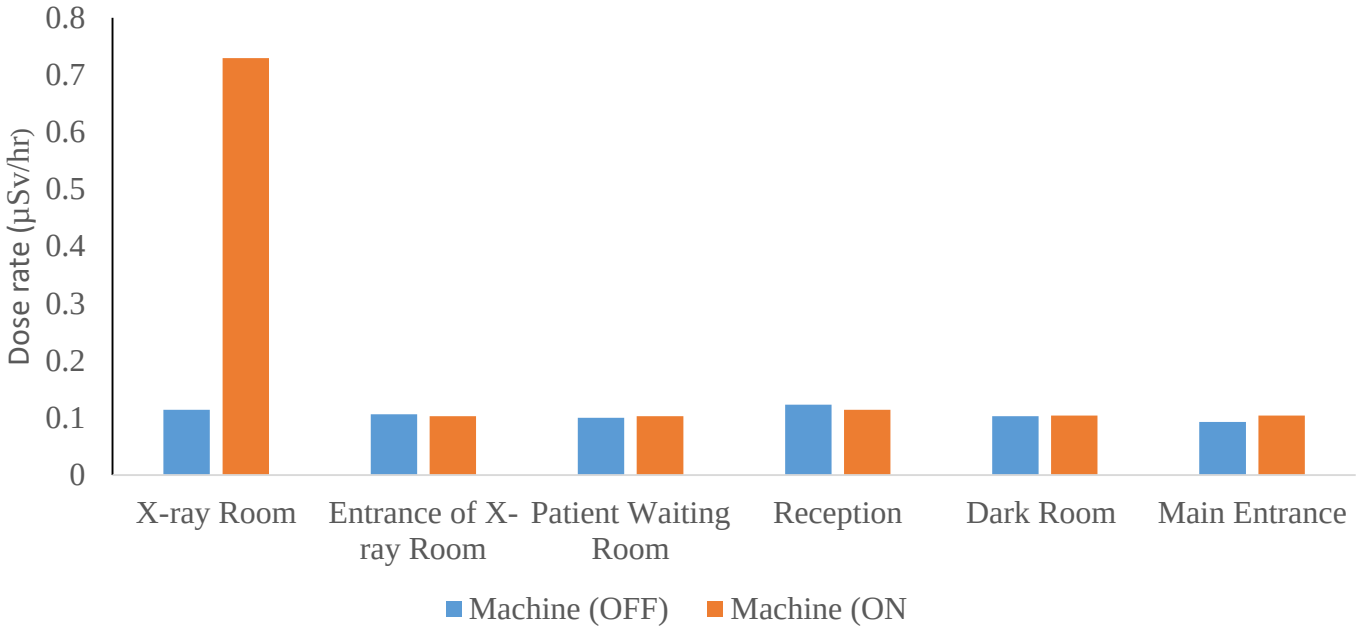

Figure 2: Dose rate measurements at Lagos Island General Hospital

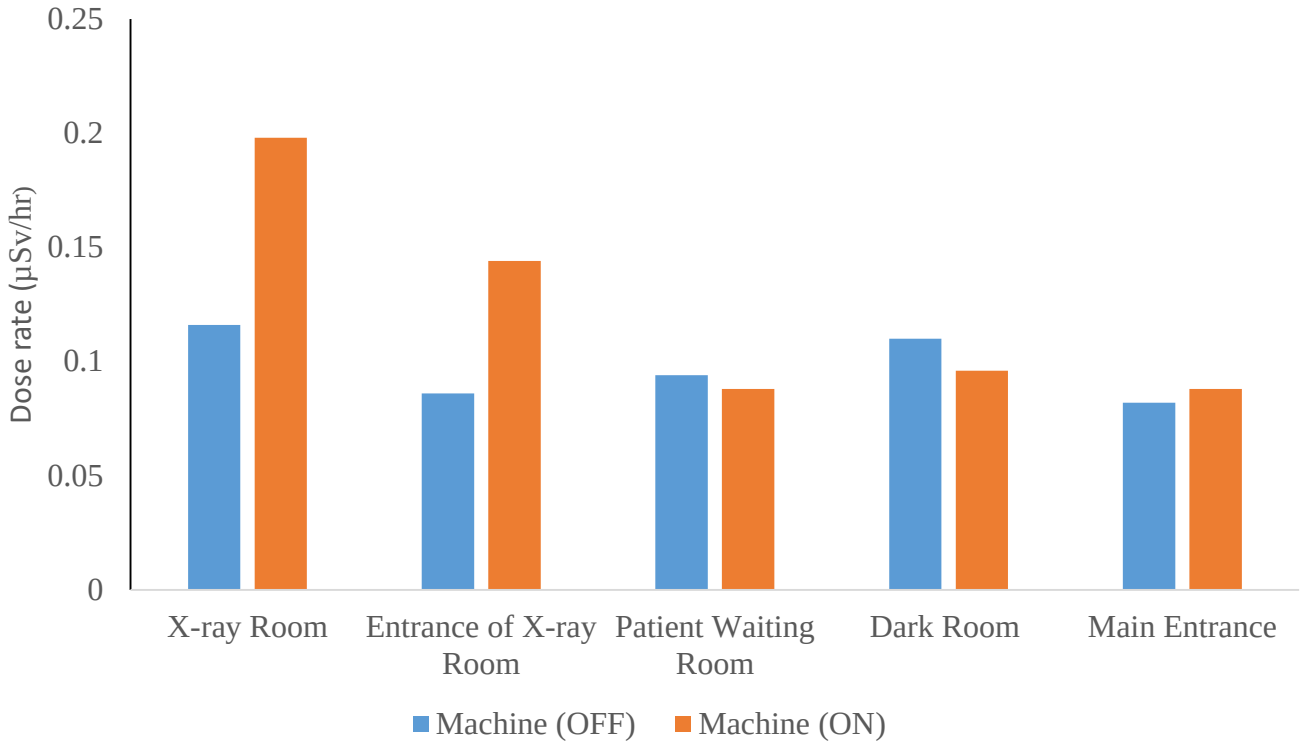

Figure 3: Dose rate measurements at Isolo General Hospital

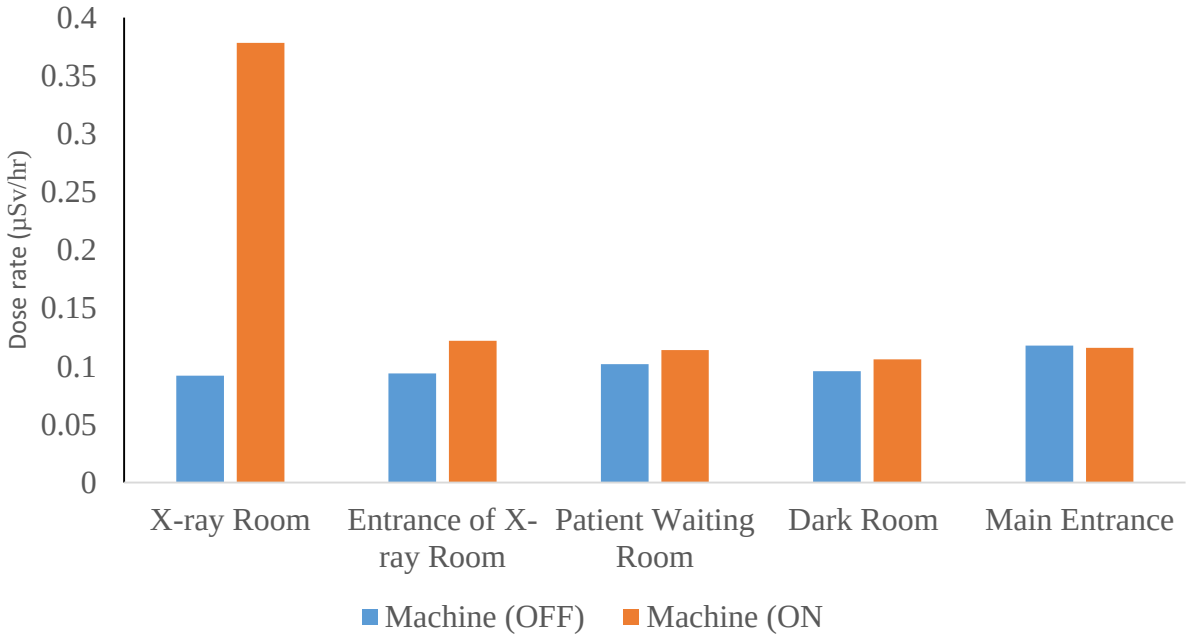

Figure 4: Dose rate measurements at Igando General Hospital

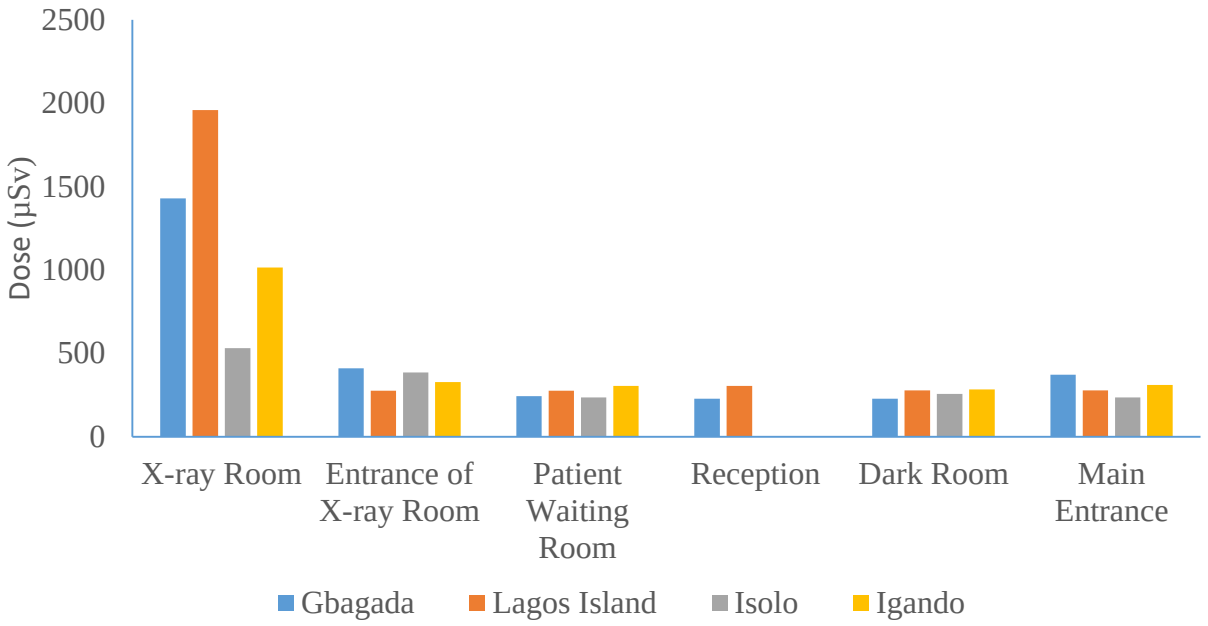

Figure 5: Effective doses when the x-ray machine is ON

The mean dose values in the four hospitals were within the range of 0.53 – 1.96 mSv inside the x-ray rooms, 0.28 -0.41 mSv at the x-ray room entrances, 0.24 -0.31 mSv at the patients' waiting room, 0.24 -0.37 mSv at Main Entrance. The noticeable variations inside the x-ray rooms in the four hospitals when the x-ray machine is in use could be as a result of the type of x-ray generator, the energy of the x-ray machines used and the frequency of usage of x-ray machine (Sharma, 2015; Hassan et. al, 2012). The effective doses in the hospitals are much lower than those obtained in Haval Zaroka hospital, Iraq (Dindar *et. al*., 2015).

The fraction of the dose level to which the public is exposed to outside the x-ray room compared to the dose level inside the x-ray room when the machine is in use is an indication of the extent of containment of the ionizing radiation inside the x-ray room. This is shown in table 2.

Table 2: Containment of x-ray radiation within the hospital environment (expressed in %)

Hospital	x-ray room entrance	Patients' waiting room	Reception	Dark room	Main entrance
Gbagada	28.76	17.11	15.98	15.98	26.05
Lagos Island	14.13	14.13	15.64	14.27	14.27
Isolo	72.73	44.44	N/A	48.48	44.44
Igando	32.28	30.16	N/A	28.04	30.69

N/A –Not available

Clearly, the best containment of the x-radiation is seen in Lagos Island General Hospital, with lowest possible values recorded in all the sample points outside the x-ray room compared to what would have been emanating from the inside of the x-ray room. The least containment of the x-radiation is seen at Isolo General Hospital, which at the same time has the lowest exposure level

inside its x-ray room when compared to the exposure levels inside the x-ray rooms of the other hospitals (figure 5).

The international commission for radiation protection (ICRP) set the annual limit of exposure to ionizing radiation to be 1mSv and 20mSv (ICRP,1990) respectively for the public and radiation workers. Inside the x-ray room, the 1 mSv dose limit to the public was exceeded at Gbagada and Lagos Island General hospitals, but this is of no serious threat to the public, since the x-ray room is rarely being frequented by the public. At Igando General Hospital, the effective dose is approximately same as the recommended safe limit to the public. The mean annual effective dose at the reception, patients' waiting room and main entrance to the x-ray buildings in all the hospitals sampled where the public readily have access to, is well below 1 mSv, thus putting the public and radiation workers in the hospitals at no radiological risks.

CONCLUSION

The radiation dose levels within four General Hospitals in Lagos State have been computed following the measurement of the dose rates at specific locations inside the x-ray buildings. Exposure to radiation x-ray emission is greatest inside the x-ray room at Lagos Island General Hospital and least at Isolo General Hospital. A good containment of the radiations emitted from the x-ray rooms exists in all the sampled General hospitals but most especially at Lagos Island General Hospital. The exposure to ionizing radiation from x-ray emission poses no radiation health risk to both workers and the public as mean annual dose were below the I.C.R.P. regulatory limit.

Notwithstanding the present radiological safety associated with the use of the x-ray machines in the hospitals where this research was conducted, there is still need for periodical monitoring of environmental dose level around the hospitals so that the recommended limit of exposure is not exceeded at any time.

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REFERENCES

Abubakar A., Sadiq A A., Musa M.G., Hassan J., Malgwi DF., (2017). Assessment of Indoor Ionizing Radiation Profile in Radiology Department FMC Asaba Delta State, Nigeria. *Journal of Dental and Medical Sciences*, ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 16, Issue 1

Dindar S. Bari, Pshtiwan M. Amin, Nawzad A. Abdulkareem (2015) Measurement of the Effective Dose Radiation at Radiology Departments of Some Hospitals in Duhok Governorate. Journal of Modern Physics, 2015, 6, 566-572

Hassan G.M., Rabie N., Mustafa K.A. & Abdel-Khalik S.S. (2012) Study on the quality assurance of diagnostic X-ray machines and assessment of the absorbed dose to patients, Radiation Effects and Defects in Solids, 167:9, 704 711, DOI: 10.1080/10420150.2011.559238

International Atomic Energy Agency, (1996). International basic safety standards for protection against ionizing radiation and for the safety of radiation sources. Safety series no. 115

International Commission on Radiological Protection (1990). Recommendations of the International Commission on Radiological Protection. Annals of the ICRP 21(1-3). ICRP Publication 60.Pergamon Press, Oxford, 1991

James, I. U., Moses, I.F, Vandi, J.N, Ikoh U.E Measurement of Indoor and Outdoor Background Ionising Radiation Levels of Kwali General Hospital, AbujaJ. Appl. Sci. Environ. Manage. *March*, 2015 Vol 19(1) 89-93

Okoye, P. C. and Avwiri, G. O (2013). Evaluation of background ionising radiation levels of Braithwaite Memorial Specialist Hospital Port Harcourt, Rivers State. Am. J. Sci. Ind. Res. 4(4): 359-365.

Olubosede O., Akinnagbe O and Adekoya O. (2012) Assessment of Radiation Emission from Waste Dumpsites in Lagos State of Nigeria. *International Journal of Computational Engineering Research 2(3): 806-811*

Ribeiro A., Husson O., Drey N., Murray I., May K., Thurston J. and Oyen W. (2020). Ionising radiation exposure from medical imaging – A review of Patient's (un) awareness. *Radiography 26(2): e25 -e30*

Sharma R. (2015) Radiation dose to patients from X-ray radiographic examinations using computed radiography imaging system. *Journal of Medical Physics 40(1):29-37*

United Nations Scientific Committee on the Effects of Atomic Radiation, UNSCEAR (2008). Effects of Ionizing Radiation. Volume I: Report to the General Assembly, Scientific Annexes A and B; Volume II: Scientific Annexes C, D and E., New York.

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IONISING RADIATION MEASUREMENTS FROM ACTIVE WASTE DUMPSITES IN LAGOS STATE

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ABSTRACT

Improper management of wastes is a leading cause of environmental pollution and degradation in many cities, especially in developing countries. It could also result in the destruction of the ecosystem. In this work, the radiation levels at Epe, Ikorodu, Solus 2, Solus 3 and Olusosun dumpsites were investigated in line with proper waste management guidelines, using a radiation survey meter PRM 9000. The instrument was calibrated electronically using a pulse generator relative to Cs-137 with permissible error of 5%. The radiation survey meter was set for 10 minutes and readings were taken by placing it at gonad level i.e. about 1 m above the ground for each dumpsite. Measurements were done at an interval of 15 m away from the point source up to 150 m for eleven readings. The dose rates (µSv/hr) range between 0.008 and 0.010 at the various dumpsites and between 0.040 and 0.140 at a distance of 150 m away from the dumpsites. The annual dose equivalents for the various locations were seen not to exceed the recommended International Commission for Radiation Protection (ICRP) limit for both radiation workers and the public. The results indicate that the radiations emanating from the dumpsites pose minimal health risks to the waste management workers at the site and the public within these vicinities.

Keywords: Wastes, Dumpsites, Active, Equivalent Dose, Radiation

1.0 INTRODUCTION

There has been increasing global concern over the public health impacts attributed to environmental pollution (Briggs, 2003; Remoundou and Koundouri, 2009). The 2006 news release by World Health Organization (WHO), estimates that about a quarter of the diseases facing mankind today occur due to prolonged exposure to environmental pollution (WHO,2006). Most of these environment-related diseases are however not easily detected and may be acquired during childhood and manifested later in adulthood.

Pollution, according to the European Public Health Alliance, (EPHA, 2009) is described as the harm that results because of the presence of a substance(s) where they would not normally be found or because they are present in larger than normal quantities. Solid waste could be infectious, toxic or radioactive (Onibokun and Kumuyi, 1996; Wong *et al*., 2003; UNDP, 2006; Kimani, 2007). According to World Nuclear Association, (2015), in solid waste, low level radioactive waste is generated from hospitals and industry, as well as the nuclear fuel cycle. Low-level wastes include paper, rags, tools, clothing, filters, and other materials which contain small amounts of mostly short-lived radioactivity. Improper management of such wastes is a main cause of environmental pollution and degradation in many cities, especially in developing countries as well as the destruction of the ecosystem (Navarro and Vincenzo, 2019). Poor waste management poses a great challenge to the well-being of city residents, particularly those living adjacent the dumpsites due to the potential of the waste to pollute water, food sources, land, air and vegetation.

In Lagos, for instance a number of waste dumpsites are located in the city, into which uncontrolled wastes and refuse from industries, health institutions, homes, etc. are continuously dumped. Several residences and make-shift eateries are situated around these dumpsites thus exposing the people to the risks of contamination by inhalation or ingestion of the pollutants, some of which could be radioactive. Some of such wastes are air-borne and could be

deposited on exposed water bodies, displayed edible wares as well as on farm crops grown on lands in these areas. The health implication due to such continuous exposure could be very fatal to the local people residing around these dumpsites.

In order to assess the extent of radiation exposure of both waste management workers and the locals around such dumpsites, there is the need to determine the effective doses due ionizing radiation from such dumpsites.

2.0 METHODOLOGY

The study was carried out in five active dumpsites in Lagos state, Nigeria in 2016.

2.1 STUDY AREA

Lagos state lies within latitude 6º and 35ºN and longitude 3º and 45ºE, with population of about 17,553,924. It covers an area of approximately 3,475.1km^2 (Olubosede *et al.*, 2012). The state is divided into five administrative divisions which are further divided into 16 local government areas.

The sampling areas where the dumpsites are located are Solus 2 & 3 in Igando, Ewu-elepe in Ikorodu, Epe and Olusosun, in Ojota as depicted in figure 1.

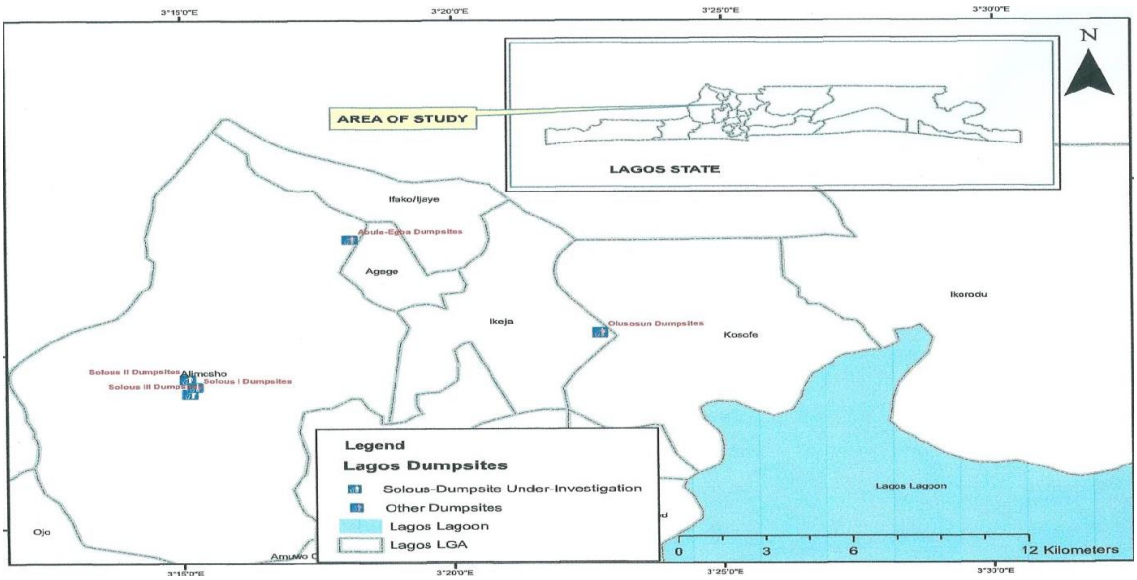

Figure 1: Spatial distributions of major dumpsites in Lagos State

2.2 INSTRUMENTATION

A PRM-9000 Handheld Alpha, Beta, Gamma, X-Ray Geiger Counter - Nuclear Radiation Contamination Detector & Monitor was used in measuring the ionizing radiation being released from the dumpsite and around it. The instrument is suitable for regulatory inspections, and for the detection, measurement and monitoring of broad spectrum, low energy radionuclides, including Naturally Occurring Radioactive Material (NORM). It was calibrated electronically using a pulse generator "relative" to CS -137, with a maximum error that did not exceed 5%.

The instrument is designed to continuously collect measurements and statistics enabling complete environment assessment. This nuclear detector not only records the maximum radiation measured, but also displays the time and date at which the maximum reading occurred.

2.3 MEASUREMENT TECHNIQUE

The PRM-9000 handheld digital radiation meter was held at gonad level of 1.0m above the ground and directed to the dumpsite point source to measure the equivalent dose rate in air. It was preset to take measurements for ten minutes at

each of the sampled points. Afterwards, other measurements at ten other points each separated by a distance of approximately 15 metres up to a total distance of 150 metres away from each dumpsite was done. The measurements were taken twice at each location points and the mean values determined. The mean dose equivalent rates obtained with the PRM-900 survey meter was used in calculating the annual equivalent dose being received by individuals living around the dumpsite areas using equation 1 (Marilyn and Maguire, 1995)

$$D = \sigma \times \mu \times 24 \times 365.25 \text{ (mSv/yr)} \quad (1)$$

where D is the annual equivalent dose rate in micro sievert per year, σ=equivalent dose rate in micro sievert per hour and μ, the occupancy factor is 0.2.

3.0 RESULTS AND DISCUSSIONS

The dose rate measurements at the dumpsites and its vicinities are shown in table 1. The highest dose rates were recorded at the dumpsites at Epe, Ikorodu, Solus 2 and Solus 3. This is expected, since the source of the ionizing radiations being determined are the dumpsites themselves. At Olososun, it was observed that from a distance of about 45 m away from the dumpsite, the measured dose rates increased steadily. A diesel sales point is cited around the area which might have contributed to the enhanced level of radiation within that vicinity thereby resulting in the higher dose rate measurements in comparison to the measured dose rate at the dumpsite. The dose rates measured at Solus 2 and 3 in the present study are higher than those reported for the same dumpsites by Olubosede *et al*., (2012) by a factor of 4.

The annual equivalent dose is depicted in figure 2. An annual radiation dose of 0.14 mSv was noticed at Epe, Ikorodu, Solus 2 and Solus 3 dumpsites, implying that exposure level to ionizing radiation at the four dumpsites is approximately same. At Soulus 2 & 3, the associated risk with exposure to radiation from the dumpsite is fairly constant up to a distance of about 75meters away from the dumpsite. This implies that residential apartments situated within that range have radiation exposure levels that are about same as what obtains at the dumpsites, particularly via inhalation. Radiation exposure level is also same from a distance of 15m from the dumpsites to about 75m and 105 m respectively at Epe and Ikorodu. This however does not call for serious concerns to the public as there are no residential apartments situated within those distances from the dumpsites.

At Olososun, the impact of environmental ionizing radiation is more beyond the dumpsite than it is at the dumpsite. The computed annual equivalent doses from about 45m beyond the dumpsite are higher than the 0.18 mSv obtained at the dumpsite. The radiation wastes from the dumpsite could not have contributed significantly to the annual dose recorded at various points away from

Table 1. Dose rate measurements at dumpsites

	DOSE RATE (μSv/hr)				
DISTANCE (m)	**Epe**	**Ikorodu**	**Soulus 2**	**Soulus 3**	**Olososun**
0	0.080	0.080	0.080	0.080	0.100
15	0.070	0.080	0.080	0.080	0.080
30	0.070	0.080	0.080	0.080	0.080
45	0.070	0.080	0.080	0.080	0.110
60	0.070	0.070	0.080	0.080	0.150
75	0.070	0.080	0.080	0.080	0.150
90	0.060	0.080	0.080	0.070	0.160
105	0.060	0.080	0.070	0.070	0.180
120	0.050	0.070	0.070	0.070	0.140
135	0.050	0.060	0.070	0.070	0.140
150	0.040	0.060	0.070	0.080	0.140

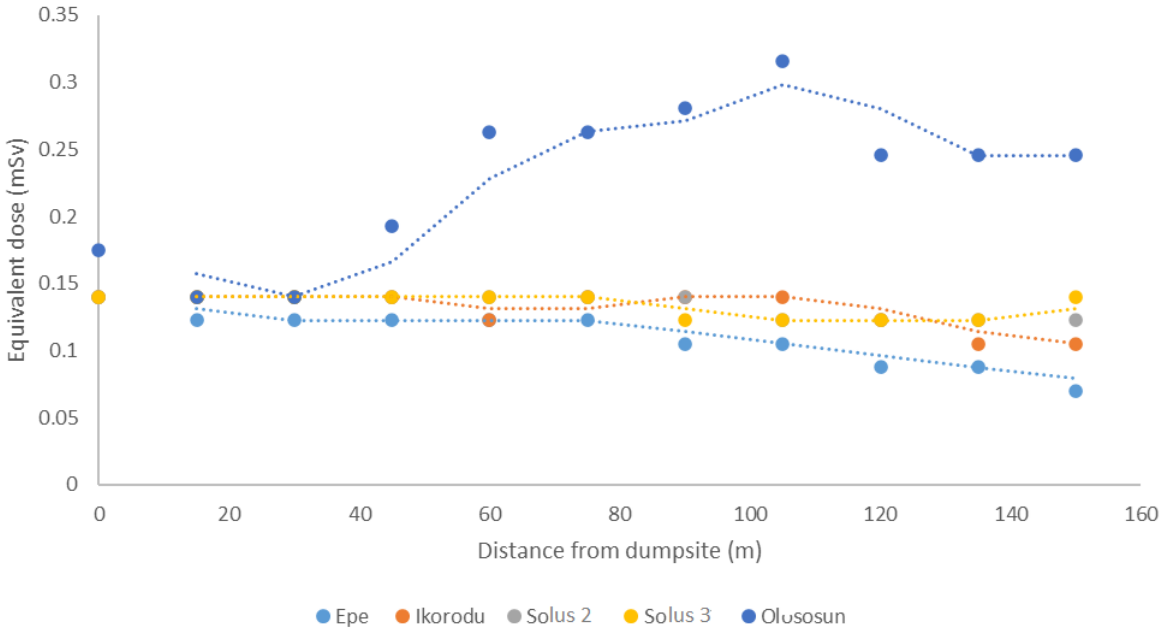

Figure 2: Annual dose measurements at the dumpsite

the dumpsite. The least annual equivalent doses of 0.07, 0.11 and 0.12 mSv are recorded at the farthest distance from the dumpsites at Epe, Ikorodu and Solus 2 respectively.

The dose values obtained in all the dumpsites are higher than the world's average of 0.07 mSv (UNSCEAR 2000; Ademola, 2008) and those reported in some selected dumpsites at Abeokuta (Odunaike *et al.*, 2009), but lower than those reported at dumpsites in Ota and its environs (Usikalu *et al*., 2017).

The International Commission for Radiation Protection set recommended safe limit of 1mSv radiation dose to the public (ICRP, 2007). This value is seen not to be exceeded in all the dumpsites and their immediate vicinities.

The annual dose equivalents in the vicinity of five active waste dumpsites in Lagos State have been determined, following the measurements of the dose rates at several points around the dumpsites. The results indicate that the radiations emanating from the dumpsites pose minimal radiation health risks both to the waste management workers at the site and the public living within the areas. There is still need however for continuous monitoring of the level of radiation in these dumpsites as continuous exposure even to low levels of radiation over an extended period of time can be carcinogenic (Gofman, 1990). It is also advisable for waste management workers to be placed on shift duties so that their levels of exposure to radiation from these dumpsites are minimized.

REFERENCES

Ademola J.A (2008) Exposure to high background radiation level in the tin mining area of Jos Plateau, Nigeria *J. Radiol. Protect, 28 (2008), pp. 93-99*

David B. (2003) Environmental pollution and the global burden of disease. *British Medical Bulletin, 68(1): 1-24*

European health report 2009: health and health systems. http://www.euro.who.int/data/assets/pdf_file/0009/82386/E93103.pdf Accessed on 28-11-19

Gofman J. W. (1990). Radiation- induced cancer from Low Dose Exposure: An independent analysis. 1st edition. Committee for social responsibility, Inc. CNR Book Division.

P.O. Box 11207 San Francisco California 94101 USA

International Commission on Radiological Protection (2007). The system of radiological protection of humans. Ann ICRP. 37 (2–4):81–101.

Kimani, N.G. (2007). Implications of the Dandora Municipal Dumping Site in Nairobi, Kenya. Environmental Pollution and Impacts on Public Health, Kenya: United Nations Environment Programme (UNEP) report

Marilyn, E.N. and G.Q. Maguire Jr., (1995). Radiation Protection in the Health Sciences. 1st Edn. World Scientific Publishing, Singapore, pp: 296.

Navarro F. and Vincenzo T. (2019). Waste Mismanagement in Developing Countries: A Review of Global Issues. *Int J Environ Res Public Health. 16(6): 1060.*

Odunaike R.K., Alausa S.K., Fasunwon O.O., Orunsolu B.A, Ijeoma G.C. (2009) Radiation dose survey of refuse dumpsites in Abeokuta in Ogun state in the Southwestern zone of Nigeria *Res. J. Environ. Sci., 3 (2), pp. 262-266*

Olubosede. O, Akinnagbe .O.B., Adekoya O (2012) Assessment of Radiation Emission from Waste Dumpsites in Lagos State of Nigeria. *International Journal Of Computational Engineering Research Vol. 2 | Issue No.3 pp 806-811*

Onibokun A.G and Kumuyi A.J. (1996) Urban poverty in Nigeria: towards sustainable strategies for its alleviation. Centre for African Settlement Studies and Development, Ibadan, Nigeria. CASSAD Monograph Series.

Remoundou K. and Koundouri P. (2009). Environmental Effects on Public Health: An Economic Perspective. *Int J Environ Res Public Health 6(8): 2160–2178.*

UNDP 2006. Practical Action. Technology Challenging Poverty. United Nation Development Programme Report.

United Nation Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) Sources and Effects of Ionizing radiation. Report of the United Nations Scientific Committee On the Effects of Atomic Radiation to the General Assembly United Nations, New York (2000)

Usikalu M.R., Ola O.O., Achuka J.A., Babarimisa I.O and Ayara W.A Background radiation dose of dumpsites in Ota and Environs *J. Phys.: Conf. Ser. 852 012045*

WHO,2006. 'Preventing disease through healthy environments - towards an estimate of the environmental burden of disease' https://www.who.int/mediacentre/news/releases/2006/pr32/en/ Accessed on 09/10/2020

Wong, C.S.C., Li, X.D., Zhang, G., Qi, S.H., Peng, X.Z (2003). Atmospheric depositions of heavy metals in the Pearl River Delta, China. *Atmosphere and Environment, 37, 767-7*

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RADIONUCLIDES CONCENTRATION IN SELECTED VEGETABLES FROM FARMS IN ODOGUNYAN AND MARYLAND AREAS OF LAGOS STATE

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ABSTRACT

Radionuclides such as Uranium-238, Thorium-232 and Potassium-40 and their isotopes which are the products of natural radioactivity decay series are regarded as naturally occurring radioactive materials (NORMS). The radionuclide concentration of six vegetable samples selected from farms Maryland and Odogunyan was determined using a well calibrated Nal(TI) and well shielded detector coupled to a computer resident quantum MCA2100R Multichannel analyzer for 36000s. The radionuclide concentration of ^{40}K, ^{238}U and ^{232}Th varies is 238.16±6.68 Bq/kg, 12.39±0.72 Bq/kg and 10.57±0.02 Bq/kg respectively. The total annual effective dose due to was 1.313±0.285 µSv/yr and 1.359±0.464 µSv/yr in Odogunyan and Ikorodu respectively. The estimated Excess Life Cancer Risk ranges from 4.59×10^{-6} to 5.21×10^{-6} which is much less than the world's average value of 0.29×10^{-6}. There is no radiological hazard as a result of consumption of vegetables from these two locations because the mean effective dose values are lower than 0.3 mSv/yr recommended by International Commission on Radiation Protection.

KEYWORDS: Vegetables, Isotopes, Radionuclides, Effective Dose

1.0 INTRODUCTION

Human exposure to radiation from natural sources is an unending part and unpreventable phenomenon on earth. The amount of radiation exposure to human originates from artificial and natural sources. The two main contributors to natural radiation exposures are: high-speed cosmic ray particles incidents in the earth's atmosphere and the primordial radionuclides present in the earth's crust which are present everywhere, including the human body. Naturally occurring radioactive materials have always been a part of our daily life and still remains so. These are radionuclides and their isotopes which are the products of natural radioactivity decay series. The series naturally occurring radionuclide found in food chain are Uranium-238 (^{238}U), Thorium-232 (^{232}Th) and their progenies while the non-series one is the potassium-40. The main elements that contribute to natural terrestrial radioactivity are Uranium-238 (^{238}U), Thorium-232 (^{232}Th) and Potassium-40 (^{40}K) (UNSCEAR, 1993). These naturally occurring radionuclides and their daughters undergo decay to release gamma rays, alpha and beta particles into the environment (UNSCEAR, 1993a). The ingested radionuclides could be concentrated in certain parts of the body. For example ^{238}U accumulated in human kidney and lungs, ^{232}Th in liver, skeleton tissue and lungs and ^{40}K in muscles (Malik, *et. al.*, 2020).

Soil-to-plant-to-human body is one of the foremost corridors for transmission of radionuclides to human being (Aswood *et. al.*, 2013). After uptake by root, radionuclides are shifted to plant along with other nutrients or minerals necessary for their growth; accumulate in several parts including the edible portions and would lead to endless radiation dose to man once consumed (Pulhani *et. al.*, 2005). The accumulation of these radionuclides in any organ in the body will affect the health condition which may result to inducing various forms of diseases, weakening the immune system and also contributing to increase in mortality rate. Radionuclides such as K-40, Ra-226 that occur naturally in soil are incorporated metabolically into plants and ultimately find their way into food and water. Potassium is normally distributed in the earth's crust and present in all

environmental media including foodstuffs and even in the human body. Under normal conditions, K-40 is the most abundant naturally occurring radioactive constituents within the human body (Asaduzzaman *et. al.,* 2015). Particularly K-40, U-238 and Th-232 and their various decay products are the most common radionuclides in food and water (Asaduzzaman *et. al.*, 2015). These radionuclides appear in plants either through direct atmospheric interception onto aerial parts of the plant surfaces or from there suspended material and absorbed metabolically by the plant surfaces and indirectly through the uptake of radionuclides from soil via the root system (Asaduzzaman *et. al.*, 2015).

Preliminary investigation of naturally occurring radionuclides in some traditional medicinal plants used in Nigeria for the treatment of some diseases by Njinga *et. al.*,(2015) revealed that the average annual committed effective doses due to the ingestion of Ra-226, Th-232 and K-40 from the plants by the consumers ranged from 0.00426 mSv/yr to 0.00686 mSv/yr with an average value of0.00538 mSv/yr which is below the worldwide provided in UNSCEAR 2000 report indicating that the consumers are radiologically safe.

Vegetables are defined as the fresh parts of plants which, either raw, cooked, canned or processed in some other way, provide suitable human nutrition. From a botanical point of view, vegetables can be divided into algae (seaweed), mushrooms, root vegetables (carrots), tubers (potatoes, yams), bulbs and stem or stalk (kohlrabi, parsley), leafy (spinach), inflorescence (broccoli), seed (green peas) and fruit (tomato) vegetables. Vegetables are a blessing for a safe and healthy life and have been in use for centuries. Production and consumption of fruit and vegetables and make them more economically accessible to consumers, while generating economic, social and environmental benefits in line with the Sustainable Development Goals. Determination of the concentration of the radioactive material in vegetables is of primary importance to ascertain the level of health risk to humans that consume the vegetable in the area of interest. The peculiarity of the farms in Odogunyan in Ikorodu and Odo Iya Alaro in Maryland is that there are heavy industrial activity going on in Odogunyan while Odo Iya Alaro is a contaminated water with a possibility that the radioactivity may be high. The motivation for carrying out this research is to ascertain the level of radionuclide activity in the vegetables planted in that environ due to the activities going on in these selected areas.

2.0 MATERIALS AND METHODS

2.1 Study Areas

Odogunyan is a farm village in Ikorodu. Odogunyan is situated in Southwest Ikorodu, which is a city found in the North East of Lagos State, South West, Nigeria bordered on the North by Ogun State and South by the Lagoon. Odogunyan is surrounded by various industries. The waste products from these factories may have negative impacts on those crops and vegetables planted within that environs. Maryland is regarded as one of the central areas of Lagos. Odo Iya Alaro river in Maryland flows under the Bridge linking Maryland to Ojota area of Lagos. Odo Iya Alaro river, characterized with unclean water, is a highly polluted with the heavy metals in sediments with high concentrations.

2.2 Sample Collection and Preparation

The vegetables chosen for this study were the commonly consumed leafy vegetables: water leaf, scent leaf, green leaf, pumpkin leaf, bitter leaf and Lagos spinach. The vegetables collected were those harvested after they have reached maturity and ready to be sold for consumption. The collected samples were kept in a well labeled polyethylene bags for identification. The edible parts (leaves) of each sample were separated from the roots and stem. They were thoroughly washed under running tap water to remove dirt, sand, insects and worms. The samples were open air dried on trays for a

period of one week and then oven dried at a moderate temperature of 60^{o} C for 2 to 4 hours in the laboratory. The oven dried samples were then grounded into five powders with a stainless steel ball grinder. The prepared samples, in powdered form were packed into weighed plastic beaker, hermetically sealed, reweighed and stored prior to counting (Scheibel and Appoloni, 2007). The containers were sealed to avoid any possibility of outgassing of radon and kept for a period of one month to make sure the samples attained radioactive equilibrium between Ra-226 and its decay products in the uranium series, and Ra-228 and its decay products in the thorium series (Tahir and Alaamar, 2009).

2.3 Radiometric Analysis of the Samples

The measurements of the radionuclide concentration of ^{40}K, ^{238}U and ^{232}Th was carried out using a well calibrated Nal(TI) and well shielded detector coupled to a computer resident quantum MCA2100R Multichannel analyzer for 36000s. An empty container under identical geometry was also counted for the same time. The 1460KeV gamma-radiation of ^{40}K was used to determine the concentration of ^{40}K in the sample. The gamma transition energy of 1764.5KeV ^{214}Bi was used to determine the concentration of ^{238}U while the gamma transition energy of 2614 keV ^{208}T. It was used to determine the concentration of ^{232}Th while ^{137}Cs was detected by its 661.6 keV gamma transition. The efficiency calibration of the detector was done using a reference standard mixed source traceable to Analytical Quality Control Service (AQCS, USA) which has a geometrical configuration identical to the sample container. The standard source contained has known radionuclide. The energy calibration was also performed by using the peaks of the radionuclide present in the standard sources. The Channel number is proportional energy, the channel scale was then converted to an energy scale. This produces an energy calibration curve i.e. energy versus channel.

2.3.1 Calculation of Annual Effective Dose

The dose received by the public from the consumption of these vegetables was estimated from the total annual effective dose AED determined using Equation 1
(Ajayi & Adesida, 2009)

$$ED = \sum_i^n A_i \times DCF_i \times I_v \text{ .....(1)}$$

where A_i (Bq/kg) is the specific activity of radionuclide *i*, DCF_i (mSv/Bq) is the dose conversion factor of radionuclide. The consumption rate of leafy vegetables for adult is 60 kg per annum (UNSCEAR, 2000). Dose conversion factor, DCF, values for radionuclides are 6.2×10^{-9} Sv/Bq, 4.5×10^{-8} Sv/Bq and 2.3×10^{-7} Sv/Bq for ^{40}K, ^{238}U and ^{232}Th respectively (Tettey-Larbi, *et. al.*, 2013; ICRP, 1996).

2.3.2 EXCESS LIFETIME CANCER RISK (ELCR)

Excess life time cancer risk was calculated using equation 2 (Thabayneh & Jazzar, 2012). This was to determine the possibility of carcinogenic effect due to long-term consumption of the selected vegetables from the study areas.

$$ELCR = AED \times RF \times DL ------(2)$$

AED is the annual effective dose, DL is the duration of life (70 years) and RF is the fatal cancer risk factor per Sievert (0.05 for the public).

3.0 RESULTS AND DISCUSSION

3.1 Activity Concentration of Radionuclides

The distribution of activity concentration of radionuclides in vegetables from farms in Odo Iya Alaro river in Maryland and Odogunyan in Ikorodu are illustrated in figure 1 and 2 respectively below. Table 1 shows that the activity concentration of ^{40}K is 221.16±30.01 Bq/kg, 240.09±20.16 Bq/kg, 212.38±15.07 Bq/kg, 268.37±30.18 Bq/kg and 215.22±47.03

Bq/kg in waterleaf, scent leaf, African spinach, Lagos spinach and bitter leaf respectively from Odo Iya Alaro, Maryland. The concentration of ^{238}U found in bitter leaf, Lagos spinach, Africa spinach, scent leaf and water leaf is 12.17±3.57 Bq/kg, 12.35±1.01 Bq/kg, 12.03±2.95 Bq/kg, 11.61±3.20 Bq/kg and 10.19±1.01 Bq/kg respectively. The concentration of ^{232}Th in African spinach, Lagos spinach, bitter leaf, scent leaf and water leaf is 10.42±3.16 Bq/kg, 10.03±3.24 Bq/kg, 10.22±1.38 Bq/kg, 10.93±5.43 Bq/kg and 11.13±2.08 Bq/kg respectively. The concentration of ^{40}K is highest in Lagos spinach while that found in African spinach, bitter leaf, water leaf and scent leaf are less by 20.1%, 19.8%, 17.6% and 10.5% respectively. The concentration of ^{238}U from Odo Iya Alaro is lowest in water leaf. It is exceeded by 13.9%, 18.1%, 21.2% and 19.4% respectively. The highest and lowest concentration of ^{232}Th is found in water leaf and Lagos spinach respectively while the average value is 10.55±0.468 Bq/kg.

Table 1 below also shows that radionuclide concentration of ^{40}K in water leaf, scent leaf, African spinach, pumpkin leaf and bitter leaf are 228.13±38.0 Bq/kg, 291.18±15.15 Bq/kg, 212.16±30.0 Bq/kg, 220.09±20.16 Bq/kg and 272.38±25.07 respectively. Scent leaf has the highest concentration while African spinach has the lowest concentration. The concentration of African spinach is lower than that of water leaf, scent leaf, pumpkin leaf and bitter leaf by 7%, 27.1%, 0.2% and 22.2% respectively. The activity concentration of ^{238}U in water leaf is 17.43±3.19 Bq/kg. It is higher than in scent leaf, African spinach, pumpkin leaf and bitter leaf which are 12.25±3.99 Bq/kg, 12.19±3.01 Bq/kg, 11.61±6.40 Bq/kg and 12.03±4.95 Bq/kg respectively. The concentration of ^{232}Th in the vegetable samples is comparably lower than that of ^{238}U. ^{232}Th has a concentration of 10.75±2.0 Bq/kg, 10.93±5.43 Bq/kg, 10.13±2.08 Bq/kg, 10.08±2.65 Bq/kg and 11.03±5.16 in bitter leaf, pumpkin leaf, African spinach, scent leaf and water leaf respectively. The concentration of water leaf is higher than that of bitter leaf, pumpkin leaf, African spinach and scent leaf by 2.6%, 0.9%, 8.9% and 9.4% respectively.

The concentration of Potassium-40 is the higher in Odogunyan than in Maryland. The lower radionuclide concentration of Uranium-238 is found in Maryland with a value of 11.67 Bq/kg while the highest value is found in Odogunyan. However, the concentration of Thorium-232 is the highest in Maryland while the least concentration is found in Odogunyan. In the two locations, Potassium-40 concentration is the highest, followed by that of Uranium-238. The concentration of Th-232 is the least. In all the cases, the specific activity of radionuclides less than the world average of 400, 35 and 30 Bq/kg for Potassium-40, Uranium-238 and Thorium-232 respectively. The activity concentration of ^{238}U and ^{232}Th is higher when compared to the reported figures of 0.6-2.6 Bq/kg and 0.7-3.4 Bq/kg in Jordan (Mohammed *et. al.,* 2016;) while the concentration obtained in this study is lower for ^{40}K. The other places where the activity concentration is higher than that obtained in this is are Tanzania and Cameron (Mohammed *et. al.,* 2016; Banzi, *et. al.,* 2000).

3.2 Annual Effective Dose and Excess Life Cancer Risk

Tables 2 and 3 shows annual effective dose and excess life cancer risk. The annual effective dose was calculated using specific activities of the natural radionuclides in the vegetables and the average consumption rate of each vegetable with the assumption that the consumption rate of vegetable per capital per annum is 60 kg for Nigeria. The annual effective dose due to consumption of African spinach, bitter leaf, Lagos spinach, scent leaf and water leaf from Odo Iya Alaro in Maryland are 0.255±0.057 µSv/yr, 0.252±0.046 µSv/yr, 0.271±0.049 µSv/yr, 0.271±0.091 µSv/yr and 0.264±0.042 µSv/yr respectively. The total annual effective dose due to consumption of vegetables from this farm is 1.313±0.285 µSv/yr. This value is much less than the total recommended annual dose of

0.3 mSv/yr recommended by ICRP for the general public (ICRP, 1996). This implies that the consumption of these vegetables do not pose any significant risk to the consumers. The total annual effective dose due to the consumption of the vegetables in Odogunyan is 1.359±0.464 µSv/yr. This figure is comparable to that obtained in Maryland and there is no significant possibility of any health risk due to ingestion of the vegetables. The contribution of ^{40}K, ^{238}U and ^{232}Th to the total effective dose are 0.43±0.052 µSv/yr, 0.16±0.031 µSv/yr, 0.73±0.211 µSv/yr respectively in Maryland. The effective dose of 0.45±0.048 µSv/yr, 0.18±0.058 µSv/yr and 0.729±0.239 µSv/yr are contributed by ^{40}K, ^{238}U and ^{232}Th in Odogunyan. The Excess Life Cancer Risk ranges from 4.59×10^{-6} to 5.21×10^{-6}. These values are much less than the world's average value of 0.29×10^{-6} (Thabayneh & Jazzar, 2012). These values only implies that the added probability of cancer incidence is about 5 in a million people.

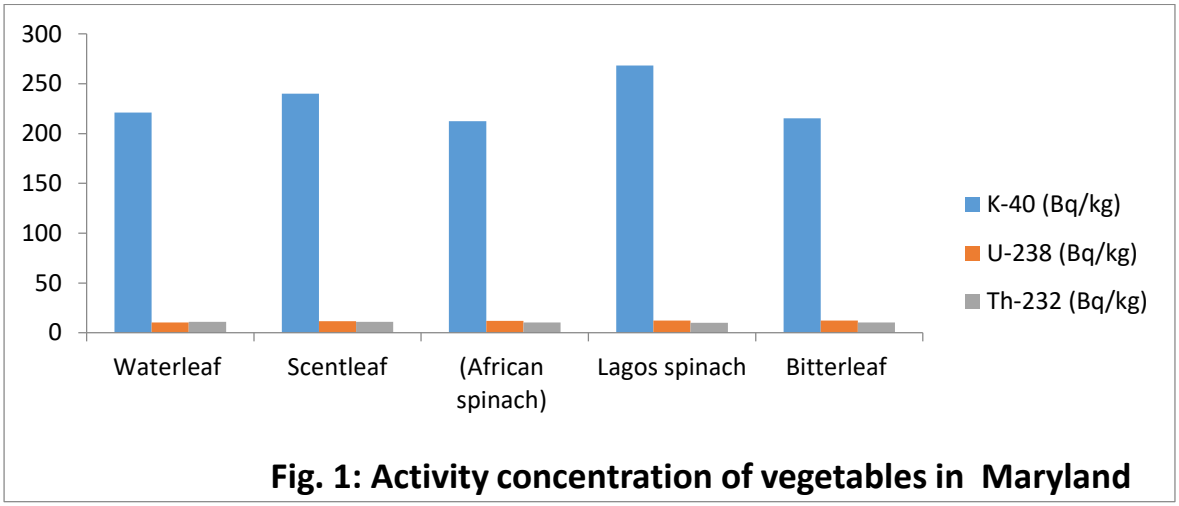

Fig. 1: Activity concentration of vegetables in Maryland

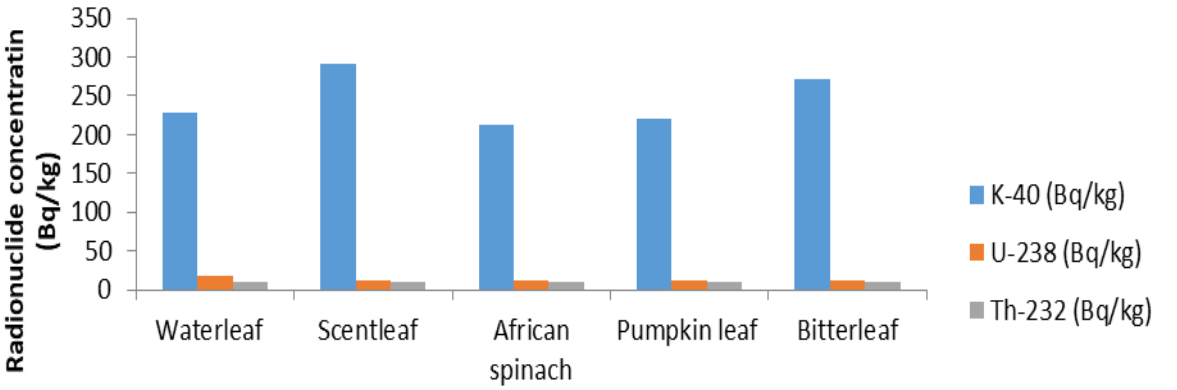

Fig. 2: Activity concentration of vegetables in Odogunyan

Table 1 Activity Concentration of ^{40}K ^{238}U ^{232}Th in the vegetable samples collected from Odo Iya Alaro, Maryland and Odogunyan.

Samples	Radionuclide Concentration (Bq/kg)					
	Odogunyan			Maryland		
	^{40}K	^{238}U	^{232}Th	^{40}K	^{238}U	^{232}Th
Waterleaf	228.13±38.0	17.43±3.19	11.03±5.16	221.16±30.01	10.19±1.01	11.13±2.08
Scent leaf	291.18±15.15	12.25±3.99	10.08±2.65	240.09±20.16	11.61±3.20	10.93±5.43
green leaf	212.16±30.0	12.19±3.01	10.13±2.08	212.38±15.07	12.03±2.95	10.42±3.16
Pumpkin leaf	220.09±20.16	11.61±6.40	10.93±5.43	NA	NA	NA
Lagos spinach	NA	NA	NA	268.37±30.10	12.35±1.01	10.03±3.24
Bitter leaf	272.38±25.07	12.03±4.95	10.75±2.0	215.22±47.03	12.17±3.57	10.22±1.38
Mean	244.79±34.88	13.102±2.43	10.58±0.449	231.44±23.30	11.67±0.871	10.55±0.468

Table 2. Annual Effective dose (AED) and Excess Life Cancer Risk (ELCR) due to Radionuclides (Maryland)

Sample	Radionuclide	Mean Activity (Bq/Kg)	Annual Effective Dose (μSv/yr)	ELCR ($\times 10^{-6}$)
Waterleaf	^{40}K	221.16 ± 30.01	0.082±0.0112	0.28811
	^{238}U	10.19 ± 1.01	0.028±0.0027	0.096357
	^{232}Th	11.13 ± 2.08	0.154±0.0287	0.537805
Scentleaf	^{40}K	240.09 ± 20.16	0.089±0.0075	0.312439
	^{238}U	11.61 ± 3.20	0.031±0.0086	0.109802
	^{232}Th	10.93 ± 5.43	0.151±0.0749	0.528201
(African Spinach)	^{40}K	212.38 ± 15.07	0.079±0.0056	0.276585
	^{238}U	12.03 ± 2.95	0.032±0.0080	0.113643
	^{232}Th	10.42 ± 3.16	0.144±0.0436	0.502591
Lagos Spinach	^{40}K	268.37 ± 30.10	0.100±0.0112	0.348933
	^{238}U	12.35 ± 1.01	0.033±0.0027	0.116845
	^{232}Th	10.03 ± 3.24	0.138±0.0447	0.483384
Bitterleaf	^{40}K	215.22 ± 47.03	0.080±0.0174	0.280107
	^{238}U	12.17 ± 3.57	0.033±0.0093	0.114924
	^{232}Th	10.22 ± 1.38	0.139±0.0192	0.486585
TOTAL			1.313±0.285	4.59

Table 3. Annual Effective dose (AED) and Excess Life Cancer Risk (ELCR) due to Radionuclides (Odogunyan)

Sample	Radionuclide	Mean Activity (Bq/Kg)	Annual Effective Dose (μSv/yr)	ELCR ($\times 10^{-6}$)
Waterleaf	^{40}K	228.13 ± 38.0	0.084±0.0140	0.3248
	^{238}U	17.43 ± 3.19	0.047±0.0086	0.18025
	^{232}Th	11.03 ± 5.16	0.151±0.0712	0.581
Scentleaf	^{40}K	291.18 ± 15.15	0.107±0.0056	0.413
	^{238}U	12.25 ± 3.99	0.033±0.0108	0.1267
	^{232}Th	10.08 ± 2.65	0.139±0.0366	0.532
African Spinach	^{40}K	212.16 ± 30.0	0.079±0.0112	0.30205
	^{238}U	12.19 ± 3.01	0.033±0.0081	0.126
	^{232}Th	10.13 ± 2.08	0.140±0.0287	0.5355
Pumpkin Leaf	^{40}K	220.09 ± 20.16	0.082±0.0075	0.31325
	^{238}U	11.61 ± 6.40	0.031±0.0173	0.12005
	^{232}Th	10.93 ± 5.43	0.151±0.0749	0.5775
Bitterleaf	^{40}K	272.38 ± 25.07	0.102±0.0093	0.3885
	^{238}U	12.03 ± 4.95	0.032±0.0134	0.12425
	^{232}Th	10.75 ± 2.0	0.148±0.0276	0.567
TOTAL			1.359±0.464	5.21

Table 4. Comparison of mean activity concentration of the sampled vegetables with the different locations

Location	Radionuclides (Bq/kg)			References
	^{238}U	^{232}Th	^{40}K	
This Study (Maryland)	11.67±0.871	10.55±0.468	231.44±23.30	
This Study (Odogunyan)	13.102±2.43	10.58±0.449	244.79±34.88	
Cameron	42	17	302	Mohammed et. al., 2016)
Jordan	0.6-2.6	0.7-3.4	698-1439	Mohammed et. al., 2016)
Tanzania	393	318	1568	(Banzi, et. al., 2000)
World Mean	35	30	400	Fisenne, I.M. 1993.

