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COMPARATIVE *IN VITRO* ANTIOXIDANT POTENTIALS OF AQUEOUS AND ETHANOL EXTRACT OF *CLERODENDRUM VOLUBILE* LEAVES

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ABSTRACT

The leaves of *Clerodendrum volubile* are commonly consumed as a vegetable, mostly blended with other vegetables as a spice with a sweet aroma and taste among South Westerners of Nigeria. *In vitro* antioxidant potential of aqueous and ethanol extracts of *clerodendrum volubile* leaves were investigated. Fresh leaves of *Clerodendrum volubile* were obtained from Mushin herbal market Lagos State. It was identified and authenticated in department of Botany of University of Lagos. The leaves of *Clerodendrum volubile* were first chopped into small pieces, dried at room temperature for three week and reduced to powder with the aid of a mortar and pestle. Two hundred gram of the powdered samples were macerated with 600 ml 70% ethanol and water for four days. After four days the extracts were then recovered by filtration using wet wash test sieve. The crude extracts obtained were then dried in a hot air oven at 45◦C to concentrate them. Reducing power assay, DPPH inhibition and nitric oxide inhibition activities were carried out in both ethanol and aqueous extracts. Tests were carried out in triplicate and data was statiscally analyzed using SPSS version 20.0. Both extracts exhibited free radical scavenging and antioxidant potential compared to ascorbic acid. The values of reducing power, DPPH activity and nitric oxide inhibition activity was higher in ethanol extract compared to the aqueous extract. Leaf extracts showed some antioxidant potentials compared to ascorbic acids; this may be due to the presence of some phytochemical in the extract.

KEYWORDS: Antioxidant, *Clerodendrum volubile*, Nitric oxide, DPPH, inhibition

INTRODUCTION.

Oxidative stress plays a crucial role in vivo tissue damage; it is important for the pathogenesis of several life-threatening diseases, as well as in the ageing of living organisms (Ejele *et al*, 2012), with documented scientific evidence to support this. Endogenous antioxidant enzymes play a significant role in protecting the biomolecules of the living organism. Excessive production of ROS (Reactive Oxidative Species) leads to a redox imbalance between free radicals and endogenous antioxidants, thereby causing oxidative stress, leading to the destruction of proteins and the structural integrity of lipid membranes of cells, as well as DNA of organisms (Erukainure *et al.*, 2011). Over the years, synthetic antioxidant drugs have been effectively employed for the treatment of diseases mediated by free radical species. However, adverse effects associated with the usage of synthetic antioxidant drugs cannot be neglected; therefore, huge consideration has been given to natural sources of antioxidants, specifically those of plant origin. (Dembinska-kiec *et al.*, 2008)

Antioxidants are believed to play a very important role in the body defense system against ROS (Reactive Oxidative Species) (Vivex and Sunrendra, 2006). An antioxidant is “any substance that, when present at low concentrations compared with that of an oxidizable substrate, significantly delays or inhibits oxidation of that substrate (Halliwel and Gutteridge, 1995). In other terms, an antioxidant is “any substance that delays,

prevents or removes oxidative damage to a target molecule. Antioxidants are inhibitors of the process of oxidation, even at relatively small concentration and thus have diverse physiological role in the body. Antioxidants are our first line of defense against free radical damage, and are critical for maintaining optimum health and well-being. Antioxidant constituents of plant materials act as radical scavengers, and helps in converting the radicals to less reactive species. An antioxidant rich diet has a very positive health impact in the long run, reducing the risk of chronic diseases (Dembinska-kiec *et al.*, 2008).

Clerodendrum volubile (Lamiaceae) is a widely distributed vegetable in the warm temperate and tropical regions of the world. The plant is popularly known as "marugbo" or "eweta" amongst the Ikale, Ilaje, and Apoi people in the southern-senatorial district of Ondo State, Southwest Nigeria. It is, however, referred to as "obnettette" in the South-Southern part of Nigeria. The leaf of *Clerodendrum volubile* is commonly consumed as a vegetable, mostly blended with other vegetables as a spice with a sweet aroma and taste. In Nigeria and other West African countries, green leafy vegetables undergo a cooking process rather than being eaten raw. Cooking is usually carried out to increase the palatability and to improve the edibility of some food (Afolabi *et al*, 2019).