CONCLUSION

This radiological study was carried out to determine the radionuclide concentration of ^{238}U, ^{232}Th and ^{40}K in selected leafy vegetable samples from farms in Odogunyan, Ikorodu and Odo Iya Alaro in Maryland areas of Lagos State. In all the cases, the specific activity of radionuclides less than the world average of 400 Bq/kg, 35 Bq/kg and 30 Bq/kg for Potassium-40, Uranium-238 and T horium-232 respectively. The activity concentration of ^{238}U and ^{232}Th is higher when compared to the reported figures of 0.6-2.6 Bq/kg and 0.7-3.4 Bq/kg in Jordan while the concentration obtained in this study is lower for ^{40}K. The other places where the activity concentration is higher than that obtained in this

is are Tanzania and Cameron. The total annual effective dose due to was 1.313±0.285 µSv/yr and 1.359±0.464 µSv/yr in Odogunyan and Ikorodu respectively. This value is much less than the total recommended annual dose of 0.1 mSv/yr recommended by ICRP for the general public (ICRP, 1996). This implies that the consumption of these vegetables do not pose any significant risk to the consumers. The estimated Excess Life Cancer Risk ranges from 4.59×10^{-6} to 5.21×10^{-6}. These values are much less than the world's average value of 0.29×10^{-6} (Thabayneh & Jazzar, 2012). These values only implies that the added probability of cancer incidence is about 5 in a million people.

REFERENCES

Ajayi, O.S. and Adesida, G. (2009). Radioactivity in some sachet drinking water samples produced in Nigeria. *Iran Journal of Radiation Research*. 7:151-158

Aswood, M. S., Jaafar, M. S. & Bauk, S. (2013) Assessment of Radionuclide Transfer from Soil to Vegetables in Farms from Cameron Highlands and Penang, (Malaysia) Using Neutron Activation Analysis. *Applied Physics Research; Vol*. 5, No. 5, 85-92

Banzi, F.P., Kifanga, L.D., Bundala, F.M. (2000) Natural Radioactivity and Radiation exposure at Minjingu phosphate mine in Tanzania. *Journal of Radiological Protection*.40:51-59

Fisenne, I.M. 1993. Tutorial session 4. Long lived radionuclides in food and in human beings. In: Fifth International Symposium on the Natural Radiation Environment. Tutorial sessions. EUR 14411 EN. 185-255

ICRP (1996) Age Dependent Doses to members of public from intake of radionuclides: compilation of ingestion and inhalation coefficients. *International Committee of Radiological Protection*. ICRP Publication 72 (Elsevier Science)

Njinga, R.L., Jonah, S.A. and Gomina, M. (2015) Preliminary investigation of naturally occurring radionuclides in some traditional medicinal plants used in Nigeria. *Journal of Radiation Research and Applied Sciences*, 1-8

Malik H. Kheder, Laith A. Najam and Hana N. Azeez (2020). Long-lived alpha emitters concentrations in the spices consumed in Iraq using CR-39 detector. *Journal of University of Babylon for Pure and Applied Sciences*, Vol. 28(1):274-287

Mohammed, N.K., Chanai, E. Alkhorayef, M. (2016) The impact of the extensive use of phosphate fertilizers on radioactive levels in farm soil and vegetables in Tanzania. *J. Radioanal Nucl. Chem*.307:2373-2379

Pulhani, V. A., Dafauti, S., Hegde, A. G., Sharma, R. M., & Mishra, U. C. (2005). Uptake and distribution of natural radioactivity in wheat plants from soil. *Journal of Environmental Radioactivity, 79*, 331-346.

Tahir, S. N. A., & Alaamer, A. S. (2009). Concentrations of natural radionuclides in municipal supply drinking water and evaluation of radiological hazards. *Environmental Forensics, 10*, 1-6.

Tettey-Larbi, L., Darko, E. O., Cyril, S. and Appiah, A. A. Natural radioactivity levels of some medicinal plants commonly used in Ghana. *Springer Plus*. 2,1-9.

Nyanda, P.B.(2012) Identification and quantification of radioactivity levels and design a database for environmental radioactive samples in Tanzania. M.Sc in Nuclear Science and Technology Dissertations. University of Alexandria, Egypt.

Shanthi, G., Kumaran, J.T., Raj, g. A & Maniyan, C.G., (2010). Natural radionuclides in the South Indian foods and their annual dose. *Nuclear Instruments & Methods in Physics Research* A .619, 435-400

Scheibel, V., & Appoloni, C. R. (2007). Survey of natural radioactivity levels in paraguariensis (St. Hil.) by Gamma-ray spectrum. *Brazil Archives Biology Technology*, *50*(5), 901-904.

Thabayneh, K.M. & Jazzar, M.M. (2012) Radioactivity levels in plant samples in Tulkarem district, Palestine and its impacts on human health. *Radiation Protection Dosimetry*, 153, 467-474.

UNSCEAR, (1993). Sources and Effects of Ionizing Radiation. (Report to general assembly with Scientific Annexes). Annex B exposure from natural radiation sources. United Nations, New York.

UNSCEAR, (1993a). Exposure of Natural Sources of Radiation. (Report to general assembly with Scientific Annexes). Annex B exposure from natural radiation sources. United Nations, New York.

UNSCEAR, (2000). Exposure from natural radiation sources. United Nations Scientific Committee on the effects of Atomic Radiation. Report to general assembly. Annex B exposure from natural radiation sources. United Nations, New York.

Varier, M. K. (2009). Nuclear radiation detection: Measurements and analysis New Delhi, India: Narosa Publishing House

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BINARY LOGISTIC ESTIMATION OF RISK FACTORS ASSOCIATED WITH CHRONIC HEPATITIS B VIRUS IN LAGOS STATE

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ABSTRACT

Hepatitis B is a viral infection that attacks the liver and cause both acute and chronic diseases, easily spread by exposure to infected body fluids. Chronic hepatitis B which is a persistence of hepatitis B surface antigen (HBsAg) for six months or more is becoming a threat and major public health challenge because of its high rate of morbidity and mortality. This study examined the risk factor associated chronic hepatitis B in Lagos. Data on demographic and behavioural factor on age, sex, marital status, alcoholic consumption, and smoking habit of the patients were extracted from the record of four hundred and six patients attending the hepatitis clinic at Lagos State University Teaching Hospital, Ikeja, Lagos. Binary logistic regression was used to examine the association between chronic hepatitis B and our variables of interest. The results shows that the prevalence of chronic cases among Hepatitis B patients in Lagos State is higher in female (39.9%) than male (34.4%). Only age was statistically significant as a predictor of chronic hepatitis B, 20 – 29 (OR = 3.167, 95% CI = 1.382 - 7.260), 30 - 39 (OR = 3.193, 95% CI = 1.658 – 6.148), 40 – 49 (OR = 2.720, 95% CI = 1.302 – 5.680). Individuals within the age 30 – 39 years show more prevalence of chronic hepatitis B (43%). The study concluded that only age is the significant predictor of chronic Hepatitis B and individuals within the age 30–39 years have the highest risk of the disease.

KEYWORDS: Binary Logistic Regression, Chronic Hepatitis B, Multivariate analysis, Risk Factors.

BACKGROUND

Hepatitis B is a vaccine-preventable liver infection that attacks the liver and cause both acute and chronic Disease. It can be contacted when blood, semen, or other body fluids from a person infected with the virus enters the body of someone who is not infected. This can happen through sexual contact; sharing needles, syringes, or other drug-injection equipment; or from mother to baby at birth. Chronic hepatitis B is the persistence of hepatitis B surface antigen (HBsAg) for six months or more, this becoming a threat and major public health challenge because of its high rate of morbidity and mortality. Despite the existence of a safe and effective vaccine, HBV continues to be a substantial and devastating health problem whose new cases are still being reported throughout the world (Shahnaz, Reza, Seyed-M Moayed, & Moayed, 2005). World Health Organization estimated that 296 million people were living with chronic hepatitis B infection in 2019, with 1.5 million new infections each year and 650, 000 people will die annually due to chronic Hepatitis B (World Heath Organization, 2017). Improving rate of vaccination coverage, especially among infants and children has reduced the burden of the hepatitis B worldwide. However, little attention has been paid to this burden of disease over the last decades and many barriers still contribute to the epidemic in Africa (Lemoine & Thursz, 2017). The continent is rated with the second largest number of individuals with chronic Hepatitis B Virus (World Heath Organization, 2017). Every individual is susceptibility except those that been vaccinated successfully or those who have developed anti-HBs antibodies after HBV infection are immune

to HBV infection (Navabakhsh, et al., 2011). Shimakawa, et al. (2014) established that about 90% to 95% mother to child transmission of the hepatitis B go on to develop chronic infection. This implies that the best way to prevent chronic hepatitis B is to get vaccinated at birth. Unfortunately, majority of African women give birth outside of medical facilities and often with the help of non-medical mid-wives. Worst still, many people that are carrier of this disease are unaware they have it, making the spread nearly uncontrollable. The objective of this study is to determine the risk of chronic hepatitis B associated with selected demographic and behavioural factors in hepatitis B patients.

2. Materials and Methods

Study population: This research was conducted among the hepatitis B patients attending clinic at the Lagos State University teaching hospital. Data on demographic and behavioural factors including age, sex, marital status, alcoholic consumption, and smoking habit were extracted from the record of four hundred and six patients.

Ethical Issues: Ethical approval was obtained from the health research ethics committee of the Lagos State University Teaching Hospital (LASUTH)

Statistical Tools: Binary logistic regression was used to examine the association between chronic hepatitis B and our variables of interest and control confounding

Logistic Regression Model

$$logit(CHBV) = \ln\left(\frac{\pi}{1-\pi}\right) = \beta_0 + \beta_1 * Age + \beta_2 * sex + \beta_3 * Marital\ status$$
$$+\beta_4 * Smoking + \beta_5 * Alcoholic\ consumption + \beta_6 * BMI$$

The outcome variable here is hepatitis B status, whether a subject has chronic hepatitis B or not (1 = Yes, 0 = No). Hence, the dichotomy of the outcome variable made the method binary logistic.

The null hypothesis of the overall model states that all regression coefficients (β_S) are zero. Rejection of this null hypothesis implies that at least one regression coefficient is non-zero meaning the logistic regression equation above predicts the probability of the CHBV status. For the interpretation convenience, we report the odds ratio of the regression coefficient.

Odds of having CHBV = $e^{\beta S}$

3. RESULTS

Table 1: Socio-demographic characteristics and behavioural factor of Hepatitis B patients

Characteristics		Frequency	Percent
Age (years)	<20	10	2.5
	20- 29	82	20.2
	30-39	158	38.9
	40-49	77	19.0
	50-90	79	19.5
	Total	406	100.0
Gender	Female	188	46.3
	Male	218	53.7
	Total	406	100.0

Marital Status	Single	109	26.8
	Married	297	73.2
	Total	406	100.0
HBV Status	HBV	256	63.1
	CHBV	150	36.9
	Total	406	100.0
Alcohol consumption	No	273	67.2
	Yes	133	32.8
	Total	406	100.0
Smoking	No	360	88.7
	Yes	46	11.3
	Total	406	100.0

Table 1 shows that only 2.5% of the patients were < 20 years, 20.2% were 20 – 29 years, 38.9% were 30 – 39 years, 19% were 40 – 49 years, and 19.5% were 50 years and above. Also, 53.7% of the patients were male while 46.3% were female, while 73.2% of them were married and 26.8% single. The table also shows that 36.9% of the patients had Chronic Hepatitis B virus. Lastly, 32.8% of the patients consume alcohol while only 11.3% smokes.

Table 2: Chi square test of association between Hepatitis B status and demographic characteristics

Characteristics		**HBV**	**CHBV**	**χ^2**	**p-value**
Age (years)	<20	7 (70.0)	3 (30.0)	12.909	0.012
	20 - 29	48 (58.5)	34 (41.5)		
	30 -39	90 (57.0)	88 (43.0)		
	40 - 49	48 (62.3)	29 (37.7)		
	50 and above	63 (79.7)	16 (20.3)		
	Total	256 (63.1)	150 (36.9)		
Gender	Female	251 (63.9)	142 (36.1)	3.487	0.080
	Male	5 (38.5)	8 (61.5)	2.828 (0.908 – 8.809)	
	Total	256 (63.1)	150 (36.9)		
Marital Status	Single	67 (61.5)	42 (38.5)	0.161	0.728
	Married	189 (63.6)	108 (36.4)	0.912 (0.58 – 1.43)	
	Total	256 (63.1)	150 (36.9)		
Alcohol consumption	No	177 (64.6)	97 (35.4)	0.863	0.381
	Yes	79 (59.8)	53 (40.2)	1.224 (0.799 – 1.876)	
	Total	256 (63.1)	150 (36.9)		
Smoking	No	230 (63.9)	130 (36.1)	0.950	0.335
	Yes	26 (56.5)	20 (43.5)	1.361 (0.731 – 2.533)	
	Total	256 (63.1)	150 (36.9)		

Table 2 shows that 30% of the Hepatitis B cases for patients under 20 years is chronic, 41.5% of those aged 20 – 29 years are chronic, 43.0% of those aged 30 – 39 years are chronic, 37.7% of patients between age 40 and 49 are chronic while only 20% of those 50 years and above are chronic and the results that chronic nature of Hepatitis B is positively and significantly associated with age **(χ^2 = 12.909; p < 0.05)**. It further shows

that while 61.5% of the male Hepatitis B patients are chronic, only 36.1% of the female are chronic. However, the association between status and gender is not significant (χ^2 = 3.487; p > 0.05). Also, 38.5% of the single patients were chronic while 36.4% of the married were and the association between marital status and status is not significant (χ^2 = 0.161; p > 0.05). Furthermore, 35.4% of patients who do not consume alcohol were chronic while 40.2% of alcohol consumers were chronic and the association between alcohol consumption and status is not significant (χ^2 = 0.863; p > 0.05). Lastly, 36.1% of patients who do not smoke were chronic while 43.5% of smokers were chronic and the association between smoking and status is not significant (χ^2 = 0.950; p > 0.05).

Table 3: Binary Logistics regression parameters

Variables	β	SE.	Wald	Df	Sig.	e^{β}	95% C.I .for e^{β} Lower	Upper
Age			12.715	4	.013			
Age (<20 years)	.837	.851	.969	1	.325	2.311	.436	12.243
Age (20 – 29 years)	1.153	.423	7.419	1	.006	3.167	1.382	7.260
Age (30 – 39 years)	1.161	.334	12.053	1	.001	3.193	1.658	6.148
Age (40 – 49 years)	1.001	.376	7.093	1	.008	2.720	1.302	5.680
Age (50 + years)	1							
Sex (Female)	-.363	.225	2.599	1	.107	.695	.447	1.082
(Male)	1							
Marital Status (Married)	.106	.311	.117	1	.732	1.112	.604	2.048
Single	1							
Alcohol Consumption (Yes)	.160	.266	.359	1	.549	1.173	.696	1.977
No	1							
Smoking (Yes)	.192	.374	.264	1	.608	1.212	.582	2.522
No	1							
Constant	-1.479	.582	6.446	1	.011	.228		

The Binary Logistic regression results in the table 3 revealed that only age was statistically significant as a predictor of chronic hepatitis B, 20 – 29 (OR = 3.167, 95% CI = 1.382 - 7.260), 30 -39 (OR = 3.193, 95% CI = 1.658 – 6.148), 40 – 49 (OR = 2.720, 95% CI = 1.302 – 5.680). However, it shows that female is 30% less likely to contract chronic HBV, married persons are 11% more likely, alcohol consumers are 17% more likely while smokers are 21% more likely to succumb to chronic HBV.

4 Discussion

The study found that individuals within the age 30 – 39 years have the highest risk of having chronic hepatitis B. They are over 3 times more likely to have chronic hepatitis B as to those in age 50 years and above. This result is consistent with several studies which have been previously reported (Zhao, *et al.*, 2021; Thanh, *et al*., 2020; Olayinka, *et al*., 2016; Hyams, 1995). Women are less likely to have chronic hepatitis B than men but not statistically significant predictor. This is in agreement with the result of Barbosa, *et al*. (2021) and Adekanle, *et al.* (2020) who found female gender offering a protective effect. Married couple are more likely to have chronic hepatitis B than single individuals, although, not a statistically significant predictor which is at variance with the conclusion of Makuza, et al. (2019). This may be attributed to the fact that they have more access to unprotected sex with their partner than single individuals. Based on our results, there is no significant association between smoking and developing chronic hepatitis B, yet, it is important to point out

that those that are smoking are more likely to develop chronic hepatitis B than those that abstained from smoking. This finding is consistent with Brahmania, *et al*. (2020) and Meier & Berger (2020) who found smoking as a pre-disposing factor to chronic Hepatitis B. Finally, patients that consume alcohol are more likely to develop chronic hepatitis B than those that are not taking alcohol, though, no statistical significance was established which in agreement with other studies (Brahmania, *et al*., 2020; Ganesan, *et al.*, 2020; Iida-Ueno, *et al.*, 2017). The study ahs shown that only age is positively associated with chronic hepatitis B. Lastly, while other studies had looked at prevalence of CHB in the population and others among specific groups in the population, this study had considered CHB among hepatitis B patients.

5 Conclusion

In this research, age has been found to be independently and statistically significant risk factor of chronic hepatitis B and positively associated with CHB. This confirms the vertical transmission of the virus arguments leading to chronic disease in most cases. Therefore, advocacy for mass immunization against the hepatitis B virus at birth should be given a rapt attention by all tiers of government in Nigeria and orientation be intensified among the populace on the risk of hepatitis B.

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REFERENCES

Adekanle, O., Komolafe, A. O., Olowookere, S. A., Ijarotimi, ,. O., & Ndububa, D. A. (2020). Hepatitis B Infection: A Mixed Methods of Disclosure Pattern and Social Problems in the Nigerian Family. *Journal of Patient Experience, 7*(2), 208 - 216. doi:10.1177/2374373519827965

Barbosa, J. V., Sahli, R., Vincent Aubert, V., Chaouch, A., Moradpour, D., & Fraga, M. (2021). Demographics and outcomes of hepatitis B and D: A 10-year retrospective analysis in a Swiss tertiary referral center. *PLoS ONE, 16*(4), e0250347. doi:10.1371/journal.pone.0250347

Brahmania, M., Liu, S., Wahed, A. S., Yim, C., Hansen, B. E., Khalili, M., . . . A., J. H. (2020). Alcohol, tobacco and coffee consumption and liver disease severity among individuals with Chronic Hepatitis B infection in North America. *Annals of Hepatology, 19*, 437 - 445. doi:10.1016/j.aohep.2020.01.005

Brahmania, M., Liu, S., Wahed, A., Yim, C., Hansen, B. E., Khalili, M. T., . . . Janssen, H. (2020). Alcohol, tobacco and coffee consumption and liver disease severity among individuals with Chronic Hepatitis B infection in North America. *Annals of Hepatology, 19*(4), 437 - 445. doi:10.1016/j.aohep.2020.01.005

Ganesan, M., Eikenberry, A., Poluektova, L. Y., Kharbanda, K. K., & Osna, N. A. (2020). Role of alcohol in pathogenesis of hepatitis B virus infection. *World Journal Gastroenterology, 26*(9), 883 - 903. doi:10.3748/wjg.v26.i9.883

Hyams, K. C. (1995). Risks of Chronicity Following Acute Hepatitis B Virus Infection: A Review. *Clinical Infectious Diseases , 20*, 992 - 1000.

Iida-Ueno, A., Enomoto, M., Tamori, A., & Kawada, N. (2017). Hepatitis B virus infection and alcohol consumption. *World Journal of Gastroenterology, 23*(15), 2651 - 2659. doi:10.3748/wjg.v23.i15.2651

Lemoine, M., & Thursz, M. R. (2017). Battlefield against hepatitis B infection and HCC in Africa. *Journal of Hepatology, 66*(3), 645- 654.

Makuza, J. D., Rwema, J., Ntihabose, C. K., Dushimiyimana, D., Umutesi, J., Nisingizwe, M. P., . . . Nsanzimana, S. (2019). Prevalence of hepatitis B surface antigen (HBsAg) positivity and its associated factors in Rwanda. *BMC Infectious Diseases, 19*, 381. doi:10.1186/s12879-019-4013-4

Meier, M. A., & Berger, C. T. (2020). A simple clinical score to identify likely hepatitis B vaccination non-responders – data from a retrospective single center study. *BMC Infectious Diseases, 20*, 891. doi:10.1186/s12879-020-05634-y

Navabakhsh, B., Mehrabi, N., Estakhri, A., Mohamadnejad, M., & Poustchi, H. (2011). Hepatitis B Virus Infection during Pregnancy: Transmission and Prevention. *Middle east Journal of digestive diseases, 3*(2), 93 - 102.

Olayinka, A. T., Oyemakinde, A., Balogun, M. S., Ajudua, A., Nguku, P., Aderinola, M., . . . Gidado. (2016). Seroprevalence of Hepatitis B Infection in Nigeria: A National Survey. *The American Society of Tropical Medicine and Hygiene, 95*(4), 902–907.

Shahnaz, S. M., Reza, B. M., Seyed-M Moayed, A. M., & Moayed, A. M. (2005). Risk Factors in Chronic Hepatitis B Infection: A Case-control Study. *Hepatitis B Monthly, V*(4), 109-115.

Shimakawa, Y., Bottomley, C., Njie, R., & Mendy, M. (2014). The association between maternal hepatitis B e antigen status, as a proxy for perinatal transmission, and the risk of hepatitis B e antigenaemia in Gambian children. *BMC Public Health , 14*, 532 - 540.

Thanh, P. N., Tho, N. T., Phu, T. D., Quang, T. D., Duong, N. T., Chien, V. C., & Lan, P. T. (2020). Prevalence and factors associated with chronic Hepatitis B infection among adults in the Central Highland, Vietnam. *AIMS Medical Science, 7*(4), 337 - 346. doi:10.3934/medsci.2020023

World Heath Organization. (2017). *Global Hepatitis B Report.* Geneva: https://www.who.int/news-room/fact-sheets/detail/hepatitis-b.

Zhao, X., SHi, X., Lv, M., Yuan, B., & Wu, J. (2021). Prevalence and factors associated with hepatitis B virus infection among household members: a cross-sectional study in Beijing. *Human Vaccines & Immunotherapeutics, 17*(6), 1818 - 1824. doi:10.1080/21645515.2020.1847951

ANTIMICROBIAL AND PHYTOCHEMICAL PROPERTIES OF OCIMUM GRATISSIMUM AGAINST SOME BACTERIA

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ABSTRACT

Medicinal plants have bioactive compounds which are used for curing and healing various human diseases. This study aimed at investigating the antimicrobial and phytochemical potentials of *Ocimum gratissimum* leaves against some bacteria Fresh leaves of *Ocimum gratissimum* were collected from Yaaru town, Kwara State, Nigeria. The leaves were washed; air dried and then pulverized using mechanical blender. Three hundred (300 gram) of the powdered leaves were weighed and soaked in 1200 ml of methanol and were extracted for seven days. The crude extracts were filtered using muslin cloth and then re-filtered using No.1 Whatman's filter paper. This was reconstituted to dryness using rotary evaporator at 40^{o}C and stored in universal bottle. The antibacterial activity was determined using agar well diffusion test while the phytochemical screening was determined using standard procedure. The antibacterial activity of *O. gratissimum* methanolic extract was active against *Escherichia coli* (22.97 mm), *Staphylococcus aureus* (18.57 mm), *Proteus mirabilis* (27.97 mm), *Klebsiella pneumoniae* (19.03 mm) and *Enterococcus faecalis* (21.47 mm), However, highest activity was observed for *Proteus mirabilis* while *Staphylococcus aureus* (18.57 mm) showed the lowest activity. The result of phytochemical constituents showed that it contained phenol (18.57 ug/mL), tannin (16.27 ug/mL), saponin (20.60 ug/mL), terpenoid (17.97 ug/mL), flavonoid (21.97 ug/mL) and cardiac glycosides (12.90 ug/mL). Saponin had the highest concentration while cardiac glycoside was the lowest. The result of the antimicrobial and phytochemical screening showed that *Ocimum gratissimum* could not just be considered as a good antibacterial agent but also a good source of flavonoid and saponin as well as an antibacterial agent.

KEYWORDS: Antimicrobial, Bacteria, Medicinal plant, *Ocimum gratissimum*,

INTRODUCTION

There is growing interest in exploiting plants for medicinal purposes especially in Africa. This stems from the fact that microorganisms are developing resistance to many drugs and as such created situation where some of the common and less expensive antimicrobial agents are losing effectiveness (Montefore *et al*., (2015); herbal medicine which uses medicinal plants primarily presents an alternative to such situation (Sofowora, 2008). These medicinal plants have immensely contributed to the development of human health and welfare. Concomitantly, there is an increase in data and huge patronage to herbal products round the world (Mahesh and Satish (2008).

One of such plants is *Ocimum gratissimu.* This plant belongs to the family *leguminocaeae*, commonly known as "alfavaca". Most members of this family are considered economically useful because of their basic natural characteristics as essential oil producers. These essential oils are composed primarily of monoterpenes and sesquiterpenes and have been the subject of extensive studies due to their economic importance (Lawrence, 1993).

Ocimum gratissimum have been asserted to provide various culinary and medicinal properties that bring about bacteriostatic and bactericidal effects on some bacteria (Okigbo and Igwe, 2007). The aim of this study is to investigate the antimicrobial potential and phytochemical properties of *Ocimum gratissimum* against some bacteria.

SAMPLE COLLECTION

Fresh leaves of *Ocimum gratissimum* were collected from the wild at Yaaru town, Kwara State, Nigeria. The leaves were identified properly and authenticated at the Department of Botany, University of Lagos, Nigeria. The leaves of *Ocimum gratissimum* were plucked, rinsed with tap water and air-dried at room temperature for three weeks.

Test organisms were bacteria obtained from Microbiology Department, College of Medicine University of Lagos Laboratory.

METHODS

The dried leaves were pulverized using a milling machine to obtain fine powder. Exactly 300g of the grinded leaves of *Ocimum gratissimum* was measured using a weighing balance and soaked in 1200 ml of methanol for 7days. The extract was then sieved using muslin clothes. The samples were filtered using Whatman's No 1 filter paper and the filtrate obtained was concentrated in water bath at 60-80°C until 10% of the entire content was obtained (Edeoga *et al.*, (2015).

The working samples were of three values, 300 mg/ml, 150 mg/ml, and 75mg/ml which were achieved by weighing 3g of extract and dissolving in the Dimethyl Sulphoxide (DMSO) and then double diluting to have other working values; 300 mg/ml, 150mg/ml and 75 mg/ml. Concentrations were varied as follows; 50 µg/ml, 25 µg/ml, 12.5 µg/ml.

TEST ORGANISMS

Test organisms were bacteria group that included *Escherichia coli, Staphylococcus aureus, Proteus mirabilus, Klebsiella pneumoniae* and E*nterococcus faecalis*. Test bacterial suspensions were standardized using sterile normal saline. The bacterial liquid cultures were added drop-wise to the normal saline until the turbidity matched that of the 0.5 McFarland turbidity standards. Mueller-Hinton agar was prepared and maintained at 45^0C so as to make it remain molten

ANTIMICROBIAL DETERMINATION

After allowing all the seeded Agar plates to set, a cork borer, size 10 mm cross-section was used to gently punch hole at different parts of the Petri dishes after it was flamed and allowed to cool. All the cut portions were thrown into a dish of disinfectant. 150 µl of each working concentrations was dispensed into each appropriate wells and allowed to stand for four hours before incubation at 37ºC.

Petri dishes were incubated in lid-up position. This was so in order to avoid spillage. After four hours on the Laboratory bench for the samples and standard concentration to diffuse, the plates for antibacteria studies were incubated at 37^0C and observed after 24 hours and observed for zones of inhibition as a result of growth of the bacteria.

Agar well technique of was used because of the colorful nature of the extracts. One (1) ml of calibrated organisms was seeded into the warm agar and was mixed thoroughly using the roll-palm method before pour-plating. After solidifying under sterile condition in a biological safety cabinet, they were prepared for cork boring.

PHYTOCHEMICAL SCREENING

TANNIN DETERMINATION

It was carried out following the method of Elba *et al*. (2008). Five hundred (500) mg of the sample was weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 h in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then, 5 ml of the filterate was pipetted out into a test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.I NHCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 540 nm within 10 minutes.

DETERMINATION OF TOTAL PHENOLS

The fat-free sample was boiled with 50 ml of ether for the extraction of the phenol component for 15 min. 5 ml of the extract was pipette into a 50 ml flask and 10 ml of distilled water was added. Ammonium hydroxide solution (2 ml) and 5 ml of concentrated amyl alcohol were also added. The samples were made up to mark and left to react for 30 minutes for color development. This was measured at 505 nm (Edeoga *et al*., 2007).

Alkaloid Determination

Five grams of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added. The mixture was covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop-wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

Saponin Determination

The method used was that of Chinelo *et al*. (2014). The samples were ground and 20 g of each was put into a conical flask and 100 cm^3 of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4hours with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated.60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight; the saponin content was calculated as percentage.

Flavonoid Determination

Ten grams of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman's filter paper No. 42 (125 mm). The filtrate was later transferred into a crucible, evaporated into dryness over a water bath and weighed to a constant weight (Elba *et al*., 2008).

Cardiac Glycoside Determination

Glycosides of the ethanol plant extracts were determined by adding 10% of each extracts with 10 ml freshly prepared Baljet's reagent (95 ml of 1% picric acid + 5 ml of 10% NaOH). After an hour, the mixture was diluted with 20 ml distilled water, and the absorbance was measured at 495 nm.

RESULTS

In the present study, antimicrobial activity of *Ocimum gratissimum* was identified. The results of the antibacterial activity of the extract against the test organisms namely *Escherichia coli, Staphylococcus aureus, Proteus mirabilus, Klebsiella pneumoniae,* and *Enterococcus faecalis* are shown in Table1. It also showed the mean zones of inhibition of growth of the isolates are a function of relative antibacterial activity of the extracts. The extract of *Ocimum gratissimum* showed selective levels of activities against the isolates. It was indicated in Table 1 showed that the plant extract of *Ocimum gratissimum* showed the highest antibacterial activity with the diameter of zone of inhibition of 22.97 mm as observed against *Escherichia coli*, while the rest were 18.57mm against *Staphylococcus aureus,* 27.97 mm against *Proteus mirabilus*, 19.03 mm against *Klebsiella pneumoniae*, 21.47 mm against *Enterococcus faecalis* at 300 mg/ml Concentration.

The results of the quantitative and qualitative phytochemical analysis of *Ocimum gratissimum* extract investigated are summarized in Table 2. The qualitative result represented in Table 2 shows that saponin, tannins, flavonoid, alkanoids, steroids, terpernoids were observed in moderate level while anthraquinones, phenols and cardiac glycosides were absent.

The secondary metabolites revealed in the plant extract (*Ocimum gratissimum*) were quantitatively estimated and the result was presented with Saponins having the highest concentration of 20.60, followed by flavonoid 21.97, Terpernoid with 17.97, Tannins with 16.27, Phenol with 18.57, Cardiac glycoside with 12.90 at concentration of mg/100g.

Table 1 shows the antimicrobial activity of *Ocimum gratissimum* (mg/100g). It reveals that *Proteus mirabilus* has the highest mean zone of inhibition of *Ocimum gratissimum* of 27.97 mg/100 g (se = 0.26 mg/100g) while *Staphylococcus aureus* has the least mean zone of inhibition of *Ocimum gratissimum* of 18.57 mg/100 g (se = 0.23 mg/100 g) and these means are significantly different from each other ($p < 0.05$).

Table 1: Antimicrobial activity of *Ocimum gratissimum* with the mean ± standard error of the zone of inhibition (mm) (mg/100g)

Microbial organisms	**Mean Values**
Escherichia coli	22.97
Staphylococcus aureus	18.57
Proteus mirabilis	27.97
Klebsiella pneumonia	19.03
Streptococcus faecalis	21.47

Table 2 shows the quantitative phytochemical composition of *Ocimum gratissimum* (mg/100g) using methanolic extract. It reveals that Flavonoid has the highest mean *Ocimum gratissimum* of 21.97 mg/100g (se = 0.09 mg/100g) while cardiac glycocides has the least mean *Ocimum gratissimum* of 12.90 mg/100g (se = 0.32 mg/100g) and these means are significantly different from each other ($p < 0.05$).

Table 2: Quantitative phytochemical composition of the leaf of *Ocimum gratissimum* using methanoic extract

Chemical constituents	Composition (mg/100g)
Saponin	20.60 ± 1.12
Tannin	16.27 ± 0.50
Phenol	18.57 ± 3.09
Flavonoid	21.97 ± 0.09
Cardiac glycocides	12.90 ± 0.32
Terpernoid	17.97 ± 0.45

Value represent mean ±SEM

DISCUSSION

The phytochemical composition and antimicrobial properties of the leaves of *Ocimum gratissimum* were studied. The data obtained from this study indicate that the methanolic extract of the leaf *Ocimum gratissium* possesses variable degrees of antimicrobial properties activity against some bacteria such as *Escherichia coli, Staphylococcus aureus, Proteus mirabilis, Klebsiella pneumoniae and Enterococcus faecalis.* The antimicrobial sensitivity test indicated that *Proteus mirabilus* had the highest zone of inhibition of 27.97 mm and *Staphylococcus aureus* with the least zone of inhibition of 18.57 mm at 100mg/ml. These establish a good support for the use of this plant in herbal medicine and a base for the development of new drugs and phytomedicine (Edeoga, *et al.,* 2015).

The positive qualitative results confirm the presence of alkaloid, saponins, tannins, flavonoids, steroids and terpenoids as secondary metabolites in the leaf extract of *O. gratissimum.* This is in line with Nweze *et al.* (2004); Ajose and Okozi (2017) where they reported the presence of tannins, saponin.

This present study indicates the absence of anthraquinones which is in line with Nsor *et al.* (2012) who stated the absence of anthraquinones.

Antimicrobial effects of plant extracts have been attributed to the presence of these secondary metabolites Nsor *et al.* (2012). The presence of these metabolites in the investigated plant part shows this plant as a medicinal plant. *Ocimum gratissimum* is known for its several uses in traditional medicine. Indeed, this extract has been reported for its action against gastrointestinal infections (diarrhea or dysentery), infections of the skin (dermatitis, eczema, scabies), infections of the upper respiratory tract, associated with cough, asthma and bronchitis, wounds and sores, insect bites, nosebleeds, stroke, anaemia (Edeoga *et al.*, 2015). This could be linked to the presence of essential oil. The quantitative phytochemical screening revealed that saponins had the highest concentration with 20.60 followed by flavonoid with 21.97 while the least concentration was phenol with 18.57. These chemicals evolved in plants to protect them against herbivorous insects, vertebrates, fungi, pathogens, and parasites (Nweze *et al*., 2004).

CONCLUSION

Ocimum gratissimum has a broad spectrum antibacterial activity against all tested isolates and thus has a potential as a source of natural drug that may be employed in combating ailments and inconveniences resulting from microbial infections. The result from this study indicates that the plant has tannins, saponins, terpernoids, steroid, alkaloid and flavonoid but lacks anthraquinones. The absence of anthraquinones and cardiac glycoside in this study however contradicts some past studies and this may be due to the method used to obtain extract used for the phytochemical analysis.

RECOMMENDATION

- Therapeutic potentials of this plant should be accessed from the traditional African system of medicine as it could give us insight on what and how these plants can be used in the treatment of diseases.
- Combination studies on the plant (*Ocimum gratissimum*) are also recommended using different isolates to test the Antimicrobial potentials of the plant.

REFERENCES

Chinelo, A., Ezeabara, C. U., Okeke, B. O. , Aziagba, C. V. and Adaeze, N. E. (2014). Determination of Saponin Content of Various Parts of Six *Citrus* Species. *International Research Journal of Pure and Applied Chemistry* 4(1): 137-143.

Edeoga, H.O., Okwu, D.E., and Mbaebre, B.O. (2007). Phytochemical Constituent of Some Nigerian Medicinal Plants. *African J*ournal *of Biotechnology*. 7:685-68.

Elba, L. C., Amorim, J. E., Nascimento, J. M., Tadeu, J. S., Peixoto S.T., Araujo, A. S. and Ulysses, P. A. (2008). A Simple and Accurate Procedure for the Determination of Tannin and Flavonoid Levels and Some Applications in Ethnobotany and Ethnopharmacology. *Functional Ecosystems and Communities* 2 (1), 88-94.

Mahesh, B. and Satish, S. (2008). Antimicrobial Activity of Some Important Medicinal Plant Extract Against Plant and Human Pathogens. *World Journal of Agri*. *Sci*., **4**: 839-843.

Montefore, D., Rotimi, Y.O. and Adeyemi-Doro, F.A. (2015). The Problem of Antibacterial Resistance to Antibiotics among Strains from Hospital Patients in Lagos and Ibadan. *Nigeria Journal of Antimicrobial Chemotherapy* 23: 604.

Nsor, O. A., Emmanuel, M. I., Arikpo, I. O., Kimboline, D. E., and Matthew, E. E. (2012). Phytochemical and Nutritional profiles of *Lasianthera africana*, *Heinsia crinata* and *Gongronema latifolium New York Science Journal* 5(3) 45 – 48.

Nweze, E. I., Okafor, J. I. and Njoku, O. U. (2004). Antimicrobial Activities of Methanolic Extracts of *Trema guineensis* (Schumm and Thorn) and *Moringa lucida* Benth Used in Nigerian Herbal Medicinal Practice. *Journal of Biological Research and Biotechnology* 2(1): 39 -46.

Okigbo, R.N. and Igwe, M. (2007). The Antimicrobial Effects of *(Piper) Guineense uziza* and *Phyllantus amarus ebe-benizo* on *Candida albicans* and *Streptococcus faecalis*. *Acta Microbiologica et. Immunologica. Hungarica.* 54 (4)**:** 353-366.

Sofowora, A. (2008). Medicinal Plants and Traditional Medicine. Spectrum books Ltd. Ibadan, Nigeria. pp. 224 -227.

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ANALYSIS OF STUDENT'S PERCEPTIONS ON FACTORS AFFECTING ACADEMIC PERFORMANCE: A FACTORIAL EXPERIMENTAL DESIGN APPROACH

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ABSTRACT

This study examines the impact of some selected factors on academic performance. A survey design was adopted for collecting data with a self-designed questionnaire administered among 384 students of Yaba College of Technology randomly selected using multi-stage sampling technique from seven schools in the institution. Reliability of the scale having 28 items was done by experts and through pilot study with Cronbach Alpha value measured at 0.920. The data collected was analyzed using descriptive statistics, Yates Algorithm method, and ANOVA technique. The main and interaction effects were run simultaneously and factorial effects total were obtained by Yates Algorithm method, analysis of variance approach of factorial experimental design was then applied to confirm the statistical significance of both the main and interaction effects. Results showed that Females perform better academically than their Male counterparts ($p < 0.05$) in the studied population while there was no statistical difference in academic performance of those who were admitted based on their career option and those based on option of second choice or those who changed their career option ($p < 0.05$). The study also found out that the main effects of time of study, Career Choice and their interaction effects are all statistically significant ($p < 0.05$) while other effects like Gender and the interaction between Gender and Career Choice are not statistically significant ($p > 0.05$). Thus, each of the level of factors influence significantly academic performance of students while Gender and the interaction between Gender and Career Choice do not. Therefore the study concluded that more emphasis should be placed on equipping students academically and psychologically so as to function effectively as a professional in their chosen field of discipline.

KEYWORDS: Academic Performance, Experimental Factors, Factorial Design, ANOVA, Yates Method

1.1 INTRODUCTION

Education is an important instrument of human resource development in any given society. Its importance cannot be overemphasized because it is a basic element for human, social and economic development. Every nation strives towards the provision of quality education for its citizens because national development is initiated by those who are professionals in their field of practice. Tobih (2012) emphasized that there are numerous ways of evaluating student's knowledge or proffiency in a skill, this include continuous assessment, examination, grade point average, graduation and retention rate, all which takes place in a formal school setting. An advantage of a test or examination is to show the credibility of the certificate awarded by any tertiary institution, Anikwese (2005) extends this definition that the objective of any evaluation is to ascertain what an individual is capable of doing under controlled condition, hence the examiner must adhere strictly to the purpose upon which the assessments are based which implies that examinations serve as an appraisal purpose and are meaningful to all parties involved if it is used to motivate average learners. Different investigators have shown that the academic performance of students

depend on several factors like learning facilities, age and gender differences and different conclusions have been reached via various studies. Byrne and Flood (2008) finds out that that there is no association between gender and performance of students offering allied accounting courses in the university. Meanwhile, Schmidt & Wartick (2014) established a relationship between gender variation and student performance in the course of accounting information systems, while investigating the factors that contribute to students' academic performance in accounting principles, cost accounting, advanced accounting, accounting information systems, auditing and tax accounting courses. The results further show that female students perform better than their male conterparts. (Wally-Dima & Mbekomiza 2013) (Baard et al. (2010) (Arthur & Everaert 2012; Gracia & Jenkins, 2002), however other specie studies show a varied outcome i.e. Weil, Oyelere & Rainsbury (2004) reveal that boys have the better achievement of learning outcomes than girls. Waters & Marzano (2006) further suggest that other factors need to be taken into consideration. Yisa (2010) affirm that teaching method, level of study and their interaction have significant effects on students' academic performance while gender and each of the other interactions have no effect. Okolie et al (2014) assert that parents' support and type of family background can also influence student's academic performance. The students who are properly guided by their guardians may excel in their examination. Epstein (1987) affirm that direction is a crucial element that assist student in achieving their aspiration, personal and academic development and can be provided by parents and academic support staff. Kochhar (2000) supports this assertion that appropriate guidance is required to help the learners having issue of merging ability with accomplishment, flawed study practice, poor methods of learning. Noble, Roberts & Sawyer (2006), found that academic activities of students, perceptions of their coping techniques and upbringing potentials are secondarily related to their aggregate scores during academic achievement in secondary school. Robert and Sampson (2011) also observed students who are serious about their studies have the prospects of improving their cumulative grade point average. (Abdullah, 2011) affirm that competence of students in English is statistically significantly related to student's academic performance. The introduction of Post-JAMB examination which is meant to serve as entrance examination is common in many tertiary institutions, but the ability of this test to forecast student achievement has been quite limited. Akinseinde (2016) investigates the reasons for yearly reduction in number of candidates applying for Quantity Surveying education and observes that the students' choice of career is as a consequence of parental / family influence rather than their sincere interest and aspiration. Hence, this paper examines mode of career choice, hours of study and gender on academic performance of students using a factorial design approach. Factorial designs allow the effects of a factor to be estimated at several levels of the other factors, yielding conclusions that are valid over a range of experimental conditions. The simplest type of factorial designs involve only two factors or sets of treatments combinations. The significance of this study lies in its contribution to the existing works while its limitations are challenge in obtaining information perceived by the respondents concerning their academic performances and the restriction on movement imposed by the federal government across the nation due to the ripple effect of the COVID-19 pandemic.

2.0 MATERIALS AND METHOD

A survey design was adopted for collecting data with a self-designed questionnaire administered among 384 students of Yaba College of Technology randomly selected using multi-stage sampling technique from seven schools in the institution. Reliability of the scale having 28 items was done by experts and through pilot study with Cronbach Alpha value measured at 0.920. Multi-stage sampling technique was employed and the rationale behind the choice of the sampling technique was that the researcher

intends to make the selection as random as possible and since the population is grouped into homogeneous groups called "schools" and each school is also divided into departments, and also levels; hence the justification of multi-stage sampling technique, and the sampling frame at each stage of sampling was easily obtained. The data obtained was analyzed using descriptive statistics and ANOVA method of 2^3 factorial design technique to determine the effect of the three explanatory variables on academic performance of students in Yaba College of Technology. The design technique involves varying several factors simultaneously and drawing out the individual effect of the factors and looking for any possible combination (interaction) effects (Montgomery, 1996). This type of design provides the smallest number of runs for which k factors can be studied in a complete factorial design. Each factor has two levels (often labeled + and −). In order to obtain an optimal sample size for the study, the mathematical formula below was used:

$$\mathbf{n}= \frac{Z^2_{\alpha/2}}{d^2}\text{P (1-P)}$$

Where n is the sample size, $Z_{\alpha/2}$ is the standard normal value (1.96), and d is the margin of error
α = 0.05,

$$\frac{(1.96)^2 \times 0.5 \times 0.5}{(0.05)^2} = 384.2$$

A general two-factor factorial model is defined as:

$$y_{ijk} = \mu + \alpha_i + \beta_J + (\alpha\beta)_{ij} + £_{ijk} \begin{cases} i = 1,2, \dots \dots \dots \dots, a \\ j = 1,2, \dots \dots \dots \dots, b \\ k = 1,2, \dots \dots \dots \dots \dots, n \end{cases}$$

Where μ is the overall mean effect α_i is the effect of the ith level of the factor A, β_J is the effect of the jth level of factor B, $(\alpha\beta)_{ij}$ is the effect of the interaction between α_i and β_J and $£_{ijk}$ is a random error component. Both factors are assumed to be fixed, and the treatment effects are defined as deviations from the overall mean, $\sum_{i=1}^{a} \alpha_i$=0 and $\sum_{i=1}^{b} \beta_j$=0. Similarly, the interaction effects are fixed and are defined such that,

$$\sum_{i=1}^{a}(\alpha\beta)_{ij} = \sum_{j=1}^{b}(\alpha\beta)_{ij} = 0$$

Because there are n replicates of the experiment, there are abn total observations. In the two-factor factorial, both treatments A and B are of equal interest. Specifically, we are testing hypotheses about the equality of treatment A effects,

H_O: $\alpha_1 = \alpha_2 = \dots\dots\dots\dots\dots\dots \alpha_a$
H_1 : at least one $\alpha_i = 0$

and the equality of treatments B effects,
H_O: $\beta_1 = \beta_2 = \dots\dots\dots\dots\dots\dots \beta_a$
H_1 : at least one β_i

The total corrected sum of squares may be written as:

$$\sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{n=1}^{n}\left(y_{ijk}-\bar{y}_{...}\right)^2 =$$

$$=\sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{n=1}^{n}\left[\left(\bar{y}_{i..}-\bar{y}_{...}\right)+\left(\bar{y}_{.j.}-\bar{y}_{...}\right)+\left(\bar{y}_{ij.}-\bar{y}_{i..}-\bar{y}_{.j.}+\bar{y}_{...}\right)\right.$$
$$\left.+\left(y_{ijk}-\bar{y}_{.j.}\right)\right]^2$$

$$SST = SS_A + SS_B + SS_{AB} + SSE$$

Table 2.1 The Analysis of Variance Table for the Two-factor factorial Design

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F-Value
A treatments	SS_A	a-1	$MS_A = \frac{SS_A}{a-1}$	$F_o = \frac{MS_A}{MS_E}$
B treatments	SS_B	b-1	$MS_B = \frac{SS_B}{b-1}$	$F_o = \frac{MS_B}{MS_E}$
Interaction	SS_{AB}	(a-1)(b-1)	$MS_{AB} = \frac{SS_{AB}}{(a-1)(b-1)}$	$F_o = \frac{MS_{AB}}{MS_E}$
Error	SSE	ab(n-1)	$MS_E = \frac{SSE}{ab(n-1)}$	
Total	SST	abn-1		

Hence for a 2^3 factorial Experiment, where k=3, we have the following contrast coefficients:

Table 2.2

Symbol	A	B	AB	C	AC	BC	ABC
(1)	-	-	+	-	+	+	-
A	+	-	-	-	-	+	+
B	-	+	-	-	+	-	+
Ab	+	+	+	-	-	-	-
C	-	-	+	+	-	-	+
ac	+	-	-	+	+	-	-
bc	-	+	-	+	-	+	-
abc	+	+	+	+	+	+	+

2.1 RESEARCH HYPOTHESES

Hypothesis 1

H_{O1}: Gender disparity does not significantly affect students' academic performance in Yaba College of Technology.

Hypothesis 2

H_{O2}: Hours of Study has no significant effect on students' academic performance in Yaba College of Technology.

Hypothesis 3

H_{O3}: Course choice does not significantly affect students' academic performance in Yaba College of Technology.

Hypothesis 4

H_{O4}: Each level of the interaction effects does not significantly affect students' academic performance in Yaba College of Technology.

3.0 RESULTS

Table 1: Descriptive Analysis of Demographic information of Respondents

Variable	Category	Frequency	Percentage (%)
Gender	Male	193	50.5
	Female	189	49.5
Age Group	Below 18years	11	2.9
	18-20	93	24.3
	21-23	127	33.2
	24-26	97	25.3
	27-29	52	13.6
	30 and above years	3	0.8
Ethnic Group	Igbo	96	25.6
	Yoruba	239	62.2
	Hausa	21	5.5
	Others	28	7.3
Mode of Accomodation	On-Campus	124	32.3
	Off-Campus	258	67.2

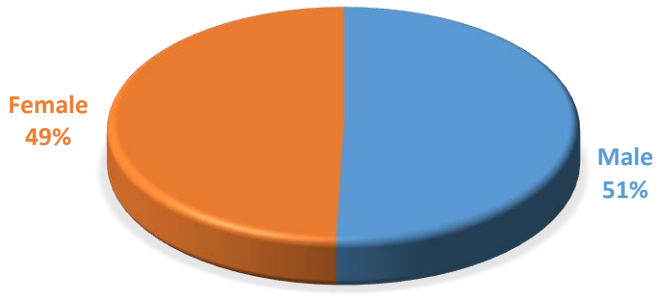

Figure 1: Gender of Respondents

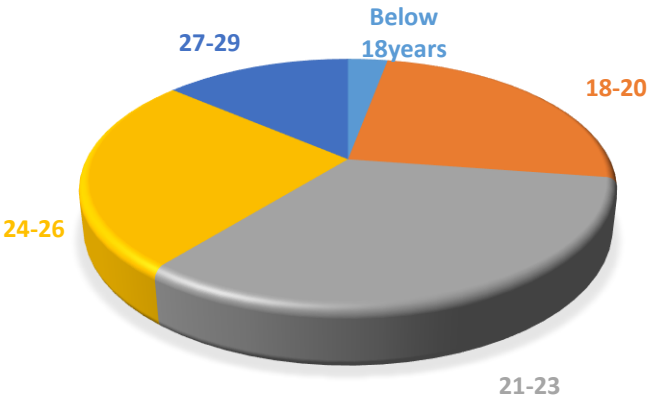

Figure 2: Age of Respondents

The table above shows that 50.5% of the studied respondents are males and 49.5% are females. The largest proportion of the respondents are within the age range of 21-23years (33.2%) while those that are 30 years and above accounted for only 0.8%. Dominant among the study respondents are students of Yoruba ethnic group, accounting for 62.2%, Igbo (25%) and Hausa (5.5%). In terms of mode of accommodation, 32.3% of the students reside on campus while 67.2% live off-campus. It is observed that there are variations in the values of the variables and these variations are due to different reasons particular to each variable in the study.

Table 2: Descriptive Analysis of Determinants of Educational Attributes

Variable	Category	Frequency	Percentage
Academic Level	ND	170	44.4
	HND	213	55.6
Type of Secondary School	Public School	219	57.0
	Private School	185	43.0
Career Choice	Initial Career Choice	225	58.6
	Second Choice/Change of Course	159	41.4
CGPA Level	Distinction	32	8.3
	Upper Credit	156	40.6
	Lower Credit	172	44.8
	Pass	22	5.7
Daily Study Period	1-2 hours	218	56.8
	3-4 hours	166	43.2
Learning Preference	Theory	156	41.1
	Practical Session	224	58.9

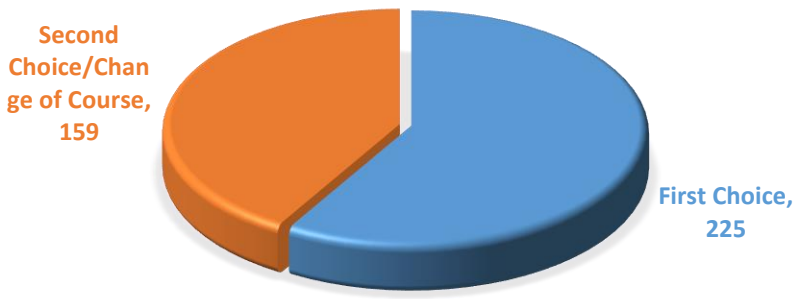

Figure 3: Initial Career Option/Change of Course

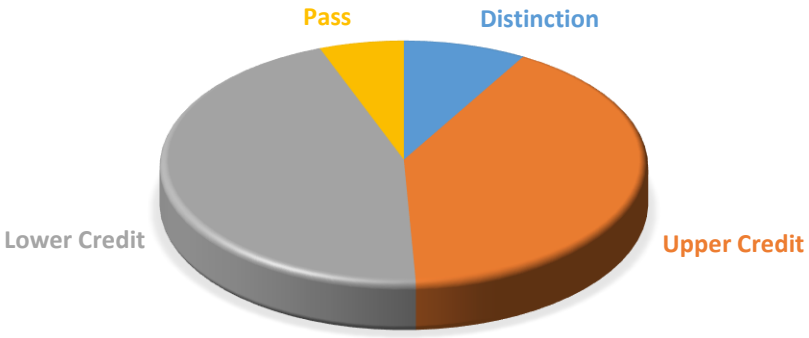

Figure 4: CGPA Level

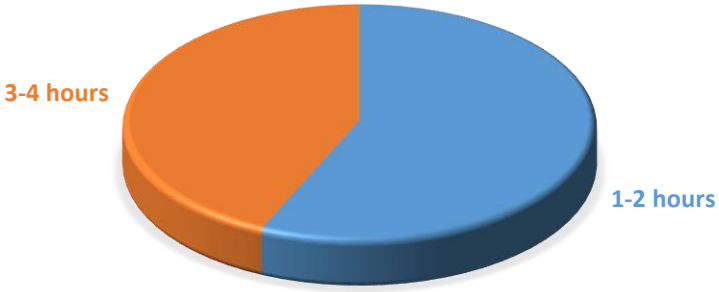

Figure 5: Daily Study Period

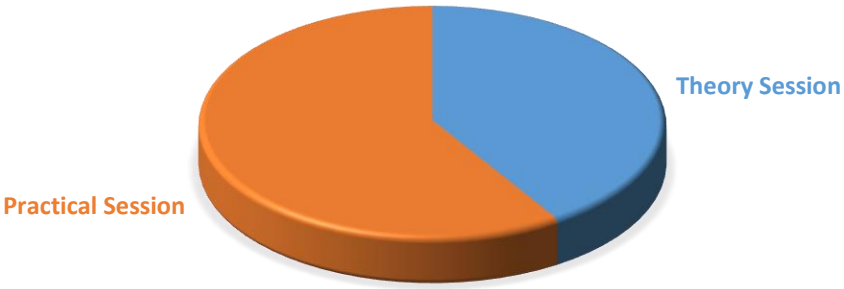

Figure 6: Learning Preference

Table 2 shows that 44.4% of the respondents register for National Diploma programme while 55.6% are on Higher National Diploma programme, 58.6% of the respondents do not change their career option as against 41.4% of the respondents who change their career option or institution. The distribution of the academic grades appear to be normally distributed as 44.8% of the respondents are on a lower credit grade, 40.6% on upper credit, 8.3% on distinction while 5.7% are currently on pass. 56.8% of the respondents reports that they spend between 1 to 2 hours daily to study while 43.2% spend between 3 to 4 hours. In terms of learning preference, 58.9% of the students prefer practical session to 41.4% who choose theoretical lesson.