Clerodendrum volubile is a promising source of minerals and vitamins, which can be used to fight malnutrition if correctly exploited. In southwestern Nigeria, when consumed, the leaves are often noted for stimulating lost appetite as well as replenishing vitality for pregnant women and mothers of new born babies (Ogunwa *et al.*, 2016). It is also used in the management of arthritis, swellings, rheumatism, gout, dropsy, and oedema, while also possessing anti-abortifacient and sedative properties. The plant had been used in the treatment of inflammation and pain by traditional medical practitioners, but with no scientific evidence to support this (Adefegha and Oboh, 2018). Preliminary studies indicated that *clerodendrum volubile* is rich in nutrients and minerals such as carbohydrates, proteins, crude fat, ash, fibre, calcium, sodium, potassium, iron, zinc, copper, magnesium and manganese Further studies on phytochemical composition indicated that the aqueous and ethanol extracts of *clerodendrum volubile* leaves consists of phytochemicals such as tannins, phenols, alkaloids, saponins, steroids, reducing sugar, flavonoids and cardiac glycosides etc. with the ethanol extract having more phytochemicals compared to the aqueous extracts.

Phytochemicals being naturally occurring are believed to be effective in combating or preventing disease due to their antioxidant properties which produce the definite physiological actions on human body (Ejele *et al.*, 2012). The medicinal values of plants lie in their component phytochemicals; hence this study was designed to evaluate the comparative *in vitro* antioxidant potential of aqueous and ethanol extracts of *clerodendrum volubile* grown in Lagos south western Nigeria.

MATERIALS AND METHODS

COLLECTION OF PLANT MATERIAL

Fresh leaves of, *Clerodendrum volubile were* collected at Mushin market, Lagos State, Nigeria. The fresh leaves were botanically identified and authenticated at the Department of Botany, University of Lagos Nigeria with voucher specimen 8915.All reagents used were of analytical grade.

SAMPLE PREPARATION

Clerodendrum volubile leaves were dried at room temperature for three weeks and then kept dry for further use. The leaves of *Clerodendrum volubile* were first chopped into small pieces and then dried similarly.

The fresh leaves were air dried and reduced to powder with the aid of a mortar and pestle. Two hundred gram of the powdered samples was macerated with 600 ml of ethanol and water for four days. After four days the extracts were then recovered by filtration using wet wash test sieve . The crude extracts obtained were then dried in a hot air oven to concentrate the extracts by evaporating the solvents. The semi-solid material obtained was accurately weighed and then used for antioxidant activity analysis.

DETERMINATION OF ANTIOXIDANT ACTIVITIES

DETERMINATION OF TOTAL ANTIOXIDANT CAPACITY:

Solution of the sample extracts (1 ml) was mixed with 3 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a boiling water bath at 95^0 C for 90 minutes. After the samples had been cooled to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm. The total antioxidant capacity was expressed as equivalent of ascorbic acid.

ASSAY OF 2,2-DIPHENYL-1-PICRYLHYDRAZY (DPPH) RADICAL SCAVENGING ACTIVITY.

An aliquot of 0.5 ml of each extract in ethanol (95%) at different concentrations (25, 50, 75, 100µg/ ml) was mixed with 2.0 ml of reagent solution (0.004 g of DPPH in 100 ml methanol). The control contained only DPPH solution in place of the sample while methanol was used as the blank. The mixture was vigorously shaken and left to stand at room temperature. After 30 minutes the decrease in absorbance of test mixture (due to quenching of DPPH free radicals) was read at 517 nm. The scavenging effect was calculated using the expression:

% inhibition = $[A^0\text{-}A^1] \times 100/A^0$

Where A^0 is the absorption of the blank sample and A^1 is the absorption of the extract

ASSAY OF NITRIC OXIDE SCAVENGING ACTIVITY

Four milliliter sample of each plant extracts or standard solution of different concentrations (25, 50, 75, 100 µg/ml) were taken in different test tubes and 1 ml of Sodium nitroprusside (5 mM in phosphate buffered saline) solution was added into the test tubes. They were incubated for 2 hours at 30 °C to complete the reaction. A 2 ml sample was withdrawn from the mixture and mixed with 1.2 ml of Griess reagent (1% Sulphanilamide, 0.1% naphthylethylene diamine dihydrochloride in 2% H_3PO_4). The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and its subsequent coupling with napthylethylene diamine was measured at 550 nm. Ascorbic acid was used as standard. The percentage (%) inhibition activity was calculated from the following equation:

$[(A^0 - A^1)/A^0] \times 100$.

Where, A^0 is the absorbance of the control and A^1 is the absorbance of the extract or standard.

ASSAY OF REDUCING POWER ACTIVITY

Various concentrations of each extract (20 to 100 µg/ml) in 1.0 ml of deionized water were mixed with phosphate buffer (2.5 ml) and potassium ferricyanide (2.5 ml). The mixture was incubated at 5^0C for 20 min. Aliquots of trichloroacetic acid (2.5 ml) were added to the mixture, which was then centrifuged at 3000 rpm for 10 min. the upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared ferric chloride solution (0.5 ml). The absorbance was measured at 700 nm. A blank was prepared without adding extract. Ascorbic acid at various concentrations (1 to 16µg/ml) was used as standard.

RESULTS.

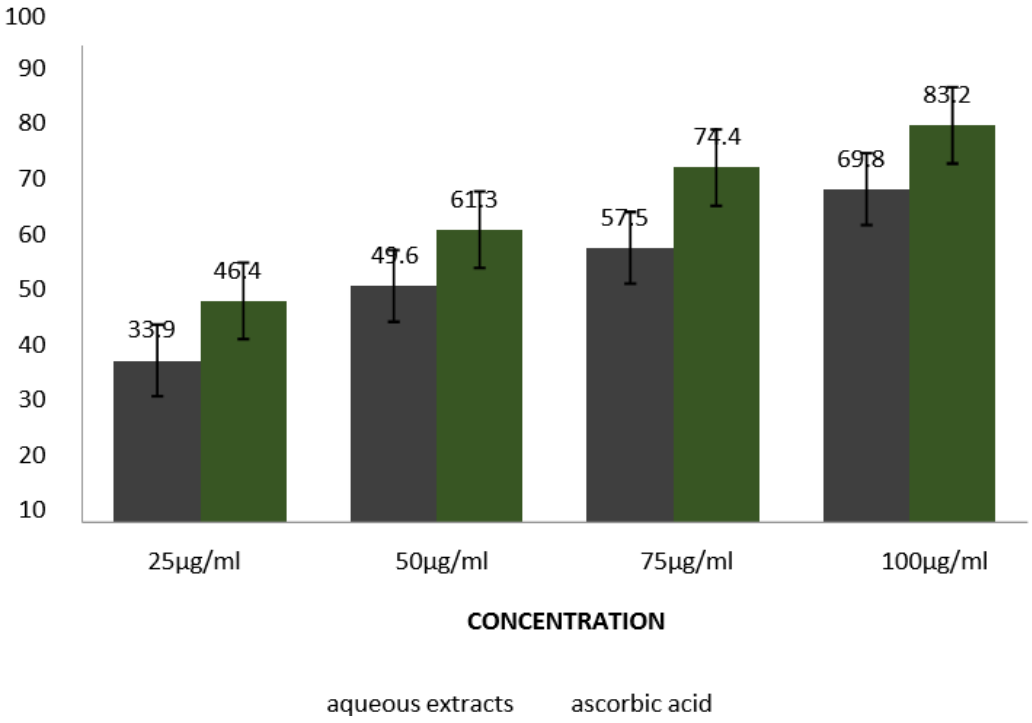

Fig 1. DPPH Scavenging activity (%Inhibition) of aqueous extract of *Clerodendrum volubile* leaves

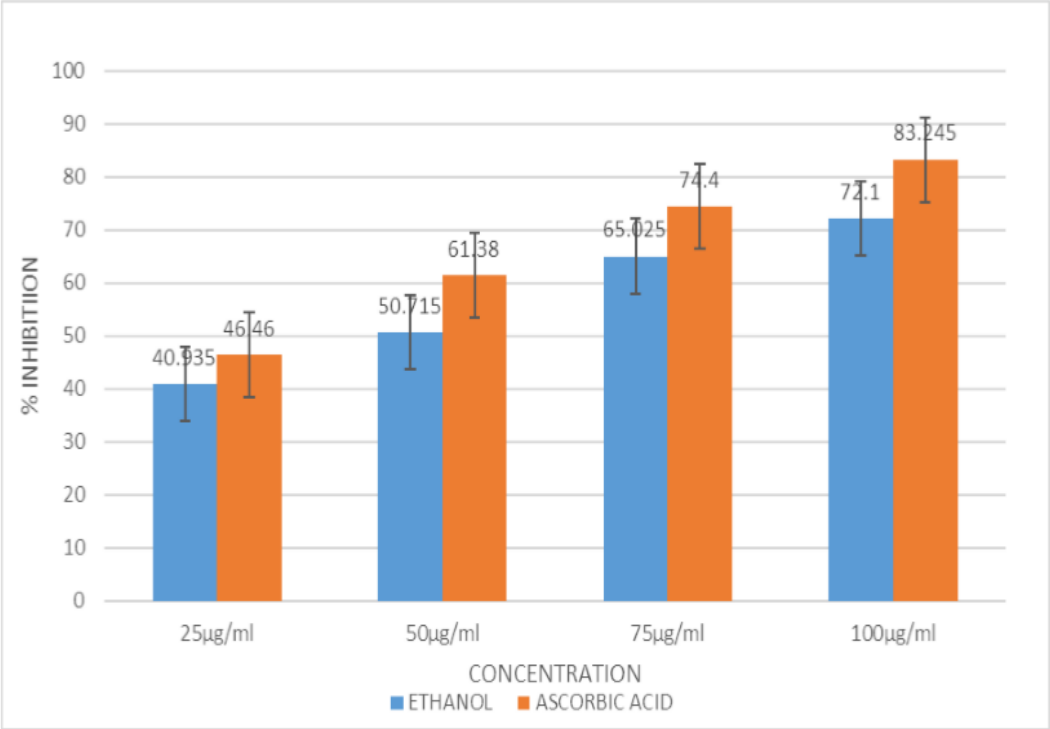

Fig. 2. DPPH Scavenging activity (%Inhibition) of ethanol extracts *Clerodendrum volubile* leaves