Table 3: Descriptive Analysis of School related factors contributing to academic performance

School related factors	Strongly agree (%)	Agree (%)	Undecided (%)	Disagree (%)
Class time table is duly followed	107 (27.9%)	205 (53.4%)	55 (14.3%)	17 (4.4%)
There is fast internet access in the school	23 (6%)	92 (24%)	162 (42.2%)	107 (27.9%)
The school library is conducive for reading and assimilation	67 (17.4%)	238 (62%)	69 (18%)	10 (2.6%)
The larger the number of students in class, the better the learning	34 (8.9%)	89 (23.2%)	180 (46.9%)	81 (21.1%)
Communication is key in improving learning in higher institution	173 (45.1%)	183 (47.7%)	20 (5.2%)	8 (2.1%)
Lecturers' manner of teaching in the school aids easy understanding of concept	112 (29.2%)	198 (51.6%)	64 (16.7%)	10 (2.6%)
Class attendance helps improve academic performance	95 (24.7%)	195 (50.8%)	80 (20.8%)	14 (3.6%)
The classroom is conducive for learning.	72 (18.8%)	184 (47.9%)	98 (25.5%)	30 (7.8%)

Table 3 reports the perception of the respondents on school related factors contribution to academic performance. It is observed that majority of the respondents (81.3 %) agreed that class time table is duly followed in the schools implying that there is check and balances in the institution as each unit is playing its role to achieve a common goal. 30% of the respondents agree that the school has fast internet access, this implies that there is the need to provide a functional broadband internet facility to aid learning while only 2.6 % agreed that the school library is not conducive for reading and assimilation. This means that the college operate a good library system that support learning with the adoption of electronic library system. However, 21.1% of the respondent disagreed that student's learn better when their population is large. Most of the respondents (98.2%) also agreed that communication is key in improving students learning in higher institution, 80.8% agreed that lecturers' manner of teaching aids easy understanding of the concept, 75.5% of the respondents agreed that class attendance helps in improving academic performances and 66.7% of the respondents agreed that the classrooms are comfortable enough for adequate learning.

Table 4: Descriptive Analysis of Family related factors contributing to academic performance

Family related factors	Strongly agree (%)	Agree (%)	Undecided (%)	Disagree (%)
My family give me the freedom to read	133 (34.6%)	162 (42.2%)	68 (17.7%)	21 (5.5%)
Studying at home aids my understanding	83 (21.6%)	168 (43.8%)	102 (26.6%)	31 (8.1%)
Family stress affects students' academic performance	149 (38.8%)	164 (42.7%)	52 (13.5%)	19 (4.9%)
Having educated parents influences students in their academic quest	137 (35.7%)	180 (46.9%)	53 (13.8%)	14 (3.6%)
Having a single parent affects the academic performance of student	45 (11.7%)	125 (32.6%)	160 (41.7%)	54 (14.1%)
Family size influences academic performance of students	51 (13.3%)	130 (33.9%)	150 (39.1%)	53 (13.8%)
Financial status can determine how well a student perform in higher institution	100 (26%)	140 (36.5%)	110 (28.6%)	34 (8.9%)

In table 4, Most of the respondents 76.8 % agreed that their family give them the freedom to study while 65.4% also agreed that studying at home aids their understanding. 4.9% of the respondents claimed that family stress affects students' academic performance, in the same vein, majority of the respondents 82.6% agreed that having educated parents influence student academic achievements while 3.6% disagreed with the assertion. Meanwhile, 14.1% of the respondents disagreed that having a single parent could affect the academic performance of students. Similarly, 13.8% disagreed that family size influences student performance in school while 62.5% claimed that financial status of the family can determine how well a student perform in higher institution

Table 5: Summary Statistics of Factors Affecting Academic Performance

Variables	Factors	N	Mean	Std. Deviation
Gender	Male	193	2.9215	0.35045
	Female	189	2.9953	0.29456
Career Choice	First choice	225	2.9713	0.31362
	Second choice/change of course	159	2.9385	0.34110
Duration of study per day	1-2 hours	218	2.9366	0.33966
	3-4 hours	166	2.9855	0.30406

Table 5 showed the summary statistics respectively for each of the factors considered. It revealed that females performed better than males academically while those who opted for their career option also performed better than those who choose second choice/ change their course and cumulative grade point average of those who spend between 3-4 hours studying is also more than those who read between 1-2 hours daily. Further t-test to confirm this result is given below:

Table 6: Independent sample t-test for the factors

CGPA		N	Mean	SD	t	df	p
Gender	Male	193	2.9215	0.35045	-2.225	371	0.027
	Female	189	2.9953	0.29456			
Time of Study	1-2 hours	218	2.9366	0.33966	-1.462	372	0.331
	3-4 hours	166	2.9855	0.30406			
Career Choice	First Choice	225	2.971	0.313	0.973	322	0.144
	Second Choice/Change of Course	159	2.938	0.341			

An independent samples t-test was conducted to compare cumulative grade point average (CGPA) of Males and Females. There was a significant difference in the scores for Males (M=2.9215, SD = 0.35045) and Females (M = 2.9953, SD = 0.29953); $t = -2.887$, $p < 0.05$. This implies that there is difference between males and females in relation to their academic performance. The t-test conducted to compare (CGPA) of respondents who study between 1-2 hours and those that study between 3-4 hours daily revealed that there was no significant difference in the scores for the respondents

(M = 2.9855, SD = 0.30406); $t = -1.462$, $p > 0.05$. This results suggest that there is no difference between academic performance of those that study between 1-2 hours and 3-4 hours among the respondents. Also there is no difference between academic performance of those who were admitted based on their career option and those who changed their course or opted for second choice with

(M=2.9713, SD=0.31362); $t = 0.973$, $p > 0.05$.

Table 9: Summary of 2x3 Analysis of Variance on Factors Contributing to Academic Performance

Source	Sum of Squares	Df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	1.964	7	0.281	2.729	0.009	0.049
Intercept	3120.109	1	3120.109	30350.895	0.000	0.988
Gender (A)	.570	1	0.570	5.541	0.15	0.015
Time of study (B)	.114	1	0.114	1.107	0.03	0.003
Career Choice (C)	.012	1	0.012	.114	0.01	0.00
Gender *Time of Study (AB)	.160	1	0.160	1.554	0.004	0.004
Gender* Career Choice (AC)	.542	1	0.542	5.274	0.14	0.014
Time of study * Career Choice (BC)	.035	1	0.035	.343	0.001	0.001
Gender * Time of Study * Career Choice (ABC)	.396	1	0.396	3.853	.010	0.010
Error	38.448	374	0.103			
Total	3382.845	382				
Corrected Total	40.411	381				

Table 5 revealed the effect of Time of study of the respondents (factor B) $F(1, 374) = 1.107$, $P < 0.05$, CareerChoice (factor C), $F(1, 374) = 0.114$, $P < 0.05$, and the interaction effects between Gender *Time of Study (factor AB), $F(1, 374) = 1.554$, $P < 0.05$, Time of study * CareerChoice (factor BC) $F(1, 374) = 0.343$, $P < 0.05$ and Gender * Time of Study * CareerChoice (factor A BC) $F(1, 374) = 3.853$, $P < 0.05$, are statistically significant. Also, other effects like Gender (factor A) $F(1, 374) = 5.541$, $P > 0.05$ and Gender* CareerChoice (factor AC) $F(1, 374) = 5.274$, $P > 0.05$ are not statistically significant. Thus, each of factors B, C, AB, BC, and ABC influences significantly academic performance of students while A and AC do not. Based on Partial Eta Squared Statistic, It was found that each of the main effects and interaction effects have positive impact on students' academic performance while main effect of Career Choice does not $F(1, 374) = 0.00$.

4.0 DISCUSSIONS

The distribution of the social-demographic characteristics showed that 50.5% of the studied respondents are males as against 49.5% that are females which implies that there are more males in the study than females. The largest proportion of the respondents are within the age range of 21-23 years (33.2%), 24 and 26 years (25.3%), 18 and 20 years (24.3%), 27 – 29 years (13.6%), <18 years (2.9%), while >30 years (0.8%) of the studied population. 44.4% of the respondents register for National Diploma while 55.6% are for Higher National Diploma programme. 81.3% of the respondent agreed that they normally adhere strictly to the class time table while 79.4% however agreed that the school library is conducive for reading and assimilation. This implies that there are checks and balances in the system for efficient service delivery. 30% of the respondents agreed that the school has fast internet access, this point out the need for the management to provide an efficient broadband internet facility for effective learning. 92.8% of the respondents agreed that communication is key in improving learning in higher institution, this result is in line with the position of Abdullah (2011) who affirmed that good communication skill help improve student academic performance. 80.8% of the respondents agreed that lecturers' manner of

teaching aids easy understanding of the concept. 75.5% agreed that class attendance helps in improving academic performance while only 7.8% of the respondents claimed that the classroom is not conducive for learning. This outcome supports the claim of Robert and Sampson (2011) who stated that students that effectively participate in learning procedure are seen to have a higher CGPA (cumulative grade point average). 76.8% of the respondents agreed that they have the freedom to read at home while 64.4% also agreed that studying at home aids their understanding. Only 4.9% of the respondents disagreed that family stress affects their academic performance. 82.6% of the respondents agreed that having educated parents influence wards academic performance while 14.1% of the respondents disagreed that having a single parent could affect the academic performance of students. Similarly, 13.8% disagreed that family size influences student performance in school while 62.5% claimed that financial status of the family can determine how well a student performs in higher institution. These results validate earlier work of Okolie et al (2014) who affirmed that parents' support and type of family background might influence student's performance. Also, 58.6% of the respondent choose their career option as against 41.4% of the respondents who change either their career option or institution. This result is in line with Akinsehinde (2016) who revealed that the students' choice of career in Quantity surveying is as a result of change of career choice by the students or by the institution, different from the initial choice of career desire. However, the t-test analysis results show that the cumulative grade point average (CGPA) of students who maintain their career choice is not statistically significantly different from those who either change their career option or institution. Similarly, the average CGPA of students who study between 1 and 2 hours daily is not statistically significantly difference from those that spend 3-4 hours to read. This implies that the variation in the number of study hours does not affect student academic performance. In terms of study preference, more students (58.9%) prefer practical session in relation to theoretical lessons (41%). This is a varied outcome as outline by Smith and Renzulli (1984) in their work on learning styles where they affirm that learning style may vary but there are no clear-cut and systematic differences in learning style preference with any given classroom of students.

The findings also revealed that each of the main effects of Time of study, Career Choice and their interaction effects, Gender and Time of Study, Time of study and Career Choice, and Gender/ Time of Study/Career Choice are statistically significant while other effects like Gender and Gender and Career Choice are not statistically significant which implies that all the factors influence significantly academic performance of students while Gender and the interaction between Gender and Career choice do not. This work is in line with Yisa (2010) who affirm that teaching method, level of study and their interaction have significant effects on students' academic performance while Gender does not.

5.0 CONCLUSION

Education holds the key to any significant development in a nation. It is the process of receiving systematic instruction, or the acquisition of knowledge, and personal development. Academic success is imperative because it is a product of positive outcomes for the individual and the society at large because individuals who are educated are likely to be successful, have stable employment or have more job prospects, receive higher incomes, have health insurance, less reliance on social assistance, and are less likely to commit crime, are more active as citizens and give back to their community and live a prosperous and happy life. This study therefore conclude that gender is a key determinant of academic performance and students can increase their chances of doing well in their academics if they can effectively participate in learning procedure which will lead to better individual and better nation.

6.0 RECOMMENDATIONS

Emotional aspects of teaching should be included in the teaching model presently in use to equip students academically and psychologically so as to function effectively in their chosen field of discipline. The college management should provide an efficient internet facility for effective learning. It can improve the quality of education in many ways by opening a pathway to abundance of information, scholarly and educational resources, and more prospects for learning in and beyond the classroom. Special attention should be paid to the issue of initial choice of career of candidate applying for admission because following a career path lead to building of confidence that will open the way for economic security and job satisfaction. More weight should be allocated to practical oriented courses to empower and equip the student in becoming a professional of the future. Parents and Guardian should be more proactive in order to have a lasting impact on their children's learning because of the longer-term economic and social benefits.

REFERENCES

Abdullah, A. (2011). Factors affecting business students' performance in Arab Open University. *The case of Kuwait. International Journal of Business and Management, 6*(5), 146.

Akinseinde, O. A. (2016). Factors Responsible for the Decline in Youth Participation in Quantity Surveying Education in Nigeria. *5th National Conference of the Academic Staff Union of Polytechnics (ASUP) Ilaro Chapter,16th-19th October, 2016.*

Anikweze. (2005). *Measurement and Evaluation in Education.* Enugu: Snaap Press Ltd.

Arthur, N., & Everaert, P. (2012). Gender and performance in accounting examinations, exploring the impact of examination format. *Accouting Education*, 471-487.

Baard, R., Steenkamp, L., Frick, B., & Ve Kidd, K. (2010). Factors influencing success in first-year accounting at a South African University, the profile of a successful first-year accounting student. . *South African Journal of Accounting Research* , 129-147.

Byrne, M., & Flood, B. (2008). Examining the Relationships among Background Variables and Academic Performance of First Year Accounting Students at an Irish University. *Journal of Accounting Education*, 202-212.

Epstein, J. L. (1987). Parent Involvement: What Research Says to Administrators. *Education and Urban Society, 19*(2), 119–136. doi:10.1177/0013124587019002002

Gracia, L., & Jenkins, E. (2002). An exploration of Student Failure in an Undergraduate Accounting Programme of Study. *Accounting Education, An international Journal*, 93-107.

Kochhar, S. (2000). Educational and Vocational Guidance in Secondary Schools. *Sterling Publishers Private Limited*.

Montgomery, D. (1996). *Design and Analysis of Experiments* (4th ed.). USA: John Wiley & Sons, Inc.

Noble, J., Roberts, W., & Sawyer, R. (2006, October). Student achievement, behavior, perceptions and other factors affecting ACT scores. *ACT Research Report Series*.

Okolie, U., Inyiagu, E., Elom, E., Ndem, J., & Nwuzo, A. (2014). Effect of Home Background on academic Performance of Technical College Students in Ebonyi State, Nigeria. *The International Journal of Humanities and Social Studies, 2*(5), 76-82.

Roberts, K., & Sampson, P. (2011). School board member professional development and effects on student achievement. *International Journal of Education Management*, 701-713.

Schmidt, D., & Ve Wartick, M. (2014). Performance in upper-level accounting courses: The case of transfer students . *Advances in accounting education, teaching and curriculum innovations* , 171-192.

Tobih, D. (2012). Students appraisal of the conduct of undergraduate examinations in Obafemi Awolowo University Ile-Ife, Nigeria. *Ife Journal of Theory and Research in Education*, 86-97,.

Wally-Dima, L., & Mbekomiza, C. (2013). Causes of Gender Differences in Accounting Performances. *International Education Studies*, 13-26.

Waters, T., & Marzano, R. (2006). School District Leadership that Works: The Effect of Superintendent Leadership on Student Achievement. *Mid-Continent Research for Education and Learning*.

Weil, S., Oyelere, P., & Rainsbury, E. (2004). The Usefulness of Case Studies in Developing Core Competencies in a Professional Accounting Programme . *Accounting Education,*, 139-169.

Yisa, Y. (2010). Design and Analysis of 2^3 Factorial Experiments of Variables Affecting Students'Academic Performance. *African Journal Online, 8*(2). doi:104314/jorind.v8i2.66843

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GERMINATION AND GROWTH OF *Luffa aegyptiaca* (Mill) ON SPENT ENGINE OIL CONTAMINATED SOIL: EFFECT OF *Alternaria altanata*

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ABSTRACT

The indiscriminate disposal of Spent Engine Oil (SEO) from engines after servicing is recently on the increase in Nigeria due to increasing urbanization and industrialization. Resulting in gross pollution of the environment with its negative consequence on plants and other life forms both on terrestrial and aquatic organisms. The effect of *Alternaria altanata* on germination and growth of *Luffa aegyptiaca* grown on spent engine oil polluted soil was assessed *in-vivo* on experimental plot of land. The effect of SEO on *L. aegyptiaca* was assessed on plant height, leaf area, stem girt, internode length and number of leaves. Using Randomized Complete Block Design (RCBD), eight treatments were applied including SEO, *A. altanata* and *L. aegyptiaca*. Results show that the spent engine oil-contaminated soil affected the growth of *L aegyptiaca* negatively, resulting in poor/delayed germination, reduced heights, leave chlorosis, decrease in number of leaves among others. The introduction of *Alternaria altanata* on the polluted soil however, increased seed germination from 20% to 60% in 5 days in addition to improving other agronomic parameters. *Alternaria altanata* is therefore, shown by this result to improve the germination, growth and survival of *L. aegyptiaca* in spent engine oil contaminated soil

KEY WORDS: *Luffa aegyptiaca,* Phytoremediation, *Alternaria altanata,* germination,

INTRODUCTION

Luffa is the genus name of a group of gourds also known as vegetable sponges, dish cloth gourds, Chinese okra and loofa. It is a member of *Cucurbitacea* family. (Ani *et al.,* 2020, Dairo *et al.,* 2007). The leaf is 13cm and 30cm in length and width respectively and has the acute-end lobe. It is hairless and has serrated edges. The flowers of *L. aegyptiaca* is yellow and blooms to August- September (Mazali *et al.,*2005). Its fruit is green and has a large cylinder like shape. He outside of the fruit has vertical lines and a reticulate develops inside of the flesh. The stem is green and pentagonal and grows climbing other physical solid. Sponge gourd prefers pH of around 6 to 6.8 and the seeds need to be germinated at 25°c, grown on and transplanted when the soil temperature is about 18°C (Partap *et al.,* 2012). In traditional African medicine, *L. aegyptiacac* or its extracts are use in the treatment of constipation, as a diuretic, nose cancer, as an abortifacient, to promote wound healing, oedemas, for the treatment of malaria, enterobiasis, filariasis, whooping cough, stomach-ache, to facilitate childbirth and as a purgative. Young fruits are used as vegetable. (Sangh *et al.,* 2012).

Since the dawn of the industrial revolution, mankind has been introducing numerous hazardous chemicals into the environment at an exponential rate. These hazardous pollutants consist of a variety of organic compounds and heavy metals which pose serious risks to human health. The economic growth that Nigerian has enjoyed as a result of oil revenue has exposed the population in oil producing areas to environmental contamination (Ani *et al.,* 2021, Anoliefo *et al.,* 2006, Sharddha *et al.,* 2015). Plants especially legumes have been identified to play important role in remediating oil polluted soil. (Mojiri *et*

al., 2012, Parrish *et al*., 2005). A number of researches have been carried out on the effect of pollutants on plants. Agbogidi *et al.*, 2005 and Abii *et al.*,2009 studied the effect of crude oil spillage on soil fertility and plant growth. Odjegba *et al.*, 2002; Agbogidi *et al.*, 2010; showed the effect of spent engine oil on plants. Some of the most commonly observed symptoms of oil pollution on plants include; deformation of chlorophyll, alteration in the stomata mechanisms and reduction in photosynthesis and respiration, increase in the production of stress related phytohomones (Larcher *et al.*, 2003), accumulation of toxic substances or their by product in vegetal tissue, decrease in size and less production of biomass (Adenipekun *et al*.,2008). This study aims at investigating the influence of *Alternaria altanata* on *Luffa aegyptiaca* grown on spent engine oil polluted soil.

MATERIALS AND METHODS

Sample collection

Mature and dried seeds of *Luffa aegyptiaca* were collected at Somolu, Lagos State (latitude 5º 33N, latitude 3º20E) while spent engine oil was collected at local Author Mechanic workshops.

Isolation of fungi from rhizosphere of *L. aegyptiaca*

For the isolation of fungi from rhizosphere of *L. aegyptiaca*, Volume Displacement Technique as suggested by Reyes & Mitchell (1962) was used. Fungi were cultured in sterilized Petri dishes containing Potato Dextrose Agar supplemented with streptomycin (0.2 g/l) to prevent bacterial growth and incubated at room temperature.

Physio chemical analysis of soil and spent engine oil

Physico-chemical characteristics of the experimental soil and spent engine oil used was analyzed. The particle size distribution was determined, soil pH was determined in distilled water using soil liquid ratio of 1:1, electrical conductivity was measured using conductivity bridge. Phosphate- phosphorous, total nitrogen was also determined, Nitrate-nitrogen was determined by the phenol-disulphonic, Organic carbon was measured using wet combustion method and converted to organic matter by multiplying the values of organic carbon by a factor of 1.722. Exchangeable calcium was determined on atomic absorption spectrophotometer.

Experimental design

The research work was carried out in a plot of land measuring $20m^2$ (latitude 8º33N, longitude 4º25E) at the Botanical/Zoological garden, University of Lagos. The plot was divided into 4 blocks, Block1 (B_1), Block 2 (B_2), Block 3 (B_3) and Block 4 (B_4) which was further divided into cells of $1m^2$ each with inter-blocking/intercellular space of 70cm. Within each block, using Randomized Complete Block Design, eight treatments were randomly assigned using a random number table.

Treatment 1 (T1) = Remove weeds with plant
Treatment 2 (T2) = Allow weeds with plant
Treatment 3 (T3) = Remove weeds with plant with oil
Treatment 4 (T4) = Allow weeds with plant with oil
Treatment 5 (T5) = Remove weeds with plant with oil with fungus
Treatment 6 (T6) = Allow weeds with plant with oil with fungus
Treatment 7 (T7) = Remove weeds with plant with fungus
Treatment 8 (T8) = Allow weeds with plant with fungus

Each of the treatments were replicated 4 times. Seeds *L. aegyptiaca* were planted in the plot polluted seven days after pollution at the rate of $1L/1m^2$ following a modified method of Adu *et al*., 2015. *Alternaria altanata* isolated from the rhizosphere of *L. aegyptiaca* was introduced 24hours after planting. For some of the treatments, weeding was done while for others, weeding was not done. The spent engine oil was applied on the soil surface without mixing with the soil to provide a scenario as close to real life as possible.

Planting and germination

viability of the seeds was tested by floatation method as described by Jephris *et al.*, 2015. The seeds were sown to the depth of 3cm. The study was conducted for 6 months (May-November, 2016). The germination percentage was calculated according to Oyedeji *et al.*, 2012.

$$\textbf{Percentage germination} = \frac{\textbf{No of germinated seeds}}{\textbf{No of seeds sown}} \textbf{X} \frac{\textbf{100}}{\textbf{1}}$$

Data collection

Growth study of the plant was studied under experimental condition by measuring the effect of spent engine oil on Leaf area, stem girth, internode length, plant height and number of leaves of *L. aegyptiaca* at two weeks' interval for 24weeks. Leaf area was calculated using the method described by Okon and Mbong (2013).

Statistical Analysis

Result were analyzed statistically using student general linear model (GLM) which incorporates the univariate analysis of variance (ANOVA) and the pair wise test comparison at ($P<0.05$) which was considered significant compare to the control

RESULTS

The physicochemical parameters of the soil and spent engine oil used for this experiment are presented in tables 1 and 2 respectively.

Table1: Physicochemical properties of the soil sample used

Properties	Percentage
Soil PH	6.20
Total organic carbon (TOC)	1.57
Organic matter %	2.74
Clay %	33.00
Silt %	50.00
Sand %	21.00
Exchangeable cation (cmo/kg-1)	185.24
Available phosphate(mg/kg)	10.20
Available sulphate(mg/kg-1)	117.60
NO_3 (mg/kg)	44.00

Table 2: Physicochemical properties of spent engine oil used

Parameters	Results
PH	6.04
Moisture content (%)	13.92
Temperature (C^0)	29.80
Density g/cm^3	0.94
Viscosity	24.00
Flash point	122.00
Sulphur mg/kg	0.97
Iron (mg/kg)	26.92

Effect of *Alternaria altanata* and spent engine on seed germination

Results of the effect of SEO and *Alternaria altanata* on germination of seeds of *L. aegyptiaca* are presented on table 3. *Luffa aegyptiaca* planted in soil treated with SEO started germinating after 5 days compared to 2 days for plants in untreated soil (control) and 3 days for seeds planted in soils treated with SEO and *A. altanata* (fungus). Germination efficiency for seeds planted an unpolluted soil was up to 90% but reduced to 20% when the soil was polluted with SEO. However, the introduction of *Alternaria altanata* increased seed germination in the oil polluted soil from 20% to 60%.

Table 3: Effect of *Alternaria altanata* (fungus)and spent engine on germination

Treatments	No of seeds planted	No of seeds germinated	Germination (%)
Remove weeds with plant (T_1)	10	9	90
Allow weeds with plant (T_2)	10	8	80
Remove weeds with plant with fungus (T_7)	10	9	90
Allow weeds with plant with fungus (T_8)	10	8	80
Remove weeds with plant with oil (T_3)	10	3	30
Allow weeds with plant with oil (T_4)	10	2	20
Remove weeds with plant with oil with fungus (T_7)	10	5	50
Allow weeds with plant with oil with fungus (T_8)	10	6	60

Effect of *Alternaria altanata* and spent engine on Growth of *L. aegyptiaca*

The effect of SEO and *A. altanata* on the height, leaf area, internode, number of leaves and stem girth of *L. aegyptiaca* are presented on table 4. Statistical analyses show that plant grown in non-oil contaminated soil performed better than those in Spent Engine Oil-polluted soil. Some of the observed effects of SEO on the growth of *L. aegyptiaca* include reduced heights, internodes and leaves areas in addition to chlorosis on the leaves. The introduction of *Alternaria altanata* on the polluted soil however, improved these agronomic parameters.

Table 4: Minimum and maximum of different parts by factors

NB: values in parenthesis are the means and std. errors preceded by the minimum and followed by the maximum. Treatments with the same superscripts are not significantly different from each other at 5%

	Plant heights	Number of leafs	Stem girt	Internodes	Leaf area
Treatments	**Mean ± Se**	**Mean ± Se**	**Mean ± Se**	**Mean ± Se**	**Mean ± Se**
R + P	3.9 (23.25 ± 1.7) 46.9^{b}	2 (19.98 ± 1.7) 47^{c}	0.2 (0.32 ± 0.01) 0.4^{c}	0.2 (2.5 ± 0.13) 4.1^{ab}	1.91 (11.09 ± 0.77) 24.6^{de}
R + P + O	3.3 (15.23 ± 0.99) 33.5^{a}	1 (13.33 ± 1.06) 34^{a}	0.1 (0.2 ± 0) 0.2^{a}	0.4 (2.11 ± 0.12) 3.7^{a}	3 (8.34 ± 0.41) 14.2^{bc}
R + P + O + F	4.3 (16.78 ± 1.03) 33.6^{a}	2 (14.97 ± 1.08) 33^{ab}	0.1 (0.19 ± 0) 0.2^{a}	1 (2.47 ± 0.11) 4.3^{ab}	3.31 (7.88 ± 0.38) 15.4^{ab}
A + P	5.2 (26.17 ± 1.76) 46.8^{b}	3 (21.27 ± 1.72) 43^{c}	0.2 (0.3 ± 0.01) 0.4^{c}	0.6 (3.51 ± 0.2) 5.6^{d}	3.64 (11.81 ± 0.79) 24.4^{e}
A + P + O	4.3 (16.63 ± 0.99) 33.2^{a}	2 (15.01 ± 1.08) 39^{ab}	0.1 (0.2 ± 0) 0.3^{a}	0.4 (2.61 ± 0.15) 5.3^{bc}	3 (6.71 ± 0.3) 12.9^{a}
R + P + F	4.9 (22.84 ± 1.38) 39.6^{b}	3 (18.52 ± 1.28) 38^{bc}	0.2 (0.37 ± 0.01) 0.4^{d}	0.6 (2.68 ± 0.11) 4.1^{bc}	3.71 (11.45 ± 0.6) 21.6^{de}
A + P + F	4.5 (22.3 ± 1.47) 41.4^{b}	2 (19.42 ± 1.6) 42^{c}	0.2 (0.27 ± 0.01) 0.4^{b}	0.2 (3 ± 0.18) 5.3^{c}	3.25 (9.23 ± 0.56) 20.4^{bc}
A + P + O + F	3.1 (16.88 ± 1.01) 31.6^{a}	1 (14.54 ± 1.21) 32^{ab}	0.1 (0.2 ± 0.01) 0.4^{a}	0.4 (2.31 ± 0.16) 4.4^{ab}	3.8 (9.89 ± 0.52) 19.6^{cd}
F – statistics	$F_{7,447}$ = 9.556; p < 0.001	$F_{7,448}$ = 4.827; P < 0.001	$F_{7,448}$ = 158.969; P < 0.001	$F_{7,447}$ = 8.273; P < 0.001	$F_{7,448}$ = 12.074; P < 0.001

KEY:
R=Remove natural vegetation, A=Allow natural vegetation, P=Plant (L. aegyptiaca), F=Fungus (Aleranaria altanata), O=Oil (spent engine oil)

DISCUSSION

Soil pollution by petroleum and its derivatives especially spent engine oil is posing a great challenge to agricultural productivity in Nigeria and thus resulting in increasing poverty and hunger among the populace. The negative influence of oil pollution to agricultural crops and soil micro-organisms has been highlighted by several authors. (Odjegba *et al.*, 2002; Adam *et al.*, 2003; Agbogidi *et al.*, 2010). Germination of *Luffa aegyptiaca* was delayed in SEO polluted soil

similar to the finding of (Adenipekun *et al.*, 2009) that oil in soil above 2% concentration adversely affects the growth of agricultural crops. This study demonstrated that used motor oil has significant effect on the germination and growth of *L. aegyptiaca.* Delayed germination of seeds of *L. aegyptiaca* was observed in SEO polluted soil similar to the finding of Nwite and Alu 2015 in a similar research using maize seeds. The effect of used motor oil on *L. aegyptiaca* was observed in reduction in the number and leaf area, reduction in heights, internodes and stem girth. Agbogidi *et al*., 2006 showed that crude oil application to soil significantly reduced the growth of okra. Reduction in number of leaves for *Parkinsonia aculeate* gown in an oil polluted soil was observed by Igbal *et al.*, 2016 similar to the finding of this research. The response of seeds and seedlings of *L. aegyptiaca* to soil treatment with fungi (*Alternaria altanata)* is of particular importance because it enhanced the ability of the plant to withstand oil polluted environmental. Treatment of soil polluted with used motor oil with *A. altanata* significantly ($p<0.05$) increased the agronomic parameters compared with polluted soil without *A. altanata*. The ability of rhizopheric fungi to increase the supply of mineral nutrients to plant, stimulate plant growth indirectly by preventing the growth or activity of pathogens and promote the growth of plants through the production of phytohomones have been highlighted by (Prakash and Sheela 2016). Gamal, 2005 showed that fungi especially Mycorrhizal fungi assists plant during water stress. Fungi have been known to improve the performance of plant under environmental stress through modifying root architecture (Hooker and Atkinson, 1996), improving membrane function (Graham *et al*., 1981) and by enhancing production of oxidative enzymes (Salzer *et al.*, 1999). The Inoculation of *L. aegyptiaca* with *Alternaria altanata* increased the survival and growth of *L. aegyptiaca* in SEO contaminated soil. This is similar to the finding of Gamal, 2005 who reported reduction in the negative effect of oil pollution on plant when a fungus is introduce. This study shows that the introduction of spent engine oil on agricultural soil has considerable negative effects on the germination and agronomic growth and development *L. aegyptiaca*

CONCLUSION

The role of rhizospheric fungi in germination, survival and growth *Luffa aegyptiaca* is highlighted in this study. A better understanding of the basic principles of the rhizosphere ecology, including the function and diversity of rhizospheric fungi is needed to optimize soil microbial technology to the benefit of plants in an oil polluted environment.

REFERENCES

Abii, T.A., and Nwosu, P.C. (2009). The effects of oil spillage on the soil of Eleme in Rivers state of Nigeria. *Research Journal of Environmental Toxicology.***1**(3): 116-123

Adenipekun, C.O., Oyetunji, O.J. and Kassim, L.Q. (2009). "Screening of *Abelmoschus esculentus* L. Moench for Tolerance to Spent Engine," *J. of Applied Biosciences,* 20:1131-1137

Adenipekun, C.O., Oyetunji, O.J. and Kassim, L.S. (2008). Effect of spent engine oil on the growth parameters and chlorophyll content of *Cocchorus olitorus* Linn. Environmentalist. 28: 446-450.

Adu, A. A.; Aderinola, O. J. and Kusemiju, V. (2015). Comparative effects of spent engine oil and unused engine oil on the growth and yield of *Vigna Unguiculata* (Cowpea). *International Journal of Science and Technology*, 4(3):105-118.

Agbogidi O.M, Onosode A.T, Okonta B.C. (2006). Susceptibility of *Dennettia tripetala* (Bak.) F. seeds to crude oil. *Journal of Food, Agriculture and Environment,* 4(2): 350– 352.

Agbogidi, O.M. and Nweke (2005). Effect of crude oil polluted soil on the performance of Okra (*Abalmoschus esculentus L.*). Moench in Delta State Afr. J. Nat. Sci. 8: 31- 35

Ani, E., Adekunle, A. A., Aboluwade J. B. and Ibrahim, O. A. (2020). *Ex-situ* Characterization of *Luffa aegyptaica* in Lagos State, Nigeria. *Baghdad Science Journa*l, **17**(3):946- 952.

Ani, E., Adekunle, A.A., Kadiri, A. B. and Njoku, K. L. (2021). Rhizoremediation of hydrocarbon contaminated soil using Luffa aegyptiaca (Mill) and associated fungi. International Journal of Phytoremediation. DOI: 10.1080/15226514.2021.1901852

Anoliefo, G.O. and vivioko, D.E. (2006). Tolerance of chromolaena odorata (L) grown on soil contaminated with spent lubricating oil. Journal of Tropical Bioscience **1**: 20 - 24.

Dairo, F.S., Aye, P.A. and Oluwasola, T.A. (2007). Some functional properties of loofa gourd *(Luffa cylindrical*) seed. *Journal of food Agriculture and Environment* **5**:97-101.

Gamal, H. R. (2005). Role of Arbuscular Mycorrhizal Fungi in Phytoremediation of Soil Rhizosphere Spiked with Poly Aromatic Hydrocarbons. *Mycobiology*, 33: 41-50

Graham, J. H., Leonard, R. T. and Meng, J. A. (1981). Membrane mediated decrease in root exudation responsible for phosphorus inhibition of vesicular-arbuscular mycorrhiza formation. *Plant Physiol.* 68: 549-552.

Hooker, J. E. and Atikinson, D. (1996). Arbuscular mycorrhizal fungi- induced alteration to tree- root architecture and longevity, *J. Pflanz. Boden.* 159: 229-234.

Iqbal, M. Z., Khursheed, S. and Shafiq, M. (2016). Effects of motor oil pollution on soil and seedling growth of *Parkinsonia aculeate*. *Sci. Agri.* 13 (3): 130-136

Jephris, G., Rangariray, K., Victor, N., Tendayi, N., Silas, M.M. and Alfred, K. (2015). Filing considerably breaks seed dormancy of *Berchemia discolour* Hemsley. *African Journal of Plant science*, **9**(6):274-278

Larcher, W. (2003). *Physiological Plant Ecology* (fourth edition), SpringerVerlag, Berlin, Heidelberg, New York,

Mazali, I.O. and Alves, O.L. (2005). Morphosynthesis: high fidelity inorganic replica of the fibrous network of loofa sponge (*Luffa Cylindrica*). *An Academy Base Journal* **77**(1): 25-31.

Mojiri, A., Abdul Aziz, H., Zaman, N.Q. and Azi, S.Q. (2012). A Review on Anaerobic Digestion, Bio-reactor and Nitrogen Removal from Wastewater and Landfill Leachate by Bio-reactor. *Advances in Environmental Biology*, 6(7): 2143-2150,

Nwite, J. N. and Alu, M. O. (2015). Effect of different levels of spent engine oil on soil properties, grain yield of maize and its heavy metal uptake in Abakaliki, Southeastern Nigeria. *Journal of Soil Science and Environmental Management*, 5(4):44-51

Odjegba, V.J. and Sadiq, A.O. (2002). Effect of spent engine oil on the growth parameters, chlorophyll and protein levels of *Amaranthus hybridus* L. *Environ.*, 22: 23-28

Okon, J. E. and Mbong, E. O. (2013). Effects of Nutrient Amendments of Spent Engine Oil Polluted Soil on Some Growth Parameters of *Abelmoschus esculentus* (L.) Moench. in South-South Nigeria. *Bull. Env. Pharmacol. Life Sci.*, 2 (5): 75-78

Oyedeji,A. A., Adebiyi, A. O. Omotoyinbo, M. O. and Ogunkunle. C. O. (2012). Effect of Crude Oil-Contaminated Soil on Germination and Growth Performance of Abelmoschus esculentus L. Moench—A Widely Cultivated Vegetable Crop in Nigeria. *American Journal of Plant Sciences*, 3:1451-1454

Parrish, B. D., Luzadis, V.A. and Bentley, W.R. (2005). What Tanzania's Coffee Farmers Can Teach the World: A Performance-Based Look at the Fair Trade–Free Trade Debate. *Sust. Dev.* 13:177–189

Partap, S., Kumar, A., Sharma, N.K. and Jha, K.K. (2012). *Luffa Cylindrica*: An important medicinal plant. *Journal of*

National Products of Plant Resources **2**: 127-134.

Prakash Deshmukh, Sheela Shinde (2016). Beneficial Role of Rhizosphere Mycoflora in the Field of Agriculture: An Overview. *International Journal of Science and Research*, 5(8):529-533

Salzer, P., Corbiere, H. and Boller, T. (1999). Hydrogen peroxide accumulation in *Medicago truncatula* roots colonized by the arbuscular mycorrhiza-forming fungus *Glomus intradices. Planta*. 208: 319-325.

Sangh, P., Amit, K., Neeraj, K.S., Jha K.K. (2012). *Luffa Cylindrica*: An important medicinal plant. *International Journal of Agricultural Science* **2**:127-134.

Shraddha, T. Raman, T. and Jata, S. (2015). Role of Heat-Shock Proteins in Cellular Function and in the Biology of Fungi. *Biotechnology Research International*, 10:1-11

Reyes, A.A. and J.E. Mitchell. (1962). Growth response of several isolates of *Fusarium* in rhizosphere of host and non-host plants. *Phytopathol.*, 52: 1196-1200.

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BIOCHEMICAL EVALUATION OF PAINT EFFLUENT ON THE ANTIOXIDANT ENZYMES OF *TYMPANOTONUS FUSCATUS VAR RADULA* UNDER SUBLETHAL CONDITION.

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ABSTRACT
In Nigeria, paint production makes use of large volume of water lacking adequate waste water treatment plant. Therefore, enormous quantities of both hazardous and non hazardous wastes are naturally released to the environment, thus causing health-related problems, ecological imbalance and bioaccumulation in aquatic organisms. This study aims at examining the biochemical changes of the effect of paint effluent on the antioxidant enzymes of *Tympanotonus fuscatus* under sublethal condition. Healthy test organisms (*Tympanotonus fuscatus)* were handpicked at the mud flat area of lagoon front at University of Lagos. Bioassay test procedure in the laboratory followed standard methods. A sublethal varying concentration of 1.80ml, 0.50ml, 0.20ml, 0.13ml, 0.10ml and a control experiment was used. The paint effluent was analysed for physicochemical and heavy metal characteristics. The oxidative state of the tissues of *Tympanotonus fuscatus* was examined after chronic toxicity testing using GSH, CAT, SOD and MDA as a biomarker for oxidative stress. The oxidative state examination revealed that there were significant reductions in GSH, CAT and SOD activities compared to the activities in the control experiment. Significant increase was observed in MDA content of the tissue compared to the control. The assessment of the oxidative state of *Tympanotonus fuscatus* revealed that the organism experienced oxidative stress which can be attributed to the effect of the paint effluent. Conclusively, this study has shown that the paint effluent induced oxidative stress in the tissue of *Tympanotonus fuscatus*.

KEYWORD: Paint effluent, *Tympanotonus fuscatus*, Toxicity, Antioxidant enzyme, Oxidative stress.

1.0 INTRODUCTION
Coastal and estuarine ecosystems have been, and still are, severely influenced by humans through pollution and habitat loss worldwide. Industrial waste water is one of the important pollution sources in the contamination of the aquatic environment. During the last century, a huge amount of industrial waste water was discharged into river, lakes and coastal areas. This resulted in serious pollution problem in the aquatic environment and caused negative effect to the ecosystem and human life (Hanchang, 2004).
Wastewater is produced primarily due to cleaning operations of mixers, reactors, blenders, packing machines and floors. Owing to the varying degree of chemicals used, the wastewater contains palpable concentrations of carbon (biological oxygen demand (BOD) or chemical oxygen demand (COD)), suspended solids, toxic compounds and colour (Aboulhassan *et al.*, 2014).
Water-based paints commonly consist of organic and inorganic pigments and dyestuffs, extenders, cellulosic and non-cellulosic thickeners, latexes, emulsifying agents, antifoaming agents, preservatives, solvents and coalescing agents, which, due to their high persistence and toxicity, have shown to be difficult to treat effectively. Latex paints commonly consist of organic and inorganic pigments and dyestuffs, extenders, cellulosic and non-cellulosic thickeners, latexes, emulsifying agents, antifoaming agents, preservatives, solvents and coalescing agents

(Aboulhassan *et al.,* 2014). The release of such wastewater into the environment inhibits light penetration, injury to the quality of the receiving streams and could be toxic to treatment processes, to food chain flora and fauna and to overall aquatic life (Aboulhassan *et al.,* 2006).

Antioxidant enzymes are capable of stabilizing, or deactivating free radicals before they attack cellular components. They act by reducing the energy of the free radicals or by giving up some of their electrons for its use, thereby causing it to become steady. In addition, they may also interrupt with the oxidizing chain reaction to lessen the damage caused by free radicals. For the past decade, numerous studies have been devoted to the beneficial effects of antioxidant enzymes. It has been found that a considerable link exists between free radicals and more than sixty different health conditions, together with the aging process, cancer, diabetes, Alzheimer's disease, strokes, heart attacks and atherosclerosis. By reducing exposure to free radicals and increasing the intake of antioxidant enzyme rich foods or antioxidant enzyme supplements, our body's potential to reducing the risk of free radical related health problems is made more significant (Chitra and Pillai, 2002).

This antioxidant system includes, antioxidant enzymes (e.g., SOD, GPx and reductase, CAT, etc.), nutrient-derived antioxidants (e.g., ascorbic acid, tocopherols and tocotrienols, carotenoids, glutathione and lipoic acid), metal binding proteins (e.g., ferritin, lactoferrin, albumin, and ceruloplasmin) and numerous other antioxidant phytonutrients present in a wide variety of plant foods (Praveen and Ashish, 2012).

Most of the literature on the genus *Tympanotonus* in the Lagos lagoon is concerned with classification, geographic distribution and population dynamics (Oyenekan, 1998). Recent studies have focused on the use of *Tympanotonus* species for the biomonitoring of coastal water pollution (Egonmwan, 2008; Edeghagba and Badru, 2019)

This study was aimed at examining the biochemical effect of paint effluent on the antioxidant enzymes of the tissues of *Tympanotonus fuscatus var radula* under sublethal condition.

2.0 MATERIALS AND METHOD

2.1 Study Area

The study area was Lagos Lagoon front of University of Lagos, Lagos State. The location (6° 30'N and 3° 23'E) of the sample collection and its co-ordinates was done with the Global positioning Application on a mobile phone.

2.2 Collection and Acclimatization of Test Organism

Healthy periwinkles (*Tympanotonus fuscatus)* were handpicked at the mud flat area of Lagos Lagoon front at University of Lagos. The collection of test organisms was done early in the morning. The organisms were placed in a sterile polyethylene bag and they were transported to the laboratory for acclimatization. In the laboratory the organisms were transferred from the polyethylene bag into a clean sterile transparent plastic container, with sediment, providing substrate and food for the organisms. The periwinkles were acclimatized for five days at ambient temperature in the laboratory.

2.3 Characterization of Paint Effluent

The physico-chemical parameters (pH, DO, BOD, COD, TS, TSS, TDS, Salinity, Nutrients) of the effluent and heavy metals such as arsenic, copper, chromium, cadmium, mercury, lead and zinc levels were determined with Atomic Absorption Spectrophotometer model (SOLAR SERIES S2).

2.4 Bioassay Test Procedure

The bioassay was carried out in 12 sterile plastic containers. The sediment (substrate) used was collected from the same source in which the test organisms were collected. Using sensitive weighing balance, 20g of sediment was weighed into each bioassay container. Healthy test organisms of approximately the same size and length were selected for the bioassay. The weight of the selected organisms was determined using sensitive weighing balance. The selected organisms were transferred from

the acclimatization tank into the bioassay containers using sterile materials.

2.4.1 Chronic Toxicity Test

The test media were prepared in the different concentrations of the chemical using fractions of LC_{50} at 96 hour obtained from the probit analysis of 7.51 ml (Edeghagba *et al..,* 2019) acute toxicity testing. A sub-lethal varying concentration of 1.80 ml, 0.50 ml, 0.20 ml, 0.13 ml and 0.10 ml with control experiment was used. 20 healthy test organisms of the same size were introduced into each bioassay tanks. The test organisms were exposed to the chemical for 14days. The treatment was renewed at 72hours interval.

2.5 Biochemical Test

After sublethal exposure, the periwinkles were taken to the biochemistry laboratory in the Biochemistry Department of Lagos University Teaching Hospital (LUTH) at Idi-Araba, Lagos State for antioxidant test.

2.5.1 Tissue Preparation

In the laboratory, the shells of the periwinkles were broken; the tissues were collected and placed in universal bottles. The organs of the test organisms were washed in an ice cold 1.15% KCL solution, blotted and weighed. They were then homogenized with 0.1M phosphate buffer (pH 7.2), putting the organs each into the mortar; laboratory sand (acid washed) was added to it and blended in the mortar with pestle together. The resulting homogenate was centrifuge at 2500rmp speed for 15mins then it was removed from the centrifuge and the supernatant was decanted and stored -20^{o}C until analysis. The supernatant was analyzed for activity of CAT, GSH, SOD, and MDA.

Superoxide Dismutase activity was ascertained by its ability to inhibit the auto-oxidation of epinephrine determined by the increase in absorbance at 480nm as defined by Sun and Zigma (1978). Catalase activity was determined according to Sinha (1972). It was assayed colorimetrically at 620nm and expressed as µmoles of H_2O_2 consumed/min/mg protein at 25^{0}C. The reduced glutathione content of liver tissue as non-protein sulphydryls was assessed according to the method described by Sedlak and Lindsay (1968). Malondialdehyde an index of lipid peroxidation was established using the method of Buege and Aust (1978).

3.0 RESULTS

Table 1 shows the biochemical test results of the activities of antioxidant enzymes of *T. fuscatus* under sublethal exposure to paint effluent.

Table 1. Oxidative stress markers of *Tympanotonus fuscatus var radula* due to exposure to different concentrations of paint effluent

Samples	GSH (µmol/ml/min)	SOD (µmol/ml/min)	CAT (µmol/ml/min)	MDA (µmol/ml/min)
Control	34.39 ± 3.22^{a}	132.77 ± 12.45^{a}	585.99 ± 54.94^{a}	1.06 ± 0.099^{b}
1.8ml	32.65 ± 3.37^{a}	135.43 ± 13.96^{a}	489.17 ± 50.41^{a}	1.48 ± 0.153^{a}
0.50ml	30.76 ± 1.97^{ab}	129.74 ± 8.30^{a}	254.78 ± 16.29^{b}	2.02 ± 0.129^{a}
0.20ml	31.86 ± 2.35^{ab}	124.43 ± 9.18^{a}	519.75 ± 38.35^{a}	1.52 ± 0.112^{a}
0.15ml	24.61 ± 2.45^{ab}	120.64 ± 12.03^{a}	570.70 ± 56.93^{a}	1.12 ± 0.111^{bc}
0.10ml	19.72 ± 2.07^{b}	125.19 ± 13.12^{a}	596.18 ± 62.47^{a}	0.87 ± 0.091^{c}
F (p)	4.608 (0.014)	0.228 (0.943)	6.835 (0.003)	12.46 (<0.001)

NB: mean of samples with the same superscript are not significantly different at 5%

Table 1 shows that control has the mean GSH of 34.39 (se = 3.22) while 0.10ml has the least GSH of 19.72 (se = 2.07) and the means are significantly different from each other ($p < 0.05$). Also, 1.80ml has the highest mean SOD of 135.43 (se = 13.96) while 0.15ml has the

least SOD of 120.64 (se = 12.03) and the means are not significantly different ($p > 0.05$). In addition, 0.10ml has the highest mean CAT of 596.18 (se = 62.47) while 0.50ml has the least CAT of 254.78 (se = 16.29) and the means are significantly different ($p < 0.05$). Lastly, 0.50ml has the highest mean MDA of 2.02 (se = 0.129) while 0.10ml has the least MDA of 0.87 (se = 0.091) and the means are significantly different ($p < 0.05$).

3.1 Effect of the Paint effluent on enzyme activity

Glutathione (GSH) level significantly decreased in response to the varying concentration of the test compound when compared to the control (Table 1). Superoxide dismutase (SOD) activity significantly increased in response to the highest concentration (1.8ml) of the test compound (Fig. 2). A significant reduction in SOD activity was observed in other concentrations of the test compound, with concentration 0.15ml having the lowest SOD activity. Catalase (CAT) activity significantly increased in response to the lowest concentration (0.1ml) of the test compound. Significantly low activities were observed in other concentrations of the test compound with concentration (0.50ml) having the lowest catalase activity (Fig. 3). Significant enhancement in the level of lipid peroxidation (MDA content) was observed in all concentration of the test compound except the lowest concentration (0.10ml) which had the lowest value recorded in Fig. 4 accordingly.