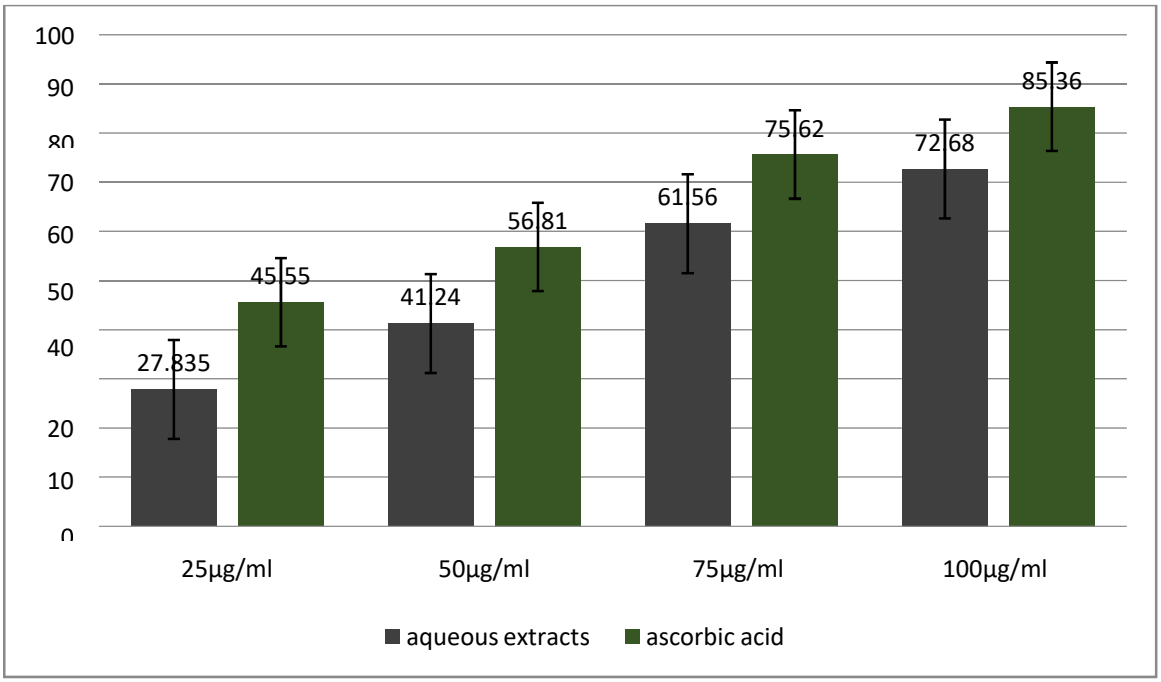

Figure 3. Nitric Oxide Scavenging activity (%Inhibition) of aqueous extracts *Clerodendrum volubile* leaves

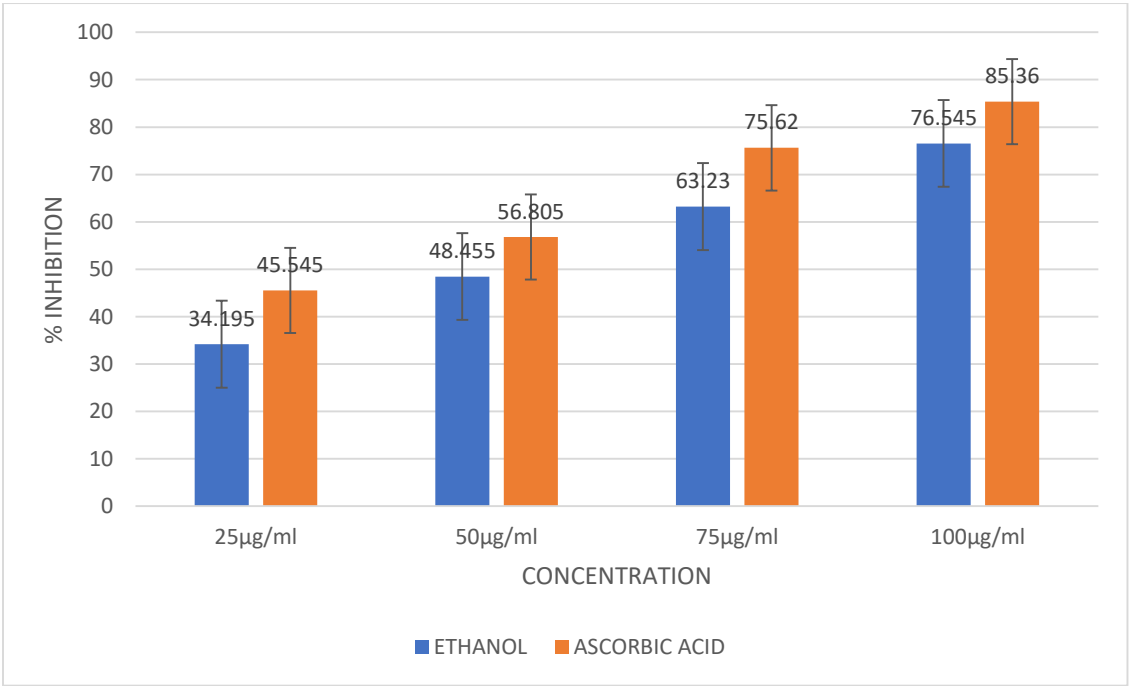

Fig 4. Nitric Oxide Scavenging activity (%I inhibition) of ethanol extracts *Clerodendrum volubile* leaves