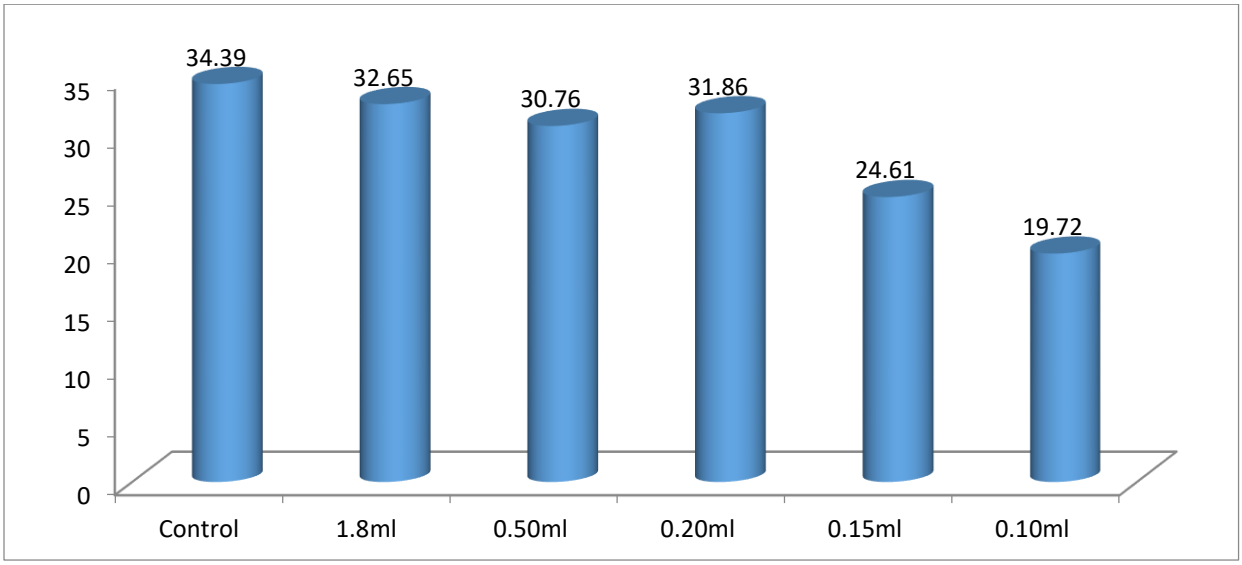

Fig. 1 Glutathione activity in the tissue of T. fuscatus after chronic exposure to varying concentration of the paint effluent

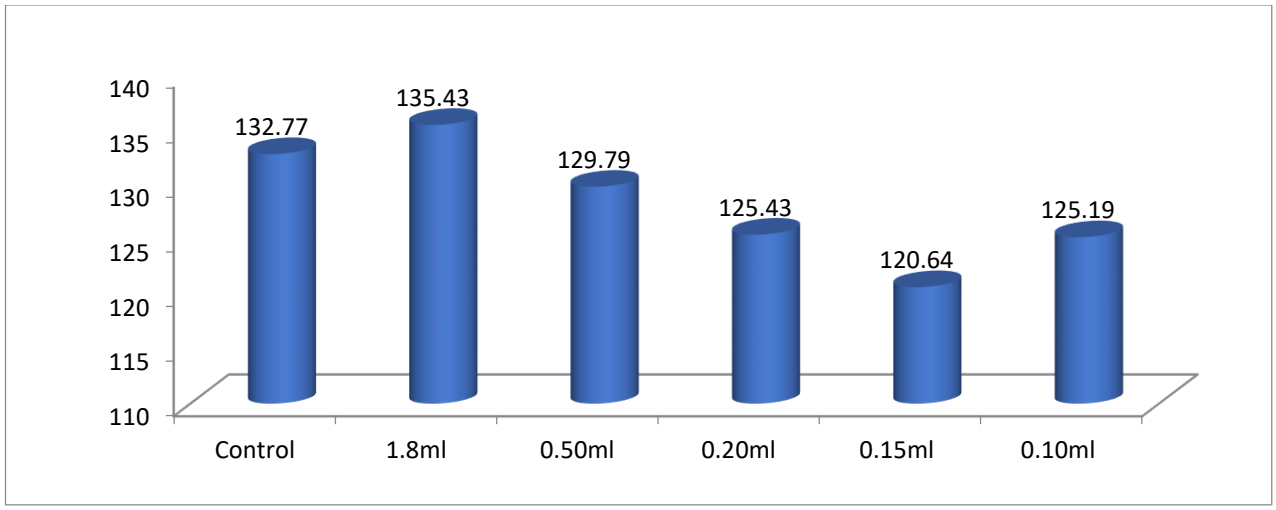

Fig. 2 Superoxide dismutase activity in the tissue of T. fuscatus after chronic exposure to varying concentration of the paint effluent

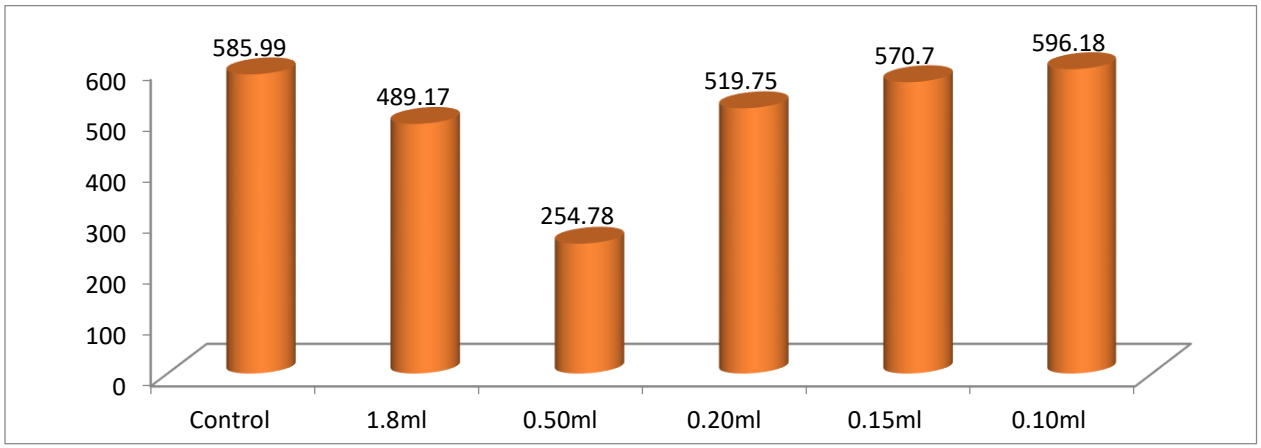

Fig. 3 Catalase activity in the tissue of T. fuscatus after chronic exposure to varying concentration of the paint effluent

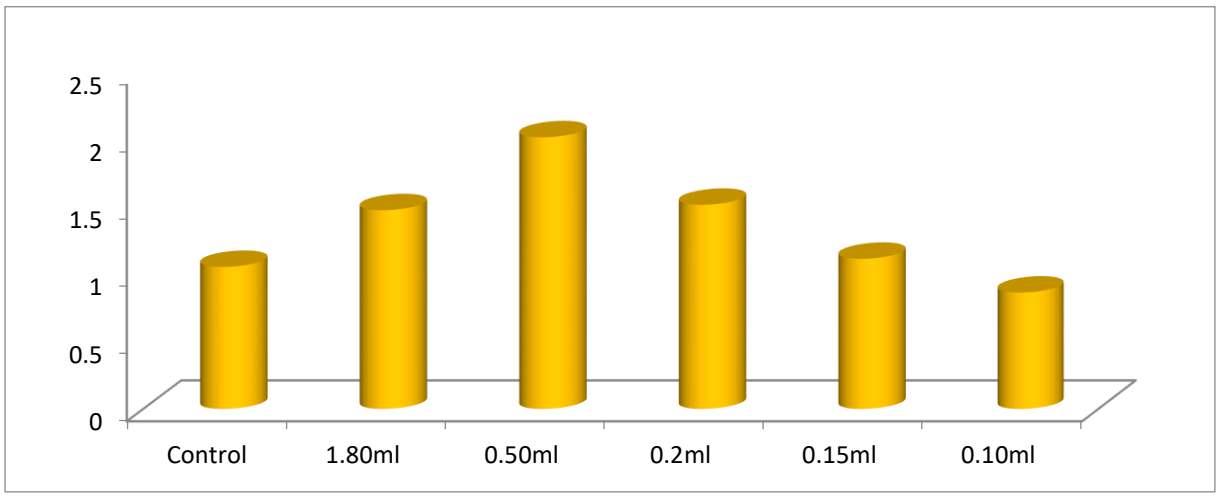

Fig. 4 Malondialdehyde (Protein content) in the tissue of *T. fuscatus* after chronic exposure to varying concentration of the paint effluent

4.0 DISCUSSION.

In this study, the periwinkles were exposed to sublethal concentration of the paint effluent that seem to exert stressful effects as reflected by marked alterations in the activities of the antioxidant enzymes. In an effort to adapt to oxidative stress induced by various xenobiotics, eukaryotes and prokaryotes are able to up-regulate their antioxidant defense mechanisms in response to low concentration of those xenobiotics (Davies, 1995).

The results of the physico-chemical parameters, heavy metal levels and acute toxicity testing of the paint effluent used in this study have already been determined and published (Edeghagba *et al.,* 2019)

In this study, significant decrease was observed in the level of reduced Glutathione when compared with the control experiment in the tissue of the test organisms exposed to sublethal concentration of paint effluent (Fig. 1). Reduced glutathione (GSH) is the major non-protein thiol and plays a fundamental role in cell capability, protecting cells against lipid peroxidation either alone or in conjugation with other protein (Anjum *et al.,* 2011). This decrease may be due to glutathione being used to scavenge the free radicals and reactive oxygen species (ROS) generated or induced by the paint effluent. Depletion of reduced Glutathione may also result from direct conjugation of the molecule with electrophiles or redox cycling or indirectly through inhibition of its biosynthesis and regeneration (Reed, 1994). Also the significant decrease in reduced glutathione level observed in the tissue of *Tympanotonus fuscatus* indicates that there was an alteration in the oxidative state of the tissue. This result is in agreement with the findings of Temitope *et al.,* (2014) who reported diminution in the Glutathione activity in the periwinkles collected from the oil impacted area of Port-Novo creek. Faramobi *et al.,* (2007) also reported decrease in Glutathione level in the gills of *Clarias gariepinus* due to the continued exposure of the organ to contaminant. Exposure to pollutants or stressful condition can result in elevated glutathione levels; there is evidence that adverse effects are associated with glutathione exhaustion in marine bivalves. Studies by Hassoun and Periandri (2010) found significant clampdown in glutathione level in the brain tissue of rat exposed to subchronic concentration of PCB126. Yazdani *et al.,* (2016) reported a significant decrease in GSH mRNA level in Atlantic salmon kidney cell exposed to 100uM BPA. Decreased GSH level was also observed in hepatocytes of pearl mullet exposed to 200uM BPA for 24 hours. (Kaya and Kaptaner, 2016).

In this report, SOD and CAT showed significant decreases following sublethal exposure to paint effluent. Only SOD at concentration 1.8ml had the highest concentration (Fig. 2), similarly, CAT showed decrease in values except at 0.10ml which had the lowest concentration (Fig. 3). Superoxide dismutase (SOD) and Catalase (CAT) comprise the first line defense against oxygen toxicity and serve as early indicators of exposure to pollutants that trigger oxidative stress. The similarity in the trend of activities of SOD and CAT may be linked to other mode of action. SOD protects the cell against free radicals induced damage by converting superoxide radicals (O^{2-}) generated in the peroxisomes and mitochondria to hydrogen peroxidase. The hydrogen peroxidase is then removed from the system by the enzymes catalase which converts it to water and molecule oxygen (O^2) {Timbrell, 2000}.

Thus the direct relationship in their activities could be used to explain the similarity in their responses. In the trend of this result, Vieira *et al.,* (2012) reported that the decreased CAT activity was related with stimulated SOD activity in Gold fish under acute toxicity following manganese exposure. Zheng *et al.,* (2016) also reported significant decrease in SOD and CAT activity following PCB exposure on juvenile Tilapia.

Consequently, catalase activity was observed to be reduced except the lowest concentration of the test compound (0.10 ml) which showed significant increase (Fig 3). Catalase is an active

and first enzyme that shows alteration following oxidative stress (Jin *et al.,* 2010). The decreased activity of CAT in *Tympanotonus fuscatus* indicate a changed defense against increased generation of hydrogen peroxide (H_2O_2) induced by the paint effluent. The reason for the decrease in Catalase activity may be the inactivation of enzyme by overproduction of reactive oxygen species (ROS). However, the observed highest value in the lowest concentration of the test compound (Fig. 3), indicate improved defense against increased generation of hydrogen peroxide (H_2O_2) induced by the paint effluent.

Findings showed Malonaldehyde (MDA) level observed in the tissues of *Tympanotonus fuscatus* exposed to the test compound were significantly elevated than MDA level in the control (Table 1 and Fig. 4). This result indicates that *T. fuscatus* exposed to paint effluent experienced oxidative stress due to lipid peroxidation in response to generation of ROS. Lipid peroxidation level may also increase when antioxidant defense is no longer capable of coping with increased ROS (Faheem and Lone, 2017). This result corroborates the findings of Achuba and Osakwe (2003), who reported an increase in lipid peroxide in the tissues of fish exposed to petroleum hydrocarbons. Chitra *et al.,* (2003), reported related findings in rats orally administered with BPA for 45days. Increased level of lipid peroxidation was recorded in common carp (*Cyprinus carpio*) exposed to sublethal concentration of BPA (Qui *et al.,* 2016). Faheem and Lone (2017) also reported increased lipid peroxidation in *C. idella* after 14 days of exposure to bisphenol-A. The increase in lipid peroxides is usually linked to an inhibitory effect on the mitochondrial electron transport system leading to stimulation in the production of intercellular reactive oxygen species (ROS). The ROS produced during oxidative stress reacts with unsaturated fatty acids that are present in membranes and cause lipid peroxidation. Therefore, increased lipid peroxidation is a signal of high level of ROS generated (Thiele *et al., 1995).* Thus organisms inhabiting polluted aquatic environments are exposed to variety of oxyradicals leading to oxidative damage of lipid or protein biomolecules. Oxidation damage reflects the imbalance between the production of oxidants and sifting or removal of these oxidants. The intensity of oxidative damage suffered by an organism depends on the acceptable balance amongst its individual antioxidant enzymes (Lenartova *et al.,* 1997).

5.0 CONCLUSION

The exposure of *Tympanotonus fuscatus* to sublethal concentration of paint effluent used in this study resulted in a significant increase in lipid peroxidation and significant decrease in GSH, CAT and SOD activities in the tissue of the test organisms. This suggests that the oxidative state of the periwinkles was changed by the effect of the paint effluent. This therefore, can be considered as a threat to the oxidative state of aquatic organisms especially molluscs.

REFERENCES

Aboulhassan, M.A., Souabi, S.,Yaacoubi, A. and Baudu, M. (2006). Improvement of Paint Effluents Coagulation Using Natural and Synthetic Coagulant Aids. *Journal of Hazardous Materials,* **138**(1): 40-45.

Aboulhassan, M., Souabi, S., Yaacoubi, A. and Baudu, M. (2014). Treatment of Paint Manufacturing Waste Water. *International Journal of Science, Environment and Technology,* **3**(5): 1747-1758.

Achuba, F.I. and Osakwe, S.A. (2003). Petroleum Induced Free Radical Toxicity in The African Catfish (*Clarias gariepinus*). *Journal of Fish Physiology and Biochemistry,* **29**: 27-103

Anjum, S., Rahman, S., Kaur, M., Ahmad, F., Rashid, H., Ahmad, R. and Raisuddin, S. (2011). Melatonin Ameliorates Bisphenol-A Induced Biochemical Toxicity In Testicular Mitochondria of Mouse. *Journal of Food and Chemical Toxicology,* **4**(3): 31-38

Buege, J. A, Aust, S. D.(1978). Microsomal lipid peroxidation. *Methods Enzymol*.52: 302–31

Chitra, K., and Latchoumycandane, C. and Mathur P. (2003). Induction of Oxidative Stress by Bisphenol-A in the Epididymal Sperm of Rats. *Journal of Toxicology and Pharmacy,* 185(1-2):119-127

Chitra, K.P. and Pillai, K.S. (2002). "Antioxidants in Health". *Indian Journal of Physiology and Pharmacology*, **46** (1): 1-5

Davies, K.J.A. (1995). Oxidative Stress: The Paradox of Aerobic Life. Free Radicals and Oxidative Stress in Environment, Drug and Food Additives. Portland Press, London. **Pp** 7-18.

Edeghagba, B. O. and Badru, T. I. (2019). Chemical Evaluation of Effluent from a Pharmaceutical Industry Using *Tympanotonus fuscatus. Nigerian Journal of Botany,* Volume **32**(1):1-9.

Edeghagba, B. O., Jolly, F. N., Ajipe, J. O and Babatunde, O. D. (2019). Toxicological Investigation, Physico-Chemical Determination and Histopathological Changes of Paint Effluent against *Tympanotonus fuscatus var radula*. *International Journal for Research in Applied Science & Engineering Technology*, Volume 7 Issue XI.

Egonmwan, R. I. (2008). The Ecology and Habits of *Tympanotonus fuscatus var radula* (L.) (Prosobranchia: Potamididae) .*Journal of Biological Science,* **8**(1): 186-190

Faheem, M. and Lone, K. (2017). Oxidative Stress and Histopathologic Biomarker of Exposure to Bisphenol-A in the Fresh Water Fish, *Cteriopharyngodon idella*. *Brazillian Journal of Pharmaceutical Sciences*, **53**(3): 170-179

Faramobi, E. O., Adelowo, O.A. and Ajimoko, Y.R. (2007). Biomarker of Oxidation Stress and Heavy Metal Levels as Indicators of Environmental Pollution in Catfish (*Clarias guriepinus*) from Ogun state, Nigeria. *International Journal of Environmental Research and Public Health*, **4**(2): 158-165

Hanchang, S. (2004). Industrial Waste Water; Types, Amounts and Effluent. *Journal of Environmental Science*, **4**(1): 1-6

Hassoun, E. A. and Periandri, S. S. (2010). Assessment of The Roles of Antioxidant Enzymes and Glutathione in 3, 3', 4, 4', 5-Pentachlorobiphenyl (PCB 126) Induced Oxidative Stress in The Brain Tissues of Rat after Subchronic Exposure. *Journal of Environmental Toxicology and Chemistry*, **92**(2): 301-316

Jin, X.Y., Zhang, Y. X., Shu, L .J., Chen, L . F., Sun, L. W., Quan, H. F., Liu, W. P. and Fu, Z. W. (2010). Oxidation Stress Response and Gene Expression in Adult Zebra fish (*Danio rario*). *Journal of Chemosphere*, **78**(7): 846-852

Kaya, O. and Kaptaner, B. (2016). Antioxidant Defense System Parameters in Isolated Fish Hepatocytes Exposed to Bisphenol-A. Effect of Vitamin C. *Journal of Science Total Environment*, **67**(3): 225-235

Lenartova, V., Holovska, K., Pedrajas, J., Martinez, E., Peinado, J., Lopez, B., Rosival, I. and Kosuta, P. (1997). Antioxidant and Detoxifying Fish. Enzymes as Biomarker of River Pollution. *Journal of Environmental Pollution* **2**(4): 247-252

Oyenekan, J.A. (1998). Benthic Macro Fauna Communities of Lagos lagoon, Nigeria. *Nigerian Journal of Science*, **21**: 45-51

Praveen, K. and Ashish, W. (2012). Antioxidant Enzymes and Human Health In: Antioxidant Enzymes, Muhammed Elmissiry (ed), inTech Prepress, Novi Sad, Croatia. **Pp** 3-14

Qui, W., Chen, J., Li, Y., Chen, Z., Jiang, L., Yang, M. and Wu, M. (2016). Oxidative Stress and Immune Disturbance After Long Term Exposure to bisphenol-A in Juvenile Common Carp (*Cyprinus carpio). Journal of Ecotoxicology and Environmental Safety*, 130:93-102

Reed, J. D. (1994). Mechanism of Chemically Induced All Injury and Cellular Protection Mechanism. In: introduction to Biochemical Toxicology, Norwalk, Connecticut Appleton and Lange. **Pp** 265-295.

Sedlak, J. Lindsay, R. H (1968). Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Anal Biochem* 25: 1192–1205.

Sinha, K. A .(1972). Colorimetric Assay of Catalase. *Analytical Biochemistry*, 47: 389-394

Sun, M. Zigma, S. An improved spectrophotometric assay of superoxide dismutase based on ephinephrine antioxidation. *Anal Biochem* 1978, 90:81-89.

Temitope, O. S., Ochuwa, O. G., Nnamdi, H. A. and Adebayo, A. O. (2014). Biomarker Response in *Tympanotonus fuscatus var radula* [L] Inhabiting an Oil Impacted and Fire Ravaged Mangroove Ecosystem. *Journal of Current Advances in Environmental Science*, **2**(3): 101-111

Thiele, J. J., Freisleben, H. J., Fuchs, J. I. and Ochsendof, F. R. (1995). Ascorbic Acid and Urate in Human Seminal Plasma: Determination and Interrelationship with Chemilumiscence in Washed Semen. *Journal of Human Reproduction.* **10**(1): 110-115

Timbrell, J. (2000). Toxic Responses to Foreign Compounds. In: Principles of Biochemical Toxicology. 3rd Edition, Taylor and Francis. **Pp** 175-253

Vieira, M., Torronteras, R., Cordoba, F. and Canalejo, A. (2012). Acute Toxicity of Manganese in Goldfish *(Carassius auratus)* is Associated with Oxidative Stress and Organ Specific Antioxidant Response. *Journal of Ecotoxicology and Environmental Safety*, 78: 212-217

Yadzani, M., Andresen, M. and Gjøen, T. (2016). Short Term Effect of Bisphenol-A on Oxidative Stress Responses in Atlantic Salmon Kidney Cell Line: A Transcriptional Study. *Journal of Toxicology Mechanism and Method*, **26**(4) 295-300

Zheng, Y., Qui, L., Meng, L., Fan, M., Song, C., Li, D., Zheng, C. and Chen, J. (2016). Effect of Polychlorinated biphenyls on Oxidation Stress in the Liver of Juvenile GIFT Tilapia. *Journal of Genetics and Molecular Research*, **15**(3): 150-159.

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A REVIEW OF DRUG DISCOVERY PROCESSES.

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ABSTRACT

Developing a new drug from original idea to the launch of a finished product is a complex process which can take a number of years. The idea for a target can come from a variety of sources including academic and clinical research and from the commercial sector. It may take many years to build up a body of supporting evidence before selecting a target for a costly drug discovery program. Once a target has been chosen, the pharmaceutical company and more recently some academic centers have streamlined a number of processes to identify molecules which possess suitable characteristics including efficacy, pharmacokinetics and safety needs, which have to be optimized in parallel, to provide drug candidates and to make acceptable drugs. Recent advances in areas such as microfluidics-assisted chemical synthesis and biological testing, as well as artificial intelligence systems that improve a design hypothesis through feedback analysis are now providing a basis for the introduction of greater automation into aspects of drug design and development. This could potentially accelerate time frames for compound discovery and optimization and enable more effective searches of chemical space. However, such approaches also raise considerable conceptual, technical and organizational challenges, as well as skepticism about the current hype around them. This review will look at key preclinical stages of the drug discovery process, from initial target identification and validation, through assay development, high throughput screening, 'HITS' identification, lead optimization and finally the selection of a candidate molecule for clinical development.

KEYWORDS: Drug discovery, Target identification, pharmacokinetics.

INTRODUCTION:

Definition of the term 'DRUG': Different class of people and profession defined drugs differently. To a layman: A drug is a chemical compound administered in order to treat or prevent an illness or disease. In this review, we shall be presenting the various definitions of the term to update the knowledge of readers. Listed below are some of the definitions:

- To a pharmacist: A drug is a chemical substance that is used to treat, cure, prevent, or diagnose a disease or to promote well-being.
- To a chemist: A drug is a chemical substance, typically of known chemical structure, which, when administered to a living organism, produces a biological effect
- To National Drug Law enforcement Agency (NDLEA): A drug is any substance that causes a change in an organism's physiology or psychology when consumed.
- To Food and Drug Administration (FDA): A drug is a substance designed for use in and on the body for the diagnosis, cure, treatment, or prevention of disease. (Munos *et al*.,2010)

For this discussion, we shall use the definition; a drug is any substance that causes a change in an organism's physiology or psychology when consumed. Drugs are typically distinguished from food and substances that provide nutritional support. Consumption of drugs can be *via* inhalation, injection, smoking, ingestion, absorption *via* a patch on the skin, or dissolution under the tongue (Anson *et al*., 2009).

In pharmacology, a drug is a chemical substance, typically of known structure, which, when administered to a living organism, produces a biological effect. A pharmaceutical drug, also called a medication or medicine, is a chemical substance used to treat, cure, prevent, or diagnose a disease or to promote well-being. Traditionally drugs were obtained through extraction from medicinal

plants, but more recently also by organic synthesis. Pharmaceutical drugs may be used for a limited duration, or on a regular basis for chronic disorders. (Warren *et al*.,2011).Pharmaceutical drugs are often classified into drug classes, groups of related drugs that have similar chemical structures, the same mechanism of action (binding to the same biological target); a related mode of action, or that are used to treat the same disease, (Munos *et al*.,2010). One major way of classifying drugs is the Bio pharmaceutics Classification System. This classifies drugs according to their solubility and permeability or absorption properties. (Mehta, 2016)

Psychoactive drugs are chemical substances that affect the function of the central nervous system, altering perception, mood or consciousness. These drugs are divided into different groups like stimulants, depressants, antidepressants, anxiolytics, antipsychotics, and hallucinogens. These psychotropic drugs have been proven useful in treating wide range of medical conditions including mental disorders around the world. The most widely used drugs in the world include caffeine, nicotine and alcohol, which are also considered recreational drugs, since they are used for pleasure rather than medicinal purposes. Abuse of several psychoactive drugs can cause psychological or physical addiction. It's worth noting that all drugs can have potential side effects. Excessive use of stimulants can promote stimulant psychosis. Many recreational drugs are illicit and international treaties such as the Single Convention on Narcotic Drugs exist for the purpose of their prohibition. (Geoffrey *et al*., 1998).

1.1 WHY ARE NEW DRUGS NEEDED?

New Drugs are needed for several reasons, some of which are;

- Prevention of diseases. These are prophylactic drugs, used to guard against the occurrence of diseases, e.g. Fansidar, antimalarial prophylactic.
- Treatment of diseases (old & new). Presently the search is on for HIV-Aids drug, as well as the Covid-19 pandemic that is ravaging humanity globally, there is a frantic search for vaccine and or drugs.
- Improvement in the treatment of Old diseases (safer drugs). For older diseases like Cancer, malaria, diabetics, high blood pressure, (to mention but a few), the search for safer drugs is a continuous task.
- To combat (fight) drug resistance.
- Improvement of health care and general well-being.
- Management of disease conditions.
- And sometimes for recreation. (Paul *et al*., 2010).
- Drugs are designed based on different techniques, which includes:
- TARGET-BASED DRUG DESIGN.
- STRUCTURE-BASED DRUG DESIGN.
- COMPUTER-AIDED DRUG DESIGN (CADD).

In CADD, Molecular modeling and molecular dynamics simulations are being used as a guide to improve the potency and properties of new drug leads. (Gareth, 2007).

Drug discovery and development can broadly be divided into two different approaches: structure based drug discovery and target-based discovery. In structure based drug discovery, a compound is identified by one of several methods and its biological profile is explored. In structured based drug design, if the compound displays desired pharmacology activity, it is refined and developed further; where as in target based strategy supposed drug target is identified first. The potential target could be a receptor thought to be involved in a disease process or a critical enzyme, or another biologically, important molecule in the diseases pathway. The genomics (genomics the study of the complete genetic information either DNA or RNA of an organism) revolution has been the main driver of the target-based strategy over the last decade. Target validation requires the confirmation that whether the particular target is involved in the disease or not. Such as ulcer which is caused by an excess acid in the stomach and it can be treated by using proton pump inhibitor and anti-acid. Also, blood pressure which occur when the heart muscle is stiff to pump the blood due to low electrolyte (K^+). This can be treated by blood tinning and by taking drugs that contains much electrolyte

and also fruits and vegetables that contain potassium (K^+). Though, blood tinning has disadvantages of excess bleeding and it also wears off when it expires. (Jinlong *et al.*, 2009).

1.2 DRUG DISCOVERY AND DEVELOPMENT PROCESS.

The drug discovery process began in the nineteenth century by John Langley in 1905 when he proposed the theory of receptive substances. The first rational development of synthetic drugs was carried out by Paul Ehrlich (father of modern chemotherapy) and Sacachiro Hata who produced arsphenamine (Salvarsan) in 1910 by structure activity relationship from atoxyl previously used in the treatment of sleeping sickness (trypanosomiasis) and syphilis. (Heyse *et al.*, 2005). The following general steps are followed in the discovery of a new drug for a particular disease. The steps are;

- ❖ Basic research into the disease process and its causes, such as it is presently going for the Covid-19 pandemic.
- ❖ Assessment of the biochemical and biological processes of the disease and/or its causes.
- ❖ Team decides where intervention is most likely to bring about the desired result.
- ❖ Team decides the structure of a suitable lead compound.
- ❖ Design of the synthetic pathway to produce the lead compound.
- ❖ Initial biological and toxicological testing and synthesis of analogues.
- ❖ Selection of the analogue with the optimum activity.
- ❖ Phased Clinical trials.
- ❖ Then MAA(Marketing Authorisation Application)

(Gareth, 2007).

The Chart below summarizes the drug discovery cycle:

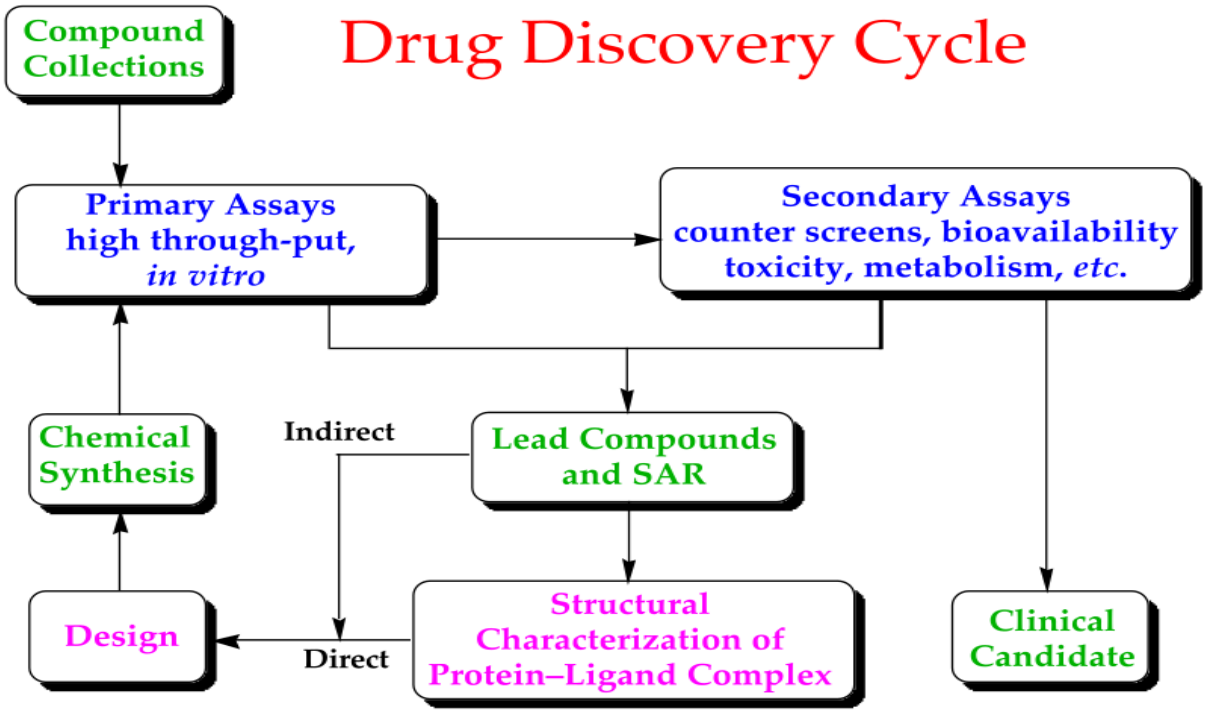


Figure 1.0 (en.wikipedia.org. 22 June, 2015).

The activities involved in the discovery of a new, potent and viable drug for a particular disease, takes very long period as indicated in the timeline chart below:

EXAMPLE OF DRUG DISCOVERY/DEVELOPMENT TIMELINE

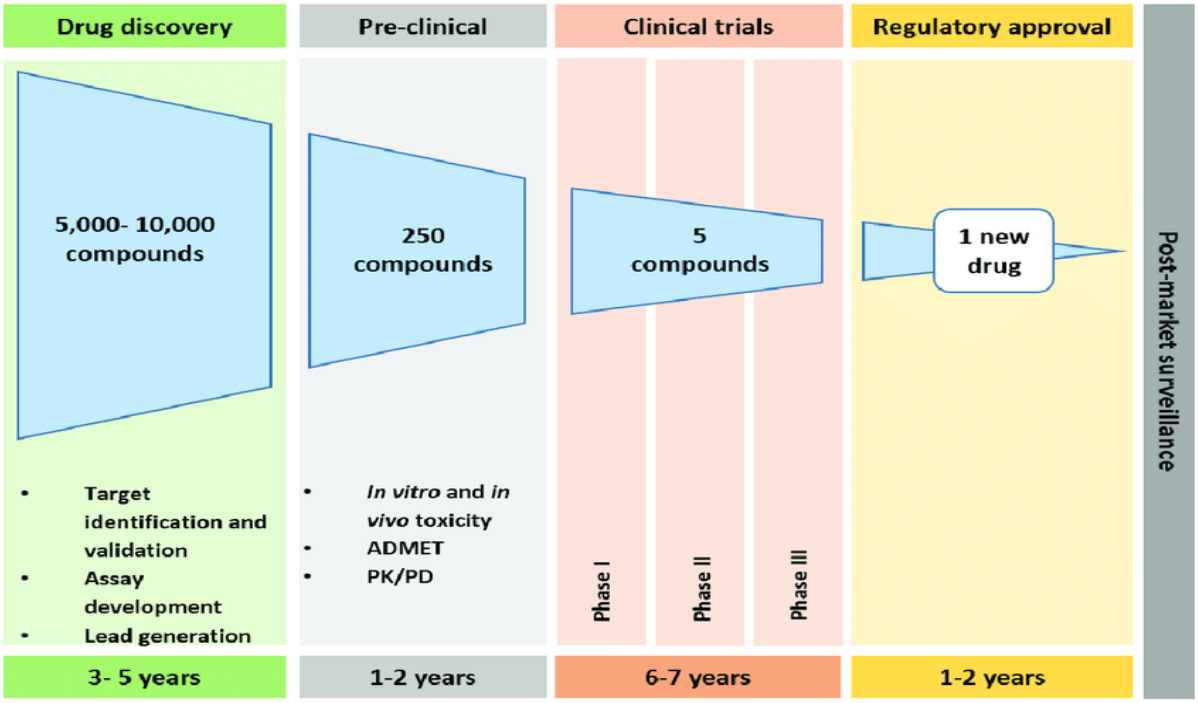
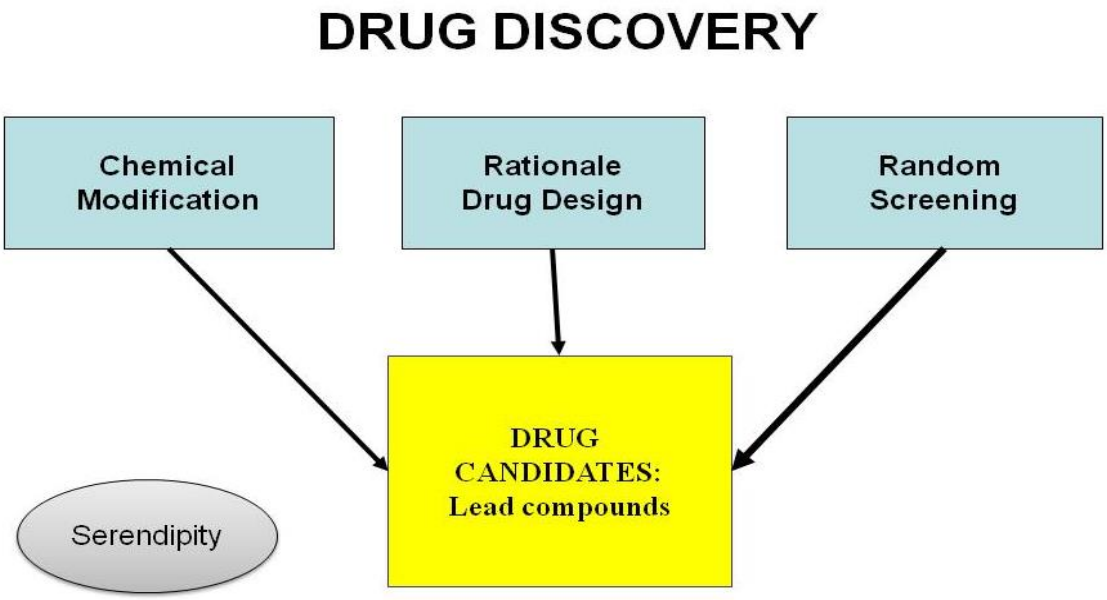


Figure 1.1 (Researchgate.net. 2018.)

When new drugs are discovered, they are subjected to clinical trials and at different stages (Robert *et al* 2012). Below is a chart that summarizes the pre-clinical drug making processes.



1.3 ECONOMIC IMPORTANCE OF DRUG DISCOVERY

- Classical drug discovery has largely been a process of trial and error, with a myriad of failures for every minor breakthrough and a significant period between discovery, development and launch.
- Because of the increasingly competitive environment in which pharmaceutical companies operate, it is important to provide strong, transparent economic arguments for new products, especially if a premium price is sought.
- Increases in expenditure on medicines that are higher than the level of increases in healthcare are generally a feature of all Western health systems.

Health economics is becoming a much better understood case. more healthcare providers as well as those in the industry recognize that high quality, relevant health economic evaluations can add considerable value to the decision making process. As this has become more universally accepted, so has the use of health economic evaluation at all levels of product development. (Robert *et al*.2012). The full cost of bringing a new drug (i.e., new chemical entity) to market from discovery through clinical trials to approval is complex and controversial. Typically, companies spend tens to hundreds of millions of U.S. dollars. One element of the complexity is that the much-publicized final numbers often not only include the out-of-pocket expenses for conducting a series of Phase I-III clinical trials, but also the capital costs of the long period (10 or more years) during which the company must cover out-of-pocket costs for preclinical drug discovery. Additionally, companies often do not report whether a given figure includes the capitalized cost or comprises only out-of-pocket expenses, or both. Another element of complexity is that all estimates are based on voluntary releases of otherwise confidential information which may not be easily independently verified. One 2010 study assessed both capitalized and out-of-pocket costs for bringing a single new drug to market as about US$1.8 billion and $870 million, respectively (Paul *et al*,..2010).

In an analysis of the drug development costs for 98 companies over a decade, the average cost per drug developed and approved by a single-drug company was $350 million. But for companies that approved between eight and 13 drugs over 10 years, the cost per drug went as high as $5.5 billion, due mainly to geographic expansion for marketing and ongoing costs for Phase IV trials and continuous monitoring for safety.

Alternatives to conventional drug development have the objective for universities, governments, and the pharmaceutical industry to collaborate and optimize resources.

In the fields of medicine, biotechnology and pharmacology, drug discovery is the process by which new candidate medications are discovered. Historically, drugs were discovered by identifying the active ingredient from traditional remedies or by serendipitous discovery, as with penicillin. (Dahlin *et al* 2014 & Warren, 2011).

1.4. CONCLUSION: Since 1990s, the drug discovery process has been revolutionized with the introduction of some newer techniques in molecular biology, bio techniques, genomics, and bioinformatics. Very high expectations from newer trends in drug discovery to its speed, cost, and greater success. High Throughput Screening (HTS) is a powerful technique which speedup the screening process. The wealth of potential drugs has two potential sources: either from the laboratory or rainforest. We hold that the future of drug discovery lies in neither of these options alone, rather it embodies both the rainforest and the laboratory. Combinatorial laboratory techniques (is a method where molecular constructions can be synthesized and tested for biological activity), though relatively a recent trend, is growing in efficiency with support from pharmaceutical companies. And as the combinatorial libraries grow, the ability to quickly synthesize derivatives of biologically active compound increases. But even with technology, there cannot be a substitution for the biodiversity that can be found within the boundaries of the rainforest.

RECOMMENDATION

Given the high attrition rates, substantial costs and slow pace of new drug discovery and development, repurposing of ‘old’ drugs to treat both common and rare diseases is increasingly becoming an attractive proposition because it involves the use of de-risked compounds, with potentially lower overall development costs and shorter development timelines. Thus, drugs repurposing and discovery is presented, the challenges faced by the repurposing community

is discussed and innovative ways by which these challenges can be addressed is recommended and it is helpful to realize the full potential of drug repurposing and discovery.

REFERENCES

Andalibi A, Austin C, Baltezor M, Beckloff M, Coussens N.P, Hughes M, Inglese J, Kurtz A, Patton L, Sittampalam S, Strovel J, Weingarten M, Weir S. (2016). Early Drug Discovery and Development Guidelines: For Academic Researchers, Collaborators, and Start-up Companies. Assay Guidance Manual. Eli Lilly & Company and the National Center for Advancing Translational Sciences.

Anson D, Ma J, He JQ (2009), Identifying Cardiotoxic Compounds. Genetic Engineering & Biotechnology News. TechNote. 29 (9). Mary Ann Liebert. pp. 34–35. ISSN 1935-472X. OCLC 77706455.

Arkin M.R, Wells J.A. (2004), Small-molecule inhibitors of protein-protein interactions: progressing towards the dream. Nat Rev Drug Discov.;3(4):301-17.

Auld D.S, Austin C.P, Inglese J, Johnson R.L, Simeonov A, Xia M, Zheng W. (2007), High-throughput screening assays for the identification of chemical probes. Nat Chem Biol.;3(8):466-79

Buchdunger E, Deininger M, Druker B.J. (2005), the development of imatinib as a therapeutic agent for chronic myeloid leukemia. Blood. 1;105 (7):2640-53

Cayen M.N, Jurima-Romet M, Mortimer E, Pritchard J.F, Reimer M.L, Rolfe B. (2003), Making better drugs: Decision gates in non-clinical drug development. Nat Rev Drug Discov.;2(7):542-53.

Jinlong C; Mengmeng M; Yanwei L; Lishen W; Chutse W. & Haifen D. (2009). Rhaponticin from rhubarb rhizomes alleviates liver steatosis and improves blood glucose and lipid profiles in KK/Ay diabetic mice. Planta Med. 75 (5):472–7.

Dahlin J.L, Walters MA (2014). The essential roles of chemistry in high-throughput screening triage. Future Medicinal Chemistry. 6 (11): 1265–90.

Gareth Thomas, (2007). An introduction in Medicinal Chemistry. ISBN 978-0-470-02597 printed and bound in Great Britain by Anthony Rowe ltd., Chippenham Wiltshire.

Institute of Medicine (US) Forum on Drug Discovery, Development (2009).

Mehta M. (2016). Biopharmaceutics Classification System (BCS): Development, Implementation and Growth. Wiley. ISBN 978-1-118-47661-1.

Miseta, Ed (2018). Gene Therapies Create Moral Dilemma For Clinical Researchers. Clinical Leader. Pennsylvania, United States: Vert Markets, Inc.

Paul S.M, Mytelka D.S, Dunwiddie C.T, Persinger C.C, Munos B.H, Lindborg S.R, , Schacht A.L, (2010); How to improve R&D productivity: the pharmaceutical industry's grand challenge. Nature Reviews Drug Discovery. 9 (3): 203–14.

Researchgate.net (2018) Drug discovery/development timeline.

Robert M.C.; Gary L.F. & Micheal R. (2012): The Clinical trial enterprise in the United States: A call for disruptive innivation, a discussion paper, p. 4-13.

Takenaka T (2001), Classical vs reverse pharmacology in drug discovery. BJU International. 88 Suppl 2: 7–10, discussion 49–50.

Warren J. (2011), Drug discovery: lessons from evolution. British Journal of Clinical Pharmacology. 71 (4): 497–503.

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A REVIEW OF NEGLECTED TROPICAL DISEASES (NTD's).

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ABSTRACT

The primary aim of this paper is to create awareness on the existence of Neglected Tropical Diseases, their health effect as well as the imminent threat posed to the economies of nations where they exist. Neglected Tropical diseases (NTD's) which are also known as rare or orphan diseases are a diverse group of communicable diseases that prevail in tropical and subtropical conditions in numerous countries of the world. They affect more than a billion people globally and cost developing economies billions of dollars in cash and man hours every year. The World Health Assembly and the WHO Regional Committee, through a series of resolutions, have expressed regional and global commitments for the elimination of these diseases as public health problems. Such action is expected to have a quick and dramatic impact on poverty reduction and to contribute to the achievement of the Millennium Development Goals. A host of human-sourced and environmental factors complicate these actions, such as societal influences, human susceptibility to infection, demographics, and availability of health care, food production, human behavior, trade and travel, environmental and ecological changes, economic development, war and famine, adequacy of public health infrastructures, man-made events with intent to harm, and pathogen adaptation or evolution.

The paper highlights some of the common examples of these diseases and their symptoms, the mode of transmission and the carrier vector of the pathogens of each disease; with a view to helping in the understanding of the preventive measures that could be taken to avoid being infected.

KEYWORDS: Filariasis, Leishmaniasis, Schistosomiasis, Dengue fever.

1.0 INTRODUCTION

A rare disease is any disease that affects a small percentage of the population. Sometimes referred to as an orphan disease, especially in cases where a rare disease, whose rarity means there is a lack of a market large enough to gain support and resources for discovering treatments for it, except by the government granting economically advantageous conditions to creating and selling such treatments (Eurordis, 2009).

Most rare diseases are genetic and thus are present throughout the person's entire life, even if symptoms do not immediately appear. Many rare diseases appear early in life, and about 30% of children with rare diseases will die before reaching their fifth birthday (Eurordis, 2009).

2.0 LITERATURE REVIEW-

Neglected Tropical Diseases

Neglected tropical diseases (NTDs) are a diverse group of tropical infections which are common in low-income populations in developing regions of Africa, Asia, and the Americas. They are caused by a variety of pathogens such as viruses, bacteria, protozoa and helminths. These diseases are contrasted with the big three infectious diseases (HIV/AIDS, tuberculosis, and malaria), which generally receive greater treatment and research funding (Hotez, 2013). In sub-Saharan Africa, the effect of these diseases as a group is comparable to malaria and tuberculosis (Hotez and Kamath, 2009). NTD co-infection can also make HIV/AIDS and tuberculosis more deadly (*Shanahan, 2006).*

Neglected tropical diseases (NTDs) also known as rare or orphan disease is a diverse group of communicable diseases that prevail in tropical and subtropical conditions in 149 countries which affects more than one billion people and

cost developing economies billions of dollars every year. Populations living in poverty, without adequate sanitation and in close contact with infectious vectors and domestic animals and livestock are the worst affected (WHO, 2010). Neglected diseases are also considered as conditions that inflict severe health burdens on the world's poorest people. Many of these conditions are infectious diseases that are most prevalent in tropical climates, particularly in areas with unsafe drinking water, poor sanitation, substandard housing and little or no access to health care (Shanahan, 2006). These diseases are said to be neglected if they are often overlooked by drug/vaccine developers or by other agents or personnel, instrumental in drug/vaccine access, such as government officials, public health programs and the news media.

Typically, private pharmaceutical companies cannot recover the cost of developing and producing treatments for these diseases. Another reason neglected diseases are not considered high priorities for prevention or treatment is because they usually do not affect people who live in the developed countries like the United States and others (Niaid, 2015).

Neglected diseases also lack visibility because they usually do not cause dramatic outbreaks that kill large numbers of people. Rather, such diseases usually exact their toll over a longer period of time, leading to crippling deformities, severe disabilities and/or relatively slow deaths (Niaid, 2015).

In some cases, the treatments are relatively inexpensive. For example, the treatment for schistosomiasis is US$0.20 per child per year. Nevertheless, in 2010 it was estimated that control of neglected diseases would require funding of between US$2 billion and US$3 billion over the subsequent five to seven years (Hotez, 2010). Some pharmaceutical companies have committed to donating all the drug therapies required, and mass drug administration (for example, mass deworming) has been successfully accomplished in several countries (Reddy *et al.,* 2007). However, preventive measures are often more accessible in the developed world, but not universally available in poorer nations of the world (Hotez, 2009). Listed below are a few examples of such diseases, as released by WHO:

2.1. Chagas Disease

Also called *American trypanosomiasis*; it is a parasitic disease which occurs in the Americas, particularly in South America. Its pathogenic agent is a flagellate protozoan named *Trypanosoma cruzi*, which is transmitted mostly by blood-sucking assassin bugs, however other methods of transmission are possible, such as ingestion of food contaminated with parasites, blood transfusion and fetal transmission.

Between 16 and 18 million people are currently infected (Niaid, 2015).

2.2. Dengue

This is a mosquito-borne viral infection occurring in tropical and sub-tropical regions of the world. Dengue fever (as is sometimes called) causes a severe flu-like illness and, at other times, a potentially lethal complication called severe **dengue**. The incidence of **dengue** has increased 30-fold over the last 50 years (Niaid, 2015).

Figure 1: Mosquito, the Dengue disease vector.

2.3. Helminths

Helminths are worm-like parasites that survive by feeding on a living host to gain nourishment and protection, sometimes resulting in illness of the host. There are a variety of different helminths from the very large to the microscopic. 'Helminth' is a general term meaning worm. There are many different kinds of helminth ranging in length from less than one

millimeter to over one meter. Helminths infect a range of hosts, including humans (Niaid, 2015).

2.4. African Trypanosomiasis or Sleeping sickness.

This is a parasitic disease, caused by protozoa called trypanosomes.

The two responsible for African trypanosomiasis are Trypanosoma *brucei gambiense* and Trypanosoma *brucei rhodesiense*. These parasites are transmitted by the tsetse fly (Niaid, 2015).

Figure 2: Tse-tse fly- Trypanosome Vector

2.5. Leishmaniasis

Caused by protozoan parasites of the genus Leishmania, and transmitted by the bite of certain species of sand fly (Niaid, 2015).

2.6. Leprosy or Hansen's disease

This is a chronic infectious disease caused by Mycobacterium leprae. Leprosy is primarily a granulomatous disease of the peripheral nerves and mucosa of the upper respiratory tract; skin lesions are the primary external symptom. Left untreated, leprosy can be progressive, causing permanent damage to the skin, nerves, limbs, and eyes. Contrary to popular conception, leprosy does not cause body parts to simply fall off, and it differs from tzaraath, the malady described in the Hebrew Scriptures and previously translated into English as *leprosy* (Niaid, 2015).

Figure 3: A picture of a person infected with the Leprosy or Hansen disease.

2.7. Lymphatic Filariasis

This is a parasitic disease caused by thread-like parasitic filarial worms called nematodes, all transmitted by mosquitoes. Loa is another filarial parasite transmitted by the deer fly. 120 million people are infected worldwide. It is carried by over half the population in the most severe endemic areas. The most noticeable symptom is elephantiasis: a thickening of the skin and underlying tissues. Elephantiasis is caused by chronic infection by filarial worms in the lymph nodes. This clogs the lymph nodes and slows the draining of lymph fluid from a portion of the body (Niaid, 2015).

Figure 4: One of the most noticeable symptoms of persons with lymphatic filariasis.

2.8. Malaria

Caused by a Protozoan parasites transmitted by female *Anopheles* mosquitoes, as they are the blood-feeders. The disease is caused by species of the genus Plasmodium. Malaria infected an estimated 190-311 million people in 2008 and 708,000-1,003,000 died mostly in Sub-Sahara Africa (Niaid, 2015).

Figure 5: Female anopheles mosquito feeding on human blood.

2.9. Onchocerciasis or (river blindness)

This is the world's second leading infectious cause of blindness. It is caused by *Onchocerca volvulus*, a parasitic worm. It is transmitted through the bite of a black fly. The worms spread throughout the body, and when they die, they cause intense itching and a strong immune system response that can destroy nearby tissue, such as the eye. About 18 million people are currently infected with this parasite. Approximately 300,000 have been irreversibly blinded by it (Niaid, 2015).

Figure 6: A patient infected with the Night blindness.

2.10. Schistosomiasis (also known as *schisto* or snail fever)

This is a parasitic disease caused by several species of flatworm in areas with freshwater snails, which may carry the parasite. The most common form of transmission is by wading or swimming in lakes, ponds and other bodies of water containing the snails and the parasite. More than 200 million people worldwide are infected by schistosomiasis (Niaid, 2015).

2.11. Sexually Transmitted Infections (STIs)

These are infections you can get by having sex with someone who has an infection. These infections are usually passed from person to person through vaginal intercourse (Niaid, 2015).

2.12. Tuberculosis (abbreviated as TB)

This is a bacterial infection of the lungs or other tissues, which is highly prevalent in the world, with mortality over 50% if untreated. It is a communicable disease, transmitted by aerosol expectorant from a cough, sneeze, speak, kiss, or spit. Over one-third of the world's population has been infected by the TB bacterium (Niaid, 2015). Although leprosy and tuberculosis are not exclusively tropical diseases, their high incidence in the tropics justifies their inclusion.

Figure 7: Tuberculosis Epidemic.

2.13. Lassa Fever

Lassa fever, also known as Lassa hemorrhagic fever (LHF), is a type of viral hemorrhagic fever caused by the Lassa virus. Many of those infected by the virus do not develop symptoms. When symptoms occur, they typically include fever, weakness, headaches, vomiting, and muscle pains. Less commonly there may be bleeding from the mouth or gastrointestinal tract. The risk of death once infected is about one percent and frequently occurs within two weeks of the onset of symptoms. Among those who survive about a quarter have hearing loss, which improves within three months in about half of these cases (W.H.O. 2016).

Figure 8: Symbol of Lassa fever outbreak as contained by Nigerians genome team.

3.0: GLOBAL HEALTH AND NTDs

In global health, critical challenges have arisen from infectious diseases, including the emergence and reemergence of old and new infections, example is the on-going Covid-19 pandemic. Others include Ebola fever, SARS, MERS to mention but a few. Emergence and reemergence are accelerated by rapid human development, including numerous changes in demographics, populations, and the environment. This has also led to the occurrence of zoonoses (infectious diseases that jumped from non-human animal to humans) in the changing human-animal ecosystem, which are impacted by a growing globalized society where pathogens (zoonotic or not) do not recognize geographical borders (WHO July, 2020).

Within this context, neglected tropical infectious diseases have historically lacked adequate attention in international public health efforts, leading to insufficient prevention and treatment options. Infectious tropical diseases disproportionately impacts the world's poorest, representing a significant and underappreciated global disease burden, and is a major barrier to development efforts to alleviate poverty and improve human health. Neglected tropical diseases that are also categorized as emerging or reemerging infectious diseases are an even more serious threat and have not been adequately examined or discussed in terms of their unique risk characteristics. This review sets out to identify emerging and reemerging neglected tropical diseases and explore the policy and innovation environment that could hamper or enable control efforts. Through this examination, we hope to raise awareness and guide potential approaches to addressing this global health concern (Mackey, 2014).

The 21st century has ushered in an era when globalization of infectious diseases is occurring frequently and at an unprecedented speed (Mackey, 2014). In this "globalized" environment of interdependent trade, travel, migration, and international economic markets, many factors now play an important role in the rise, emergence, and reemergence of infectious disease, which necessitates a coordinated, global response (Frieden 2014 & Mackey, 2014).

Zoonotic diseases account for the majority of emerging and reemerging infectious diseases occurring due to increased contact between humans and animals as a by-product of development, industrialization, and encroachment on wildlife habitats, resulting in a dynamic upward trajectory of these diseases (Jones 2008 & Heymann 2013). Yet many of these emerging and reemerging infectious diseases are also "neglected," meaning they impact the world's poorest and lack adequate funding and innovation for prevention and treatment, with some not adequately identified or studied (WHO, 2010).

Emerging infectious diseases (EIDs) and reemerging infectious diseases (ReIDs) can arise due to a multitude of factors and influences and must be addressed dynamically by diverse sectors of society; including public health, medicine, environmental science, animal health, food safety, economics, and public policy stakeholders.

A host of human-sourced and environmental factors complicate these actions, such as societal influences, human susceptibility to infection, demographics, and availability of health care, food production, human behavior, trade and travel, environmental and ecological changes, economic development, war and famine, adequacy of public health infrastructures, man-made events with intent to harm, and pathogen adaptation or evolution (Morens, 2004 ; Jones, 2008). Striking examples of these EID events in play can be seen throughout history, with the

majority originating from zoonotic pathogens (Jones 2008). These include the black plague of the 14th century, caused by *Yersinia pestis*. That plague event was largely attributable to regional trade and societal influences, with overcrowding, poor hygiene, and destruction of the predator of the animal reservoir being leading cause for the rapid transmission of the illness (Tognotti, 2013). Human behavior and mobility can further be implicated in sexually transmitted diseases such as HIV disease (also originally caused by cross-species transmission), hepatitis, gonorrhea, syphilis, and others, including in the context of rural and low-income settings (Dean 2010). Immunosuppression due to HIV/AIDS coincided with the rise of opportunistic EIDs and ReIDs in the 1980s (Jones, 2008).The invention of new drugs to fight cancer or autoimmune disease has also led to immunosuppression. This, along with the development of antimicrobial resistance, has resulted in the emergence of diseases that were otherwise rare (Naggie 2009 & Kumar 2010)Most notably, the development of antimicrobial resistance with new pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *S. aureus*(VRSA), extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli*, multidrug-resistant (MDR) and extensively drug-resistant (XDR) Mycobacterium tuberculosis, and a multitude of other microbial pathogens that were once easily treated is now leading to new infectious disease threats (CDCP, Centers for Disease Control and Prevention, 2013.) and (Gould 2008).

Beyond human action, extremes of weather and natural disasters have also influenced vector-borne infectious disease spread, suggesting a role for climate change in these events (Haines 2006 & Berirtzoglou 2011). Environmental changes such as the introduction of a new insect or plant vector into a region or population have also led to rapid transmission of diseases that were not previously prevalent, such as in the case of Rift Valley fever, dengue, and malaria (Jones, 2008; Mackenzie, 2013).