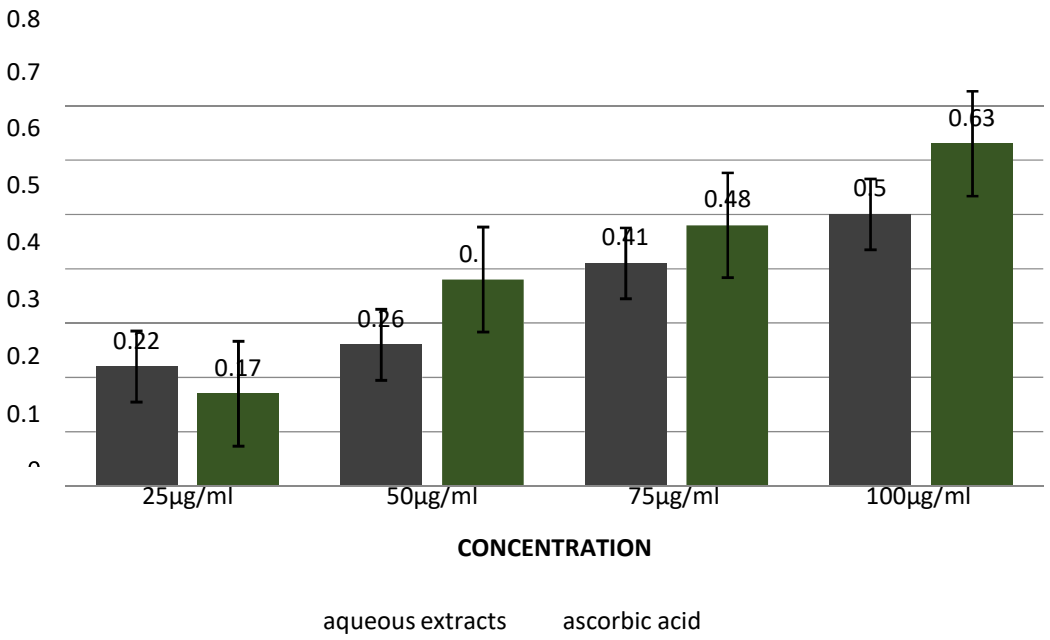

Figure 5. Reducing power of aqueous extracts *of Clerodendrum volubile* leaves

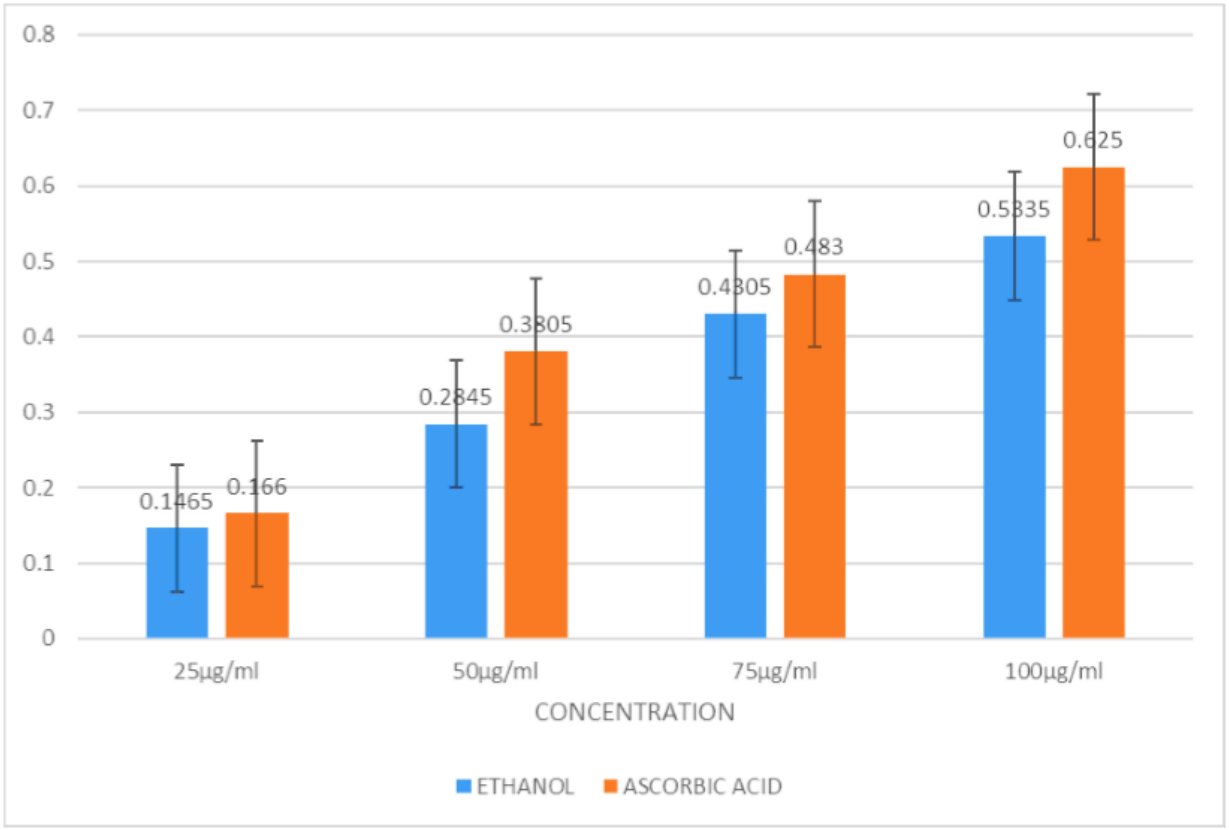

Fig. 6. Reducing power of ethanol extracts *Clerodendrum volubile* leaves

DISCUSSION

One of the important steps ever taken when it comes to the use of medicinal plants involves screening for phytochemicals for possible antioxidant activities of the plants extracts. The antioxidant activity of aqueous and ethanol extract of *clerodendrum volubile* leaves were investigated. DPPH for aqueous extract ranged from 33.94± 0.24 at 25µg/ml to 69.87 ± 1.83 at 100µg/ml while for the ethanol extract, the % inhibition of DPPH ranged from 40.94±0.78 at 25µg/ml to 72.10±0.57% at 100µg/ml. According to Ogunwa *et al.*, (2016), the DPPH model is a simple, precise, relatively quick and acceptable method which is widely used to measure free radical scavenging activity. It is a stable radical even at room temperature and becomes a stable diamagnetic molecule upon receiving an electron or hydrogen radical. It has been employed in the determination of antioxidant ability of numerous natural products.

The interaction of DPPH with antioxidants is visually noticeable because the purple color changes to yellow, demonstrating the reduction of the stable DPPH radical to its diphenyl picryl hydrazine. This reaction is used to reveal the capacity of neutral products to scavenge free radicals. It is believed that antioxidants act on DPPH via hydrogen donation or electron transfer mechanism (Zihad *et al*, 2019) and this is often measured as a decrease in absorbance taken spectrophotometrically at 517nm. This study is in agreement with the report by Ogunwa *et al*., (2016) on the antioxidant activities of *Clerodendrum volubile* showed the increasing order of 50µg/ml > 100µg/ml >200µg/ml > 500µg/ml. The nitric oxide activity for the aqueous extract ranged from 27.83±0.69 at 25µg/ml to 72.68± 0.34 at 100µg/ml compared to the ethanol extract which ranged from 34.20±0.49 at 25µg/ml to 76.55±0.36 at 100µg/ml. The reducing power followed a similar trend as observed with scavenging activities. The antioxidant activity for reducing power for the aqueous extract ranged from 0.22± 0.12 at 25µg/ml to 0.50±0.005 at 100µg/ml while for the ethanol extract it ranged from 0.15±0.0021 at 25µg/ml to 0.63±0.0014 at 100µg/ml. This study showed a lower reducing power compared to the report by Ogunwa *et al.*, (2016).

This reducing property makes them safer in administration compared to synthetic antioxidants that have been reported to be carcinogenic. Some phytochemicals have been reported to possess antioxidant activity by either acting singly or by interacting with polyphenols (Ogunwa *et al*, 2016).

From the results, we observed that the values of DPPH, Nitric oxide and reducing power activity was higher in ethanol extract compared to the aqueous extract with ascorbic acid as the positive control. This is an indication that the ethanol extract of *clerodendrum volubile* possess more antioxidant potential compared to the aqueous extract.

The antioxidant properties, as observed in this investigation, could be as a result of the phytochemicals such as phenolic compounds, flavonoids, saponins and tannins present in this plant as they have been shown to possess potent antioxidant potentials. They have high redox potentials which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers. Previous study has indicated that the ethanol extract of *clerodendrum volubile* possess higher content of phytochemicals such as phenols, flavonoids, alkaloids, cardiac glycosides, reducing sugar, tannins etc. compared to the aqueous extract of the plant which has lower content of phytochemicals with tannins completely absent in the aqueous extract. This is an indication that ethanol is a better solvent at extracting the important constituents of this plant compared to water.

It can be said that both aqueous and ethanol extract of *clerodendrum volubile* leaves possess free radical scavenging and antioxidant activities, however, ethanol extract has comparatively more potent *in vitro* free radical scavenging activity and antioxidant capacity than aqueous extract. This capacity may be correlated with the total phenolic contents in the plant. The free radical scavenging and antioxidant potentials of this plant suggest that it could be potential natural source of antioxidants which may possess better implications at reducing oxidative stress and degenerative diseases.

CONCLUSION

Our findings revealed the potentials of *Clerodendrum volubile* leaves for treatment of many free radical-mediated life-threatening diseases and their complications, thus validating its use in folkloric and ethnomedicines. It is therefore suggested that maximum potential of these plant should be explored in pharmaceutical sciences and medicinal field for their appropriate application.

REFERENCES

Adefegha S. A. and Oboh G. (2018) Antioxidant and inhibitory properties of *Clerodendrum volubile* leaf extracts on key enzymes relevant to non-insulin dependent diabetes mellitus and hypertension. J*ournal of Taibah University for Science*, vol 10 no 4: pp 521–533.

Afolabi S., Olorundare O. and Gyebi G. (2019). Cytotoxic potentials of *Clerodendrum volubile* against prostate cell lines and its possible proteomic targets. *Journal of Clinical Nutrition and Food Sciences*, vol 2 (2): pp 46–53.

Ajao A. A., Oseni O. M., Oladipo O. T., Adams Y. A., Mukaila Y. O., Ajao A. A. (2018) *Clerodendrum volubile* P. Beauv (Lamiaceae), an underutilized indigenous vegetable of utmost nutritive and pharmacological importance. *Beni-Suef University Journal of Basic and Applied Sciences*, vol 7 no 4: pp 606–611

Apata, J.T.; Ogunleye, S.G.; Ogunbiyi, O.J.; Babalola, O.O. GC-MS (2017) Analysis and Phytochemical Screening of n-Hexane Fraction Constituents from the Leaf of *Clerodendrum volubile* P. Beauv. *Inter. J. Biosci. Technol* 3 (1):

Bassam, H. (2012). Medicinal Plants (Importance and Uses). *Pharmaceutica Analytica Acta*. vol 3 no 1 pp1-10

Chukwujekwu, J.C., Lategan, C.A., Smith, P.J., Van Heerden, F.R. and Van Staden, J. (2009). Antiplasmodial and cytotoxic activity of isolated sesquiterpene lactones from the acetone leaf extract of *Vernonia colorata*. *South Afr. J. Bot*, vol 75: pp 176-179.

Dai, J. and Mumper, R. (2010). Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules*, vol 15: pp 7313-7352.

Dembinska-Kiec, A., Mykkanen, O., Kiec-Wilk, B. and Mykkanene H. (2008). Antioxidant Phyto-chemicals against Type 2 Diabetes. *British J. Nutri*, vol 99: pp 109-117

Ejele, A.E., C.I. Akalezi, I.C. Iwu, L.N. Ukiwe, C.K. Enenebaku and S.U. Ngwu, (2014). Bioassay-guided isolation, purification and characterization of antimicrobial compound from acidic metabolite of *Piper umbellatum* seed extract. *Int. J. Chem.*, vol 6: pp 61-70.

Ejele, A.E., I.C. Iwu, C.K. Enenebeaku, L.N. Ukiwe and B.N. Okolue, (2012). Bioassay-guided isolation, purification and partial characterization of antimicrobial compound from basic metabolite of *Garcinia kola*. *J. Emerg. Trends Eng. Applied Sci.*, vol 3: pp 668-672

Erukainure O. L., Ebuehi O. A. T. and Choudhary I. M. (2011). Iridoid glycoside from the leaves of *Clerodendrum volubile* Beauv. shows potent antioxidant activity against oxidative stress in rat brain and hepatic tissues. *Journal of Dietary Supplements*, 11 (1): pp 19–29.