Food-borne illnesses are another area of emergence, with outbreaks of *Salmonella, E. coli,* and *bovine spongiform encephalitis* all occurring due to poor food processing practices (Skovgaard 2007). Hence, understanding the cause of a disease's emergence can be critical to its prevention and treatment.

3.1: Prevention and control of Neglected Tropical Diseases

More than one billion of the world's poorest people are affected by neglected tropical diseases (NTDs), which are a group of parasitic, viral and bacterial infections that each year cause an estimated 534 000 deaths and a disease burden of 57 million disability-adjusted life–years (DALYs) (Hotez, 2006). The World Health Organization (WHO) advocates five strategies for preventing and controlling NTD: Prevention strategies, including combined preventive chemotherapy (i.e. the treatment of more than one disease by the mass administration of more than one drug concurrently), intensified case management, control of disease vectors, provision of clean water and sanitation and veterinary public health measures (WHO, 2010).

3.2. Preventive Chemotherapy

Concentrates on illnesses for which a system exists and also on devices and the accessibility of sheltered and successful medications that make it plausible to execute expansive scale preventive chemotherapy. Preventive chemotherapy focuses on diseases for which a strategy exists as well as on tools and the availability of safe and effective drugs that make it feasible to implement large-scale preventive chemotherapy. Chemotherapy prevents the production of new blood cells. When white cell counts get too low, the body loses the ability to fight infection. A common side effect of chemotherapy is increased susceptibility to infections. These infections can be very serious and often require hospitalization.

3.3. Intensified Case Management

This is to encourage the rapid development and implementation of better control tools and to ensure the full involvement of national control

programmes. To advocate for health service development in affected areas and allows these diseases to be easily managed within the primary health-care system and ultimately eliminated as a public health problem ((WHO), 2008.)

3.4. Vector control.

This is a vital component of malaria control and elimination strategies as it can be highly effective in preventing infection and reducing disease transmission. The 2 core interventions for malaria vector control are insecticide-treated nets (ITNs) and indoor residual spraying (IRS). In specific settings and under special circumstances, these core interventions can be supplemented by larval source management and personal protection measures (WHO, Working to overcome the global impact of neglected tropical disease, 2010). Vector-borne NTDs—those that are spread by worms, flies, mosquitoes, or other hosts—can be prevented through control of the vectors themselves. This can include mass spraying of insecticides in areas where the vectors breed or gather, killing them before they become parasite carriers. Scientists are also exploring ways to genetically alter the vectors so that they cannot carry the parasite. The genetically altered vectors would then be released into the population to breed and spread their genetic abnormalities to future generations.

3.5 Provision of Clean Water

Neglected tropical diseases (NTDs) are largely preventable, even without vaccines. Clean water, sanitary food handling, and good hygiene can prevent diseases such as guinea-worm disease, schistosomiasis, soil-transmitted helminthiasis, and trachoma. Water is in continuous motion by the processes of the hydrological cycle. Improved access to clean water can reduce diarrhea and waterborne diseases. The provision of safe water and sanitation is a key mechanism required to break the cycle of poverty, particularly for women and girls (open.edu).

3.6 Veterinary Public Health

VPH has been defined as that part of public health action which is committed to the protection and improvement of human health through application of the capabilities, knowledge and professional resources of veterinary science. Being animal-derived, zoonotic diseases are among the most relevant problems posed in VPH. Zoonoses control has long been a major VPH objective and health authorities have devoted great efforts to the development of programmes and strategies on a global scale, with identification of specific targets associated with regional and individual needs (Rev. sci. tech. Off. int. Epiz, 1992).

Historically, the development of drugs for these diseases has been limited by a lack of market incentives (Trouiller, 2002). . More recently, the formation of public–private partnerships for drug development has increased investment in research and development but the results have been uneven, with some diseases benefiting more than others (Cohen, 2010). For some NTDs, such as geohelminth infection, affordable and effective treatments do exist but their availability for people living in highly endemic areas is often limited (Cohen, 201). For many others, treatment is inconvenient, poorly tolerated and expensive.

Educating the at-risk population is also an important aspect of NTD prevention. By controlling environmental factors that invite NTDs, people can reduce their risk. For instance, eliminating areas of standing water, where mosquitoes like to breed, will reduce the risk of mosquito-borne diseases, or sleeping under a treated bed net will reduce the risk of diseases carried by flies that circulate at night. Travelers to areas where insect-borne NTDs are widespread should take care to wear protective clothing, use bug repellant, and sleep under a treated bed net.

Many NTDs have similarities in treatment measures, epidemiology, and geographic distribution (Kabatereine NB, 2010). Accordingly, many NTDs have similar strategies for control and eradication. Among the 15 most common NTDs, seven are controlled using preventative chemotherapy in

NTD endemic countries (Hanson C, 2012). Traditional approaches to NTD control often relied on the aforementioned vertical programs within these countries working in parallel to one another, using the same treatments in the same areas and populations (Molyneux DH, 2005). As a result, although vertical control programs are effective tools in combating specific diseases, integrated disease control programs could enhance control efforts by combining efforts to control multiple diseases into a single intervention.

WHO now recognizes the integration of NTD efforts as a crucial activity for tracking progress, ensuring accountability, and informing the development of policies and strategies (WHO, World Health Organization. Integrating neglected tropical diseases into global health and development: fourth WHO report on neglected tropical diseases Geneva, 2017). It is in this context that NTD control programs may be incorporated into broader public health systems providing opportunities for countries to advance their NTD control by increasing efficiency, improving the overall quality of health services, covering a larger percentage of the population, and reducing the disparities associated with control programs (Mitashi, 2015). Recent disease integration efforts have also yielded considerable savings both financially and in personnel time (Brady 2006), and modeling efforts have identified opportunities for epidemiological benefits at a population level under some conditions (Standley, 2018). Thus, the positive impacts of large-scale integrated disease control programs– both for the burden of NTDs, as well as the cost-effectiveness of interventions–may render them the best option for many countries (Molyneux, 2005). However, there is no standardized approach to integration, allowing for substantial heterogeneity at the country-level in the implementation, administration, and oversight of integration efforts.

Generally, integrated disease control efforts are administratively placed within Ministries of Health (MOH), and thus the leadership, management, and organizational structures of the ministry can impact the ability to integrate programs. The goal of this work was to understand and present the various was by which NTD endemic countries have approached the integration of NTD control from an administrative standpoint. By observing the different approaches taken by NTD-endemic countries, we hoped to be able to extract common elements which might serve as recommendations or lessons learned that could be provided as a model to other countries that have yet to integrate their NTD control programs.

4.0: CONCLUSION: Prevention and eradication of these diseases are important because "of the appalling stigma, disfigurement, blindness and disabilities caused by NTDs."(Fenwick, 2012) The possibility of eliminating or eradicating dracunculiasis, leprosy, lymphatic filariasis, onchocerciasis, trachoma, sleeping sickness, visceral leishmaniasis, and canine rabies within the next ten years was the principal aim of the London Declaration on Neglected Tropical Diseases, which is a collaborative effort involving the WHO, the World Bank, the Bill & Melinda Gates Foundation, the world's 13 leading pharmaceutical companies, and government representatives from US, UK, United Arab Emirate, Bangladesh, Brazil, Mozambique and Tanzania. It was launched in January 2012 (Hotez, 2013).

While the current era has had a noticeable uptick in biological research into neglected tropical diseases, prevention may be supplemented by social and development outreach. Spiegal and his coauthors advocated for this to take the form of "social offset." Social offset entails the delegation of some of the funding for biotechnological research to social programs. The attempts to alleviate some of the surrounding factors (such as poverty, poor sanitation, overcrowding, poor healthcare etc.) that greatly exacerbate the conditions brought on by neglected tropical diseases. Projects such as these also strengthen the goal of sustained eliminations, rather than quickly addressing symptoms (Spiegel, 2010).

Neglected tropical diseases (NTDs) are a group of parasitic and bacterial diseases that cause substantial illness for more than 2 billion people globally. Affecting the world's poorest people, NTDs impair physical and cognitive development, contribute to mother and child illness and death, and make it difficult to farm or earn a living, and limit productivity in the workplace. As a result, NTDs trap the poor in a vicious cycle of poverty and disease.

However, control of NTDs has gained momentum in the last couple of years. The burden of these diseases on over 2 billion people in the poorest communities, mostly in developing countries and the importance of controlling these NTDs has gained some major level of recognition by the international community. Researches are being carried out relentlessly to study the behavioural patterns of the pathogens in order to develop vaccines. More efforts are needed to systematically fill in the knowledge gaps in relation to the broad range of NTDs. Thus developing vaccines is only a curative measure most times this only happens when the breakout of the disease becomes epidemic or a pandemic like Covid-19, hence, efforts on preventive measures should be fortified by the World Health governing bodies to sensitize and create awareness to the public on the ways of prevention especially environmental control measures. For a long-term and sustainable effort, control of neglected tropical diseases should also be integrated into national primary healthcare systems.

REFERENCES:

Bezirtzoglou C, Dekas K, Charvalos E. (2011). Climate changes, environment and infection: facts, scenarios and growing awareness from the public health community within Europe. Anaerobe 17:337–340.

Brady MA, Hooper PJ, Ottesen EA (2006). "Projected benefits from integrating NTD programs in sub-Saharan Africa". Trends in Parasitology. **22** (7): 285–91. doi:10.1016/j.pt.2006.05.007

Centers for Disease Control and Prevention (2013). Antibiotic resistance threats in the United States. Centers for Disease Control and Prevention, Atlanta, GA.

Colatrella B (2008). "The Mectizan Donation Program: 20 years of successful collaboration - a retrospective". Annals of Tropical Medicine and Parasitology. 102 Suppl 1: 7–11.

Conteh L, Engels T, Molyneux DH (2010). "Socioeconomic aspects of neglected tropical diseases". Lancet. **375** (9710): 239–47.

Dean HD, Fenton, KA. (2010). Addressing social determinants of health in the prevention and control of HIV/AIDS, viral hepatitis, sexually transmitted infections, and tuberculosis. Public Health Rep. 125(Suppl 4):S1–S5.

Disease control priorities in developing countries (2nd ed.)(2006). Oxford University Press. 2006. ISBN 978-0-821-36179-5

Feasey N, Wansbrough-Jones M, Mabey DC, Solomon AW (2010). "Neglected tropical diseases". British Medical Bulletin. **93** (1): 179–200.

Feasey N, Wansbrough-Jones M, Mabey DC, Solomon AW (2010). "Neglected tropical diseases". British Medical Bulletin. **93** (1): 179–200.

Fenwick A (2012). "The global burden of neglected tropical diseases". Public Health. **126** (3): 233–236.

Frieden TR, Tappero JW, Dowell SF, Hien NT, Guillaume FD, Aceng JR. (2014). Safer countries through global health security. Lancet 383:764–766..

Gillis, J (2006). "Cure for Neglected Diseases: Funding". Washington Post.

Gould IM. (2008). The epidemiology of antibiotic resistance. Int. J. Antimicrob. Agents 32(Suppl 1:S2–S9.

Haines A, Kovats RS, Campbell-Lendrum D. (2006). Climate change and human health: impacts, vulnerability and public health. Public Health 120:585–596.

Heymann DL, Dixon M. (2013). Infections at the animal/human interface: shifting the

paradigm from emergency response to prevention at source. Curr. Top. Microbiol. Immunol. 366:207–215.

Hotez P (2009). "Neglected diseases amid wealth in the United States and Europe". Health Affairs. **28** (6): 1720–5.

Hotez P, Ottesen E, Fenwick A, Molyneux D (2006). "The neglected tropical diseases: the ancient afflictions of stigma and poverty and the prospects for their control and elimination". Advances in Experimental Medicine and Biology. **582**: 23–33.

Hotez PJ (2010). "A plan to defeat neglected tropical diseases". Scientific American. **302** (1): 90–4, 96.

Hotez PJ (2013). "NTDs V.2.0: "blue marble health"--neglected tropical disease control and elimination in a shifting health policy landscape" PLoS Neglected Tropical Diseases. **7** (11): e2570.

Hotez PJ (2014). "Neglected parasitic infections and poverty in the United States". PLoS Neglected Tropical Diseases. **8** (9): e3012.

Hotez PJ, Kamath A (2009). Cappello M (ed.). "Neglected tropical diseases in sub-saharan Africa: review of their prevalence, distribution, and disease burden". PLoS Neglected Tropical Diseases. **3** (8): e412.

Jones KE, Patel NG, Levy MA, Storeygard A, Balk D, Gittleman JL, Daszak P. (2008). Global trends in emerging infectious diseases. Nature 451:990–993.

Kealey A, Smith R (2010). "Neglected tropical diseases: infection, modeling, and control". Journal of Health Care for the Poor and Underserved. **21** (1): 53–69.

Kirchhoff LV (1993). "American trypanosomiasis (Chagas' disease)--a tropical disease now in the United States". The New England Journal of Medicine. **329** (9): 639–44.

Kumar D. (2010). Emerging viruses in transplantation. Curr. Opin. Infect. Dis. 23:374–378.

Lindoso JA, Lima AC, Cunha MA, Gomes CM (2015). "Diagnosing Neglected Tropical Diseases in HIV Coinfection". Human Parasitic Diseases. **2015** (7): 11–18.

Litt E, Baker MC, Molyneux D (2012). "Neglected tropical diseases and mental health: a perspective on comorbidity". Trends in Parasitology. **28** (5): 195–201.

Mackenzie JS, Jeggo M. (2013). Reservoirs and vectors of emerging viruses. National Library of Medicine. National Center for Biotechnology Information.

Mackey TK, Liang BA. 2012. Lessons from SARS and H1N1/A: employing a WHO-WTO forum to promote optimal economic-public health pandemic response. J. Public Health Policy. 33:119–130.

Mackey TK, Liang BA. (2012). Threats from emerging and re-emerging neglected tropical diseases (NTDs). Infect. Ecol. Epidemiol. 2:75–88.

Mike Shanahan (2006). "Beat neglected diseases to fight HIV, TB and malaria". SciDev.Net. Archived from the original on 19 May 2006

Mike Shanahan (2006). "Beat neglected diseases to fight HIV, TB and malaria". SciDev.Net. Archived from the original on 19 May 2006.

Molyneux DH (2004). ""Neglected" diseases but unrecognized successes--challenges and opportunities for infectious disease control". Lancet. **364** (9431): 380–3.

Morens DM, Folkers GK, Fauci AS. (2004). The challenge of emerging and re-emerging infectious diseases. Nature 430:242–249.

National Institute of Allergy And Infectious Diseases (NIAID). www.niaid.nih.gov.(2015)

Naggie S, Perfect JR. (2009). Molds: hyalohyphomycosis, phaeohyphomycosis, and zygomycosis. Clin. Chest Med. 30:337–353.

Payne L, Fitchett JR (2010). "Bringing neglected tropical diseases into the spotlight". Trends in Parasitology. **26** (9): 421–3.

Reddy M, Gill SS, Kalkar SR, Wu W, Anderson PJ, Rochon PA (2007). "Oral drug therapy for multiple neglected tropical diseases: a systematic review". JAMA. **298** (16): 1911–24.

Rilkoff H, Tukahebwa EM, Fleming FM, Leslie J, Cole DC (2013). "Exploring gender dimensions of treatment programmes for neglected tropical diseases in Uganda". PLoS Neglected Tropical Diseases. **7** (7): e2312.

Singer M, Bulled N (2012). "Interlocked Infections: The Health Burdens of Syndemics of Neglected Tropical Diseases". Annals of Anthropological Practice. **36** (2): 328–345.

Skovgaard N. (2007). New trends in emerging pathogens. Int. J. Food Microbiol. 120:217–224.

Spiegel JM, Dharamsi S, Wasan KM, Yassi A, Singer B, Hotez PJ, et al. (2010). "Which new approaches to tackling neglected tropical diseases show promise?". PLoS Medicine. **7** (5): e1000255.

Taylor-Robinson DC, Maayan N, Soares-Weiser K, Donegan S, Garner P (2015). "Deworming drugs for soil-transmitted intestinal worms in children: effects on nutritional indicators, haemoglobin, and school performance". The Cochrane Database of Systematic Reviews. **7** (7): CD000371.

Tognotti E. (2013). Lessons from the history of quarantine, from plague to influenza A. Emerg. Infect. Dis. 19:254–259.

Trouiller P, Olliaro P, Torreele E, Orbinski J, Laing R, Ford N (2002). "Drug development for neglected diseases: a deficient market and a public-health policy failure". Lancet. **359** (9324): 2188–94.

van den Berg H, Kelly-Hope LA, Lindsay SW (2013). "Malaria and lymphatic filariasis: the case for integrated vector management". The Lancet. Infectious Diseases. **13** (1): 89–94.

Weiss MG (2008). "Stigma and the social burden of neglected tropical diseases". PLoS Neglected Tropical Diseases. **2** (5): e237.

WHO. (2010). Working to overcome the global impact of neglected tropical diseases: first WHO report on neglected tropical diseases. WHO, Geneva, Switzerland. whqlibdoc.who.int.

World Health Organization (WHO) (2015): Water Sanitation and Hygiene for accelerating and sustaining progress on Neglected Tropical Diseases. A global strategy 2015 – 2020 Archived 25 September 2015 at the Wayback Machine. Geneva, Switzerland, p. 26.

World Health Organization (WHO) (2016). Archived from the original on 1 November 2016.

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SIMULATING A CONFINED AQUIFER PERFORMANCE FROM CONSTANT-RATE PUMPING TEST DATA USING COOPER-JACOB METHOD AND MATHEMATICAL EQUATION.

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ABSTRACT

The optimum performance of the aquifer assessed from constant discharge rate pumping test using the cooper-Jacob method and mathematical equation has been carried out in part of the North central Nigeria. Two (2) distinctive drilled boreholes constructed within Gosa area of FCT were subjected to a Constant discharge rate pumping test in order to have an overview of the approximate value of hydraulic parameters in the study area. The Cooper Jacob's Straight-line Equation was used to analyze the pumping test results of drawdown against time. This method shows that Borehole 2 has a low yield while Borehole 1 has a high yield due to the nature of the aquifer in the study area. The average borehole yield in the area is 3.57Liters/Seconds for borehole 1 and 1.27 Liters/Seconds for borehole 2. The pump installed cannot maintain the yield at Borehole 2 and it was advised to install a smaller pump so as to achieve continues/constant pumping, if the yield does not increase in a later stage due to the nature of the geology. It was deduced from the test that quantifying and assessing the yield of any borehole or well can be achieved using Cooper-Jacob method for optimum aquifer optimization.

KEYWORDS: Aquifer performance, Cooper-Jacob Mathematical equation, Borehole, Hydraulic parameters.

1.0 INTRODUCTION

Gosa is a community in the suburb of Abuja along the Abuja airport road and it is surrounded by farm land and dwellers living within this area. However; the study area is Gosa farm market which featured a large areal extent of Land. It is much closer to the airport expressway. A commercial town known as Lugbe is very close to Gosa along the expressway to the main town. Majority of dwellers in the Gosa domain rely heavily on private boreholes as a supplement to the public water supplies which are not readily available. There has been shortage of water in the study area. Some boreholes around the study have been reported to experience low yield, moderate yield and some not producing at all due to the complex geology of the area. This necessitates the fundamental factor why pumping test was carried out on some selected boreholes in the area to test their efficiency.

Determining the characteristics of the aquifer in terms of the hydraulic conductivity and transmissivity is predominantly attained on data acquired from well pumping tests. In essesnce, the aquifer parameters knowledge is very important for the optimum and effective management of groundwater resource. The pumping test is also known as aquifer test that is most widely used for determining the hydraulic behaviour of aquifers such as transmissivity (T), hydraulic conductivity (K), storage coefficient (S), specific yield (S_y) and leakage factor (B). It is a cognitive test that is practically and most reliable method of estimating borehole functionality, borehole yield, the influence zone of the Borehole and aquifer behaviours using different Mathematical equations developed by Cooper and Jacob, Theis,Hantush,Walton equation approach for unsteady flow situation(Abramowitz *et al*,2001). Cooper and Jacob method is a modification of the Theis non-equilibrium concept of pumping test and it demonstrated that when plotted on semi-

logarithmic paper, the theoretical drawdown curve approaches a straight line when sufficient time has elapsed after pumping started. The most widely used method is based on the semi-log representation of the drawdown versus the time. This method was first introduced by Cooper and Jacob (Kruseman, De Ridder, 1990) for drawdown tests and extended by Theis (Theis, 1935) for recovery analysis. Cooper and Jacob (1946) suggested a straight-line graphical method based on the Theis equation for the evaluation of aquifer parameters. Later, Chow (1952) developed a graphical method that has the advantages of avoiding curve fitting and being unrestricted in its application

The constant-rate test is the most common type of pumping test performed on a borehole and the concept is very simple. The borehole is pumped at a constant rate for an extended period (from several hours to several days or even weeks) while the water levels and pumping rates are monitored at a predetermined time. If the most value is to be gained from constant-rate tests, water levels should be monitored in an observation borehole as well as in the pumping borehole (or better still, several observation boreholes at different distances from the pumping borehole). The pumping rate for the test can either be the actual pumping rate when the pump is switched on, or the average long-term pumping rate (including the operational non-pumping periods).The test is carried out by pumping at a constant rate for a much longer period of time than the step test, and majorly designed to make available information on the hydraulic characteristics of the aquifer. Aquifer tests are important and a very veritable tools that proffer information on the hydraulic characterization of a borehole and aquifer parameters (Todd 1980; Turner et al 1991). The "constant-rate" test is usually used when observation wells are available and the test is aimed at determining the aquifer characteristics of the borehole. Halford and Kuniansky 2002 evaluate the aquifer parameters using Microsoft Excel spreadsheet tool developed. Various commercial pumping test software e.g. AQTESOLV (Duffield, 2002) and Aquifer Test Pro (Waterloo Hydrogeologic, 2002) have also been developed for analyzing pump test data.

Herweijer, 1996 simulates pumping and tracer tests in T fields corresponding to both a deterministic sedimentological facies model and a Gaussian geostatistical model. He concludes that early time portions of pumping test data reveals high conductivity interwell pathways, which dominate solute transport. (Naderi and Gupta, 2020) find out the variations in discharge rates occurring during pump tests and showcased their impacts on transmissivity (T) and storage Coefficient(S) values. Suprapti and Pongmanda, 2019 investigated ten (10) observation wells in part of Indonesia named hotel Makassar paradise area. They study the aquifer parameters Using pumping test through the use of theis mathematical concept and the study concluded that the investigation area to have the following: Transmissivity (T) ranging between 124.27 - 966.58 m^2/day, Hydraulic Conductivity (K) ranges between 0.012 - 0.094 cm/s, Storativity (S) ranges between 0.084 - 0.472. b) The cross-section B-B has a value of Transmissivity (T) ranging between 230.14 - 621.37 m^2/day, Hydraulic Conductivity (K) ranges between 0.022 - 0.060 cm/s, Storativity (S) ranges between 0.125 - 0.416.

Many researchers include numerical calculations for the determination of aquifer parameters from pumping tests. Some of these methods depend on matching the observed aquifer response during the whole, early or late times of the pumping to a type curve (Wikramaratna 1985; Sen 1986, 1988, 1996; Srivastava and Guzman-Guzman 1994; Shapiro and Oki 2000; Singh 2001). Other works depend on numerical solutions of the differential equations (Rushton and Holt 1981; Patel and Mishra 1983; Rai 1985; Singh and Gupta 1986; Yeh 1987).

In view of the above, this paper is aimed at carrying out a constant yield or constant drawdown test on two (2) drilled boreholes within the vicinity of Gosa farm market area of Abuja, Nigeria to simulate the performance of the boreholes for groundwater sustainability and optimum yield using the cooper-Jacob straight

line mathematical concept in a Precambrian confined aquifer region.

2.0 STUDY AREA, GEOLOGY AND HYDROEGOLOGICAL SETTING

The investigated area is situated along the Nnamdi Azikwe international Airport, Abuja with geographic coordinate between latitudes 08˚56' 15.5″N and 08˚ 56' 26.9″N and longitudes 07˚17'41.4″E and 07˚17' 55.6″ E as seen in Figure 1. It is accessible through the Airport road, linking Lugbe area along the expressway. It is relatively on a basement rock and several outcrops are present geologically. The area is predominantly on an elevation which spans between 330m to 328m characterized by undulating topography and sparsely populated area where people live. The area is accessible through a pedestrian bridge to the left of the expressway. Geologically, the study area consists of Biotite, Amphibolites, Granite Gneiss, and Migmatite Gnesiss as evidenced during the course drilling the boreholes as seen in Figure 1 below. Hydrogeologically, drilling a borehole has not been very easy in this area as most of the well produced only small amount of water from the weathered layer and fractured zones geologically. The water bearing zones or units in the study area is weathered basement and the fracture basement. The static water level in the study area ranges from 5.37m to 5.00m respectively as measured during the course of this investigation.

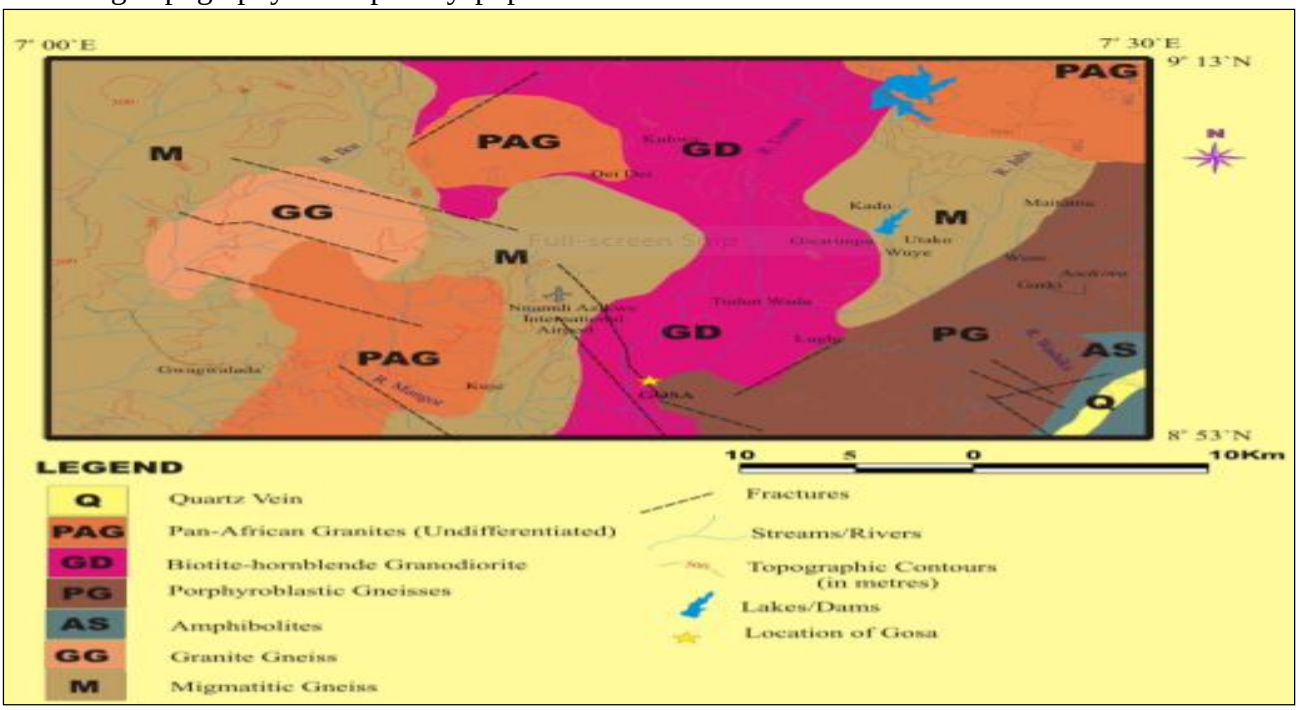

Figure 1: Map of the study area showing the geology and the Location of the study.

3.0 PUMPING TEST FIELD METHODOLOGY

Concise information regarding the drawdown and aquifer characteristics culminating from the specific pumping rate in difference to the corresponding time has been ascertained from constant rate test in the study area. The pumping test data were evaluated using constant pumping rate discharge method. Two (2) distinctive Boreholes were used for the study where Boreholes 1 was drilled to depth of 350m and Borehole 2 was drilled to the depth of 200m due to the complex geology eschew on the site (Figure 2).The constant discharge test was done for one (1) to two and half (2.5) hours for Borehole 1 and three (3) hours and thirty minutes through the use of single well pumping test principle. In analyzing the pumping test results of drawdown with respect to time for the two (2) boreholes in order to estimate

Drawdown, transmissivity, storativity and specific discharge, the Jacob's straight-line method was used and equations. Prior to pumping, the borehole head was removed and the static water level measurement was taken and recorded using the water level indicator (made by PASI Geophysical in Italy) which in length is 500m. After taken the groundwater level at that point, the submersible pump of 3Hp was lowered to a desired depth, then connected to a stand by generator and the pumping test operation commence. A known twenty (20) liters volume of bucket was made available to receive the discharge and a standard stop watch set to zero. The Pumping was then started, drawdown measured base on scheduled time on the calibrated data sheet. The time taken for the bucket to be full was noted and also discharge of the water in terms of the yield was measure and record concurrently.

3.1 Constant rate test Discharge stage

The pumping test was done in a constant rate norms for the two (2) boreholes and the level of the water was noted at regular intervals in the pumped boreholes .The constant discharge rate test was strictly embarked upon between two (2) and half hours and three hours and thirty minutes which was experimented for a day to understand the dynamics of the discharge or yield of the boreholes. This was used to calibrate the performance of the Borehole and it was inferred from the test that Borehole 2 has a low yield, and Borehole 1 has a high yield. The Boreholes were subjected to Constant rate pumping test for five times in a day respectively in which the experiments started at exactly 9.10 am daily and average yield were taken. At the initial of pumping process, the static water level was 5.37m and the discharge of the borehole was 12Liter/Seconds. Pumping continued with same yield but after the 50th minute it started dropping till 6Liter/Seconds in 1hr 15mims and pumping stopped as evidenced for borehole 2. Obviously the pumping level dropped to the pump installation level. This was an indication that the volume of water recharging the borehole was less than the pumping and couldn't maintain constant flow.

3.2 Mathematical Background of Cooper-Jacob drawdown time Method.

According to Todd, 2004, the differential equation that better explains the phenomenon of the rate of water level decrease due to the effect of pumping increases in a region is given as

$$\frac{\delta^2 h}{\delta d^2} + \frac{1}{d}\frac{\delta h}{\delta d} = \frac{S}{T}\frac{\delta h}{\delta t} \quad \text{----------equation1}$$

S denote the storage coefficient, T denote the transmissivity, h is the head, d denote distance from the pumped well and t denote the time of pumping respectively.

By assuming that the well can be replaced by a sink of constant strength and the conditions that h = ho for t = 0 and h→ho as d→∞ for t≥0, Theis obtain a solution for Equation 1 written as:

$$l = \frac{Q}{4\pi T}\int_{u}^{\infty}\frac{e^{-u}}{u}du \quad \text{.....equation2}$$

L denote the drawdown, Q denote the constant well discharge and u is written as the equation 3

$$u = \frac{d^2 S}{4Tt} \quad \text{.....equation3}$$

According to Fetter (2007), Equation 2 is expanded as a convergent series which gives the Equation 4 below as express by Theis as :

$$l = \frac{Q}{4\pi T}\left[-0.5772 - In\,u + u - \frac{u^2}{2\bullet 2!} + \frac{u^3}{3\bullet 3!} + \ldots\right] \quad \text{.....equation4}$$

Equation 3 and 4 was simplified by **Theis** to give Equation 5 and 6 respectively as below:

$$\frac{d^2}{t} = \left(\frac{4T}{S}\right)u \quad \text{.....equation5}$$

$$l = \left(\frac{Q}{4\pi T}\right)W(u) \quad \text{.....equation6}$$

where, W(u) is called the well function. According to Todd (2004), the Theis Equation was re-defined by Cooper and Jacob as:

$$l = \frac{Q}{4\pi T}\left[-0.5772 - In\frac{d^2S}{4Tt}\right] \quad \text{.....equation7}$$

Converting Equation 7 to decimal logarithms gives:

$$l = \frac{2.30Q}{4\pi T} log_{10}\frac{2.25Tt}{d^2S} \quad \text{.....equation8}$$

Cooper and Jacob extended the work of Theis analytically to obtain equations which solves the problem of curve matching. The graph of the drawdown against the logarithm of time will yield a straight line which can be extended to give t = to as h-ho = 0. Applying these conditions to Equation 8 gives Equation 9:

$$\frac{2.30Q}{4\pi T} log_{10}\frac{2.25Tt}{d^2S} = 0 \quad \text{.....equation9}$$

And Equation 9 can thus be written as below to give equation 10:

$$\frac{\quad}{d^2S} = 1 \quad \text{.....equation10}$$

Hence, the storage coefficient is obtained as Equation 11:

$$S = \frac{2.25Tt_o}{d^2} \quad \text{.....equation11}$$

Considering that t/to = 10, then the log10 t/to = 1. If *l or S* is the drawdown and ΔL *or* ΔS *is* the drawdown per log circle of time, the transmissivity is expressed by Equation 12 as:

$T = 2.3\ Q/4\pi\Delta L$ or $T = 2.3Q/4\pi\Delta S$ equation12

For the Cooper-Jacob straight-line method, drawdown is plotted with an arithmetic scale on the y-axis versus time plotted with a logarithmic scale on the x-axis. Transmissivity (T) is estimated from the pumping rate (Q) and the change in drawdown per log-cycle (Δs) from the following equation above equation. Where, Δs is change in drawdown per log-cycle (L).
Hydraulic conductivity (K) is given by **K =T/h** ,where T is the transmissivity and h corresponds to the drawdown from the cooper-jacob straight line graph.

3.3 Discharge or Yield of the Borehole in Litre per second (L/S)

It was observed that in the investigated area, the discharge or the yield of the Borehole 1 is between 4.8 Liter/seconds to 1.3 Liter/Seconds. The average borehole yield in the area for Borehole 1 is 3.52 Liter/Seconds (Table 1.). This is a diagnostic feature of a high yield aquifer that has the tendency of recharging at a high rate while borehole 2 yielded a low value between 0.55 Liter/Seconds to 2.0 Liter/Second and the average yield is 1.27 Liter/Seconds

pointing that the borehole production was not encouraging.

4. RESULTS AND DISCUSSION

The pumping test results of the wells are plotted on semi-logarithmic paper, the slope of the line Δs with initial time of pumping test t_o were determined, as presented in Figure 1 and 2 below. Due to the purpose of the borehole, the Boreholes in the investigated area were drilled to the depth of 200m depth for the borehole 1 and to the depth of 350m for borehole 2(Table 1).The measurement of the groundwater water level during the course of carrying out the pumping test spans from 5.0 m to 5.37m while the depth of installation ranges from 180m to 210m for the two (2) boreholes signifying that the borehole in the experimental field are relatively borehole at deeper depth. From the aquifer behavior results mathematically calculated via the constant rate pumping test data from Borehole 1 and Borehole 2 signifies that the transmissivity of Borehole 1 imprints a value of $0.03m^2$/day and Borehole 2 imprints a lower transmissivity of $0.0122m^2$/day(Figure 2, Figure 5,Table 1). It was observed that in the investigated area, the discharge or the yield of the Borehole 1 is between 1.23 Liter/Seconds to 5.8 Liter/Seconds. The average borehole yield for borehole 1 in the area is 3.52 Liter/Seconds (Figure 8,Table 1.) while the yield of Borehole 2 is between 0.55 Liter/Seconds to 2.0Liter/Seconds with average yield of 1.27 Liter/Seconds indicating that Borehole 2 has a very low yield despite the depth of the borehole (table 1).This also point out that the yield of the borehole does not depend on the depth of the borehole geologically. The Borehole 1 has more potential hydro-geologically to have a high yield because of the weathered zone penetrated during the drilling operation and the thickness of the aquifer units. The specific discharge of the boreholes resulted into a value spanning from a low value of $1.2m^2$/day for Borehole 2 and to a high value of 2.0 m^2/day for Borehole 1 with average of $1.60m^2$/day (Figure 4) .Hydraulic conductivity parameters were also calculated for the boreholes and it was seen that Borehole 1 hydraulic conductivity value is 0.00136m/day(Table 1,Figure 2) while that of the Borehole 2 is 0.00064m/day as seen in table 1,Figure 3 below. This shows that Borehole 1 is performing well hydraulically. Draw down was observed to between 3m to 40m for Borehole 1 which indicate a very unprolific groundwater regime while the drawdown for Borehole 1 ranges from 1m to 20m (Table 1).Recovery of the borehole were not calculated during the course of study but there was an observation where it took borehole 2 several hours to recharge meaning the recovery rate is not promising and the Borehole might not be able to meet the water demand in the investigated area while Borehole 1 despite its drawdown values recovered very quickly.

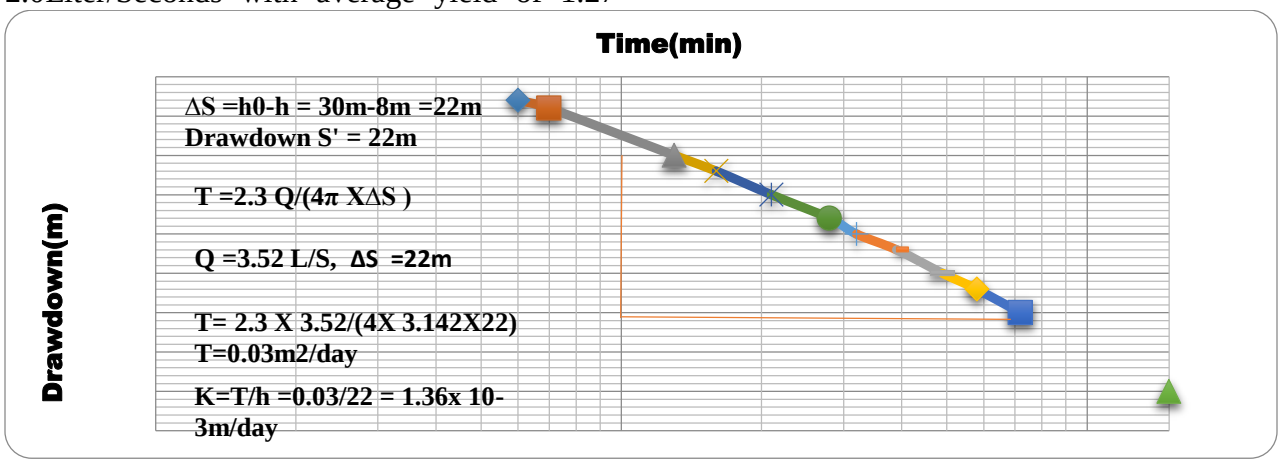

Figure 2: Graph of Drawdown S' against time using the Cooper-jacob mathematical equation for Borehole 1

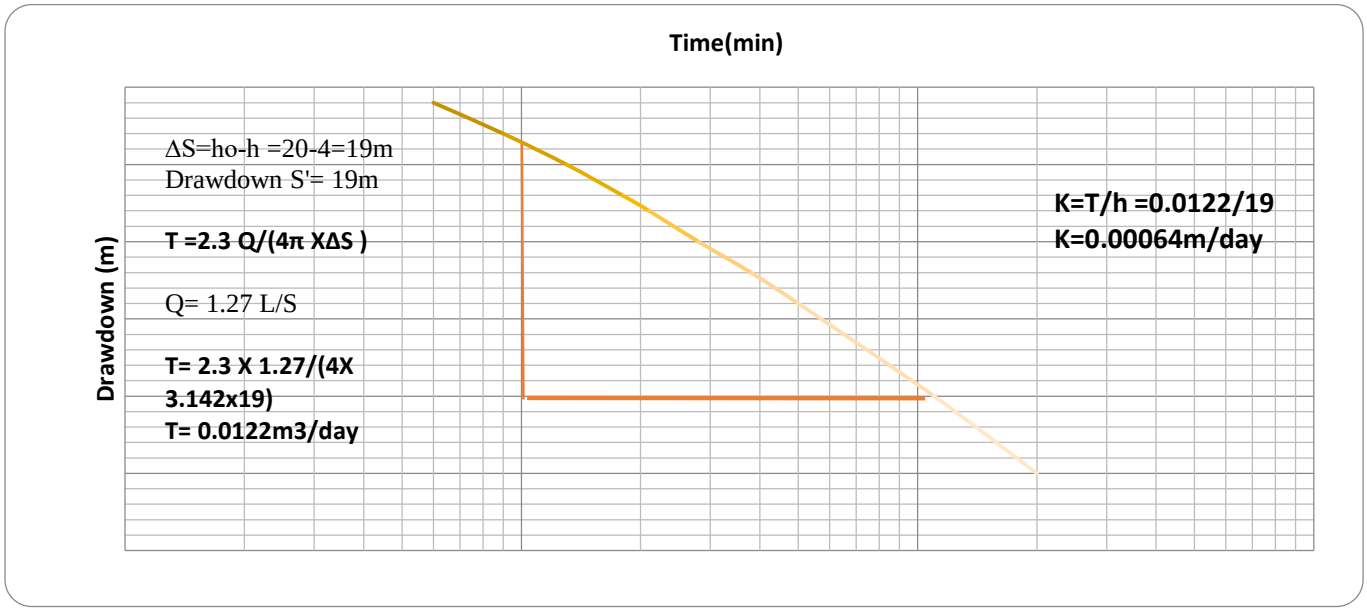

Figure 3: Graph of Drawdown S' against time using the Cooper-jacob mathematical equation for Borehole 2

Table 1: Synopsis of aquifer parameters acquired on the field and by the use of Mathematical equation developed by Cooper-Jacob straight line method.

S/N	Location of Borehole	Borehole Depth(m)	Initial Static Water Level (m)	Transmissivity (m^2/day)	Average yield(L/S)	Specific discharge(m^2/day)	Hydraulic conductivity (m/day)	Average Drawdown (m)	Borehole Coordinate (Decimal Degree)	Elevation (m)
1	Gosa	200	5	0.33	3.52	2.0	0.00136	18.8	Lat: 8.93875 , Long:7.29661	330
2	Gosa	350	5.37	0.0122	1.27	1.2	0.00064	10.5	Lat:8.93964 , Long:7.29503	328

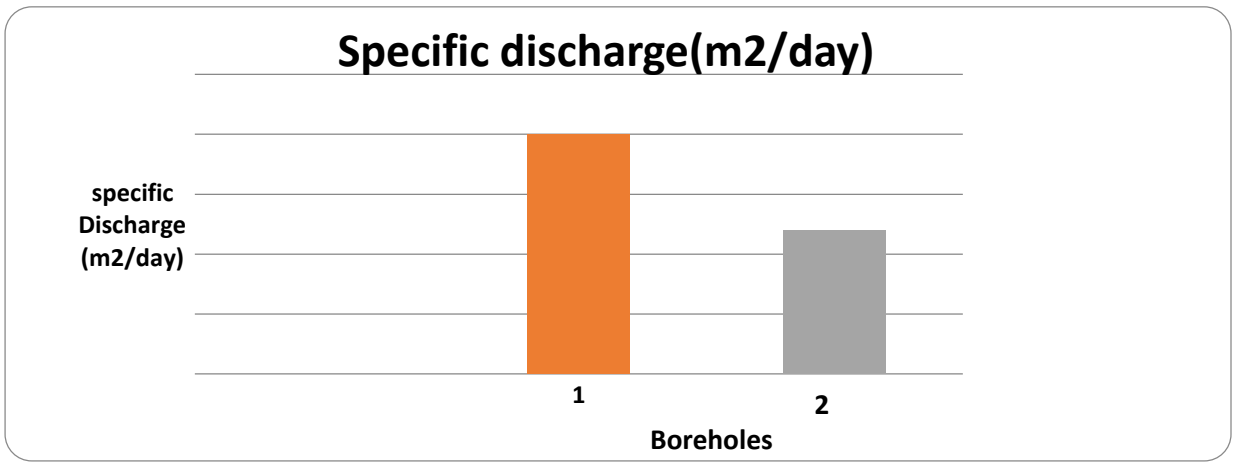

Figure 4: chart showing the specific discharge value of the boreholes in the study area.

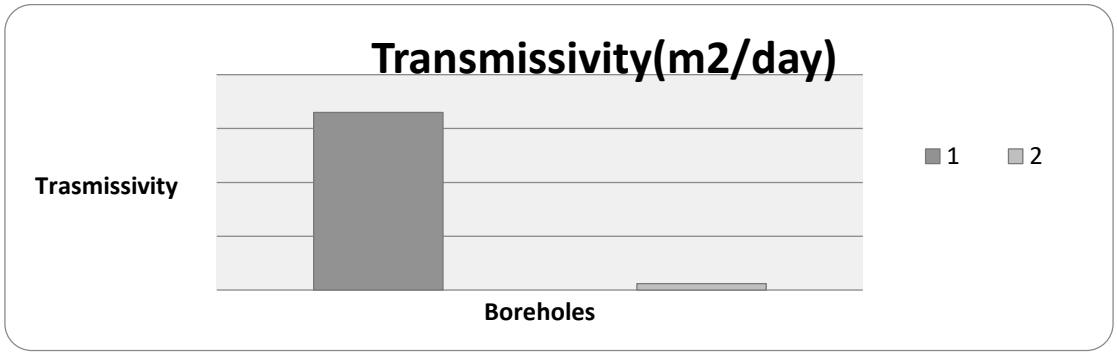

Figure 5: chart showing the transmissivity value of the boreholes in the study area.

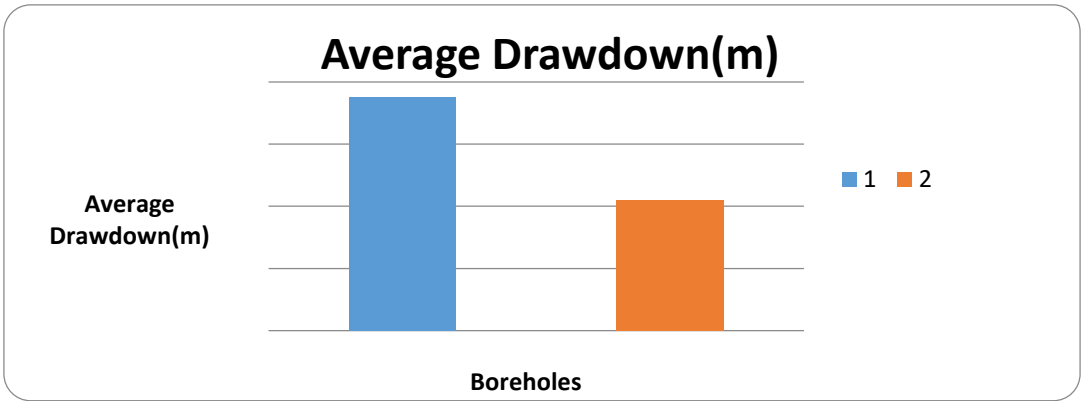

Figure 6: chart showing the Average drawdown value of the boreholes in the study area.

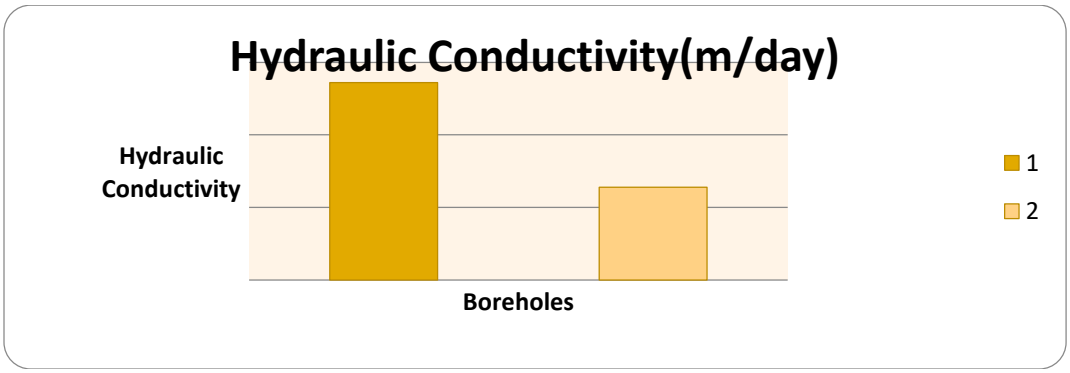

Figure 7: chart showing the hydraulic conductivity value of the boreholes in the study area.

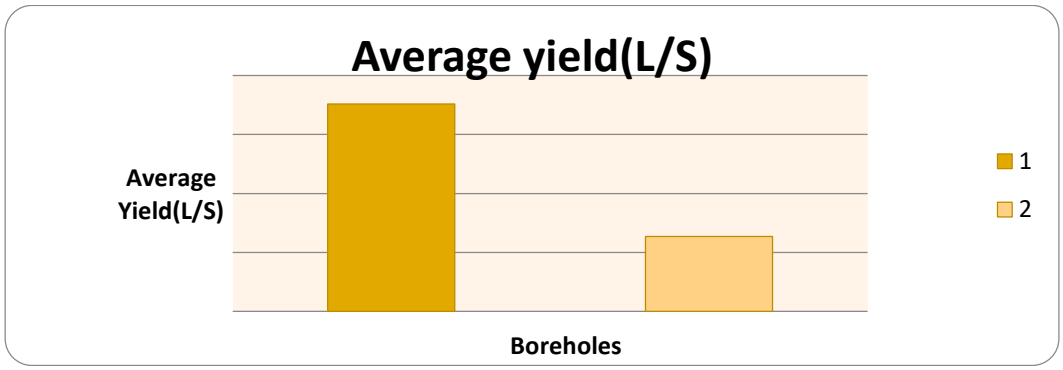

Figure 8: chart showing the Average yield value of the boreholes in the study area.

4.0 CONCLUSION

The pumping test guidelines involving the use of Cooper-Jacob evaluation method and mathematical equation has been utilized to assess the aquifer characteristics in part of Abuja, North-central Nigeria. A straight-line equation is presented for the approximation of aquifer parameters in this study. The equation was used on the pumping test data gathered on the site and shows that the pumping well storage affected the drawdowns of observation wells tremendously especially the Borehole 2 case. The average yield of the borehole from the pumping Test was observed to be around 3.52 Liter/Seconds for Borehole 1 and 1.27 Liter/Seconds for borehole 2. It was then concluded that the efficiency of borehole 2 is not highly guaranteed and the life span might not be promising. The pump installed cannot maintain the yield and there is advisable to install a smaller pump so as to achieve continues/constant pumping, if the yield does not increase in a later stage for borehole 2. Constant discharge rate Pumping test data has been used to simulate the performance of the boreholes on the field by applying the mathematical equation developed by cooper-Jacob. In each case, the boreholes were pumped at a constant rate for extended period of time and the water level and drawdown are monitored. Only stages discharge stage was embarked upon. During the discharge stage, the boreholes were pumped at constant rate and the water level measured at intervals in the same pumped or observation well. Cooper Jacob's equation of single well was used to compute the derived aquifer parameters such as hydraulic conductivity, drawdown, discharge and the transmissivity also calculated. For instance, the hydraulic conductivity of Borehole 1 is 0.00136 m/day and Borehole 2 is 0.00064m/day which has a close familiarity to that obtained in the Ilorin Metropolis (Sule *et al* 2013). This present study uses the fundamental principle of constant rate pumping test as being scientifically evaluated through the Cooper-Jacob method and mathematical equation. The drawdown of Borehole 1 ranges from 3m to 40m and in borehole 2 ranges from 1m to 25m respectively. However, borehole 2 has a very low transmissivity value of $0.0122m^2$/day indicating that the borehole is not promising and the life span of the borehole might not be guaranteed because during the study, it took several hours before the borehole recharge and the recovery rate was not monitored while borehole 1 has a trasmissivity value of $0.03m^2$/day .The fundamental outcome of this study shows that in order to diagnose the optimum performance of a particular borehole, a pumping test is adequately desired to x-ray the efficiency of Borehole at hand.

REFERENCES

Abramowitz, M., and Stegun, I.A., eds., (2001), Handbook of Mathematical Functions, National Bureau of Standards Applied Mathematics Series 55, Washington, D.C., 1046 p.

Cooper, H. H. and Jacob, C. E., (1946), A Generalized Graphical Method for Evaluating Formation Constants and Summarizing Well-Field History, Trans. A. G. U., Vol. 27, pp. 526-534.

Chow, V.T. (1952). On the determination of transmissivity and storage coefficients from pumping test data. Trans. Am. *Geophys. U.*, 33, 397–404.

Duffield, G.M. (2002). AQTESOLV for Windows Version 4.5 User's Guide; HydroSOLVE Inc.: Reston, VA, USA,

Fetter, C.W., 2007. Applied Hydrogeology. 4th Edn., Prentice Hall, Upper Saddle River, ISBN-10: 0130882399, pp: 598

Halford, K.J.; Kuniansky, E.L. (2002.) Documentation of Spreadsheets for the Analysis of Aquifer-Test and Slug-Test Data; 2002-197; U.S. Geological Survey: Carson City, NV, USA,

Herweijer, J. C., (1996) Constraining uncertainty of groundwater flow and transport models using pumping tests, in Calibration and Reliability in Groundwater Modeling, IAHS Publ., 237, 473-482.

Kruseman, G.P. & De Ridder, N.A. (1990) Analysis and Evaluation of Pumping Test Data 2nd Edition, ILRI, Wageningen.

Naderi, M.; Gupta, H.V. (2020) On the Reliability of Variable-Rate Pumping Test Results: Sensitivity to Information Content of the Recorded Data. *Water Resour. Res.*, 56, e2019WR026961

Patel, S.C. and Mishra, G.C. (1983). Analysis of flow to a large-diameter well by a discrete Kernel approach. *Ground Wat.*, 21(5), 573–576.

Rai, S.P. (1985). Numerical determination of aquifer constants. J. *Hydraul. Engng.*, ASCE, 111(7), 1110–1114.

Rushton, K.R. and Holt, S.M. (1981). Estimation of aquifer parameters in large diameter wells. *Ground Wat.*, 19(5), 505–509.

Sen, Z. (1986). Determination of aquifer parameters by the slope matching method. *Ground Wat.*, 24(2), 217–223.

Sen, Z. (1988). Dimensionless time drawdown plots of late aquifer test data. *Ground Wat.*, 26(5), 615–624.

Sen, Z. (1996). A graphical method for storage coefficient determination from quasi-steady state flow data. *Nordic Hydrol*., 27(4), 247–254

Shapiro, A.M. and Oki, D.S. (2000). Estimating formation properties from early-time oscillatory water levels in a pumped well. *J. Hydrol.*, 236, 91–108.

Singh, S.K. (2001). Confined aquifer parameters from temporal derivative of drawdowns. *J. Hydraul. Engng., ASCE*, 127(6), 466–470.

Singh, V.S. and Gupta, C.P. (1986). Hydrogeological parameter estimation from pumping test on large diameter well. *J. Hydrol*., 87, 223–232

Srivastava, R. and Guzman-Guzman, A. (1994). Analysis of slope matching methods for aquifer parameter determination. *Ground Wat.* 32(4), 470–475.

Suprapti.A and Pongmanda.S (2019). Estimation of aquifer parameters using pumping tests: case study of hotel Makassar paradise, Indonesia, IOP Conf. Series: *Earth and Environmental Science* 419 (2020) 012118, doi:10.1088/1755-1315/419/1/012118

Theis, C. V, (1935), The relationship between the lowering of the piezometric surface and the rate and duration of discharge of a well using ground-water storage, *Transactions American Geophysical Union*, v. 16, p. 519-524.

Todd, K.D., (1980). Groundwater Hydrology. John Wiley and Sons New York 535pp.*Trans.*vol. 16, pp. 519-524,

Todd, D.K., 2004. Groundwater Hydrology. 2nd Edn., John Wiley, New York.

Waterloo Hydrogeologic.(2002) Aquifer Test Pro. Users Manual. Graphical Analysis and Reporting of Pumping Test and Slug Test Data; Waterloo Hydrogeologic: Waterloo, ON, Canada,; p. 267

Wikramaratna, R.S. (1985). A new type curve method for the analysis of pumping test in large-diameter wells. *Wat. Res. Res.*, 21(2), 261–264.

Yeh, H.D. (1987). Theis solution by non-linear least squares and finite-difference Newton's method. *Ground Wat.*, 25, 710–715.

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ATTITUDE, OPINION AND PERCEPTION OF BANK WORKERS TOWARDS THE IMPACT OF INFORMATION AND COMMUNICATION TECHNOLOGY ON STAFF PERFORMANCE AND OPERATIONS IN FINANCIAL INSTITUTIONS.

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ABSTRACT

The increasing competitive pressure as a result of technological development, globalization, changing customer demand has led to survival challenges of many banks in Nigeria and demanded for improvement in quality of customer services and speed to enhance profitability, staff performance and cost reduction. The study investigated sample of selected bank workers and customers of United Bank for Africa (UBA) headquarter, CMS, Lagos. It aimed at determining the perception of bank workers as well as identifying the extent at which Information and Communication Technology (ICT) adoption influenced staff performance and their operations. Survey research was employed in the conduct of the study since the subject of investigation centers on individual's opinion and perception. Hence, purposive sampling technique was adopted. A structured questionnaire of $24-$ item variable was administered with 91% response rate justifying the acceptability of study by the staff members. The data collated were analysed using statistical package for social science (version 23). The result reveals that about 56% ($n = 214$) were in the $30-39$ years age bracket while 39% ($n = 149$) were in the $20-29$ years age bracket. Additionally, about 96% ($n = 370$) of the staff agree to the statement that adoption of ICT has immensely influenced staff performance by increasing their productivity. In a related development, there is a significant effect of ICT on banking operations. Hence, ICT had significant impact on financial service delivery of the bank (since $p < 0.05$). It is concluded that the adoption of ICT has tremendously influenced staff performance. Likewise, banks have benefited from ICT in the area of improved efficiency and effectiveness of their operation.

KEYWORDS: Information, Communication, Technology, Staff, Financial Institution.

INTRODUCTION

Obviously, the banking industries have gone far into Information and Communication Technology (ICT) management and others in the delivery of their customers' services and staff performance.

ICT plays a very significant and unique role in the smooth running of organization all over the world. It does not only support decision making in organization/institution like banks, but also goes a long way in enhancing, promoting, corporate performance and profitability. To this end, banks with insufficient and irrelevant information will not only suffer in its decision making process, but stand the risk of running at loss.

Technology innovation has influenced the performance of all Nigerian banks. In the last ten years huge achievements were made in banks networking, service delivery, profitability and customers responses. Employees were made to cope with the demands of Information and Communication Technologies (ICT) dominated by global banking industry. Customers also benefited from improved networking and service delivery which inevitably improved banks competitiveness and profitability. But despite these achievements Nigerian banks have witnessed severe downturn in their profit and many of them have almost collapsed.

According to Woherem (2000), he claimed that only banks that renovate the whole of their payment and delivery systems and apply ICT to their operations are likely to survive and prosper in the new millennium. He advices banks to re-

examine their service and delivery systems in order to properly position them within the framework of the dictates of the dynamism of information and communication technology.

Yousafzai (2012) asserted in his study, that ICT Banking adoption is a complex and multifaceted process and joint consideration of customers' personal, social, psychological, utilitarian and behavioural aspects is more important than adoption itself and will ultimately result in the intended behaviour. It is imperative that all these innovations aimed at having a competitive edge are related to the profitability of banks. This is in accordance with (Akombo, 2011).

Roger (2016) opined that ICT is a synergy between computers and communication devices and forms an important part of the modern world. Thus the most significant shortcomings in the banking industry today is a wide spread failure on the part of senior management in banks to grasp the improvement of technology and incorporate it into their strategic plans. Research carried out by Dabwor *et al.* (2017) studied the effect of ICT adoption on the competitive performance of banks in an emerging economy. The study adopted both inferential and descriptive design using a t-test. The findings of the study revealed that a positive relationship exists between ICT and banks performance in Nigeria. This implies that a marginal change in the level of the investment and adoption of ICT such as Automated teller machine, Web based transactions, and Mobile payments in the banking industry resulted in a proportionate increase in the profit level. The study recommended that it is paramount for bank management to intensify investment in ICT products to facilitate speed, convenience, and accurate service delivery. Oyinkansola (2018) conducted a study on the impact of Information Technology on banking operations in the First bank of Nigeria PLC. The data used was primary data and the research instruments used are questionnaires and personal interview for staff and customers of the bank. Simple frequency percentage was adopted as the statistical tools and the hypothesis was analysed using Chi-square. The result revealed that IT has greatly improved the growth and performance of Nigerian commercial banks and has led to increased customers satisfaction. (Oluwagbemi *et al.* 2014) in their study on the impact of Information Technology (IT) in Nigeria banking industry, adopted a qualitative method. In their findings it was revealed that the deployment of IT facilities in the Nigerian banking industry has brought about fundamental changes in the content and quality of banking business in the country. They concluded that Nigeria banks have been rapidly transformed from being just a bank to a one-stop shop financial solution provider. Research revealed that the use of ICT tremendously increased the return on capital employed as well as the return on assets of the South African banking industry. This is established by Binuyo & Aregbesola, (2014) in their study, who assessed the impact of ICT on commercial bank performance in South Africa. The analysis of the data was achieved using the panel environment and the orthogonal transformation approach was adopted. According to Ukah (2013), Nigerian banking industry has become highly ICT-based and is reaping the benefits of a technological revolution as evidenced by its application in most of its operations. Many commercial banks are making huge investments in technology to maintain and upgrade their infrastructure, in order not only to provide new electronic information-based service, but also to take timely advantage of new off-the-shelf electronic services such as online retail banking which is making it possible for very small institutions to take advantage of new technologies at quite reasonable costs. Additionally, research has it according to Furzaneh *et al.* (2012) that customers are encouraged to utilize ICT banking as first priority. Increasing the customer's arousal by ICT advertisements to use ICT banking brings about a positive attitude toward the bank's brand, which in-turn is one of the key factors in ICT banking effectiveness. It helps the customers to validate their account numbers and receive instruction on when and how to receive their cheque books, credit and debit cards.

Banking has come of age and as such, competition has alerted banks to look for innovations that will make their staff performance better and keep their performance in check as well as service delivery. It is important to note that most banks have been in the bondage of how to manage their information, and how to satisfy their esteem customers. Despite the rapid growing adoption of

the Information Technology tools to improve banking operations through the use of Short Message Service (SMS), Internet, online banking and so on. Nigerian banks are still facing the challenges of continually modernizing their operations so as to increase their productivity, enhance quality of service delivery and also minimize the average operating cost and time. Hence, this study is aimed at establishing the link between the adoption of selected ICT tools and their impact on the performance of commercial bank workers in Nigeria, as well as, identifying the extent at which ICT adoption has influenced staff performance and determining the impact of ICT on financial service delivery.

Considering the great competition in banking industries presently, the use of ICT is of great importance to banks that adopts it as ICT has presented a turnaround in banking sector thereby creating new ways of banking operations. The study adds to the reasons why banks today abandoned their former ways of operation to choose modern banking such as e-banking and as such identify the problems arising from the operational system of commercial banks in Nigeria. Its findings show management of banks to embrace the importance and use of Information Technology in achieving the overall efficiency and effectiveness in their operation.

MATERIALS AND METHODS

Research design, study area and population

Survey research was adopted in the conduct of the study because the subject of investigation centers on individual's opinions and perception. The study was carried out at United Bank for Africa (UBA) headquarter, Lagos Island, Lagos State. The study population consisted of the staff of the bank and their customers.

DATA COLLECTION

Information was collected from respondents by means of a pre-tested $24-item$, purpose designed, self-administered anonymous questionnaire containing open and closed ended questions.

The questionnaire was divided into $four$ major sections for ease of administration. Section A containing $5\ items$ focused on demographic characteristics of respondents. Section B containing 8 $items$ focused on questions on extent to which ICT influenced the performance of staff in the bank. Section C containing 6 $items$ focused on the effect of ICT products on banking sectors while section D containing $5\ items$ focused on impact of ICT on banking service delivery.

SAMPLE SIZE DETERMINATION

The minimum sample size was calculated using Cochran formula:

$$n = \frac{z^2 pq}{e^2}$$

$$n = sample\ size$$

$$z = 1.96\ (value\ on\ the\ Z\ table\ at\ 95\%\ Confidence\ level)$$

$$e = 0.05\ (sampling\ error\ at\ 5\%)$$

$$p = 0.5\ (maximum\ variability\ of\ the\ population.)$$

$$q = 0.5 \quad i.e.(1-p)$$

$$n = \frac{[(1.96^2)(0.5)(0.5)]}{0.05^2} = 384.16.$$

To give room for an anticipated non-response rate of 9% (35 $respondents$), the sample size was increased by 35 to make 420 $respodents$. A total of 420 $questionnaires$ were administered for the study. Since the subject of investigation centers on individual's opinions and perception, survey research was adopted in the conduct of this study. From the 420 $questionnaires$ administered, 384 were returned representing 91% response rate.

4.0 RESULTS

The completed questionnaires were collated, analysed and presented using descriptive statistics of simple percentages, frequency distribution, graphs, charts and inferential statistics where applicable.

Demographic Characteristics of the Study Population

Table 1: Demographic Characteristics of the study population

What is your age bracket?				
	Frequency	Percent	Valid Percent	Cumulative Percent
Below 20 years	4	1.0	1.0	1.0
20-29 years	149	38.7	38.7	39.7
30-39 years	214	55.6	55.6	95.3
40-49 years	16	4.4	4.4	99.7
50 and above	1	.3	.3	100.0
Total	384	100.0	100.0	
Male	179	47.0	47.0	46.5
Female	205	53.0	53.0	100
Total	384	100	100	

A total number 384 staff members and customers participated in the study, $1\%\ (n = 4)$ of the respondents in the sampled population is below $20\ years$, $39\%\ (n = 149)$ of the respondents is age range $20 - 29\ years$, $56\%\ (n = 214)$ of the respondents is age range $30 - 39\ years$, $4\%\ (n = 16)$ out of the total observed respondents is age range $40 - 49\ years$, while $0\%\ (n = 1)$ out of 384 respondents is age $50\ years\ and\ above$, that is, close to retirement age. The results also showed that larger percentage of them were Female (53%) and Male (47%) as revealed in $Table\ 1\ above$.

Fig. 1

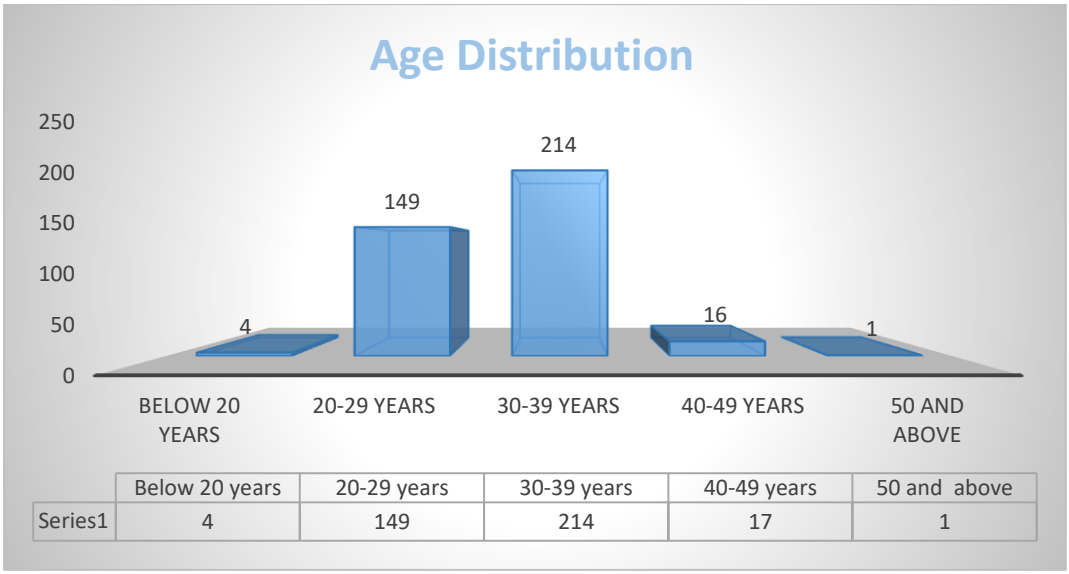

Fig. 1 summarised graphically for better clarification, the age distribution of the respondents. Most of the respondents fall within the age categories $30-39\ years$, which implies that most of the staff in the financial institution are adults.

Table 2. Extent at which ICT has increased staff productivity/performance in the organization

		Frequency	Percent	Valid Percent	Cumulative Percent
	Disagree	14	3.6	3.6	3.6
	Agree	92	24.2	24.2	27.8
	Strongly Agree	278	72.2	72.2	100
	Total	384	100	100	

It was revealed in the table above that 72% of the respondents strongly agreed and 24% agreed to the above statement. Therefore, 96% of them agreed to the statement that adoption of ICT has greatly influenced staff performance by increasing their productivity based on automated process which is however more than enough to conclude that ICT has truly increased the staff members' productivity which in turn gives a positive high influence on their performance.

Table 3. Impact of ICT on financial serviced delivery

	Disagree	**5**	**1.3**
	Agree	**75**	**19.5**
	Strongly Agree	**304**	**79.2**
	Total	**384**	**100.0**

It was shown in the table above that 79% ($n = 304$) of the respondents strongly agreed and 20% ($n = 75$) agreed that ICT has increasingly been helpful in financial serviced delivery.

*Table 4: ICT*financial service delivery*

Test Statistics	
	Less minutes/hours are spent carrying out financial transaction in the institution with the use of electronic services
Chi-Square	**99.995a**
Df	**2**
Asymp. Sig.	**.000**
a. 0 cells (0.0%) have expected frequencies less than 5. The minimum expected cell frequency is 128.3.	

It was revealed according to the result in $Table\ 4$ that ICT has significant impact on financial service delivery. Therefore, the null hypothesis is rejected since $(p < 0.05)$ and we concluded that ICT has significant impact on financial service delivery.

DISCUSSION

This study was conducted to assess the impact of information and Communication Technology (ICT) on Staff performance and operations in Financial Institutions. A total of 384 people were involved in this study, out of which $1\%\ (n = 4)$ is below $20\ years$, $39\%\ (n = 149)$ of the respondents is age range $20 - 29\ years$, $56\%\ (n = 214)$ is age range $30 - 39\ years$, $4\%\ (n = 16)$ out of the total observed respondents is age range $40 - 49\ years$, while $0\%\ (n = 1)$ is age $50\ years\ and\ above$, that is, close to retirement age. The finding revealed that most of the staff members are young adults. The results also showed that larger percentage of them were Female (53%) and Male (47%).

In addition, it is revealed that ICT has obviously influenced staff performance by increasing their productivity based on automated process which in turn enhance their speed of attending to customers. This is in agreement with Dabwor *et al.* (2017), who concluded in their study that ICT has improved productivity in banking industry. This was also supported by Oyinkansola (2018), who in his research, concluded that ICT has greatly improved the growth and performance of banks and has led to increased customers' satisfaction.

Also, findings from this study has shown that ICT has a significant impact on financial service delivery in financial institutions. This was supported by (Oluwagbemi *et al*. 2014), who affirmed in their research that the deployment of ICT facilities in Nigerian banking industry has brought about fundamental changes in the content and quality of banking businesses. Hence, customers on the other hand, stand to enjoy the benefits of quick service delivery, reduced frequency of going to the banks or institutions physically and reduced cash handling, which consequently gives rise to high rate of turnover.

CONCLUSION

The outcome of this study reveals that the adoption of ICT (an effective tool for development) has tremendously improved the performance of staff in the financial institution. Additionally, ICT has significant impact on service delivery by providing flexible and convenient services to customers.

In view of the above conclusion, the followings recommendations were made:

1. Financial institution should delve more into effective ICT training for their workers, so as to further enhance their performance.
2. The government should help in providing 24-hour uninterrupted power supply in order to facilitate effective use of ICT products.
3. Management of the institution must secure quality ICT gadgets that will enhance efficiency and customers' retention.

REFERENCES

Abubakar, A., & Rasmaini, B.T. (2012). The Impact of ICT on banks' performance and customers delivery in the banking industry. *International Journal Latest Trends Finance Economics Science, 2, 1*.

Aduda, J. & Kingoo, N. (2012). The Relationship between Electronic Banking and Financial Performance among Commercial Banks in Kenya. *Journal of Finance and Investment analysis, 1(3)*, 99-118.

Alu, A.D. (2002). Effect of information technology on customer services in the banking industry in Nigeria. *International Journal of scientific research.* 1(1), 20-34.

Amaoko, A. (2012). The Impact of Information Communication Technology (ICT) on Banking Operations in Ghana. *International*

Journal of Business and Management Tomorrow, 2(3), 34-43.

Asidok, N.O. & Micheal, A.A. (2018). Mobile banking transaction and bank profitability in Nigeria. *International Journal of Economics, Commerce and management*, 1(6), 692-716.

Balogun, E. (2016). Effect of Information Technology on Organizational Performance in Nigeria Banking Industries. *Research Journal of Finance and Accounting 7(3), 2222-2847*.

Binuyo, A. O. &. (2014). The Impact of Information Communication Technology on Commercial Bank Performance. Problems and Perspective in Management. *12(3)*, 59-68. Retrieved from http://hdl.handle.net/10500/23249

Dabwor, T. E. (2017). Effect of ICT Adoption on Competititve Performance on Banks in an Economy, the Nigeria experience. *Journal of Humanities and Social Sciences., 22(8)*, 81-89.

Daramola, G. C., Okolie, J. O. & Ogunlowore, J. A. (2014). The Challenges and Profitability of e-banking in Fidelity Bank Nigeria Plc. *International Journal of Finance and Accounting*, 2(3), 79-81.

Eze, G.P & Egoro, S. (2016). Electronic Banking and Profitability of Commercial Banks in Nigeria. *Journal of Finance and Economic Research, 3(1), 202-222.*

Furzaneh, H. (2012). An analysis on the Impact of Information and Communication Technology on deposit money banks. *International Journal of Management Sciences, 5*, 12-18.

Investopedia. (2017). Retrieved August 25, 2018, from http://www.investopedia.com/terms/b/bank-deposits.asp

Josiah A. & Nancy K. (2012). The relationship between electronic banking and financial performance among commercial banks in Kenya. *Journal of finance and invest analysis.* 1(3), 99-103.

Madueme, I. (2010). Evaluating Banking Productivity and ICT using the Translog Production Function. *International Journal of Engineering Science and Technology, 2(4)*, 400-408.

Morufu, O. &. (2012). Banker's Perception of electronic Banking in Nigeria: A review of post consolidation experience. *Research Journal of Finance and Accounting, 3(2)*, 1-11.

Ojokuku, R.M. & Sajuyigbe, A.S. (2012). The impact of Electronic Banking on Human Resources Performance in the Nigerian Banking Industry. *International Journal of Economic Development Research and Investment*, 3(2), 61-69.

Oladejo, M., (2016). E-payments adoption and Profitability performance of deposits money Banks in Nigeria. *IPASJ International Journal of Information Technology (IIJIT),* 4(3), 1-9.

Oluwagbemi, O. (2014). The Impact of Information Technology in Nigeria's Banking Industry. *Journal of Computer Science and Engineering, 7*(2), 1-8.

Oyinkansola, S. (2018). The Impact of Information Technology on Banking Operations in First bank of Nigeria PLC. Retrieved from www.researchclue.com

Ugwueze, A. C. & Nwezeaku, N .C. (2016). E-Banking and Commercial Bank Performance in Nigeria: A Cointegration and Causality Approach. *International Journal of e-Education, e-Business, e-Management and e-Learning,* 6(3), 175-185.

Wilson, U. O. (2014). The Impact of Information Technology on Bank Profitability in Nigeria. *African Journal of Science, Technology, Innovation and Development, 6(1)*, 31-37.

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ANTIMICROBIAL ACTIVITIES OF THE LEAVES AND ROOTS OF *ACALYPHA INDICA* L. DURING THE DRY AND WET SEASON

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ABSTRACT

Acalypha indica is an herbaceous plant that is usually affected by seasonal fluctuations. Therefore, this study evaluated the phytochemical composition and antimicrobial activities of leaves and root extracts of wet and dry seasons collection of *A. indica*. Matured leaves and roots of *A. indica* were collected during the wet and dry season. These were air dried, extracted, filtered and reconstituted. Phytochemical compositions of the plants were evaluated using spectrophotometry method. Antibacterial and antifungal potentials of the root and leaves extracts against some pathogenic bacterial and fungal species were investigated using agar well diffusion methods. Results showed the presence of flavonoid, tannin, cardiac glycoside, alkaloid, saponin and phenol in the root and leaves extracts of *A. indica* used in this study during the wet and dry seasons. Levels of these phytochemicals were similar with season. Flavonoid and alkaloids were significantly higher in the leaves while cardiac glycosides and saponin were higher in the root of *A. indica* during the wet and dry seasons. Level of phenol was not significantly different in the leaves and roots of *A. indica* during both seasons. Also, significantly highest zones of inhibition were recorded at 1000 µg/ml extract (root and leaves) concentration of the *A. indica* against the test organisms. During the wet and dry seasons, antibacterial activities recorded with *A. indica* root extract against MRSA, Sa95 *S. aureus, P. aeruginosa, E. coli* and *E. faecalis* were significantly higher at 1000 µg/ml concentration than those recorded with Streptomycin and Ampiclox. Similarly, antibacterial activities recorded with *A. indica* leaves extract against MRSA, Sa95, *S. aureus, P. aeruginosa, E. coli, S. typhi, S. epidermidis* and *A. baumani* were significantly higher than those recorded with Streptomycin and Ampiclox during the wet and dry seasons. Antifungal activities of *A. indica* leaves and root against *Penicillium* sp, *Aspergillus niger, Candida albicans, Aspergillus flavus, Fusarium verticillioides, Trichophyton rubrum, Trichophyton tonsurans, Candida krusei, Candida* tropicalis and *Aspergillus fumigatus* were also highest at 1000 µg/ml extract concentration during the wet and dry seasons. This study therefore affirmed that *A. indica* root and leaves contain antimicrobial properties regardless of the season of collection.

KEYWORDS: Phytochemicals, antibiotics, bacteria, antibacterial, antifungal

INTRODUCTION

Several plants have been identified and used for several purposes including medicines throughout the history of mankind (Mishra *et al.,* 2009). These plants were also described to have the capability of synthesising several chemical compounds for their biological functions and to defend against attack from predators such as insects, fungi and herbivorous mammals (Mishra *et al.,* 2009). Previous report assumed that about 12,000 chemical compounds of plant origin have been isolated thus far (Lai and Roy, 2004). Therefore, several reports have shown the effectiveness of plant extracts in the treatment of several ailments and clinical diseases such as malaria, arthritis, diabetes, hypertension, cardiovascular diseases and hepatic illness among others (Gupta *et al.*, 2004; Okoro *et al.*, 2011; Traore *et al.*, 2000;

Dongmo *et al.,* 2003). One of such plant is *Acalypha indica*. *Acalypha indica* is an erect annual herb which has been eaten as a vegetable in Africa and India, although with caution of hydrocyanic acid toxicity (Scaffidi, 2016). Previous studies have shown that the different parts of *Acalypha indica* contains some bioactive phytochemicals such as alkaloid, tannin, steroid, saponin, flavonoid, glycoside, and phenol compounds and that the extracts of this plant possess some antimicrobial activities against some bacterial species (Prem *et al.,* 2016).

According to Sagadevan *et al.* (2015), the mortality rate of human in developing countries could be directly linked to infectious bacterial diseases caused mainly by Gram-positive and Gram-negative bacteria. This is because of the ability of these organisms to survive in harsh conditions and because they are capable of adapting to the actions of the conventional and expensive antibiotics (Bibi *et al.*, 2011). Therefore, studies are increasingly focused on the use of herbal products against new resistance microbial stain (Singh *et al.*, 2008).

In a previous study, *Acalypha indica* was described as one of the most commonly used plants among the rural dwellers in Badagry area of Lagos state, South-Western Nigeria (Kolawole-Joseph, 2018). The report showed that the rate at which these people depend on *A. indica* during the wet and dry seasons was alarming because of the believe of its effectiveness against the diseases they were used for. Therefore, there is the need to scientifically study this plant especially with relation to seasonal availability. This study therefore evaluated the phytochemical composition and antimicrobial activities of leaves and root extracts of wet and dry seasons collection of *A. indica*.

MATERIALS AND METHODS

Collection of plant samples

Matured dark leaves and roots of *Acalypha indica* were collected from Badagry Local Government area, Lagos state, Nigeria during the wet and dry seasons. These were transferred to the Plant Science Laboratory, Olabisi Onabanjo University, Ago-Iwoye for further laboratory analyses. The plant was identified at the Forest Research Institute of Nigeria, Jericho, Ibadan, Nigeria (FRIN) and voucher sample deposited (herbarium No. FHI. 111007) with FRIN herbarium.

Collection of microbial samples

All the clinical fungal isolates, Gram positive and Gram negative clinical bacteria used in this research were collected as identified at the Medical Microbiology Laboratory, University College Hospital, Ibadan, Nigeria and Department of Pharmaceutical Microbiology Laboratory, Faculty of Pharmacy, University of Ibadan, Ibadan, Nigeria, and the Plant Science laboratory, Olabisi Onabanjo University, Ago Iwoye, Ogun State, Nigeria.

Sample preparation

The plant materials were thoroughly washed with tap water and sorted into roots and leaves. These were air dried at a room temperature of 27±3 ^{0}C for 14 days. Dried samples were pulverised using mechanical blender (Model EW-04242-11 Mfr # 8011EG, Cole-Parmer scientific). Three hundred grams (300 g) of each powdered sample was soaked in 1200 ml of ethanol. The mixture was agitated using an electric mixer and then poured into air-tight plastic containers and stored in the laboratory for 7 days for extraction. Mixture was filtered using Whatman's No 1 filter paper and then separately concentrated in vacuo using Rotary Evaporator (Model E52A, China) to 10% of their original volumes at 40 ^{O}C. These were concentrated to complete dryness in a water bath.

Phytochemical screening

Two grams of the plant extracts was defatted with 100 ml of diethyl ether using a soxhlet apparatus for 2 h. From the defatted samples, amount of total phenols were determined using the method of Edeoga *et al*. (2007). Level of alkaloid was determined using the method of Harborne (1973). Tannin was quantified as described by Van-Burden and Robinson (1981), saponin according to the method of Obadoni and Ochuko (2001),

flavonoids according to the method of Abyazan (1994), and cardiac glycosidase according to the method of Tofighi et al. (2016).

Antibacterial assay

The bacteria used were identified using standard methods. The stock solutions were prepared as described in the CLSI guidelines (M07-A6, 2003) for bacteria. The plant extracts were dissolved in 5 % DMSO to obtain 5,000 µg/ml stock solutions. 100 µl of stock solution was mixed with 100 µl of nutrient broth for both bacterial isolates to obtain a concentration of 1,000 µg/ml and two-fold serial dilutions were used to give a final concentration of 500, 250, 125 µg/ml.

Mueller-Hinton Agar was used for the sensitivity screening. With a sterile cork borer, wells of 5 mm diameter were dug with a previously sterilised No. 4 cork borer. The wells were aseptically filled up with the extracts avoiding splash and overfilling. The plates were incubated at 37 ^{o}C for 24-48 h. The sensitivity of the test organisms to each of the extracts were indicated by clear halo around the wells. The halo diameters were taken as an index of the degree of sensitivity.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The Minimum inhibitory concentration (MIC) of the plant extracts on the test isolates were determined by the agar micro dilution method as described by the CLSI guidelines (M07-A6, 2003) for bacteria. The minimum dilution (concentration) of the extracts completely inhibiting the growth of each organism was taken as the MIC.

The MBC of the plant extracts was derived by sub culturing portions of the agar from plates that showed no growth in the tests for determination of MICs. These agar portions were transferred respectively into plates containing freshly prepared MHA as described by the CLSI guidelines (M07-A6, 2003) for bacteria. The absence of growth at the end of the incubation period was taken as the MBC which signifies total cell death.

Antifungal activities

Potato dextrose Agar was used for the sensitivity screening. The tubes were incubated aerobically at 25°C for 48-96 hours. The plates were spiked by seeding the agar with the fungal cells and allowed to stand for 1hour in the lamina air-flow hood for the test fungal isolates to be fully embedded and well established in the seeded medium. With a sterile cork borer, wells of 5mm diameter were dug with a previously sterilised No 4 cork borer. The wells were aseptically filled up with the extracts, avoiding splash and overfilling. The plates were incubated at 25°C for 48-96 h. The sensitivity of the test organisms to each of the extracts were indicated by clear halo around the wells. The halo diameters were taken as an index of the degree of sensitivity.

Fungicidal Inhibitory Concentration (FIC) and Minimum Fungicidal Concentration (MFC)

Potato dextrose Agar was used for the sensitivity screening. The tubes were incubated aerobically at 25°C for 48-96 h. The plates were seeded with the fungal cells and allowed to stand for 1 hour in the lamina air-flow hood for the test fungal cells to be fully embedded and well established in the seeded medium. The plates were incubated at 25°C for 48-96 h. The minimum dilution (concentration) of the extracts completely inhibiting the growth of each organism was taken as the FIC.

The MFC of the plant extracts was derived by sub-culturing portions of the agar from plates that showed no growth in the tests for the determination of FICs. These agar portions were transferred respectively into plates containing freshly prepared PDA as described by the CLSI guidelines (M07-A6, 2003) for fungi. The absence of growth at the end of the incubation period was taken as the MFC which signifies total cell death.

Statistical analysis

Data obtained were subjected to statistical analyses using the Statistical Package for Social Sciences (SPSS) version 20.0 (IBM Corp., 2011). Mean values were compared

using One-Way Analysis of Variance (ANOVA). Results were presented as Mean±Standard deviation. Post hoc test was done using the Student-Newman-Keuls (SNK). Probability value (p – value) less than 0.05 was considered to be statistically significant.

RESULTS

Phytochemical composition of *A. indica* extract

Results showed the presence of flavonoid, tannin, cardiac glycoside, alkaloid, saponin and phenol in the root and leaves extracts of *A. indica* used in this study during the wet and dry seasons (Table 1). However, levels of flavonoid and alkaloids were significantly higher in the leaves than the root of *A. indica* during the both seasons. On the other hand, cardiac glycosides and saponin were significantly higher in the root of *A. indica* during the wet and dry seasons. Level of phenol recorded in both the leaves and roots of *A. indica* was not significantly different during the both seasons.

ANTIBACTERIAL ACTIVITIES OF EXTRACT

Zones of inhibition of *A. indica* leaves extract against pathogenic bacteria species

Wet season: Results showed significantly higher zones of inhibition of the *A. indica* leaves extract against the test organism at the highest concentration used (1000 µg/ml) (Table 2). Zones of inhibition recorded at this concentration of *A. indica* leaves against MRSA, Sa95 and *K. pneumonia* were not significantly different from those recorded with Ciprofloxacin. Similarly, the zones of inhibition recorded against MRSA, Sa95, *S. aureus, P. aeruginosa, E. coli, S. typhi, K. pneumonia, S. epidermidis* and *A. baumani* were significantly higher than those recorded with Streptomycin and Ampiclox.

Dry season: Similarly, zones of inhibition recorded against the test organisms were observed to be highest at 1000 µg/ml concentration of *A. indica* leaves extract (Table 2). Also, zones of inhibition recorded against MRSA, Sa95, *S. aureus, P. aeruginosa, E. coli, S. typhi, S. epidermidis, E. faecalis* and *A. baumani* were significantly higher at 1000 µg/ml concentration of *A. indica* leaves extract than those recorded with Streptomycin and Ampiclox.

Zones of inhibition of *A. indica* root extract against pathogenic bacteria species

Wet season: During the wet season, zone of inhibition recorded with *A. indica* root extract against all the test organisms were highest at 1000 µg/ml extract concentration (Table 3). However, zones of inhibition recorded against MRSA, Sa95 *S. aureus, P. aeruginosa, E. coli* and *E. faecalis* were significantly higher at 1000 µg/ml concentration than those recorded with Streptomycin and Ampiclox. Zone of inhibition recorded against *S. epidermidis* at 1000 µg/ml concentration of *A. indica* root extract was not significantly different from those recorded with Ciprofloxacin, Streptomycin and Ampiclox. Also, zones of inhibition recorded at 1000 µg/ml concentration of *A. indica* root extract against *Pro. Mirabilis* and *A. baumani* were not significantly different from those recorded with Ampiclox.

Dry season: Zones of inhibition record with the *A. indica* root extract against the test organisms were also observed to be highest at 1000 µg/ml concentration (Table 3). Zone of inhibition recorded against MRSA, Sa95, *S. aureus, P. aeruginosa, S. epidermidis* and *E. faecalis* at 1000 µg/ml concentration *Acalypha indica* root extract was significantly higher than those recorded using Streptomycin and Ampiclox. Zones of inhibition recorded against *A. baumani* at 1000 µg/ml concentration of *A. indica* root extract was not significantly different from that of Streptomycin and Ampiclox. Similarly, the zone of inhibition recorded at 1000 µg/ml concentration against *S. typhi* was not significantly different from that of Ampiclox.

Minimum Inhibitory Concentration of *A. indica* extracts against pathogenic bacteria species

The minimum inhibitory concentrations (MIC) of *A. indica* leaves and root extracts during the wet and dry seasons are shown in Table 4. The MIC recorded with the root and leaves extracts of *A. indica* against MRSA, *Sa95, S. aureus, S. epidermidis, H. influenza* and *S. typhi* were similar during the wet and dry seasons. These were significantly higher than those recorded with standard antibiotics used (Ciprofloxacin, Streptomycin and Ampiclox). In addition, MIC was significantly lower with the leaves extract against *Pro. Mirabilis, K. pneumonia* and *P. aeruginosa* during both the wet season and dry season. MIC levels recorded with root extracts of *A. indica* against *E. coli* during the wet and dry seasons were significantly lower than the leaves extract and also significantly lower than those recorded with the standard antibiotics.

Maximum Bactericidal Concentration of *A. indica* extracts against pathogenic bacteria species

The minimum bactericidal concentration (MBC) recoded with the root and leaves extract of *A. indica* during the wet and dry seasons were significantly higher against all the test organisms used than those recorded with the standard antibiotics (Table 5). However, the MBC recorded against *H. influenza* and *A. baumani* were significantly lower with root extracts than leaves extract of *A. indica*.

ANTIFUNGAL ACTIVITIES OF EXTRACT

***Acalypha indica* leaves extract**

Antifungal activities recorded with the leaves extract of *A. indica* during the wet and dry seasons followed similar trend (Table 6). Results showed significantly higher antifungal activities against *Penicillium* sp, *Aspergillus niger, Candida albicans, Aspergillus flavus, Fusarium verticillioides, Trichophyton rubrum, Trichophyton tonsurans, Candida krusei, Candida* tropicalis and *Aspergillus fumigatus* at the highest extract concentration (1000 µg/ml) used during both the wet and dry seasons.

***Acalypha indica* root extract**

Wet season: There was no significant difference recorded in the antifungal activities of the root extract of *A. indica* against *Aspergillus flavus* at 125 µg/ml, 250 µg/ml, 500 µg/ml and 1000 µg/ml extract concentrations (Table 7). In addition, antifungal activities of root extract of *A. indica* against *Trichophyton tonsurans* was significantly highest at 250 µg/ml extract concentration. On the other hand, antifungal activities was significantly highest against the other test organisms at 1000 µg/ml extract concentration.

Dry season: During the dry season, antifungal activities of the root extract of *A. indica* against *Penicillium* sp, *Candida albicans, Aspergillus flavus, Fusarium verticillioides, Trichophyton rubrum, Trichophyton tonsurans, Candida krusei, Candida* tropicalis and *Aspergillus fumigatus* were significantly highest at 1000 µg/ml extract concentration (Table 7). However, antifungal activities of *A. indica* against *Aspergillus niger* was highest at 500 µg/ml extract concentrations.

Fungicidal inhibitory Concentration of *A. indica* extract

Significantly lower fungicidal inhibitory concentration (FIC) was recorded using the root and leaves extracts of *A. indica* against *C. tropicalis, A. fumigatus, A. niger, A. flavus* and *Penicillium sp* than those of the control antibiotics during the wet and dry seasons (Table 8). The FIC recorded with the root and leaves extract of *A. indica* against *T. rubrum* during the wet and dry seasons were not significantly different from that of Hezaconizol. Similarly, FIC recorded with the root and leaves extract of *A. indica* against *F. verticillioides* during the wet and dry seasons were not significantly different from that of Hezaconizol and Myclobatanil.

Maximum fungicidal Concentration of *A. indica* extract

There was no significant difference in the maximum fungicidal concentration (MFC) recorded with the root and leaves extracts of *A. indica* during both wet and dry seasons against *C. krusei* (Table 9). These were

however significantly higher than those of the control antibiotics (Hezaconizol and Myclobatanil). The MFC of root extract of *A. indica* against *C. tropicalis* and *T. rubrum* were lower than recorded in leaves extracts and these were also significantly lower than those recorded with the control antibiotics. On the other hand, MFC recorded against *T. tonsurans, A. niger* and *F. verticillioides* with the root and leaves extracts were significantly higher than those of the control antibiotics.

Table 1: Phytochemical composition (mg/100g) of the Leaves and Roots of Acalypha indica obtained during the Wet and Dry seasons

	Wet season		**Dry season**	
	Root	**Leaves**	**Root**	**Leaves**
Flavonoid	11.57±0.30^{b}	19.00±0.50^{a}	11.81±0.01^{b}	18.17±0.10^{a}
Tannin	22.36±0.03^{a}	24.17±0.02^{a}	22.18±0.10^{a}	24.02±0.02^{a}
Cardiac glycoside	39.25±0.05^{a}	34.30±0.30^{b}	39.15±1.00^{a}	33.30±0.30^{b}
Alkaloid	21.75±0.05^{b}	28.83±0.03^{a}	21.47±0.02^{b}	28.43±0.03^{a}
Saponin	19.61±0.01^{a}	15.29±0.20^{b}	19.52±0.02^{a}	15.25±0.05^{b}
Phenol	11.45±0.05^{a}	12.85±0.05^{a}	11.65±1.00^{a}	11.95±0.05^{a}

^{abcde}Mean (±Standard deviation) in the same row having similar superscripts are not significantly different at $p < 0.05$

Table 2: Mean Zones of Inhibition of Ethanol Extract of the Leaves of Acalypha indica Obtained during the Wet and dry Seasons Against Twelve Human Pathogenic Bacterial species

	Wet season						
	Concentrations (µg/ml)				**Controls (µg/ml)**		
	125	**250**	**500**	**1000**	**Cipro.**	**Strep.**	**Amp.**
MRSA	20.0^{b}	15.0^{c}	15.0^{c}	24.0^{a}	27.0^{a}	12.0^{c}	18.0^{b}
Sa95	19.0^{b}	20.0^{b}	18.0^{b}	26.0^{a}	28.0^{a}	13.1^{c}	12.0^{c}
S. aureus	25.0^{b}	18.0^{c}	18.0^{c}	28.0^{b}	32.0^{a}	12.1^{d}	12.0^{d}
P. aeruginosa	28.0^{b}	33.0^{a}	26.0^{b}	36.0^{a}	22.0^{c}	17.1^{d}	12.0^{e}
E. coli	27.0^{a}	23.0^{b}	23.0^{b}	29.0^{a}	18.0^{c}	17.0^{c}	22.0^{b}
S. typhi	24.0^{b}	20.0^{b}	20.0^{b}	27.0^{a}	20.0^{b}	21.0^{b}	12.0^{c}
K. pneumonia	6.0^{c}	16.0^{b}	12.0^{b}	23.0^{a}	17.1^{a}	12.0^{b}	19.0^{b}
S. epidermidis	10.0^{c}	13.0^{c}	15.0^{b}	23.0^{a}	11.1^{c}	12.1^{c}	12.1^{c}
H. influenza	12.0^{b}	14.0^{b}	15.0^{b}	16.0^{b}	23.1^{a}	12.0^{b}	19.1^{b}
E. faecalis	5.0^{d}	8.0^{c}	11.0^{b}	14.0^{a}	12.0^{b}	11.1^{b}	14.1^{a}
Pro. mirabilis	10.0^{c}	12.0^{c}	13.0^{c}	16.0^{b}	12.0^{c}	22.1^{a}	12.1^{c}
A. baumani	18.0^{b}	19.0^{b}	21.0^{a}	24.0^{a}	15.0^{c}	11.0^{d}	12.0^{d}
	Dry season						
	125	**250**	**500**	**1000**	**Cipro**	**Strep.**	**Amp.**
MRSA	22.0^{b}	15.0^{d}	15.0^{d}	24.0^{b}	27.0^{a}	12.0^{e}	18.0^{c}
Sa95	19.0^{b}	20.0^{b}	18.0^{b}	26.0^{a}	28.0^{a}	13.1^{c}	12.0^{c}
S. aureus	25.0^{b}	18.0^{c}	18.0^{c}	28.0^{b}	32.0^{a}	12.1^{d}	12.0^{d}
P. aeruginosa	28.0^{b}	33.0^{a}	26.0^{b}	36.0^{a}	22.0^{c}	17.1^{d}	12.0^{e}
E. coli	25.0^{b}	21.0^{b}	23.0^{b}	29.0^{a}	18.0^{c}	17.0^{c}	22.0^{b}
S. typhi	24.0^{b}	20.0^{c}	20.0^{c}	27.0^{a}	20.0^{c}	21.0^{c}	12.0^{d}
K. pneumonia	6.0^{e}	16.0^{b}	12.0^{d}	14.0^{c}	17.1^{b}	12.0^{d}	19.0^{a}
S. epidermidis	11.0^{c}	16.0^{b}	16.0^{b}	25.0^{a}	11.1^{c}	12.1^{c}	12.1^{c}
H. influenza	13.0^{d}	16.0^{c}	17.0^{c}	20.0^{b}	23.1^{a}	12.0^{d}	19.1^{b}
E. faecalis	7.0^{e}	9.0^{d}	17.0^{a}	16.0^{a}	12.0^{c}	11.1^{c}	14.1^{b}

Pro. mirabilis	12.0^{d}	14.0^{c}	15.0^{c}	18.0^{b}	12.0^{d}	22.1^{a}	12.1^{d}
A. baumani	19.0^{c}	23.0^{b}	24.0^{b}	26.0^{a}	15.0^{d}	11.0^{e}	12.0^{e}

^{abcdef}Mean in the same row for wet and dry seasons respectively, having similar superscripts are not significantly different at $p < 0.05$; MRSA = Methycillin-Resistant *Staphylococus aureus;* Sa95 = *Staphylococccus aureus* strain of type 95; *S. aureus* = *Staphylococcus aureus*; P. aeruginosa = *Psudomonas aeruginosa*; *E. coli* = *Escherichia coli*; *S. typhi* = *Salmonella typi*; K. pneumonia = *Klebsiella pneumonia*; S. epidermidis = *Staphylococcus epidermidis*; H. influenza = *Haemophilus influenza*; E. faecalis = *Enterococcus faecalis*; Pro. mirabilis = *Proteus mirabilis*; A. baumani = *Acentobacter baumanii*; Cipro = Ciprofloxacin; Strep. = Streptomycin; Amp. = Ampiclox

Table 3: Mean Zones of Inhibition of Ethanol Extract of the Root of Acalypha indica Obtained during the Wet and Dry seasons against Twelve Pathogenic Bacterial Species

	Wet season						
	Concentrations (µg/ml)				**Controls (µg/ml)**		
	125	**250**	**500**	**1000**	**Cipro.**	**Strep.**	**Amp.**
MRSA	22.0^{b}	15.0^{d}	16.0^{d}	22.0^{b}	27.0^{a}	12.0^{e}	18.0^{c}
Sa95	13.0^{d}	16.0^{c}	18.0^{c}	23.0^{b}	28.0^{a}	13.1^{d}	12.0^{d}
S. aureus	20.0^{c}	25.0^{b}	23.0^{b}	32.0^{a}	32.0^{a}	12.1^{d}	12.0^{d}
P. aeruginosa	24.0^{b}	10.0^{d}	23.0^{b}	30.0^{a}	22.0^{b}	17.1^{c}	12.0^{d}
E. coli	24.0^{a}	19.0^{c}	22.0^{b}	24.0^{a}	18.0^{c}	17.0^{c}	22.0^{b}
S. typhi	11.0^{c}	12.0^{c}	16.0^{b}	19.0^{b}	20.0^{a}	21.0^{a}	12.0^{c}
K. pneumonia	6.0^{c}	8.0^{c}	11.0^{b}	13.3^{b}	17.1^{a}	12.0^{b}	19.0^{a}
S. epidermidis	10.0^{b}	12.0^{a}	15.0^{a}	13.0^{a}	11.1^{a}	12.1^{a}	12.1^{a}
H. influenza	6.0^{d}	8.0^{d}	10.0^{c}	14.0^{c}	23.1^{a}	12.0^{c}	19.1^{b}
E. faecalis	16.0^{d}	18.0^{c}	20.0^{b}	24.0^{a}	12.0^{f}	11.1^{f}	14.1^{e}
Pro. mirabilis	2.0^{e}	6.0^{d}	8.0^{c}	14.0^{b}	12.0^{b}	22.1^{a}	12.1^{b}
A. baumani	2.0^{d}	4.0^{c}	5.0^{c}	11.0^{b}	15.0^{a}	11.0^{b}	12.0^{b}
	Dry season						
	125	**250**	**500**	**1000**	**Cipro.**	**Strep.**	**Amp.**
MRSA	22.0^{b}	15.0^{d}	16.0^{d}	20.0^{b}	27.0^{a}	12.0^{e}	18.0^{c}
Sa95	13.0^{d}	16.0^{c}	16.0^{c}	20.0^{b}	28.0^{a}	13.1^{d}	12.0^{d}
S. aureus	18.0^{d}	25.0^{b}	21.0^{c}	32.0^{a}	32.0^{a}	12.1^{e}	12.0^{e}
P. aeruginosa	24.0^{b}	10.0^{e}	23.0^{b}	30.0^{a}	22.0^{b}	17.1^{c}	12.0^{d}
E. coli	24.0^{a}	17.0^{c}	22.0^{b}	22.0^{b}	18.0^{c}	17.0^{c}	22.0^{b}
S. typhi	10.0^{d}	12.0^{c}	14.0^{b}	19.0^{a}	20.0^{a}	21.0^{a}	12.0^{c}
K. pneumonia	6.0^{f}	8.0^{e}	11.0^{d}	15.0^{c}	17.1^{b}	12.0^{d}	19.0^{a}
S. epidermidis	12.0^{c}	13.0^{c}	17.0^{a}	15.0^{b}	11.1^{c}	12.1^{c}	12.1^{c}
H. influenza	10.0^{d}	9.0^{d}	12.0^{d}	14.0^{c}	23.1^{a}	12.0^{d}	19.1^{b}
E. faecalis	19.0^{c}	19.0^{c}	22.0^{b}	26.3^{a}	12.0^{e}	11.1^{e}	14.1^{d}
Pro. mirabilis	5.0^{e}	9.0^{d}	10.0^{c}	16.0^{b}	12.0^{c}	22.1^{a}	12.1^{c}
A. baumani	6.0^{c}	6.0^{c}	7.0^{c}	13.0^{b}	15.0^{a}	11.0^{b}	12.0^{b}

^{abcdef}Mean in the same row for wet and dry seasons respectively, having similar superscripts wet and dry seasons respectively are not significantly different at $p < 0.05$; MRSA = Methycillin-Resistant *Staphylococus aureus;* Sa95 = *Staphylococccus aureus* strain of type 95; *S. aureus* = *Staphylococcus aureus*; P. aeruginosa = *Psudomonas aeruginosa*; *E. coli* = *Escherichia coli*; *S.*

typhi = Salmonella typi; K. pneumo = *Klebsiella pneumonia*; S. epidermidis = *Staphylococcus epidermidis*; H. influenza = *Haemophilus influenza*; E. faecalis = *Enterococcus faecalis*; Pro. mirabilis = *Proteus mirabilis*; A. baumani = *Acentobacter baumanii*; Cipro = Ciprofloxacin; Strep. = Streptomycin; Amp. = Ampiclox

Table 4: Minimum Inhibitory Concentration (MIC) of the Extracts Acalypha indica Obtained during Wet and Dry seasons

	Wet season		**Dry season**		**Control**		
	Root	**Leaves**	**Root**	**Leaves**	**Cipro.**	**Strep.**	**Amp.**
MRSA	62.5^{a}	62.5^{a}	62.5^{a}	62.5^{a}	15.0^{c}	20.0^{b}	11.0^{d}
Sa95	125.0^{a}	125.0^{a}	125.0^{a}	125.0^{a}	16.0^{b}	17.5^{b}	7.0^{c}
S. aureus	250.0^{a}	250.0^{a}	250.0^{a}	250.0^{a}	25.0^{c}	200.0^{b}	12.0^{d}
S. epidermidis	31.3^{a}	31.3^{a}	31.3^{a}	31.3^{a}	20.0^{b}	15.5^{c}	6.0^{d}
H. influenza	31.3^{a}	31.3^{a}	31.3^{a}	31.3^{a}	15.0^{b}	17.0^{b}	12.0^{c}
E. faecalis	31.3^{a}	31.3^{a}	31.3^{a}	31.3^{a}	12.5^{c}	22.7^{b}	24.0^{b}
E. coli	7.8^{d}	31.3^{a}	7.8^{d}	31.3^{a}	27.5^{b}	18.5^{c}	10.0^{d}
Pro. mirabilis	31.3^{a}	7.8^{d}	31.3^{a}	7.8^{d}	4.9^{e}	12.0^{c}	25.0^{b}
S. typhi	31.3^{a}	31.3^{a}	31.3^{a}	31.3^{a}	25.0^{b}	14.0^{c}	11.0^{d}
K. pneumonia	62.5^{a}	31.3^{b}	62.5^{a}	31.3^{b}	21.0^{c}	15.0^{d}	14.0^{d}
P. aeruginosa	62.5^{a}	31.3^{b}	62.5^{a}	31.3^{b}	23.5^{c}	17.0^{d}	10.0^{e}
A. baumani	31.3^{b}	62.5^{a}	31.3^{b}	62.5^{a}	12.5^{d}	20.0^{c}	10.0^{d}

^{abcdef}Mean in the same row having similar superscripts are not significantly different at $p < 0.05$; MRSA = Methycillin-Resistant *Staphylococcus aureus;* Sa95 = *Staphylococccus aureus* strain of type 95; *S. aureus = Staphylococcus aureus*; P. aeruginosa = *Psudomonas aeruginosa*; *E. coli = Escherichia coli*; *S. typhi = Salmonella typi*; K. pneumonia = *Klebsiella pneumonia*; S. epidermidis = *Staphylococcus epidermidis*; H. influenza = *Haemophilus influenza*; E. faec = *Enterococcus faecalis*; Pro.mirabilis = *Proteus mirabilis*; A. baumani = *Acentobacter baumanii*; Cipro = Ciprofloxacin; Strep. = Streptomycin; Amp. = Ampiclox

Table 5: Minimum Bactericidal Concentration (MBC) of the Ethanol extracts of Acalypha indica Obtained during Wet Season

	Wet season		**Dry season**		**Control**		
	Root	**Leaves**	**Root**	**Leaves**	**Cipro.**	**Strep.**	**Amp.**
MRSA	125.0^{a}	125.0^{a}	125.0^{a}	125.0^{a}	30.0^{b}	35.5^{b}	22.0^{c}
Sa95	500.0^{a}	500.0^{a}	500.0^{a}	500.0^{a}	29.9^{b}	32.0^{b}	15.0^{c}
S. aureus	1000.0^{a}	1000.0^{a}	1000.0^{a}	1000.0^{a}	39.0^{b}	39.0^{b}	23.5^{c}
S. epidermidis	125.0^{a}	62.5^{b}	125.0^{a}	62.5^{b}	19.0^{c}	20.0^{c}	12.0^{d}
H. influenza	62.5^{b}	500.0^{a}	62.5^{b}	500.0^{a}	30.0^{c}	36.0^{c}	28.0^{c}
E. faecalis	125.0^{a}	125.0^{a}	125.0^{a}	125.0^{a}	29.0^{c}	42.0^{b}	45.0^{b}
E. coli	62.5^{a}	62.5^{a}	62.5^{a}	62.5^{a}	52.0^{b}	39.0^{c}	19.5^{d}
Pro. mirabilis	62.5^{a}	62.5^{a}	62.5^{a}	62.5^{a}	15.5^{d}	23.0^{c}	50.0^{b}
S. typhi	125.0^{a}	62.5^{b}	125.0^{a}	62.5^{b}	52.0^{c}	29.0^{d}	21.5^{e}
K. pneumonia	125.0^{a}	125.0^{a}	125.0^{a}	125.0^{a}	40.0^{b}	31.5^{c}	26.5^{d}
P. aeruginosa	250.0^{a}	250.0^{a}	250.0^{a}	250.0^{a}	45.0^{b}	28.3^{c}	19.5^{d}

A. baumani	62.5[b]	125.0[a]	62.5[b]	125.0[a]	22.5[d]	40.5[c]	18.5[d]

[abcdef]Mean in the same row having similar superscripts are not significantly different at $p < 0.05$; MRSA = Methycillin-Resistant *Staphylococus aureus;* Sa95 = *Staphylococccus aureus* strain of type 95; *S. aureus* = *Staphylococcus aureus*; P. aeruginosa = *Psudomonas aeruginosa*; *E. coli* = *Escherichia coli*; *S. typhi* = *Salmonella typi*; K. pneumonia = *Klebsiella pneumonia*; S. epidermidis = *Staphylococcus epidermidis*; H. influenza = *Haemophilus influenza*; E. faecalis = *Enterococcus faecalis*; Pro. mirabilis = *Proteus mirabilis*; A. baumani = *Acenetobacter baumanii*; Cipro = Ciprofloxacin; Strep. = Streptomycin; Amp. = Ampiclox

Table 6: Antifungal Activities of Ethanol Extract of the Leaves of Acalypha indica Obtained during the Wet Season against Pathogenic Fungal species