Erukainure O. L., Hafizur R.M. and Kabir N. 2018, Suppressive effects of *Clerodendrum volubile* P. Beauv. [Labiatae] methanolic extract and its fractions on type 2 diabetes and its complications. *Frontiers in Pharmacology*, 9 (8): 1-11

Erukainure O. L., Zaruwa M. Z. and Choudhary M. I. 2016, . Dietary fatty acids from leaves of *Clerodendrum Volubile* Induce cell cycle arrest, downregulate matrix metalloproteinase-9 expression, and modulate redox status in human breast cancer. *Nutrition and Cancer*. 68 (4): pp 634–645

Halliwell, B. and Gutteridge, J.M.C. (1995). The Definition and Measurement of Antioxidants in Biological Systems. *Free Radical Biology and Medicinal*, vol 18: pp 125-126

Hasler, C. M., & Blumberg, J. B. (1999). Phytochemicals: biochemistry and physiology. Introduction. *The Journal of nutrition*, 129 (3): pp 756–757.

Jeff-Agboola, Y.A. and Awe, I.B. (2016). Antifungal and phytochemical screening of some Nigerian medicinal plant extracts against toxigenic *Aspergillus flavus* Cogent Food Agric. 2 (1): 1-12

Krishnaiah, D., Nithyanandam, R. and Sarbatly, R. (2012). Phytochemical Constituents and Activities of *Morinda citrifolia* L. *Phytochemicals – A Global Perspective of Their Role in Nutrition and Health*, vol 1 pp : 127-133

Molehin O.R., Oloyede O.I. and Boligon A.A. (2017). Comparative study on the phenolic content, antioxidant properties and HPLC fingerprinting of the leaf extracts of *Clerodendrum volubile* P. Beauv. *J. Appl. Pharm. Sci*, vol 7:pp 135–140

Ogunwa T.H., Adeyelu T.T., Fasimoye R., Oyewale M.B., Ademoye T.A., Ilesanmi O.C., Awe O.B., Ajiboye S.A., Oloye B.O and Sholanke D.R. (2016). Phytochemical evaluation and in vitro antioxidant status of *Clerodendrum volubile* (An indigenous medicinal plant) *Pak. J. Pharm. Res* 2 (77): pp 11-16

Okwu, D.E. (2005). Phytochemicals, vitamins and mineral contents of two Nigerian medicinal plants. *International Journal of Molecular Medicine and Advanced Science*, 1 (14): pp 372-381

Olcese J., Cao C., Mori T., Mamcarz M.B., Maxwell A., Runfeldt M.J., Wang L., Zhang C., Lin X. and Zhang G. (2009). Protection against cognitive deficits and markers of neurodegeneration by long-term oral administration of melatonin in a transgenic model of Alzheimer disease. *J. Pineal Res*, vol 47:pp 82–96

Oloyede, K.G., M.J. Oke, Y. Raji and A.T. Olugbade, (2010). Antioxidant and anticonvulsant alkaloids in *Crinum ornatum* bulb extract. *World J. Chem.*, vol 5: pp 26-31.

Omoboyowa, D., Afolabi, F. and Aribigbola, T. (2018). Pharmacological potential of methanol extract of Anacardium occidentale stem bark on alloxan-induced diabetic rats. *Biomedical Research and Therapy*, vol5 . pp 2440-2454

Patel, K., Gadewar, M., Tripathi, R., Prasad, S. K. and Patel, D. K. (2012). A review on medicinal importance, pharmacological activity and bioanalytical aspects of beta-carboline alkaloid "Harmine". *Asian Pacific journal of tropical biomedicine*, *2* (8): pp 660–664.

Senjobi, C.T., Fasola, T. and Aziba, P. (2017): Phytochemical and analgesic evaluation of methanol leaf extract of *Clerodendrum volubile* Linn. *Ife Journal of Science*, vol 19: pp 1-14

Sofowora, A., Ogunbodede, E., and Onayade, A. (2013). The role and place of medicinal plants in the strategies for disease prevention. African journal of traditional, complementary, and alternative medicines. *African Journal of Traditional, Complementary and Alternative Medicine*, 10 (5): pp 210–229.

Vivek, K.G. and Surendra, K.S. (2006). Plants as natural antioxidants. *Natural. Production Radia*., vol 5 pp 326-334

Wright, C. I., Van-Buren, L., Kroner, C. I., & Koning, M. M. (2007). Herbal medicines as diuretics: a review of the scientific evidence. *Journal of ethnopharmacology*, 114 (1): pp 1–31.

Yudharaj, P., Shankar, R., Sowjanya, B., Sireesha, E., Ashok Naik, R. and Jasmine, P. (2016). Importance and Uses of Medicinal Plants – An Overview. *International Journal of Preclinical and Pharmaceutical Research*, **7** (2): pp 67-73

Zihad, S.M.N.K.; Gupt, Y.; Uddin, S.J.; Islam, M.T.; Alam, R.; Aziz, S.; Hossain, M.; Shilpi, J.A.; Nahar, L.; Sarker, S.D. (2019). Nutritional value, and micronutrient capacity of some green leafy vegetables commonly used by southern coastal people of Bangladesh *Heliyon Natural. Production* 3(4): pp 51-55

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