Wet season				
	Extract Concentrations (µg/ml)			
	125	**250**	**500**	**1000**
Penicillium sp	8.50±0.50[c]	9.00±1.00[c]	10.50±0.50[b]	13.50±0.40[a]
Aspergillus niger	8.00±1.00[b]	9.00±1.00[b]	9.50±0.50[b]	13.50±0.40[a]
Candida albicans	6.00±0.90[c]	6.50±0.50[c]	20.00±1.00[b]	25.00±0.30[a]
Aspergillus flavus	8.50±0.50[d]	18.00±0.70[b]	13.00±0.50[c]	20.50±0.50[a]
Fusarium verticillioides	8.50±0.50[d]	16.00±0.50[c]	30.00±0.80[b]	36.00±1.00[a]
Trichophyton rubrum	5.00±0.30[c]	8.50±0.50[b]	9.00±1.00[b]	12.00±0.60[a]
Trichophyton tonsurans	6.00±0.90[b]	7.00±0.40[b]	9.00±1.00[a]	10.00±0.50[a]
Candida krusei	8.00±1.00[b]	9.00±1.00[b]	10.00±0.50[b]	12.00±0.60[a]
Candida tropicalis	7.00±0.40[c]	8.00±0.40[c]	12.00±0.60[b]	13.00±0.50[a]
Aspergillus fumigatus	10.00±0.50[d]	12.00±0.60[c]	14.00±0.20[b]	16.00±0.50[a]
Dry season				
	125	**250**	**500**	**1000**
Penicillium sp	8.00±1.00[b]	9.00±1.00[b]	10.00±0.50[b]	13.50±0.40[a]
Aspergillus niger	8.00±1.00[b]	9.00±1.00[b]	9.50±0.50[b]	18.00±0.70[a]
Candida albicans	6.00±0.90[c]	6.50±0.50[c]	20.00±1.00[b]	25.00±0.30[a]
Aspergillus flavus	8.00±1.00[d]	16.00±0.50[b]	13.00±0.50[c]	20.00±1.00[a]
Fusarium verticillioides	16.00±0.50[c]	17.00±0.50[c]	32.00±1.00[b]	39.00±1.00[a]
Trichophyton rubrum	9.00±1.00[c]	12.00±0.50[b]	11.00±1.00[b]	14.00±0.50[a]
Trichophyton tonsurans	9.00±1.00[b]	10.00±0.50[b]	10.00±0.50[b]	12.00±0.50[a]
Candida krusei	9.00±1.00[c]	10.00±0.50[c]	12.00±0.50[b]	15.00±1.00[a]
Candida tropicalis	9.00±1.00[c]	12.00±0.50[b]	15.00±1.00[a]	15.83±0.29[a]
Aspergillus fumigatus	12.00±0.50[c]	14.00±0.50[b]	15.00±1.00[b]	19.00±0.50[a]

[abcdef]Mean (±Standard deviation) in the same row for wet and dry seasons respectively, having similar superscripts are not significantly different at $p < 0.05$

Table 7: Antifungal activities of Ethanol Extract of the Root of Acalypha indica Obtained during the wet and Dry Season against Pathogenic Fungal Species

Wet season				
	Extract Concentrations (µg/ml)			
	125	**250**	**500**	**1000**

Penicillium sp	15.00±0.50^{c}	16.00±0.50^{c}	18.00±0.70^{b}	20.00±1.00^{a}
Aspergillus niger	12.00±0.60^{d}	14.00±0.20^{c}	16.00±0.50^{b}	22.00±0.40^{a}
Candida albicans	10.00±0.50^{c}	23.00±1.00^{b}	24.50±0.00^{b}	29.00±0.00^{a}
Aspergillus flavus	6.00±0.90^{a}	6.00±0.90^{a}	6.00±0.90^{a}	6.00±0.90^{a}
Fusarium verticillioides	6.00±0.90^{c}	12.00±0.60^{b}	13.00±0.50^{b}	17.00±0.50^{a}
Trichophyton rubrum	25.00±0.30^{a}	9.00±1.00^{c}	10.00±0.50^{c}	12.00±0.60^{b}
Trichophyton tonsurans	6.00±0.90^{b}	8.00±1.00^{a}	9.00±1.00^{a}	10.00±0.50^{a}
Candida krusei	8.00±1.00^{b}	10.00±0.50^{a}	11.00±1.00^{a}	12.00±0.60^{a}
Candida tropicalis	8.00±1.00^{c}	8.50±1.00^{c}	13.00±0.50^{b}	16.00±0.50^{a}
Aspergillus fumigatus	8.00±1.00^{b}	14.00±0.20^{a}	15.00±0.50^{a}	16.00±0.50^{a}
Dry season				
	125	**250**	**500**	**1000**
Penicillium sp	31.00±1.00^{c}	34.00±1.00^{b}	34.00±1.00^{b}	39.00±1.00^{a}
Aspergillus niger	33.33±0.58^{b}	33.33±0.58^{b}	36.00±0.50^{a}	24.00±1.00^{c}
Candida albicans	10.00±0.50^{c}	21.00±1.00^{b}	20.00±1.00^{b}	25.00±0.50^{a}
Aspergillus flavus	6.00±0.90^{a}	6.00±0.90^{a}	6.00±0.90^{a}	6.00±0.90^{a}
Fusarium verticillioides	12.00±0.50^{d}	14.00±0.50^{c}	16.00±0.50^{b}	19.00±0.50^{a}
Trichophyton rubrum	9.00±1.00^{c}	10.00±0.50^{c}	12.00±0.50^{b}	14.00±0.50^{a}
Trichophyton tonsurans	7.00±0.50^{c}	10.00±0.50^{b}	11.00±1.00^{b}	13.00±1.00^{a}
Candida krusei	9.00±1.00^{c}	12.00±0.50^{b}	13.00±1.00^{b}	16.00±0.50^{a}
Candida tropicalis	9.00±1.00^{c}	14.00±0.50^{b}	15.00±1.00^{b}	19.00±0.50^{a}
Aspergillus fumigatus	9.00±1.00^{c}	15.83±0.29^{b}	17.00±0.50^{b}	22.00±0.50^{a}

^{abcdef}Mean (±Standard deviation) in the same row for wet and dry seasons respectively, having similar superscripts are not significantly different at $p < 0.05$

Table 8: Fungicidal Inhibitory Concentration (FIC) of the Ethanol Extracts of Acalypha indica Obtained during Wet Season

	Wet season		**Dry season**		**Control**	
	Root	**Leaves**	**Root**	**Leaves**	**Hezaconizol**	**Myclobatanil**
C. krusei	3.90±1.00^{c}	3.90±0.10^{c}	7.8±1.0^{b}	3.9±0.1^{c}	3.90±1.00^{c}	31.30±0.30^{a}
C. tropicalis	3.90±0.10^{b}	3.90±1.00^{b}	3.9±0.1^{b}	3.9±0.1^{b}	31.30±0.30^{a}	31.30±0.30^{a}
T. rubrum	3.90±0.10^{b}	3.90±1.00^{b}	3.9±0.1^{b}	3.9±0.1^{b}	3.90±0.10^{b}	31.30±0.30^{a}
T. tonsurans	3.90±0.10^{a}	3.90±1.00^{a}	3.9±1.0^{a}	3.9±0.1^{a}	3.90±1.00^{a}	3.90±0.10^{a}
A. fumigatus	3.90±0.10^{b}	3.90±1.00^{b}	3.9±0.1^{b}	3.9±1.0^{b}	31.30±0.30^{a}	31.30±0.30^{a}
A. niger	3.90±1.00^{b}	3.90±0.10^{b}	3.9±1.0^{b}	3.9±0.1^{b}	31.00±0.50^{a}	31.00±1.00^{a}
A. flavus	3.90±1.00^{b}	3.90±0.10^{b}	3.9±1.0^{b}	3.9±0.1^{b}	3.90±0.10^{a}	31.30±0.30^{a}
Penicillium sp	3.90±1.00^{b}	3.90±0.10^{b}	3.9±0.1^{b}	3.9±1.0^{b}	31.30±0.30^{a}	31.30±0.30^{a}
F. verticillioides	3.90±0.10^{a}	3.90±1.00^{a}	3.9±1.0^{a}	3.9±0.1^{a}	3.90±1.00^{a}	3.90±0.10^{a}
C. albicans	3.90±1.00^{b}	31.30±0.30^{a}	3.9±1.0^{b}	31.3±0.3^{a}	3.90±1.00^{b}	31.30±0.30^{a}

^{abcde}Mean (±Standard deviation) in the same row having similar superscripts are not significantly different at $p < 0.05$

Table 9: Maximum Fungicidal Concentration (MFC) of the Extracts of calypha indica Obtained during Wet and dry Season

	Wet season		Dry season		Control	
	AIR	**AIL**	**AIR**	**AIL**	**Hezaconizol**	**Myclobatanil**
C. krusei	500.00±5.00^{a}	500.00±5.00^{a}	500.0±5.0^{a}	500.0±5.0^{a}	125.00±5.00^{b}	500.00±5.00^{a}
C. tropicalis	250.00±2.00^{c}	1000.00±5.00^{a}	250.0±2.0^{c}	1000.0±5.0^{a}	500.00±5.00^{b}	500.00±5.00^{b}
T. rubrum	125.00±5.00^{c}	250.00±2.00^{b}	124.7±2.5^{c}	250.0±2.0^{b}	250.00±2.00^{b}	500.00±5.00^{a}
T. tonsurans	1000.00±5.00^{a}	500.00±5.00^{b}	1000.0±5.0^{a}	500.0±5.0^{b}	250.00±2.00^{c}	250.00±2.00^{c}
A. fumigatus	250.00±2.00^{a}	250.00±5.00^{a}	124.7±2.5^{b}	250.0±2.0^{a}	250.00±5.00^{a}	250.00±2.00^{a}
A. niger	500.00±5.00^{a}	500.00±5.00^{a}	500.0±5.0^{a}	500.0±5.0^{a}	125.00±5.00^{b}	125.00±5.00^{b}
A. flavus	250.00±2.00^{c}	1000.00±5.00^{a}	250.0±2.0^{c}	1000.0±5.0^{a}	125.00±5.00^{d}	500.00±5.00^{b}
Penicillium sp	124.67±2.52^{c}	250.00±5.00^{b}	250.0±2.0^{b}	125.0±5.0^{c}	500.00±5.00^{a}	500.00±5.00^{a}
F. verticillioides	1000.00±5.00^{a}	500.00±5.00^{b}	1000.0±5.0^{a}	500.0±5.0^{b}	124.67±2.52^{c}	125.00±5.00^{c}
C. albicans	125.00±5.00^{c}	1000.00±5.00^{a}	125.0±5.0^{c}	124.7±2.5^{c}	124.67±2.52^{c}	500.00±5.00^{b}

^{abcde}Mean (±Standard deviation) in the same row having similar superscripts are not significantly different at $p < 0.05$

DISCUSSION

This study has shown that the leaves and root of extracts *A. indica* contain antimicrobial properties regardless of the seasons of collection. During the wet and dry seasons, antibacterial and antifungal activities were significantly highest at 1000 µg/ml extract (root and leaves) concentration of the *A. indica* against the test organisms used in this study. In fact, during the wet and dry seasons, antibacterial activities recorded with *A. indica* leaves and root extracts against MRSA, Sa95, *S. aureus, P. aeruginosa*, and *E. coli* were significantly higher than those recorded with two standard antibiotics – Streptomycin and Ampiclox. These organisms have been described as being pathogenic in nature. According to CDC (2021), *Escherichia coli* are bacteria found in the environment, foods, and intestines of people and animals and although most strains of *E. coli* could be harmless, others can cause diarrhea, urinary tract infections, respiratory illness and pneumonia. Also, *Staphylococcus aureus* has been described as a leading cause of infectious diseases in sports teams (Couvé-Deacon et al., 2017). Similarly, report has shown that *P. aeruginosa* could cause infection in the urinary tract, respiratory system, dermis, soft tissue, bacteraemia, bone and joint, gastrointestine and blood, especially in patients with severe burns, tuberculosis, cancer and AIDS (Min and Xuefeng, 2015). Thus, regardless of the season of collection, the leaves and root of *A. indica* extract could be a viable potential in the production of natural and cheaper antibiotics for the control of these bacterial pathogens.

Antimicrobial properties recorded with the root and leaves of *A. indica* against the test organisms used in this study during both seasons could be as a result of the phytochemical composition of these extracts. Phytochemicals recorded in the root and leaves extracts of this study include flavonoids, tannins, cardiac glycoside, alkaloids, saponin and phenols. Previous studies also reported that the phytochemical composition of the leaves extract of *A. indica* are alkaloid, tannin, steroid, saponin, flavonoid, glycoside and phenol compounds (Mohan *et al*., 2012; Zahir and Kumaresan, 2013). The roles of these phytochemicals in plants have been well documented. According to Hasler and Blumberg (1999), phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients. The phenols have been reported to possess considerable antimicrobial properties, attributed to their

redox properties (Molan and Faraj, 2010). In addition, flavonoids have also been reported to possess antimicrobial and anti-inflammatory properties (Okwu, 2004). Cordell *et al.* (2011) also reported that alkaloids and flavonoids are responsible for the antifungal activities in higher plants. Some of the characteristics of saponins include formation of foams in aqueous solutions, haemolytic activity and cholesterol-binding properties (Eleazu *et al.*, 2012). Previous researches have shown that the tannin containing plant extracts are used as astringents, against diarrhoea, as diuretics, against stomach and duodenal tumours (David *et al.*, 1999), and as anti-inflammatory, antiseptic, antioxidant and haemostatic pharmaceuticals (Dolara *et al.*, 2005). Similarly, alkaloids are significant for the protecting and survival of plant because they ensure their survival against micro-organisms (antibacterial and antifungal activities), insects and herbivores and also against other plants by means of allelopathically active chemicals (Molyneux *et al.*, 1996). Flavonoids were also referred to as nature's biological response modifiers, because of their inherent ability to modify the body's reaction to allergies and virus and they showed their anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activities (Argal and Pathak, 2006). Hence, the presence of these phytochemicals in the ethanol extracts of the leaves and roots of *A. indica* as shown in this study is therefore an indication that this plant has great nutritive as well as medicinal benefits regardless of the season of collection.

The array of bioactive phytochemicals recorded in the root and leaves extracts of *A. indica* could also be responsible for the antifungal activities against *Penicillium* sp, *Candida albicans, Fusarium verticillioides, Trichophyton rubrum, Candida krusei, Candida tropicalis* and *Aspergillus fumigatus*. Among the phytochemicals recorded in the study extract, alkaloids and flavonoids have been reported to show antifungal properties (Cordell *et al.* 2001; Molyneux *et al.*, 1996). With the effectiveness of these plants extract as antimicrobial agents, it is therefore unexpected that the plants would be potent against the diseases caused by the pathogenic microbial species. Hence, there is the need to standardize the root and leaves extracts of *A. indica* in an attempt to producing natural antibiotics.

REFERENCES

Argal, A. & Pathak, A. K. (2006). Methanolic Root Extract of *Calotropis gigantean* InducesApoptosis in Human Hepatocellular Carcinoma by Altering Bax/Bcl-2 Expression *Journal of ethnopharmacology,* 106**,** 142-145.

Bibi, G., Haq, U., Ullah, N., Mannan, A. & Mirza, B. (2011). Antitumor, cytotoxic and antioxidant potential of Asterthomsonii extracts. *African Journal of Pharmacy and Pharmacology,* 5(2): 252-258.

CDC (2021). *Escherichia coli* Centers for Disease Control and Prevention, National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), Division of Foodborne, Waterborne, and Environmental Diseases (DFWED). https://www.cdc.gov/ecoli/index.html

Clinical and Laboratory Standards Institute (CLSI) (2003) Methods for dilution antimicro-bial susceptibility tests for bacteria that grow aerobically. Wayne, PA: CLSI; [document M07-A6].

Cordell, G.A, Quinn-Beattie, M.L. & Farnsworth, N.R. (2001). The potential of alkaloids in drug discovery. *Phytotherapy Research,* 15: 183-205.

David, J., Owen, Ornaghi, P., Ji-Chun, Y., Nicholas, L., Philip, R., Evans, P., Ballario D. N., De Bruyne T., Pieters L. & Deelstra, H. (1999). Vlietinck A. Condensed vegetables tannins: biodiversity in structure and biological activities. *Biochemical System Ecology*; 27: 445–59.

Dolara, P, Luceri, C, De Filippo, C., Femia, A.P., Giovannelli, L., Carderni, G.,

Cecchini, C., Silvi, S., Orpianesi, C. & Cresci, A. (2005). Red wine polyphenols influence carcinogenesis, intestinal microflora, oxidative damage and gene expression profiles of colonic mucosa in F344 rats. *Mutation Research*, 591: 237–46.

Dongmo, A.B., Kamanyi, A., Dzikouk, G., Nkeh, C.B., Tan, P.V., Nguelefack, T., Nole, T., Bopelet, M. & Wagner, H. (2003). Anti-inflammatory and analgesic properties of the stem bark extract of *Mitragyna ciliata* (Rubiaceae) Aubrév. & Pellegr. *Journal of Ethnopharmacology*, 84:17-21.

Eleazu, C. O., Eleazu, K. C., Awa, E., & Chukwuma, S. C. (2012). Comparative study of the phytochemical composition of the leaves of five Nigerian medicinal plants. E3 *Journal of Biotechnology and Pharmaceutical Research*, 3(2), 42-46.

Gupta, S., Kataria, M., Gupta, P.K., Murganandan, S. & Yashroy, R.C., (2004). Protective role of extracts of neem seeds in diabetes caused by streptozotocin in rats. *Journal of Ethnopharmacology*, 90, 185–189.

Hasler, C.M. & Blumberg, J.B. (1999). Symposium on Phytochemicals: Biochemistry and Physiology. *Journal of Nutrition*, 129: 756S-757S

IBM Corporation (2011). IBM SPSS statistics for Windows, version 20.0. Armonk, NY:IBM Corp.

Lai, P.K. & Roy, J. (2004). "Antimicrobial and chemopreventive properties of herbs and spices". *Current Medical Chemistry*, 11 (11): 1451–60.

Min Wu, Xuefeng Li, (2015). Chapter 87 - Klebsiella pneumoniae and Pseudomonas aeruginosa, Editor(s): Yi-Wei Tang, Max Sussman, Dongyou Liu, Ian Poxton, Joseph Schwartzman, Molecular Medical Microbiology (Second Edition), Academic Press, Pages 1547-1564

Mishra, U. S. Mishra, A. R. Kumari, P. N. Murthy, J. & B. S. Naik, (2009). "Antibacterial activity of ethanol extract of *Andrographis paniculata*," *Indian Journal of Pharmaceutical Sciences*, 4(71): 436–438.

Mohan,S.C., Dinakar, S., Anand, T., Elayaraja, R. and SathiyaPriya, B. (2012). Phytochemical, GC-MS analysis and Antibacterial activity of a Medicinal Plant Acalypha indica. International Journal of PharmTech Research, 4(3): 1050-1054

Molan, A.L. & Faraj, A.M. (2010). The effects of condensed tannins extracted from different plant species on egg hatching and larval development of *Teladorsagia circumcincta* (Nematoda: Trichostrongylidae). *Folia Parasitology Journal*, 57: 62-68.

Molyneux, R.J., Nash, R.J. & Asano, N. (1996). Alkaloids: Chemical and Biological Perspectives, Vol. 11, Pelletier SW, ed. Pergamon, Oxford; 303.

Okoro, S.O., Kawo, A.H. & Arzai, H. (2011). "Phytochemical screening, antibacterial and toxicological activities of *Acacia senegal* extracts," *Bayero Journal of Pure and Applied Sciences*, 5(1)163–1170.

Okwu, D.E. (2004) Phytochemicals and vitamin content of indigenous species of South Eastern Nigeria. *Journal of Sustainable for Agricultural Environment*, 6: 30-34.

Sagadevan, P., S.N. Suresh, R. Ranjithkumar, S. Rathishkumar, S. Sathish & B. Chandarshekar (2015). Traditional use of *Andrographis paniculata*: Review and Perspectives. *International Journal of Biosciences and Nanosciences*, 2 (5), 2123-2131

Scaffidi, A, A, D, Bohman, B, Gi, E & Flematti, G. (2016), 'Identification of the cat attractants isodihydronepetalactone and isoiridomyrmecin from *Acalypha indica' Australian Journal of Chemistry*, 69: 169-173.

Singh, M., Singh, N., Khare, PB. & Rawat, AK. (2008). Antimicrobial activity of some important *Adiantum* species used traditionally in indigenous systems of medicine. *Journal of Ethnopharmocology*, 115(2): 327-329.

Tofighi, Z., Ghazi, S., Hadjiakhoondi, A. & Yassa, N. (2016). Determination of Cardiac glycosides, total phenols in different generations of *Securigera securidaca* suspension culture. *Research Journal of Pharmacology*, 3(2) 25-31.

Traore, F., Gasquet, M., Laget, M., Guiraud, H., Giorgio, C.D., Azas, N., Doumbo, O. & Timon-David, P. (2000). Toxicity and genotoxicity of antimalarial alkaloid rich extracts derived from *Mitragyma inermis* O. Kuntze and *Nuclea latifolia*. *Phytotherapy Research.*, 4:608-11.

Zahir Hussain, A. & S. Kumaresan, GC-MS analysis and antibacterial evaluation of *Acalypha indica, Asian Journal of Plant Science and Research*, 2013, 3(6): 46-49

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POPULATION FLUIDITY APPROACH TO ESTIMATING THE RATE OF INFECTION IN A PANDEMIC USING THE S-E-I-R MODEL

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ABSTRACT

The rate at which an infectious disease spread during a pandemic is estimated with the Susceptible Exposed Infected Removed (SEIR) model and computed using Gabriel Goh's epidemic calculator. In this paper, we consider the population of a specified area, the fluidity of the people in the target population in the area under investigation, the contagious nature of the micro-organism involved together with its vector(s) and the herd immunity of the population, to formulate, using S-E-I-R Model, the different population of infected persons' ratio levels for various factors K_1, K_2, K_3, … K_n of contagious nature of micro-organisms spreading each type of disease. The essence of which is to determine the fluidity value "F" at which the population must operate for a typical disease with contagious factor K_i to minimize the effect of an outbreak. This study incorporated the fluidity metrics into the well-known model and shows that

KEYWORDS: Fluidity; Herd Immunity; Infection Rate Calculator; Pandemic; S-E-I-R Model

INTRODUCTION

The S-E-I-R model is one of the compartmental models used in the mathematics of epidemiology. The randomness of natural selection determines the qualification of any member of the population into the first compartment and the progress thereafter is subject to the four Ordinary Differential Equations (ODE) (Kermack & Mckendrick, 1927; Brauer, 2016 and Inaba, 2017; Brauer, Castillo-Chavez, & Feng, 2019).

The four ODEs are:

$$\frac{dS}{dt} = -\frac{R_t}{T_{inf}} \cdot SI \qquad (1)$$

$$\frac{dE}{dt} = \frac{R_t}{T_{inf}} \cdot SI - T_{inc}^{-1}E; \qquad (2)$$

$$\frac{dI}{dt} = T_{inc}^{-1}E - T_{inf}^{-1}; \qquad (3)$$

$$\frac{dR}{dt} = T_{inf}^{-1}I \qquad (4)$$

S = Number of Susceptible populations;
R_t= Number of Recovery in time *t*;
T_{inf}= Length of Infection Period;
E = Number of Susceptible populations that are Exposed to a disease-causing agent;
T_{inc}= Length of Incubation Period;
I = Number of Susceptible-Exposed population that are Infected; R = Number of Infected that are Removed either by Death or Recovery.

Each of the equations gives the rate people leave the four compartments. In simplifying the model, it is assumed that all the population under consideration passes through all four stages. Thus, the sum of these compartments gives the number of populations under consideration. That is, at any particular time '*t*',

$$S(t) + E(t) + I(t) + R(t) = N. \quad (5)$$

It is extremely important to note that in a disease where no one has acquired natural immunity by genetic makeup the S = N is equal to the total population of the area under investigation. So also, anyone who has recovered from the disease is no longer susceptible to that disease. The diseases with an already proven record of protection from them by vaccination automatically remove the immunised population from the susceptible group as well. An infected individual who has started receiving treatment is assumed to remain infectious until he/she leaves the infection compartment. Previous studies on the S-E-I-R model have established that R_0 the basic reproduction number/ratio, which is the average number of the susceptible person(s) an infected individual will infect during his/her

illness, is a major factor in determining the progress of a particular disease becoming an epidemic, endemic, or a pandemic (Wolfram Research, Inc., 2020; (Inaba, 2017). Depending on the mode of transmission, a human-to-human infectious disease with $R_0 < 1$ is less likely to become an epidemic and the one with $R_0 = 1$ will be around for a long time and become endemic while diseases that their $R_0 > 1$ may become a pandemic (Nishiura & Inaba, 2007)

. When vectors are involved, the values of R_0 depend on a variety of factors which are mostly environmental conditions that nurture the reproduction of the vectors. In modelling an all-encompassing S-E-I-R that provides healthcare professionals a reliable tool in tracking diseases outbreak among a specified population, the value of $R_0 = \frac{\beta}{\gamma}$ is a constant that is obtained for each disease. β is the infection rate and γ is the rate of recovery. Thus, for a human-human infectious disease with $R_0 = 10$ among a susceptible population of 1000 will become an epidemic in10^{-10} days

Goh's Epidemic Calculator

The outbreak of a new disease with no proven procedure for cure or treatment comes with the challenge of management in which the S-E-I-R model becomes very useful in controlling the vital statistics that affect the dynamics as the infection progresses. Whether for a disease that runs its course over many years as in leprosy or the one that the individual moves through all the four compartments of S-E-I-R in few days as in coronavirus disease, efforts are geared toward flattening of the curve by adjusting the infection rate and exposure of susceptible persons to infection. The model can near accurately predict the length of time (Period) within which the specific number of people will be infected within a population upon introduction of an index case. Goh's epidemic calculator provides the means of determining what other parameters will be when one or more parameter(s) is/are adjusted. For a disease with a basic reproduction ratio of $R_0 = 2.2$, number of initial infections = 1 and population size = 1,097 there will be total number of 17 fatalities in 184 days.

The Population Fluidity Levels

In curtailing physical interaction in human-to-human infectious disease like the COVID - 19 pandemic, restriction of movement of the populace to F_i (i = 1 – 5) levels of population fluidity:

Level	F_i	Attributes
1	F_1	Closure of all international borders. All activities within the country continue as usual while no one comes in and no one goes out.
2	F_2	Closure of schools, places of worship, entertainment venues where more than twenty (20) people share public space.
3	F_3	Closure of markets and offices where non-essential business transactions take place.
4	F_4	Ban on inter-state travels. Movements are only allowed within one's state of residence.
5	F_5	Total shutdown. Restriction on all movement outside the place of abode.

The Herd Immunity

Studies have shown that upon exposure to mild agents of infection disease-causing organisms individuals acquire immunity that protects them from getting infected (MFMER, 2020). This mild exposure can occur when infected persons mingled with the non-infected population. Herd immunity can also occur when a sufficient proportion of the population becomes immune to the

disease through vaccination or through being infected and recovered from the disease to the point that makes human to human infection very unlikely (www.mayoclinic.org). By reducing the population fluidity, the likelihood of acquisition of herd immunity during a pandemic is minimised while an increase in population fluidity will result in an upsurge in the number of infected persons as the susceptible population is exposed to infected persons more so, is a disease in which sizeable percentage of the infected population is asymptomatic.

Optimizing F

Here, we seek to find the optimal population fluidity level F_i at which the infection rate is minimised and the threshold for herd immunity is maximised.

Proposition1: For any given R_0 there exists F_i such that:

$$\begin{cases} \frac{dE}{dF} = \frac{dI}{dF} = \frac{dR}{dF} = 0 & (6) \\ \frac{d^2E}{dF^2} < 0; & (7) \\ \frac{d^2R}{dF^2} = 0 & (8) \\ \frac{d^2I}{dF^2} > 0 & (9) \end{cases}$$

In a pandemic where the exposure rate is kept constant by any means of regulating the movement of the infectious population having contact with susceptible people, we measure the effect of reducing the rate people entering the exposed compartment have on the infectious block and the rate at which the system removes participants. That is:

$$\frac{dE}{dF} = R_0N\text{-}R_0E\text{-}\beta\frac{1}{N}E \quad (10)$$

$$\frac{dI}{dF} = \beta\frac{1}{N}E\text{-}(\gamma + R_0)I \quad (11)$$

While

$$\frac{dR}{dF} = \frac{d\,N}{dF}\text{-}\frac{dE}{dF}\text{-}\frac{dI}{dF} \quad (12)$$

Solving equations 10 and 11 for critical points, we have:

$$\beta(F) = \frac{N}{E}(N\text{-}E)R_0 \quad (13)$$

$$\beta(F) = \frac{N}{E}(\gamma + R_0)I \quad (14)$$

Note that for existence of F_i which will also satisfy the removed compartment:

$$\beta(F) = R_0N \quad (15)$$

At this population fluidity level:

$$\frac{d^2E}{dF^2} = -\frac{E}{N}\beta'(F) < 0 \quad (16)$$

$$\frac{d^2I}{dF^2} = \frac{E}{N}\beta'(F) > 0 \quad (17)$$

The removed compartment at this point is either maximum or minimum.

CONCLUSION

The infectious compartment is minimised while the maximum number of persons will be exposed to the disease. Using the data obtained from NCDC between March and October 2020 and applying Goh's result that $R_0 = 2.2$ for COVID-19 we can scientifically advise that the population fluidity be kept at this fluidity level. This study had only generated the equations incorporating the fluidity of the population. However, empirical analysis and recommendation will be explored with the real-life data in the next stage of the study.

REFERENCES

Bailey, N. T. J. (1975). The mathematical theory of infectious diseases and its applications (2nd ed.). London: Charles Griffin & Co. Ltd.

Brauer, F. ((2016). A new epidemic model with indirect transmission. *Journal of Biological Dynamics*. doi:10.1080/17513758.2016.1207813

Brauer, F., Castillo-Chavez, C., & Feng, Z. (2019). Mathematical Models in Epidemiology. New York: Springer Nature. doi:10.1007/978-1-4939-9828-9

Capasso, V. (1993). Mathematical Structure of Epidemic Systems. Berlin: Springer.

Inaba, H. (2017). *Age-Structured Population Dynamics in Demography and Epidemiology*. Singapore: Springer Science+Business Media. doi:10.1007/978-981-10-0188-8

Kermack, W.O. & McKendrick, A.G. (1927). "A contribution to the Mathematical Theory of Epidemics" Proceedings of the Royal Society A. 115 (772): 700-721: reprinted in Bulletin of Mathematical Biology 53(1/2): 33–55 (1991)

MFMER. (2020). *Mayo Clinic Researchers Double Down on Covid - 19*. Retrieved November 01, 2020, from mayo.edu: mayo.edu/research/covid-19-research?_ga=2.78489878.328411539.1604328964-1112691114.1603956145

Nishiura, H., & Inaba, H. (2007). Discussion: Emergence of the Concept of the Basic Reproduction Number from Mathematical Demography. *Journal of Theoretical Biology, 4*: 357 – 364.

Wolfram Research, Inc. "Mathematica, Version 12.1" Champaign IL, 2020.

https://gabgoh.github.io/COVID/

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EFFECT OF ANOGEISSUS LEIOCARPUS (Combretaceae) EXTRACT ON HAEMATOLOGICAL AND BIOCHEMICAL INDICES IN MONOSODIUM GLUTAMATE INTOXICATED ALBINO RATS

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ABSTRACT

Anogeissus leiocarpus (Combretaceae) is used in folklore to treat of various forms of ailment in Nigeria and other African countries.The effect of *A. leiocarpus* ethanol leaf extract on haematological and biochemical indices of albino rats intoxicated with monosodium glutamate (MSG) was investigated. Twenty five albino rats with average weight of 110-120 gram were acclimatized and randomized into five groups. Group 1 served as control while group 2-5 received 1000 mg/kg body weight of monosodium glutamate orally for 14 days. Group 3, 4 and 5 were treated with 250, 500 and 1000 mg/kg body weight of *A. leiocarpus* respectively for 28 days. All the animals were fed with standard rat pellets and water *ad libitum* throughout the period. Results were analyzed using SPSS version 17. Administration of MSG caused changes in some haematological and biochemical indices in rats. Haemoglobin concentration (HB), Red blood cell count (RBC), and Packed cell volume (PCV) decreased significantly in MSG treated rats compared to control. Administration of varied doses of *Anogeissus leiocarpus* ethanol leaf extract was able to normalize the levels of HB, RBC and PCV. Administration of MSG in rats significantly increased the activities of the liver enzymes namely Alanine Aminotransferase (ALT), Aspartate Aminotransferase (ASP), Alkaline Phosphatase (ALP) and levels of Total Bilirubin (TB) and Direct Bilirubin (DB), but significantly reduced Albumin, Protein, Urea and Creatinine. There were significant increases in the serum activities ALT, AST, ALP, bilirubin in rats treated with varied doses *of leiocarpus* ethanol leaf extract. *A. leiocarpus* ethanol leaf extract affected haematological and biochemical indices of rats intoxicated with monosodium glutamate.

KEYWORDS: *Anogeissus leiocarpus*, Monosodium Glutamate, Haematological, Biochemical indices

INTRODUCTION

Nowadays in Nigeria and in many parts of the globe, traditional medicine is commonly used due to their availability, affordability and believe that it is safe (Mann *et al*., 2008). Traditional medicine makes use of medicinal plants with bioactive natural products that needs to be investigated for therapeutic compounds.

A. leiocarpus (Combretaceae) is a medicinal plant, locally known as *'Ayin'* or 'Orin-odan' **(local** name). It is a deciduous tree, common in West Africa were it has variety of uses (Agaie and Onyeyili, 2011). The plant is used traditionally to treat bacterial infections, diarrhea, malaria among several other uses. The plant has been have reported to have antimicrobial (Kubmarawa, 2007), anthelmintic (Soro, 2013), antiplasmodial (Akanbi, 2012), trypanocidal (Shuaibu, 2008), leishmanicidal (Shuaibu, 2008), antifungal (Mann *et al*., 2008) and antiproliferative properties (Salau *et al.*, 2013). MSG is the sodium salt of one of the most abundant amino acid glutamate (contains glutamate- 78% and sodium/water- 22%) (AL-Mosaibih and Mai,

2013). It is used as a food flavor enhancer to enhance palatability. Metabolism of MSG occurs by converting glutamate first to alanine and later to lactate in the liver and excretion is through the kidney (Bhattacharya and Bhakta, 2011). Absorption of MSG from gastrointestinal tract is fast and administration of high doses can lead to an elevated level of glutamate in blood plasma. MSG induces hepatotoxicity and oxidative stress in rats (Elagouza *et al.,* 2010). The aim of this study is to evaluate the effect of ethanol leaf extract of *A. leiocarpus* on haematological and biochemical indices of albino rats intoxicated with monosodium glutamate.

MATERIAL AND METHOD

Plant material

Fresh leaves of *A. leiocarpus* (DC) Guill and Perr were collected from a farm in Kwara state, Nigeria. The sample was identified and authenticated at the Department of Botany University of Lagos with voucher number LUH 7670.

Preparation of plant extracts

The leaves were shade dried at room temperature for three weeks. The dried leaves were crushed into coarse powder. The sample was macerated in 70 % ethanol for 48 hours. The solution was filtered, and the filtrate was evaporated to dryness under reduced pressure using a rotary evaporator to obtain the crude extract which was stored in a refrigerator at 4 ℃ until used.

Experimental animals

Twenty five matured male albino rats weighing 110-125 gram were purchased from the laboratory animal house of the College of Medicine, University of Lagos, Nigeria. After acclimatizing for fourteen days, the rats were randomly allocated into five experimental groups of five rats each. Rats in group 1 (control) received distilled water orally for 14 days while group 2, 3, 4 and 5 received 1000 mg/kg body weight of MSG for 14 days. Thereafter group 3, 4 and 5 were treated with 250, 500 and 1000 mg/kg of body weight of the extract orally, once daily for 28 days. The care of animals in this study was in conformity with NIH guideline Garber *et al.,* 2001. The ethical permission on the use of rats for this studies was granted by the College Research and Experimental ethics committee.

Animal sacrifice and collection of blood samples

After 28 days of administration of the plant extract, the rats were fasted overnight and sacrificed using chloroform as anesthetizer. The blood samples were collected by cardiac puncture into EDTA sample and plain bottles for haematological and biochemical analyses.

Determination of haematological parameters

The blood samples were analyzed for Red Blood Cell (RBC), White Blood Cell (WBC), haemoglobin (Hb), Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV) and platelets using automatic haematological analyzer Sysmex Haematology Systems (Sysmex Ameria Inc., model no. KX-21N, Kobe, Japan) (Amadu *et al.,* 2018).

Determination of Serum Biochemical parameters Serum Alanine Aminotransferase (ALT) activity was determined by measuring the concentration of pyruvate formed from α-ketoglutarate and L-alanine with 2,4, dinitrophenyl hydrazine to form colourerd hydrazone, in a reaction catalyzed by Alanine Aminotransferase (ALT) in an alkaline medium. UV-VIS Spectrophotometer Labomed UV-2502 was used to measure the colour intensity against blank at 540 nm (Reitman *et al.,* 1957).

Serum Aspartate Aminotransferase (AST) activity was determined by measuring the concentration of oxaloacetate formed from α-ketoglutarate and L-aspartate with 2,4, dinitrophenyl hydrazine to form coloured hydrazone in a reaction catalyzed by Aspartate Aminotransferase (AST) in an alkaline medium. UV-VIS Spectrophotometer

Labomed UV-2502 was used to measure the colour intensity against blank at 540 nm (Reitman *et al.,* 1957).

Serum Alkaline phosphatase (ALP) activity was analysed using spectrophotometry to monitor the concentration of p-nitrophenol. Serum alkaline phosphatase catalyses a reaction where colourless phenolphthalein monophosphate is converted to phosphoric acid and pink phenolphthalein under alkaline pH. UV-VIS Spectrophotometer Labomed UV-2502 was used to measure the colour intensity against blank at 540 nm (Reitman *et al.,* 1957).

Serum Total Protein level was determined by the method of Gornall *et al.* (1949). Copper ion reacts with polypeptide bonds to form coloured complexes. Absorbance was measured spectrophotometrically at 540 nm.

Serum Total albumin level was determined spectrophotometrically by using bromocresol green BCG dye which gives a shade of green after binding with albumin. Absorbance was measured at 578 nm (Doumas *et al*., 1971).

Serum Total Bilirubin level was determined using Randox assay kits. Diazotized sulphanilic acid react with direct (conjugated) bilirubin to form a blue coloured complex. Total bilirubin is determined when albumin bound bilirubin is released in the presence of caffeine and then reacts with diazotized sulphanilic acid. UV-VIS Spectrophotometer Labomed UV-2502 was used to measure absorbance at 545 nm (Jendrassik and Grof, 1938).
Serum Creatinine level was measured by the procedure of Tietz et al (1994) while the serum urea level was measured by the method of Kaplan (1965).

Statistical analysis
Results were expressed as mean ± SEM. One way ANOVA was used to compare differences and $p<0.05$ was considered significant (Pyke, 2014).

Results and discussion
Blood maintains homeostasis and regulates body function (Nseabasi *et al.,* 2014). Haematological indices namely RBC, PCV and WBC were therefore of diagnostic value (Afolabi *et al.,* 2010; Berinyuy *et al.,* 2015; Table 1 shows the effect of varied doses of *A. leiocarpus* ethanol leaf extract on haematology profile of MSG intoxicated albino rats. Administration of MSG significantly increased the level of WBC while levels of HGB, RBC and PCV were significantly reduced ($P < 0.05$) when compared to the control, this agrees with the result of Ashaolu, 2011 ; Ashaolu *et al.,* 2014). A change in blood level of WBC, HGB, RBC and PCV is an indication that the MSG treated rats could become anemic (Eweka and Adjene, 2007). *A. leiocarpus* ethanol leaf extract at doses of 500 and 1000 mg/kg, were able to reverse the effect of MSG on haematological parameters by significantly increasing levels of HGB, RBC and PCV ($P < 0.05$), towards the control level. This result is an indication that *A. leiocarpus* ethanol leaf extract was able to improve CO_2 and O_2 transport in the blood, increase RBC count by enhancing its rate of synthesis (erythropoiesis) or reducing its rate of destruction, and also probably improving the ability of the blood to carry and deliver O_2, thereby preventing anemia in the rats (Ugwuene, 2011; Soetan *et al.,* 2013; Holy *et al.,* 2015; Egbuonu *et al.,* 2017). The levels of WBC in groups administered MSG and *A. leiocarpus* ethanol leaf extract at different doses was high compared to the control. Significant increase in PCV value ($P < 0.05$) in rats treated with *A. leiocarpus* ethanol leaf extract after administering MSG, is an indication of the potential of *A. leiocarpus* ethanol leaf extract to increase synthesis of RBC as suggested in this study. A higher PCV value could be as a result of an increase in RBC synthesis, as suggested in this study (Peters *et al.,* 2011; Holy *et al.,* 2015; Kanu *et al.,* 2016). Platelets (PLT) play an important role in the maintenance of normal homeostasis while Mean cell volume (MCV) gives average RBC size of a blood sample. These are some of

parameters for determining anemic condition. There was no significant difference in levels of MCV and Platelets all the groups.

The liver performs a number of important functions in the body, which includes detoxification. A disruption in liver function will affect some important biochemical indices (Egbuonu *et al.,* 2010, Ozougwu, 2017). In this study the activity of the liver enzymes (AST, ALT, ALP) were significantly higher ($P < 0.05$) after administering MSG, when compared to the control group (Table 2). This is in agreement with previous studies on toxicity effect of MSG (Thomas *et al.,* 2009, Egbuonu *et al.,* 2010). This result is an indication that MSG caused leakage of some hepatic enzymes into circulation resulting in toxicity. The increase in AST, ALT and ALP could also be attributed to the kidneys, bones and musles (Egbuonu *et al.,* 2010). After treating the rats with different doses of the extracts, the activities of the liver enzymes were reduced in a dose dependent manner, with the lowest activity at a dose of 1000 mg/kg extract. The liver enzymes AST, ALT and ALP which shows functional activity of the liver, are sensitive liver damage markers (Manivasagam and Perumal, 2004). Therefore, increase in activity of these liver enzyme markers, is an indication of hepatotoxicity. This result agrees with the report of Tawfik, 2012 (Tawfik *et al.,* 2012). This study shows reduction in the serum levels of AST, ALT and ALP enzyme in the groups administered 1000 mg/Kg extract after administering MSG, when compared with the group administered MSG only (Table 2). This suggest that *A. leiocarpus* ethanol extract improved the hepatic function in rats administered MSG.

The liver is the site for protein synthesis in the body. Total protein includes albumin which is involved in the liver synthetic functions. Albumin also function in transporting bilirubin (a bye product of red blood cells catabolism), to the liver where it undergoes biotransformation to form bile before excretion (Mbah *et al.,* 2019). On exposure of rats to MSG, the serum total protein and albumin, were significantly reduced ($P < 0.05$), while total bilirubin and direct bilirubin were significantly increased ($P < 0.05$), when compared to the control group. This result is in agreement with the result of Mbah *et al.,* 2019. This is an indication of hepatic injury. The reduction in the levels of total protein and albumin is probably as a result of the synthesizing bilirubin from pigments (which has protein content), and albumin binding to bilirubin (Mbah *et al.,* 2019). However, it was observed that *A. leiocarpus* ethanol extract improved the hepatic synthetic function in a dose dependent manner (Table 2), in rats intoxicated with MSG by increasing levels of serum total protein and albumin and reducing levels of total bilirubin and direct bilirubin towards control group level. It could be inferred that *A. leiocarpus* ethanol extract has a significant ameliorating effect on rats administered MSG.

Serum creatinine and urea are markers for renal function. The levels of these markers were determined in all the groups to determine the effect of *A. leiocarpus* ethanol extract on MSG treated rats. The levels of serum creatinine were significantly ($P < 0.05$) increased in rats administered MSG when compared to the control group. MSG significantly increase in serum creatinine, by increasing its synthesis or preventing it's excretion, either of this could lead to kidney damage (Tawfik *et al.,* 2012). The creatinine levels reduced after treating MSG intoxicated rats with *A. leiocarpus* ethanol extract (Table 3). Urea is a product of protein metabolism and a measure of kidney function. The significant reduction in the concentration of urea in blood serum of rats administered MSG compared to the control group might be attributed to disruption of protein catabolism. There was an increase in urea level in the serum of rats treated with 1000 mg/Kg extract (Table 3). The result showed there was an improvement in kidney biochemical indices after treating MSG induced rats with various doses of *A. leiocarpus* ethanol extract to (Table 3).

Table 1: Effect of varied doses of A. Leiocarpus ethanol leaf extract on the hematology profile of MSG treated albino rats[1]

GROUP/ Treatment (mg/kg bd wt)	**WBC ($\times10^9$/l)**	**HGB (g/d)**	**MCV (mm^2)**	**PLT ($\times10^9$/l)**	**RBC ($\times10^{12}$/l)**	**PCV (%)**
Control	6.2 ± 0.76	13.7 ± 0.61	78.4 ± 1.53	858.0 ± 3.93	6.9 ± 0.35	47.4 ± 1.91
N. Treatment	11.9 ± 0.65*	10.7 ± 0.62*	77.4 ± 2.86	862.7 ± 7.33	5.82 ± 0.19*	38.2 ± 2.61*
250	11.1 ± 0.03*	11.3 ± 0.52	71.2 ± 1.35	867.7 ± 2.83	6.4 ± 0.15	40.9 ± 1.70*
500	9.4 ± 0.09	12.8 ± 0.43	63.0 ± 1.51	851.3 ± 1.19	6.8 ± 0.28	45.3 ± 0.92
1000	12.7 ± 0.50	13.5 ± 0.55	66.8 ± 0.61	860.0 ± 1.55	6.6 ± 0.31	44.9 ± 2.33

[1]Value represents Mean ± SEM of triplicate determinations

KEY:
WBC - White blood cell,
HGB - Haemoglobin,
MCV - Mean cell volume,
PLT - Platelets,
RBC - Red blood cell,
PCV - Packed cell volume,
AL - Anogeissus leiocarpus,
SEM - Standard Error of mean.
* - P value ≤ 0.05 – means there is statistical significant between the test groups and the control.

Table 2: Effect of varied doses of ethanol leaf extract of A. leiocarpus on some biochemical indices of MSG intoxicated albino rats[1]

Group/ Treatment (mg/kg bd wt)	**ALT (µ/L)**	**AST (µ/L)**	**ALP (µ/L)**	**T. B (mg/dl)**	**D. B (mg/dl)**	**ALB (g/L)**	**Protein (g/dl)**
Control	16.30±0.33	53.70±2.73	13.0±0.58	4.9±0.59	1.2±0.33	36.3±1.53	81.00±1.73
N. Treatment	27.70±2.3*	64.30±3.84*	23.0 ± 0.58*	6.2 ± 0.06*	2.2 ± 0.09*	32.7 ± 1.45*	74.90±3.46*
250	28.00±1.00*	59.70±4.93*	22.3± 2.03*	6.1 ± 0.23*	1.9 ± 0.18*	32.0 ± 0.58*	73.00±1.15*
500	24.70± 2.10*	61.00±0.88*	19.7 ± 0.88	5.7 ± 0.40*	1.6± 0.11	36.3 ± 1.67	79.53±2.91
1000	19.3± 2.03	54.00±4.51	15.3 ± 1.45	5.3 ± 0.21	1.4 ± 0.02	37.7 ± 2.33	78.33±3.38

[1]Value represents Mean ± SEM of triplicate determination

KEY:
ALT – Alanine Aminotransferase,
AST – Aspartate Aminotransferase,
ALP – Alkaline Phosphatase,
T.B – Total Bilirubin,
D.B – Direct Bilirubin,
ALB – Albumin, A. leiocarpus,
SEM – Standard Error of mean.
* – P value <0.05 – there is statistical significant difference between test groups and the control

Table 3: Effect of varied doses of ethanol leaf extract of Anogeissus leiocarpus on some kidney markers of MSG treated albino rats[1]

Group/ Treatment (mg/kg bd wt)	Urea (mg/dl)	Creatinine (mg/dl)
Control	43.33±2.93	4.47±0.13
N. Treatment	37.50±1.50*	5.93±0.20*
250	37.63±1.76*	5.60±0.17*
500	39.90±1.41	4.97±0.32
1000	41.60±2.90	4.53±0.09

[1]Value represents Mean ± SEM of triplicate determination

KEY:
SEM – Standard Error of mean.
** – P value <0.05 –There is statistical significant difference between test groups and the control.*

CONCLUSION

Our findings in this studies indicates that administration of *A. leiocarpus* ethanol leaf extract to MSG intoxicated rats was able to ameliorate the effects of MSG on haematological and biochemical indices at higher doses of 500 mg/kg and 1000 mg/Kg. Further research should be carried out to isolate bioactive compounds from the crude extract and elucidate its mechanism of action.

REFERENCES

Afolabi KD, Akinsoyinu AO, Olajide R, Akinleye SB. (2010). Haematological parameters of the Nigerian local grower chickens fed varying dietary levels of palm kernel cake. Proceedings of 35th Annual Conference of Nigerian Society for Animal Production. 247.

Agaie, BM, and Onyeyili PA. (2011). "In Vitro Anthelmintic Activity of the Aqueous Leaf Extract of *Anogeissus Leiocarpus* and Its Phytochemical , Proximate and Elemental Contents." *J. of Me.l Plants Res.* 5(30): 6656–61.

Ahmad HA. (2014). Review on *Anogeissus leiocarpus*. A potent african traditional drug. *Intl. J. of Res. in Pharm. and Chem.* 4(3), 496-500

Akanbi OM. (2012). The antiplasmodial activity of *Anogeissus leiocarpus* and its effect on oxidative stress and lipid profile in mice infected with Plasmodium bergheii. *Parasitology Res. Journal.* 110(1):219-226.

AL-Mosaibih and Mai A. (2013). "Effects of Monosodium Glutamate and Acrylamide on The Liver Tissue of Adult Wistar Rats." *Life Science Journal. 10(* 10(2s): 35–42.

Amadu KS, Musa TY, Oladiji AT. (2018). Effects of 1:1 Mixture of Anogeissus leiocarpus and *Terminalia avicennioides* Root Bark Extracts on Haematological Parameters, Liver and Kidney Function Indices of Male Rats. *Iranian J. of Toxicology* 12 (5):

Ashaolu JO, Ukwenya VO, Okonoboh AB, Ghazal OK, Jimoh AA. (2011). "Effect of Monosodium Glutamate on Hematological Parameters in Wistar Rats." *Intl. J. of Medicine and Medical Sci.* 3: 219–22.

Berinyuy EB, Lawal B, Olalekan AA, Olalekan IA, Yusuf AA, Sakpe S and Ossai PC. (2015). Hematological status and organs/body-weight parameters in Wistar rats during chronic administration of *Cassia occidentalis*. *Intl. Blood Res. & Rev.* 4(3): 1-7.

Bhattacharya T, and Bhakta A. (2011). "Long Term Effect of Monosodium Glutamate in Liver of Albino Mice after Neo-Natal Exposure." *Nepal Med Coll J.* 13(1): 11–16.

Doumas BT, Watson WA, and Biggs HG. (1971). Albumin standards and the measurement of serum albumin with bromcresol green. *Clin. Chim. Acta*.31(1):87-96.

Egbuonu AC, Ezeokonkwo CA, Ejikeme PM, Obidoa O, and Ezeanyika LU.(2010) Some biochemical effects of sub-cute oral administration of L-arginine on monosodium glutamate-fed Wistar rats 2:Serum alkaline phosphatase, total acid phosphatase and aspartate aminotransferase activities. *Asian J. Bioc*.5(2):89–95.

Egbuonu AC, Ekwuribe GA. (2017). Pulverized *Mangifera indica* (mango) seed-kernel modulated serum lipid profile in monosodium glutamate-challenged rats. *J. Appl. Biotechnol.* 5(2): 20-35.

Elagouza, IM, Dalia EE, and Saad SE. (2010). "The Possible Ultra Structural Ameliorative Effect of Taurine in Rat 's Liver Treated with Monosodium Glutamate (MSG)." *The Open Hepatology J.* 2: 1–9.

Eweka, A O, and J O Adjene. (2007). "Histological studies of the effects of monosodium glutamate on the medial geniculate body of adult wistar rats ." *Electron J Biomed.* 2: 9–13.

Garber J C, Barbee R W, Brelizki JT, Clayton L.A, Denovan J.L, John D.F, Lipman N.S, Locke P, Mucher J, Quinby F W, Turner P V, Wood G A, Wurbel H (2001). Guide for the care and use of laboratory animals, 8th edition the National Academy Press washindon DC 11- 60 pp

Gornall AC, Bardawill CJ, David MM. (1949). Determination of serum protein by means of the buiret reaction. *J. Biol. Chem*. 1949, 177, 751

Holy B, Kenanagha B, Onwuli DO. (2015). Haemato-pathological effects of dichlorovos on blood picture and liver cells of albino rats. *J. Toxicol. Environ. Health Sci.* 7: 18-23.

Jendrassik L, Grof P. (1938). Estimation of total serum bilirubin level by spectrophotometrically in serum and plasma. *Biochem. Zeitschrift*. 1938;297:81-89.

Kanu KC, Ijioma SN, Atiata O. (2016). Haematological, biochemical and antioxidant changes in Wistar rats exposed to Dichlorvos based insecticide formulation used in Southeast Nigeria. *Toxics.* 4(28): 1-8.

Kaplan A. (1965). Urea Nitrogen and Urinary Ammonia. In: Standard methods of clinical chemistry. *Meites S. (Ed)*. pp. 245-256.

Kubmarawa D. (2007). Preliminary phytochemical and antimicrobial screening of 50 medicinal plants from Nigeria. *Afr. J. of Biotech*. 6(14):1690-1696.

Manivasagam T and Perumal S. (2004). "Monosodium glutamate affects the temporal characteristics of biochemical variables in wistar rats." *Polish J. of Pharmacology* 56:79–84.

Mann, A, Yahaya Y, Banso, A. and Ajayi GO. (2008). "Phytochemical and Antibacterial Screening of Anogeissus Leiocarpus against Some Microorganisms Associated with Infectious Wounds." *Afr. J. of Microbiology Rese*. 060-062.

Mann, A, Banso, A. and Clifford, L.C. (2008). An antifungal property of crude plant extracts from *Anogeissus leiocarpus* and *Terminalia avicennioides*. Tanzania J. of Health Res, 10 (1):

Mbah UO, Anthony CC, Egbuonu1 NK. Achi ID, and Obinna J. (2019). Hepatoprotective Potentials of Ethanolic Extract of *Solanum melongena* Linn Fruit on Monosodium Glutamate- Intoxicated Rats' and

Liver Section Histology. *Intl. Blood Res & Rev.* 9(1): 1- 9

Nseabasi EN, Williams ME, Akpabio U, and Offiong EE. (2014). "Haematological Parameters and Factors Affecting Their Values." *Agric. Sci J.* 2(1): 37–47.

Ozougwu JC. (2017). Physiology of the liver. Intl. J. Res. Pharm & Biosci. 4(8):13-24

Peters SO, Gunn HH, Imumorin IG, Agaviezor BO, Ikeobi CO. (2011). Haematological studies on frizzled and naked neck genotypes of Nigerian native chickens. *Tropical Animal Health Production. 43*(3) 631-638.

Pyke, David, United States, and Geological Survey. (2014). "Statistical Analysis of Survival and Removal Rate Experiments." *Ecology.* 67(1): 240–45.

Reitman S, Frankel S, Amer J. (1957). "Determination of SGOT and SGPT." *American J. of Clinical Pathology* 28: 56–60.

Salau, AK, Musa TY, and Adenike TO. (2013). "Cytotoxic Activity of Aqueous Extracts of Anogeissus Leiocarpus and Terminalia Avicennioides Root Barks against Ehrlich Ascites Carcinoma Cells." *Ind. J.ournal of Pharmacology* 45(4): 381–86.

Shuaibu MN. (2008). Castalagin from Anogeissus leiocarpus mediates the killing of Leishmania in vitro. *Parasitology Research*. 103(6):1333-1338.

Shuaibu MN. (2008). Trypanocidal activity of extracts and compounds from the stem bark of *Anogeissus leiocarpus* and *Terminalia avicennoides*. *Parasitology Res.*. 102(4): 697-703.

Soetan KO, Akinrinde AS, Ajibade TO. (2013). Preliminary studies on the haematological parameters of cockerels fed raw and processed guinea corn (Sorghum bicolor). *Proceedings of 38th Annual Conference of Nigerian Society for Animal Production*. 49- 52

Soro D. (2013). In vivo anthelmintic activity of *Anogeissus leiocarpus* Guill & Perr (Combretaceae) against nematodes in naturally infected sheep. *Parasitology Res.*. 112(7):2681-2688.

Tawfik1 MS, Al-Badr N. (2012). "Adverse Effects of Monosodium Glutamate on Liver and Kidney Functions in Adult Rats and Potential Protective Effect of Vitamins C and E." *Food and Nutr. Sci.* 3(5): 9.

Thomas M, Sujatha KS, George S. (2009). Protective effect of *Piper longum* Linn on monosodium glutamate induced oxidative stress in rats. *Indian J. Expt. Bio.*47:186-192.

Tietz, NW, Prude EL. and Sirgard-Anderson O. (1994): In: Tietz Textbook of Clinical Chemistry. ed. Burtis C. A. and Ashwood, E. R. pp 1354 – 1374. W. B. Saunders Company, London.

Ugwuene MC. (2011). Effect of dietary palm kernel meal for maize on the haematological and serum chemistry of broiler Turkey. *Nig. J. Animal Sci.* 13: 93-103.

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ANTIMICROBIAL AND PHYTOCHEMICAL PROPERTIES OF *LAWSONIA INERMIS* AGAINST SOME FUNGI.

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ABSTRACT
Lawsonia inermis Linn (Lythrace), a much-branched, sometimes spiny glabrous shrub or small tree of between 2-6m height, is a very useful medicinal plant known for its antimicrobial, antioxidant and antifungal properties in all parts of the world. The present study investigated the antimicrobial potential and phytochemical properties of *Lawsonia inermis* against some fungi. Evaluation of antimicrobial potential was carried out using agar well diffusion method while phytochemical constituent was determined using standard procedures. The anti-fungal result revealed the inhibition of *Aspergillus niger* (18.70 mm), *Penicillum* sp. (0.00 mm), *Candida albicans* (10.00 mm), *Aspergillus fumigatus* (11.60 mm) and *Aspergillus flavus* (10.90 mm). *Aspergillus niger* shows the highest inhibition, followed by *Aspergillus fumigatus*, while *Penicillum sp.* show no inhibition. The result of phytochemical properties shows the presence of Alkaloids (10.40 mg/mL), Saponin (30.40 mg/ml), Steroid (04.00 mg/mL), Phlobatanin (22.30mg/mL), Terpenoid (10.40 mg/mL), Flavonoid (13.20 mg/mL), Cardiac glycoside (22.30mg/mL) and Phenol (18.00 mg/mL). Saponin has the highest value and steroid has lowest value. From the result above it shows that *Lawsonia inermis* plant can be used in treatment of infections caused by *Aspergillus niger* and *Aspergillus fumigatus*. It also shows that *Lawsonia inermis* plant is not effective against *Penicillum sp.* The study further shows that *Lawsonia inermis* plant can be used as raw material for the extraction of Alkaloids, Saponin, Phlobatanin, Terpenoid, Flavonoid, Cardiac glycoside and Phenol.

KEY WORDS: Inhibition, Constituent, Plant and Phytochemical.

1.0 INTRODUCTION
Lawsonia inermis Linn. (Lythraceae) is a very useful medicinal plant in all parts of the world. The leaf powder of henna sap is used for staining hair, nails and beard (Chengaiah *et al*., 2010). The leaves of *Lawsonia inermis* are used to treat poliomyelitis, measles among the Yoruba tribe of South Western Nigeria (Oladunmoye and Kehinde, 2011). The seeds of henna have been reported to possess deodorant action and are used in most cases of gynecological disorders such as menorrhagia, vaginal discharge and leucorrhoea (Nawagish *et al*., 2007). The leaves of *Lawsonia inermis* with those of Hibiscus *(Rosa-sinensis)*, *Ecliptaprostrata* and seeds of *Abrusprecatorius* when combined in equal quantities and ground into paste, then soaked in sesame oil for 5 days is used as hair oil by the tribes of Andra Pradesh, India (Suneetha *et al*., 2011). In Turkey, henna which is an extract of *Lawsonia* sp. is used as hair dye and nail dye, in many cultures as decorative dye for centuries (Ozaslan *et al*., 2009). Henna is widely used in the cosmetic industry as dyeing agent also in India (Chengaiah *et al*., 2010). Reports show that methanolic root extracts of *Lawsonia* is used in Nigeria for cosmetic purposes, as antimalarial (Idowu *et al*., 2010) as well as for abortifacient purposes (Aguwa, 1987). The powdered roasted seed is mixed with ginger oil to make a paste which is used for the treatment of ring worm. Decoction of the leaves is used for aseptic cleaning of wounds and healing (Kumari *et al*., 2011). *L.*

inermis is also used by some individuals as 'blood tonic', thus implying its multifaceted use (Idowu *et al*., 2010).

Lawsonia inermis plant is a much-branched glabrous shrub or small tree of about 2-6m high, which may be spiny. Young branches are quadrangular and green but these branches turn red with age. The leaves of *L. inermis* are opposite, entire, sub-sessile, elliptic to broadly lanceolate, 1.5-5 x 0.5-2cm, glabrous, acuminate; while veins on the upper surface are depressed. The flowers are small, white, numerous in large pyramidal terminals, fragrant, 1cm across with 4 petals crumpled in the bud. The calyx has 2mm tube and 3mm spread lobes; the petals are orbicular to ovate, white or red; it has 8 stamens, inserted in pairs on the rim of the calyx tube; the ovary is 4 celled and the style up to 5mm long, erect. The fruits are small, brown, globosely capsules 4-8mm in diameter, many-seeded, opening irregularly, split into 4 sections with a persistent style. The seeds are 3mm across, angular and possess thick seed coat. The specific epithet means unarmed or without spines. *L. inermis* plant is widely distributed across the Sahel and Central Africa. It also exists in the Middle East (Orwa *et al*., 2009). It grows mainly along waterways and in semi-arid regions and is adapted to a wide range of environmental conditions. It can withstand low air humidity and drought conditions. The seeds of henna plant require high temperatures for germination, growth and maximal development (Orwa *et al*., 2009).

This study investigated the antimicrobial and phytochemical properties of *Lawsonia inermis* leaves against some fungi.

2.0 MATERIALS AND METHODS

The study investigated the antimicrobial and phytochemical properties of *Lawsonia inermis* against some fungi by evaluating antimicrobial potential using agar well diffusion method while phytochemical properties were determined using standard procedures.

Plant Extraction (Maceration) and Concentration

The leaves of *L. inermis* were obtained from Aladiye Farm, Iyana Ilogbo, Ogun State, Nigeria. These were washed and drained properly. The leaves were shade dried at room temperature (25±2^{o}C) for 21days. After drying, the leaves were thoroughly ground into powder, packed and stored in an airtight bottle for the preparation of the extracts.

One hundred and fifty gram (150 gram) of ground *L. inermis* leaves was weighed and soaked in 600 ml of methanol for 7days. After 7days, muslin cloth was used to sieve the mixture and then re-sieved with No 1 Whatman's filter paper. The filtrate was then stored in a screwed cap bottle (Mada et al., 2013).

The content was poured into evaporating dish and dried in the oven at 60^{o}C. The extract after drying had green colour. It was stored in universal bottles and labeled

Preparation of stock solutions and concentrates

The stock solutions were prepared for fungi. Stock solutions of Neomycin (40µg/ml, 20µg/ml, 10µg/ml and 5µg/ml) were prepared. The plant extracts were dissolved in 5% DMSO to obtain a final concentration of 600µg/ml, 300µg/ml, and 150µg/ml.

Calibration of Assay Organisms

The fungi liquid cultures were added drop-wise to the normal saline until the turbidity matched that of the densometer, 0.5 McFarland turbidity standard. The adjusted suspension was used for the assay. All moulds were calibrated using their spores in 0.05 tween 80 in normal saline (mould spores were obtained from the Department of Pharmaceutical Microbiology, Lagos University College of Medicine, Idi Araba, Lagos). The spore load was adjusted to 10^{8} spore forming unit per milliliter (CFU/mL), using serial dilution and plating out technique. The *Candida albicans* was calibrated in normal saline.

In vitro Screening of Antimicrobial Activity of *L. inermis* Methanolic Leaves Extract

Sabouraud Dextrose Agar (SDA) was used for the sensitivity screening. The plates were seeded with 150 µl fungal cells and allowed to stand for 4 hours in the lamina air-flow hood for the test fungal isolates to be fully embedded and well established in the seeded medium. With a sterile cork borer, wells of 10mm diameter were made. The wells were aseptically filled up with the *L. inermis* methanolic extracts avoiding splash and overfilling. The plates were incubated at 25^0C for 48 hours. The sensitivity of the test organisms to each of the extracts were indicated by clear halo around the wells on the plates. The halo diameters were taken as an index of the degree of sensitivity. The fungi used are *Candida albicans, Penicillium* sp,, *Aspergillus niger, Aspergillus famigatus*.and *Aspergillus flavus.*

Minimum Fungicidal Concentration (MFC) of *L. inermis* Methanolic Leaves Extract

The Minimum Fungicidal Concentration (MFC) of the plant extracts on the test isolates were determined by the agar micro dilution method. The stock solutions were further diluted in a 2-fold serial dilution to obtain the following concentrations: 600, 300 and 150µg/ml. Agar plates were prepared by pouring SDA into sterile petri plates containing 1ml of the various dilutions of the extract. The test isolates which were grown overnight in broth were adjusted to McFarland 0.5 standard and streaked onto the surface of the agar plates containing dilutions of the plant extract. The SDA plates were then incubated at 25°C for 48 hours after which all plates were observed for growth. The minimum dilution (concentration) of the extracts completely inhibiting the growth of each organism was taken as the MFC. Several readings were taken with zone reader and average zone values were determined and recorded.

Qualitative and Quantitative Phytochemical Screening of *L. inermis* Leaves Extract (Edeoga *et al.*, 2005).

Screening was done to determine the presence of phytochemicals. These are:

Tannins: About 0.5g of the dried powdered samples was boiled in 20ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

Phlobatannins: Deposition of a red precipitate when an aqueous extract of each plant sample was boiled with 1% aqueous hydrochloric acid was taken as evidence for the presence of phlobatinins.

Saponin: About 2g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

Flavonoids: Three methods were used to determine the presence of flavonoids in the plant sample (Sofowara *et al*., 2013). 5ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H_2S0_4. A yellow colouration observed in each extract indicated the presence of flavonoids. The yellow colouration disappeared on standing.

Few drops of 1% aluminium solution were added to a portion of each filtrate. A yellow colouration was observed indicating the presence of flavonoids.

A portion of the powdered plant sample was in each case heated with 10ml of ethyl acetate over a steam bath for 3minutes. The mixture was filtered and 4ml of the filtrate was shaken with 1ml of dilute ammonia solution. A yellow colouration was observed indicating a positive test for flavonoids.

Steroids: 2ml of acetic anhydride was added to 0.5g ethanolic extract of each sample with

2ml H_2S0_4. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

Terpenoids (Salkowski test): 5ml of each extract was mixed in 2ml of chloroform, and concentrated H_2S0_4. 3ml was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids.

Cardiac glycosides (Keller-Killani test): 5ml of each extracts was treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1ml of concentrated H_2S0_4. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

Quantitative phytochemical determination

Preparation of fat free sample: 2 gram of the sample were defatted with 100 ml of diethyl ether using a soxhlet apparatus for 2 hours.

Determination of total phenols by spectrophotometric method: The fat free sample was boiled with 50 ml of ether for the extraction of the phenolic component for 15minutes. 5ml of the extract was pipetted into a 50 ml flask, then 10ml of distilled water was added. 2ml of ammonium hydroxide solution and 5 ml of concentrated amylalcohol were also added. The samples were made up to mark and left to react for 30minutes for colour development. This was measured at 505nm.

Alkaloid determination using Harborne (1973) method: 5 gram of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4hours. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

Tannin determination using Van-Burden and Robinson (1981) method: 500 mg of the sample was weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1hour in a mechanical shaker. This was filtered into a 50ml volumetric flask and made up to the mark. Then 5ml of the filtered was pipetted out into a test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.IN HCl and 0.008M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 minutes.

Saponin determination using Obadoni and Ochuko (2001) method: The samples were grinded and 2g of each were put into a conical flask and 100 cm^3 of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 hours with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40ml over water bath at about 90°C. The concentrate was transferred into a 250ml separatory funnel and 20ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight; the saponin content was calculated as percentage.

Flavonoid determination using the method of Bohn and Kocipai (1994): 10g of the plant sample was extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No 42 (125mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

3.0 RESULTS

Antimicrobial Potential of *Lawsonia inermis* against Fungi

The methanolic extract of *Lawsonia inermis* at concentration 600mg/mL, 300mg/mL, 150mg/mL was carried out on the antimicrobial activity against *Candida albicans, Penicillium* species, *Aspergillus niger, Aspergillus fumigatus* and *Aspergillus flavus.*

This result was compared to that of the standard antifungal drug called Clotrimazole. The standard antifungal drug has higher antimicrobial activity than that of the methanolic extract of *Lawsonia inermis.* A few fungi were susceptible to the methanolic extract of *Lawsonia inermis* while almost all fungi were susceptible to the standard antifungal drug, and its inhibition superseded that of *Lawsonia inermis* extract.

Table 1 shows the antifungal effect of the methanolic extract of *Lawsonia inermis* leaves at various concentrations. At 600mg/mL: *Aspergillus niger* shows the highest susceptibility (18.70mm) followed by *Aspergillus fumigatus* (11.60mm), *Aspergillus flavus* (10.90mm), *Candida albicans* (10.00mm), *Penicillium* species (0.00mm) - that is, it shows no inhibition. At 300mg/mL, *Aspergillus niger* shows the highest susceptibility (9.35mm) followed by *Aspergillus fumigatus* (5.80mm), *Aspergillus flavus* (5.45mm), *Candida albicans* (5.0mm), *Penicillium* species (0.00mm) - it has no inhibition. At 150mg/mL: *Aspergillus niger* shows the highest susceptibility (4.67mm), followed by *Aspergillus fumigatus* (2.90mm), *Aspergillus flavus* (2.72mm), *Candida albicans* (2.50mm), *Penicillium* species (0.00mm) - no inhibition.

Table 1: Average inhibition zone diameter (mm) of Lawsonia inermis leaves extract on some fungi

Assay Organism	**600mg/mL (mm)**	**300mg/mL (mm)**	**150 mg/mL (mm)**	**5% Methanol Solvent System (mm)**
Aspergillus niger	18.70	9.35	4.67	0.00
Penicillium species	0.00	0.00	0.00	0.00
Candida albicans	10.00	5.00	2.50	0.00
Aspergillus fumigatus	11.60	5.80	2.90	0.00
Aspergillus flavus	10.90	5.45	2.72	0.00

Antimicrobial Potential of Clotrimazole Against some Fungi

Table 2 shows the antifungi effect of the standard drug Clotrimazole at various concentrations. At 160mg/mL, *Aspergillus flavus* shows the highest susceptibility (35.20±0.25mm) followed by *Penicillium* species (32.10±0.37mm), *Candida albicans* (30.20±0.25mm), *Aspergillus fumigatus* (28.20±0.25mm) and *Aspergillus niger* (27.20±025mm). At 80mg/mL, *Aspergillus flavus* shows the highest susceptibility (30.30±0.25mm) followed by *Penicillium* species (29.00±0.45mm), *Candida albicans* (27.30±0.25mm), *Aspergillus fumigatus* (27.30±0.25mm) and *Aspergillus niger* (23.90±0.37mm). At 40mg/mL, *Penicillium* species shows the highest susceptibility (26.40±3.7mm) followed by *Aspergillus flavus* (25.30±0.25mm), *Candida albicans* (24.20±0.25mm), *Aspergillus fumigatus* (22.00±0.32mm) and *Aspergillus niger* (21.20±0.25mm). At 20mg/mL, *Candida albicans* shows the highest susceptibility (28.00±0.25mm) followed by *Penicillium* species (22.20±0.25mm), *Aspergillus flavus* (20.200±0.25mm), *Aspergillus niger* (18.10±0.37mm) and *Aspergillus fumigatus* (18.00±0.32mm).

Table 2: Average inhibition zone diameter (mm) of Clotrimazole control standard on fungi

Assay Organism	160mg/mL	80mg/mL	40mg/mL	20mg/mL
Aspergillus niger	27.20±0.25	23.90±0.37	21.20±0.25	18.10±0.37
Penicillium species	32.10±0.37	29.00±0.45	26.40±0.37	22.20±0.25
Candida albicans	30.20±0.25	27.30±0.25	24.20±0.25	28.00±0.25
Aspergillus fumigatus	28.20±0.25	25.30±0.25	22.00±0.32	18.00±0.32
Aspergillus flavus	35.20±0.25	30.30±0.25	25.30±0.25	20.20±0.25

Phytochemical qualitative analysis of *Lawsonia inermis* leaves extract.

Table 3 shows the qualitative analysis of the methanolic extract of *Lawsonia inermis* leave. Alkaloid, Saponin, Phlobatanin, Terpenoid, Flavonoid, Cardiac glycoside were present while Steroid was absent.

Table 3: Qualitative phytochemical composition of *Lawsonia inermis* leaves extract

Compound	Methanol extract
Alkaloid	+
Saponin	+
Steroid	-
Phlobatanin	+
Terpenoid	+
Flavonoid	+
Cardiac glycoside	+

Key – Absent + present

Phytochemical quantitative constituent analysis of *Lawsonia inermis* leaves extract

Table 4 shows the quantitative analysis of the methanolic extract of *Lawsonia inermis* leave at 100mg/mL. Alkaloid (10.40 mg/mL), Saponin (30.40 mg/mL), Steroid (04.00 mg/mL), Phlobatanin (22.30 mg/mL), Terpenoid (10.40 mg/mL), Flavonoid (13.20 mg/mL), Cardiac glycoside (22.30 mg/mL) and Phenol (18.00 mg/mL). Saponin has the highest value and steroid has the lowest value.

Table 4: Quantitative phytochemical composition of *Lawsonia inermis* leaves extract.

Compound	Methanolic extract (mg/mL)
Alkaloid	10.40±.02
Saponin	30.40±.03
Steroid	04.00±.01
Phlobatanin	22.30±.02
Terpenoid	10.40±.01
Flavonoid	13.20±.01
Cardiac glycoside	22.30±.01
Phenol	18.00±.02

4.0 DISCUSSION

In the present study, methanolic leave extract of *Lawsonia inermis* inhibit the growth of microorganisms, which are: *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus* and *Candida albicans*. Plant methanolic extract showed significant antifungal activity as compared to standard antifungal drug.

Aspergillus niger shows the maximum activity (18.70 mm) at 600mg/mL followed by (9.35 mm) at 300mg/mL and the minimum activity (4.67mm) at 150 mg/mL. *Aspergillus flavus* shows the maximum activity (10.90 mm) at 600 mg/mL followed by (5.45 mm) at 300 mg/mL and the minimum activity (2.72 mm) at 150 mg/mL. *Aspergillus fumigatus* shows the maximum activity (11.60 mm) at 600 mg/mL followed by (5.8 mm) at 300 mg/mL and the minimum activity (2.9mm). *Candida albicans* shows the maximum activity (10.±0mm) at 600 mg/mL followed by (5.±0 mm) and the minimum activity (2.±5 mm). *Penicillium species* did not show any activity. This study agreed with the reported work of Hadef and Boufeldja (2020) which proved that the phytochemical constituent of *Lawsonia inermis* exhibited antimicrobial activity. Also, Kannahi M. and Vinotha K. (2013) reported that the methanol extracts of *Lawsonia inermis* leaves showed maximum activity at 100% level (2.5±2.3mm) and minimum activity at 25% (0.3±1.1mm) against *Aspergillus flavus*, maximum activity at 100% (2.5.0±8.1mm) and minimum activity at 25% (8.3±9.1mm) against *Aspergillus niger* and f*or Fusarium*, maximum activity at 100% (4.7±4.4mm), followed by 25% (1.3±1.8mm), 50% (2.2±2.1mm) and 75% (1.3±1.8mm).

Khan and Nasreen (2010), tested the antifungal activity of methanolic extracts of *Lawsonia inermis* leaves and five others against human pathogenic fungi. *L. inermis* showed inhibition of mycelial growth of target fungi (76.47 - 87.77%) among all the extracts tested. But the protein fractions of *L. inermis* exhibited four to five times more percentage inhibition of mycelial growth of fungi.

The phytochemical quantitative analysis study confirmed the presences of Alkaloid (10.40mg/mL), Saponin (30.40 mg/mL), Steroid (04.00 mg/mL), Phlobatanin (22.30 mg/mL), Terpenoid (10.40mg/mL), Flavonoid (13.20 mg/mL), Cardiac glycoside (22.30 mg/mL) and Phenol (18.00 mg/mL). The phtyochemical qualitative analysis study confirmed the presence of Alkaloid, Saponin, Phlobatanin, Terpenoid, Flavonoid, Cardiac glycoside while Steroid is absent.

The phytochemical quantitative and qualitative analysis confirmed in this study was in agreement but with slight deviation from the reported work of Amit *et al.,* (2012) which confirmed the presence of cardio-glycosides, terpenoids and phenols compounds but the absence of the remaining carbohydrates, oils and fats, tannins, saponins and amino acids.

Also, Nirmala *et al.,* (2016) in their experiment conducted the phytochemical analysis with the crude extract of *Lawsonia inermis L* (Henna plant) reported the presence of various residues or components such as tanins, flavanoids, anthraquinones, alkaloids, terpenoids, saponins, cardiac glycosides, glycosides, reducing sugars, phlobatanins, steroids, phenolic, aminoacids, proteins and quinones while Wasim *et al.,* (2013) confirmed the presence of glycosides, phytosterol, steroids, saponins, tannins and flavonoids in the extract. These phytochemical constituents are good sources of antimicrobial and antioxidant activity (Kumari *et al*., 2011; Oladunmoye and Kehinde, 2011).

5.0 CONCLUSION

In this study, the investigation of anti-fungal effects of *L. inermis* leaves revealed the inhibition of *Aspergillus niger* (18.70 mm), *Penicillum* sp. (0.00 mm), *Candida albicans* (10.00 mm), *Aspergillus fumigatus* (11.60 mm) and *Aspergillus flavus* (10.90 mm). *Aspergillus niger* shows the highest inhibition, followed by *Aspergillus fumigatus*, while *Penicillum sp.* show no inhibition. The result of phytochemical properties shows the presence of Alkaloids (10.40 mg/mL), Saponin (30.40 mg/ml), Steroid (04.00 mg/mL), Phlobatanin

(22.30mg/mL), Terpenoid (10.40 mg/mL), Flavonoid (13.20 mg/mL), Cardiac glycoside (22.30mg/mL) and Phenol (18.00 mg/mL). Saponin has the highest value and steroid has lowest value. These show that *Lawsonia inermis* plant can be used in treatment of infections caused by *Aspergillus niger* and *Aspergillus fumigatus*. It also shows that *Lawsonia inermis* plant is not effective against *Penicillum sp.* The study further shows that *Lawsonia inermis* plant can be used as raw material for the extraction of Alkaloids, Saponin, Phlobatanin, Terpenoid, Flavonoid, Cardiac glycoside and Phenol.

This plant can be used in the future as alternative medicine for treating many diseases such as migraine, albinism, skin abrasions and ulcers, burns, smallpox, leprosy boils, wounds and some mycotic infections. Also, further evaluation needs to be carried out on *L. inermis* in order to discover the concealed areas and their practical clinical applications which can be used for the welfare of mankind.

REFERENCES

Aguwa, C.N. (1987). Toxic Extract of *Lawsonia inermis* Roots. *Journal of Pharmaceutical Biology*. (25) 241-245

Amit, P., Yatendra, K. and Sudeep, K. T. (2012). Evaluation of Antimicrobial Activity and Phytochemical Analysis of Leaves and Stems of *Lawsonia Inermis. Journal of Pharmacy Research.* 5(**3**):1802-1805

Bohn, C. and Kocipai, G. (1994). *Method of Analysis.* Washington DC, U.S., 8, 1233-1246

Chengaiah, B., K.M. Rao, K.M. Kumar, M.Alagusundaram and C.M. Chetty, (2010). Medicinal Importance of Natural Dyes. *International Journal of Pharmaceutical Technology Research.* (2): 144-154.

Edeoga, H.O., Okwu, D. E., and Mbaebie, B. O. (2005). Phytochemical Constituents of Some Nigerian Medicinal Plants. *African Journal of Biotechnology.* Vol. 4 (**7**), 685-688

Hadef K. Z. and Boufeldja W., (2020). Antimicrobial Activity of Lawsonia inermis Leaf Extract Collected from South of Algeria Touat (Adrar) and Tidikelt (In Salah). Journal of Plant Sciences, 15: 9-16.

Idowu, O.A., Soniran, O.T. Ajana O. and Aworinde, D.O. (2010). Ethnobotanical Survey of Antimalarial Plants Used in Ogun State, Southwest Nigeria. *International Journal of Pharmacology* (40): 055-060.

Kannahi, M. and Vinotha, K (2013). Antimicrobial activity of *Lawsonia inermis* leaf extracts against some human pathogens. *International Journal of Current Microbiology and Applied Sciences* 2(5): 342-349

Khan, Z.S. and Nasreen, S. (2010) Phytochemical Analysis of Antifungal Activity and Mode of Action of Methanol Extracts from Plants Against Pathogens. *International Journal of Agricultural Technology*.(6): 793-805

Kumari, P., Joshi, G.C. and Tewari, L.M. (2011). Diversity and Status of Ethno-medicinal Plants of Almora District in Uttarakhand, India. *International Journal of Biodversity Conservation*. (30): 298-326.

Mada S. B., Garba A., Mohammed H. A., Muhammad A., Olagunju A., and Muhammad A. B. (2013) Antimicrobial activity and phytochemical screening of aqueous and ethanol extracts of Momordica charantia L. leaves. *Journal of Medicinal Plants Research* Vol. 7(10), pp. 579-586.

Nawagish, M., Ansari,S.M and Ahmad,S.(2007). Preliminary Pharmacognostical Standardisation of *Lawsonia inermis* Linn. Seeds. *International Journal of Biotechnology* (2): 161-164.

Nirmala, B. R., SitaKumari, O. and Rajesh, G. G. (2016). Phytochemical Analysis and Antimicrobial Activity of *Lawsonia*

inermis (Henna). *Journal of Plant Science & Research.* 3(**2**)

Obadoni, B.O. and Ochuko, P.O. (2001) Phytochemical Studies and Comparative Efficacy of the Crude Extracts of Some Homeostatic Plants in Edo and Delta States of Nigeria. *Global Journal of Pure and Applied Science*, 8, 203-208.

Oladunmoye, M.K. and Kehinde, F.Y (2011). Ethnobotanical Survey of Medicinal Plants used in Treating Viral Infections among Yoruba Tribe of South Western Nigeria. *African Journal of Microbiological Research*. (5): 2991-3004.

Orwa, C., Mutua, A., Kindt, R., Jamnadass, R and Simons, A. (2009). Agroforestree Database: A Tree Reference and Selection Guide, version 4.0. World Agroforestry Centre, Kenya.

Ozaslan, M., Zumrutdal, M., Daglioglu, K., Kilic, I.H and Karagoz, I.D (2009). Antitumoral effect of *L. inermis* in Mice with EAC. *International Journal of Pharmacology* (5): 263-267.

Sofowora, A., Ogunbodede, E. and Onayade, O. (2013). *African Journal of Traditional Complementary and Alternative Medicines* 10(5), 210-229 2: 213-221

Suneetha, J., Koteswara, J., Rao, T.V.V and Seetharami, R. (2011). Herbal Remedies for Hair Disorders by the Tribals of East Godavari District, Andhra Pradesh. *International Journal of Experimental Science*. (20): 30-32.

Van-Burden, T.P. and Robinson, W.C. (1981).Formation of complexes between protein and Tannic acid. *Journal of Agricultural Food Chemistry*, 1:77

Wasim, R., Ovais, M. and Amit, D. (2013). Phytochemical Screening and Antibacterial Activity of *Lawsonia inermis* Leaf Extract. *International Journal of Microbiological Research* 4 (**1**): 33-36.

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ASSESSMENT OF INTRASPECIES RELATIONSHIPS AMONG GARLIC (*Allium sativum* L.) GENOTYPES FOUND IN SOME PARTS OF LAGOS, NIGERIA USING RAPD MARKERS.

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ABSTRACT

This study was designed to evaluate intraspecies relationships among garlic (*Allium sativum* L.) genotypes found in some Local Government Areas of Lagos, Nigeria, using molecular methods. Thirty-two (32) samples of *A. sativum* were collected from six locations covering five (5) local government areas (LGA) and one (1) Local Council Development Area - LCDA (Bariga) in Lagos State and coded namely; Ajeromi/ Ifelodun (AI), Alagbado (AL), Agbado (AG), Bariga (BA), Ikorodu (IK), and Somolu (SO). The molecular study was carried out using eight (8) Random amplified polymorphic DNA (RAPD) Primers. Results from the molecular study showed that only (4) RAPD Primers (OPA 03, OPA 07, OPA 12, and OPY 20) were amplified. These results obtained from the molecular study were scored as 1 (present) or 0 (absent) and used to generate a dendrogram using NTsys (2.0j) computer programme which showed complete clustering at about 0.67 and a similarity coefficient of 0.76 for all thirty-two (32) garlic samples used in the study. This analysis revealed that RADP markers are useful tools in assessing the level of genetic diversity of garlic germplasm in Nigeria.

KEYWORDS: *Allium sativum*, dendrogram, genetic diversity, molecular, Random Amplified Polymorphic DNA (RAPD).

INTRODUCTION

Garlic (*Allium sativum* L.*)* is a spicy vegetable crop widely cultivated throughout the world (Abraham *et al*., 2019). It is considered a rich source of volatile compounds, which are responsible for the distinct flavour and the bioactive properties of dry bulbs (Petropoulos *et al*., 2018). It is found in middle Asia where it has been used as medicine since 2700 BC (Petrovska and Cekovska, 2010). There is a general belief that the health-related properties of fresh garlic extract are mostly attributed to sulfur compounds within the plant (Amagase, 2006; Bhandari, 2012). However, the most common non-sulphur compounds of garlic including polyphenols also display health benefits (Baeto *et al*., 2011; Bhandari, 2012). Phenolic compounds are known to be powerful antioxidants. Phenolics, in contrast to several garlic sulphur compounds, are more stable and might be extracted from fresh, frozen or dried plant samples (Dai and Mumper, 2010). Interestingly, studies attribute the biological properties of garlic to the synergistic action and proportion of different phytochemicals contained in a garlic clove (Bhandari, 2012). Several health benefits of garlic depend on its antioxidant activity.

Garlic extracts and components obtained from garlic bulbs were shown to prevent oxidative modification of DNA, protein and lipids by scavenging reactive oxygen species (ROS), increasing the cellular antioxidant enzymes and enhancing glutathione levels inside the cells (Belloir *et al.*, 2006). Phenolic compounds are powerful antioxidants abundant in fruit, vegetable and spices. Several epidemiological studies indicate a significant correlation between a high intake of plant polyphenols in diet and preventive effects in terms of cancer, cardiovascular and neurodegenerative diseases. Interestingly, contents of biologically-active compounds in garlic vary between cultivars grown in

different geographical regions (Beato *et al.*, 2011; Gorinstein *et al.*, 2005).

Plant molecular biology is the study of the molecular basis of plant life. It is particularly concerned with the processes by which the information encoded in the genome is identified and known. Molecular biology concerns the molecular basis of biological activities between biomolecules in the various systems of a cell, including the interaction between DNA, RNA, and proteins and their biosynthesis, as well as their interactions. Various molecular marker techniques such as Random Amplified Polymorphic DNA (RAPD), Inter Simple Sequence Repeats (ISSRs) and Simple Sequence Repeats (SSRs) can be used in the molecular characterization of garlic species. This study aimed to evaluate genetic diversity in some garlic genotypes found in six locations including five (5) Local Government Areas and one (1) Local Council Development Authority (LCDA) of Lagos, Nigeria using Random Amplified Polymorphic DNA (RAPD) markers.

METHODOLOGY

Sample Collection and Preparation

Garlic samples were collected from six different locations in Lagos, Nigeria namely; Somolu (SO), Ajeromi Ifelodun (AJ), Ikorodu (IK), Bariga (BA), Alagbado (AL), Agbado (AG). These samples were packed in a zip-lock bag and were all transported to the College Central Research Laboratory, Yaba College of Technology. These garlic samples were each labelled using codes SO1 to SO22, AJ 31 to AJ32, IK 41 to IK42, BA 51 to BA52, AL61 to AL62, AG71 to AG72. This was done to facilitate better recognition of samples to be used, or used sample identification (Table 1).

Table 1: Location details of sample collection.

	LOCATION	LONGITUDE	LATITUDE	ALTITUDE
1.	Somolu	6°38'6" N	3°23'7" E	20 m
2.	Ajeromi/Ifelodun	6°27'47" N	3°19'19" E	32 m
3.	Ikorodu	6°31'31" N	3°22'11" E	20 m
4.	Bariga	6°92'7" N	3°52'4" E	10 m
5.	Alagbado	6°38'46" N	3°18'24" E	20 m
6.	Agbado	6°38'12" N	3°18'3" E	20 m

DNA Extraction

DNA was extracted following the Cetyl Trimethyl Ammonium Bromide (CTAB) method (Murray and Thompson, 1980; Ogunkanmi *et al*., 2010). Sample materials were thoroughly washed with detergent and water as well as rinsed properly. Afterwards, the glass container having the Eppendorf tubes and tips were autoclaved at a standard temperature of 121°C and left to cool for 15 minutes. The valves were released and materials removed and kept in the oven to dry. 500 ml of pre-warmed CTAB isolation buffer was added into the mashed garlic samples and was further crushed to mix. After it had been ground, it was then transferred into pre-labelled Eppendorf tubes which were vortexed to further mix properly. These crushed samples were incubated in a water bath at 60 °C and each sample was brought out after 5 minutes, shaken again, and returned in the water bath for another 5 minutes. 100 ml of chloroform/Isoamyl alcohol was added, and it was capped and inverted severally to mix. Samples were then transferred into the micro-centrifuge and set at a speed of 14,000 rpm for 10 minutes. The DNA-containing upper phase (the aqueous solution) was decanted and placed in fresh tubes. 170 ml of isopropanol was added to the aqueous upper phase and mixed to precipitate DNA and the tubes were placed in the refrigerator to stand for 5 minutes. Thereafter, samples were placed in the centrifuged for 10 minutes at 14,000 rpm. Afterwards, samples were removed and the supernatant was

discarded. 1 ml of wash buffer was added to the immiscible DNA pellet, swirled using the vortex mixer and incubated for 5-10 minutes at room temperature. The DNA pellets were suspended in 40 ml of sterile distilled water.

PCR Optimization and Primer Selection

Polymerase chain reaction using eight (8) Random Amplified Polymorphic DNA (RAPD) primers (Table 2) was performed in a thermal cycler, programmed for 40 cycles of 1 minute at 92 °C, 1 minute at 35 °C and 2 minutes at 72 °C. These primers were selected randomly in no particular order. The PCR reaction products were separated by electrophoresis on 1.2% agarose gel using a Tris-borate-EDTA buffer system, stained with ethidium bromide.

Checking the quality of DNA by Agarose Gel Electrophoresis

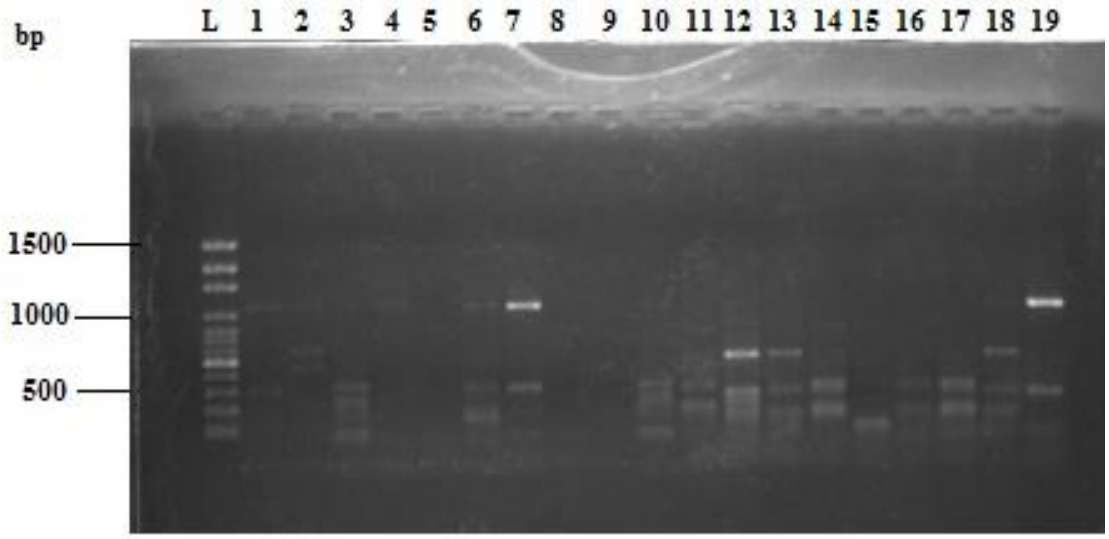
50 ml of TBE (Tris-borate-EDTA) was measured. 950ml of distilled water was also measured and mixed in a glass bottle 2.5 grams of agarose powder was poured into a conical flask, then 100ml of the mixture was also added and stirred. The gel tray was then collected and wrapped on either side using paper tape. This was done carefully and tightly to avoid the spillage of the prepared gel. It was also ensured that hand gloves were worn to avoid contact with the skin because of ethidium bromide which was a necessary ingredient for the work. Combs were then placed in the gel, one at the top of the gel tray, and the other at the middle of the gel and allowed to cool. These combs are used to create wells in the gel for the process. A few drops of ethidium bromide was added carefully to avoid contact with the skin. Ethidium Bromide (also known as homolous bromide) is carcinogenic which means that it causes cancer. Hence, it is advised that it should be handled with caution. It is a fluorescent dye that intercalates between bases of nucleic acids and allows very convenient detection of DNA fragments in gels. It can be incorporated into agarose gels or added to samples of DNA before loading to enable visualization of fragments within the gel. As might be expected, the binding of ethidium bromide to DNA alters its mass and rigidity, and therefore its mobility.

Table 2: RAPD Primers used in this molecular study and their genetic sequences

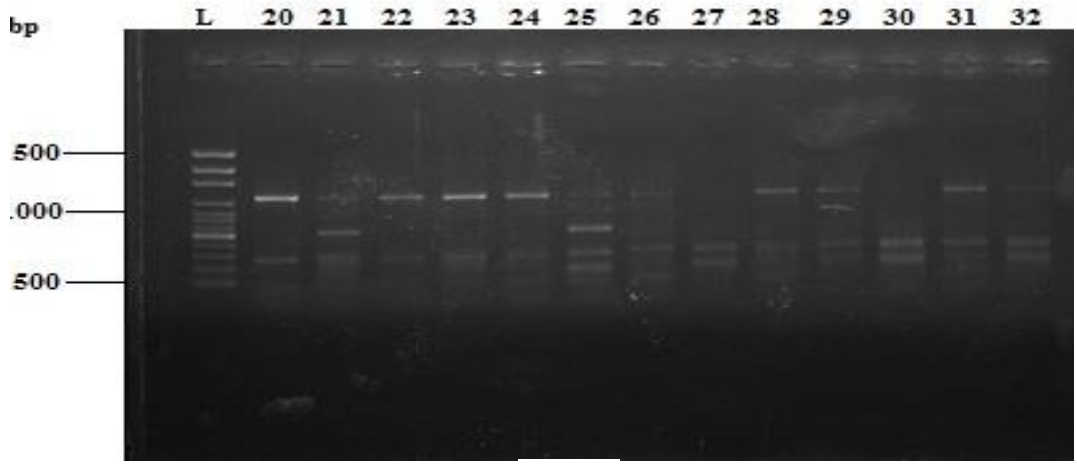
S/N	RAPD PRIMERS	SEQUENCES
1	OPA O3	AGTCAGCCAC
2	OPA 05	AGGGGTCTTG
3	OPA 06	GGTCCCTGAC
4	OPA 07	GAAACGGGTG
5	OPA 08	GTGACGTAGG
6	OPA 12	TCGGCGATAG
7	OPE 03	CCAGATGCAC
8	OPY 20	AGCCGTGGAA

RESULTS

Eight (8) RAPD primers were used namely, OPA03, OPA05, OPA06, OPA07, OPA08, OPA12, OPE03, and OPY20. However, only 3 primers (OPA03, OPA12 and OPY20) amplified while OPA07 gave partial amplification (not all samples presented amplicons). Plates 1(a and b) show the series of band flow through the agarose gel. These bands were scored as present (1) or absent (0) and used to generate a dendrogram using NTsys (2.0j) computer programme (Figure 1).



(a)



(b)

PLATE 1(a and b): Electrophoregram showing the result of a randomly amplified polymorphic DNA (RAPD) using OPA-12 marker. In these plates above thirty-two samples were used and were further scored for the construction of the dendrogram using NTsys version 2.02j computer program.

(a)

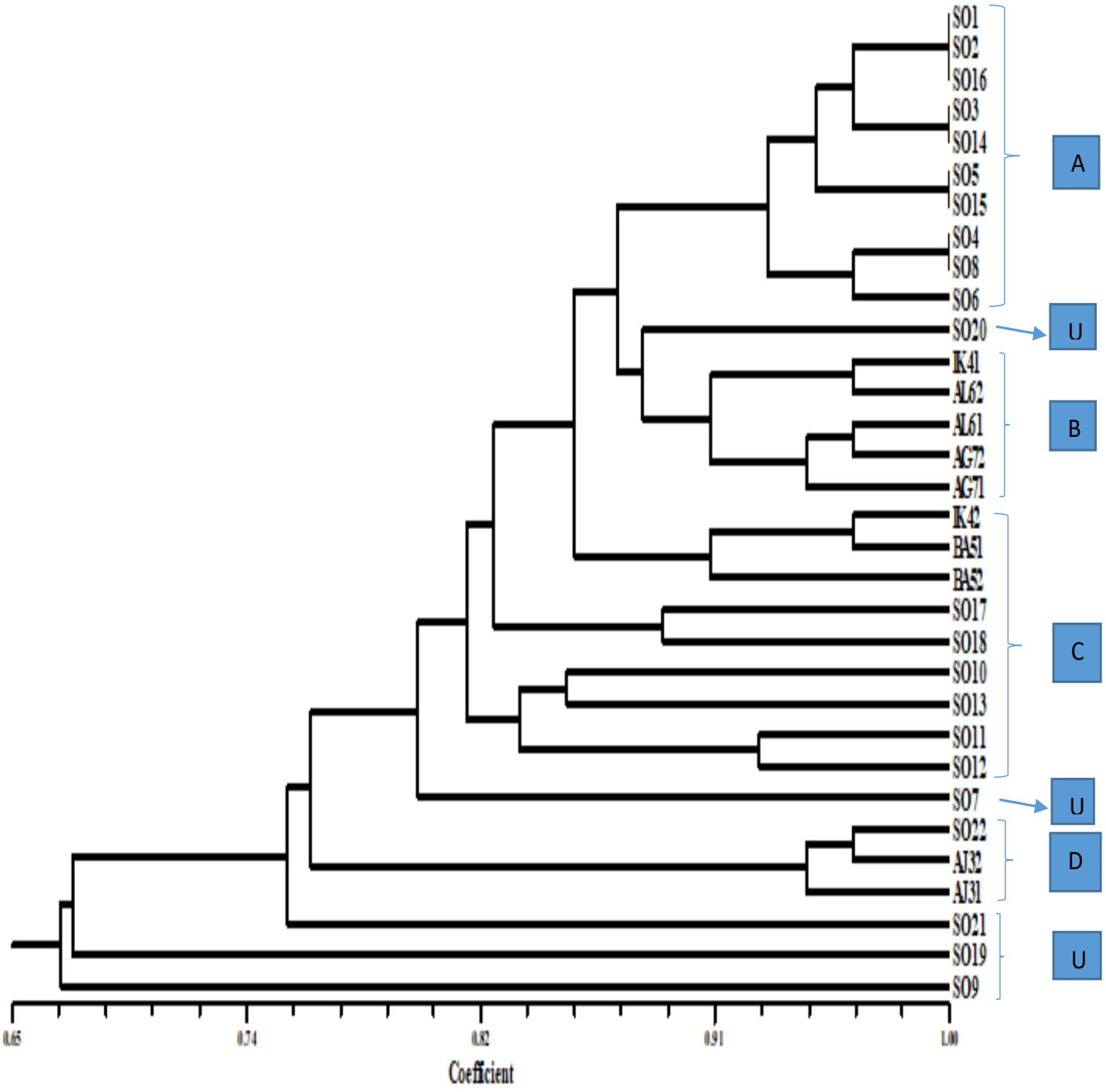

SO1
SO2
SO16
SO3
SO14
SO5
SO15
SO4
SO8
SO6
SO20
IK41
AL62
AL61
AG72
AG71
IK42
BA51
BA52
SO17
SO18
SO10
SO13
SO11
SO12
SO7
SO22
AJ32
AJ31
SO21
SO19
SO9
A
U
B
C
U
D
U
0.65
0.74
0.82
0.91
1.00
Coefficient

Figure 1: Dendrogram of 32 samples of *A. sativum* from six locations involving six (5) Local Government Areas (Shomolu (SO), Ajeromi Ifelodun (AJ), Ikorodu (IK), Alagbado (AL), Agbado (AG) and one (1) LCDA - Bariga (BA) with RAPD Primer OPA 03, OPA 07, OPA 12, and OPY 20 using NTsys (2.0J) computer programme showed complete clustering at about 0.67 and similarity coefficient of 76% among the 32 samples collected from the six (6) locations. Genotypes segregated into four (4) Groups (A-D) with about five (5) genotypes ungrouped (U).

DISCUSSION

Genetic similarity levels of all *A. sativum* samples on the dendrogram ranged from 0.65 – 1.00. The clustering pattern of the dendrogram from the 32 samples was observed to segregate into 4 Groups (A – D). Samples SO1, SO2, SO16, SO3, SO14, SO5, SO15, SO4, SO8 and SO6 formed Group A and clustered at 0.97 similarity coefficient which implies that they have 97% similarity and 3% genotypic variation. Samples IK41, AL62, AL61, AG71 and AG72 formed Group B and clustered at 0.91 similarity coefficient which implies that they have 91% similarity and 9% genotypic variation. Samples IK42, BA51, BA52, SO17, SO18, SO10, SO13, SO11 and SO12 formed group C and clustered at 0.82 similarity coefficient which implies that they have 82% similarity and 18% genotypic variation. Samples SO22, AJ32 and AJ31 formed group D and clustered at 0.96 similarity coefficient which implies that they have 96% similarity and 4% genotypic variation. Samples SO20, SO7, AG71, SO21, SO19, and SO9 appeared ungrouped which implies that they have wide genotypic variations. The high intraspecific similarities exhibited by these Groups A-D (97%, 91%, 82%, 96%) could point to a common ancestry within the species.

Nwakanma *et al.,* (2021) reported that indicators of resemblance that are higher designate that samples in the population have tighter genetic links, whereas lower similarity indices indicate that samples in the population have a greater genetic distance between them. Similarly, the quantity and purity of isolated genomic DNA are important factors in determining molecular diversity and optimizing PCR settings (Weeden *et al.,* 1992).

The present study showed complete clustering of all samples at 67% and a similarity coefficient of 76%. This means that there is a 24% variation among the 32 samples collected from six (6) different locations in Lagos. This work is in agreement with the work on genetic diversity of *Vernonia* as revealed by RAPD markers that clustered at 64% with also a similarity coefficient of 72% (Nwakanma *et al*., 2018). Earlier, Azeez *et al.,* (2009) used the RAPD technique in the assessment of genetic diversity among 30 accessions of the *Santalum album*. All accessions were grouped into two major groups at about 0.70 coefficient, having a distinct sub-cluster of about 0.85 coefficient and genetic similarity of 45%. Also, Ray and Roy, (2009) who studied genetic diversity and relationships among 6 *Amaranthus species* from 8 phytogeographic regions of the Indor Gangetic Plains calculated a mean genetic similarity coefficient of 0.56 using RAPD marker and had a genetic variation of about 43% among all the *Amaranthus species* studied. RAPD marker analysis carried out by other researchers on other plants had also reported that it is a very good tool for the investigation of genetic diversity (interspecies and intraspecies genetic diversity) among plants. For example, studies in Stokes Aster (*Stokesia* spp.) showed that they are very closely related, with values for all pairwise comparisons of cultivars of stokes asters ranging from 0.92 – 0.68 while similarity indices between stokes aster and *Vernonia* and between stoke aster and *Rudbeckia* were between 0.44 and 0.50 respectively (Gettys and Werner, 2001). Other plants, whose genetic relationships had been studied using RAPD include Jute plants (Ogunkanmi *et al.*, 2010), *Amaranthus* species (Tony-Odigie *et al.,* 2012) and *Gladiolus* (Malik and Pal, 2014).

CONCLUSION

The study here leads us to conclude that RAPD marker analysis is very useful for assessing intra genetic diversity (intraspecific similarity and variation) among Garlic (*A. sativa*) genotypes found in these parts of Lagos, Nigeria. Therefore, selection could be made from the diverse genotypes as parents for crosses designed for breeding improved cultivars and for producing mapping populations for QTL analysis.

REFERENCES

Abraham, A. D., Kidanemariam, D. B and Holton, T. A. (2019). Molecular identification, Incidence and Phylogenetic analysis of seven viruses infecting garlic in Ethiopia. *European Journal of Plant Pathology*, **155**(1): 181-191.

Amagase, H. (2006). Clarifying the Real Bioactive Constituents of Garlic. *The Journal of Nutrition* **136:** 716S-725S.

Azeez, A. S., Nelson, R., Prasadbabu, A. and Srinivas, R.M. (2009). Genetic diversity of *Santalum album* using RAPD. *African Journal of Biotechnology*, **8**(13): 2943-2947.

Beato, V. M., Orgaz F., Mansilla F. and Montaño A. (2011). Changes in phenolic compounds in garlic (*Allium sativum* L.) owing to the cultivar and location of growth. *Plant Foods and Human Nutrition*, **66:**218–223.

Bhandari, P. R. (2012). Garlic (*Allium sativum*): A review of potential therapeutic application. *International Journal of Green Pharmacy*, **6**(2): 118–129.

Belloir, C., Singh, V., Daurat, C. Siess, M. H and Le Bon, A. M. (2006). Protective effects of garlic sulfur compounds against DNA damage induced by direct and indirect acting genotoxic agent in cells. *Food and Chemical Toxicology*, **44**(6): 827-834.

Dai, J and Mumper, R. J. (2010). Plant phenolics: Extraction, Analysis and their Antioxidant and Anticancer properties. *Molecules*, **15**(10): 7313-7352.

Gettys, L. A. and Werner, D. J. (2001). Genetic Diversity and Relatedness Among Cultivars of Stoke Asters. *Horticultural Science*, **36** (7): 1323 – 1326.

Gorinstein, S., Drzewiecki, J., Leontowicz, H., Leontowicz, M., Najman, K., Jastrzebski, Z., Zachwieja, Z., Barton, H., Shtabsky, B., Katrich, E. and Trakhtenberg S. (2005). Comparison of the bioactive compounds and antioxidant potentials of fresh and cooked Polish, Ukrainian, and Israeli garlic. *Journal of Agriculture and Food Chemistry*, **53:** 2726–2732.

Ogunkanmi, L.A., Okunowo, W.O., Oyelakin, O.O., Oboh, B.O., Adesina, O.O., Adekoya, K.O. and Ogundipe, O.T. (2010). Assessment of Genetic Relationships between Two Species of Jute Plants Using Phenotypic and RAPD Markers. *International Journal of Botany*, **6**(2): 107 – 111.

Malik, K., and Pal, K. (2014). Genetic Divergence and Relationship Analysis among Twenty Two Population of Gladiolus Cultivars by Morphological and RAPD-Per Tool. *International Journal of Education and Science Research*, **1**(6): 1-8.

Murray, M.G. and W. F. Thompson (1980). Rapid isolation of high molecular weight DNA. *Nucleic Acids Research,* **8**: 4321 – 4325.

Nwakanma, N. M. C., Ofodile, L.N., Adedoyin, D., Ibegbunam, I.C., Saliu, G.Y. , Anderson I. Okafor, A.I., Oluwaseyifunmi A. Omotayo, O.A., Egbuchua, F. F. and Aboyade-Cole, O. (2021). Genetic Diversity of Newbouldia laevis as Revealed by Random Amplified Polymorphic DNA (RAPD) Markers. *AKSU Journal of Agriculture and Food Sciences*, **5** (3): 26-34.

Nwakanma, N. M. C., Adekoya, K.O., Ogunkanmi, L. A. and Oboh, B. O. O (2018). Genetic diversity of *Vernonia* as revealed by Rapid Amplification Polymorphic DNA Marker (RAPD). *Egyptian Journal of Biological Sciences*, **9(**1): 27-38.

Petropoulos, S.A.; Fernandes, Â.; Ntatsi, G.; Petrotos, K.; Barros, L.; Ferreira, I.C.F.R. (2018). Nutritional Value, Chemical Characterization and Bulb Morphology of Greek Garlic Landraces. *Molecules* ***23***, 319. https://doi.org/10.3390/molecules23020319

Petrovska, B. B and Cekovska, S. (2010). Extracts from the History and Medical properties of garlic. *Pharmacognosy reviews*, **4**(7): 106-110.

Ray, T and Roy, S. C. (2009). Genetic diversity of *Amaranthus species* from the Indo-Gangetic plains revealed by RAPD analysis leading to the development of Ecotype specific SCAR marker. *Journal of Heredity*, **100**(3): 338-347.

Tony-Odigie, A.E., Adekoya, K.O., Makinde, S.C.O., Oboh, B.O., Ogunkanmi, L.A. and Fowora, M.A. (2012).Assessment of Genetic Interspecies Relationships among Five Selected Amaranthus Species Using Phenotypic and RAPD Markers. International Journal of Botany, **8**(3):145 – 152.

Weeden, N. F., Timmerman, M., Hermmat, M., Kneen, B. E., & Lodhi, M. A. (1992). Applications of RAPD technology to plant breeding symposium proceeding (pp. 12–17). Minneapolis.