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Phytochemistry and Antioxidant Assays of *Entandrophragma angolense* (Welw.) C.DC. (Meliaceae) using DPPH and Nitric Oxide Free Radical Scavenging Methods

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Background: Medicinal plants have always been a veritable source of bioactive compounds and also serve as 'lead molecules' and templates for the design of medicinally useful synthetic molecules. *Entandrophragma angolense* Meliaceae, chemically characterized by synthesis of modified triterpenes known as limonoids which are powerful antioxidants and may help to prevent certain cancers and cardiovascular disease.

Objectives: To carry out phytochemical screening of the methanol bark and leaf extracts of *Entandrophragma angolense*, evaluate and compare the antioxidant activity of the methanol bark and leaf extracts of *Entandrophragma angolense*.

Method: The different phytochemicals in *E. angolense* were screened for using standard methods. The antioxidant activity of the plant was accessed using two DPPH free radical scavenging and Nitric oxide scavenging activities.

Result: The presence of alkaloids, flavonoids, condensed and hydrolysable tannins, saponins, steroidal nucleus, anthraquinones and reducing sugars in the methanolic bark extract were confirmed, with similar result for the methanolic leaf extract, except for the absence of alkaloids, anthraquinone and reducing sugars. The antioxidant assays indicated that both extracts from the two morphological plant parts showed considerable antioxidant activities (bark IC_{50} 0.45, leaf IC_{50} 0.41 for DPPH assay and bark IC_{50} 0.42, leaf IC_{50} 0.38 µg/ml for Nitric oxide assay) when compared with the standards (Ascorbic acid IC_{50} 0.40 and IC_{50} 0.40 µg/ml for DPPH and Nitric oxide assay, respectively, and Gallic acid, IC_{50} 0.25 and IC_{50} 0.35 µg/ml for DPPH and Nitric oxide assay, respectively).

Conclusion: *E. angolense* could be a potential source of natural antioxidant that could have greater importance as therapeutic agent in preventing or slowing accumulation of lipid peroxidation, and thereby oxidative stress related degenerative diseases.

Keywords: Phytochemical screening, *Entandrophragma angolense*, Antioxidant, DPPH, Nitric oxide

INTRODUCTION

The use of herbal medications for curing diseases is almost as old as man itself, and it is still widely used either as a drug in it raw form or starting materials in production of new drugs. More than 200,000 out of the 300,000 plants species so far on our planet are found in Africa and other tropical countries (Odugbemi, 2008). About 80% of the population worldwide use traditional medicine which has compounds derived from medicinal plants (Bodeker *et al.*, 2005).

About 25% of the drugs prescribed worldwide and found in modern pharmacopoeias come from plants, and about 150 such active compounds are still in current use (Mohamed *et al.*, 2012). Of the 252 drugs considered as basic and essential by the World Health Organization (WHO), 11% are exclusively of plant

origin and a significant number are synthetic drugs obtained from natural precursors (Mohamed *et al.,* 2012). It is estimated that 60% of anti-tumour and anti-infectious drugs already on the market or under clinical trial are of natural origin (Rates, 2001).

Again there has recently also been an increasing reliance on the use of medicinal plants in the an industrialized societies and this has been traced to the extraction and development of several drugs and chemotherapeutics from these plants as well as from traditionally used herbal remedies (Tiwari *et al.,* 2014). The medicinal properties of these plants could be based on the antioxidants, antimicrobial, antipyretic effects, etc. of the phytochemicals or secondary metabolites in them (Soetan *et al.,* 2009).

Entandrophragma angolense is a deciduous tree in the mahogany family Meliaceae, restricted to tropical Africa. At least some of the species attain large sizes, reaching 40–50 m tall, exceptionally 60 m, and 2 m in trunk diameter. Bark surface pale greyish brown to orange-brown, smooth but becoming scaly with irregular scales up to 20 cm in diameter leaving concave, often mussel-shell-shaped scars, inner bark pinkish red with whitish streaks, finely fibrous. The leaves are pinnate, with 5-9 pairs of leaflets, each leaflet 8–10 cm long with an acuminate tip (Prota, 2008).

Chemically, the Meliaceae is characterized by synthesis of modified triterpenes known as limonoids (Seigler, 1998). The leaves of *E. angolense* consists of Tirucallane tripenoidal compounds 3,23-dioxotirucalla-7,24-dien-21-al, 3,4-secotirucalla-23-oxo-4(28),7,24-trien-21-al-3-oic acid and 3,4-secotirucalla-23-oxo-4(28),7,24-trien-3,21-dioic acid (21-methyl ester) (Orisadipe *et al.,* 2005). The roots and bark consists of two gedunin type limonoids 5-hydroxy-7-deacetoxy-7-oxogedunin and 5,6-dehydro-7-deacetoxy-7-oxogedunin, and three methyl angolensate derivatives, 6-deacetoxydomesticulide D, 6-deacetoxydomesticulide D 21-methylether, and entangosin, together with known compounds, methyl angolensate, 6-acetoxymethyl angolensate and secomahoganin (Nsiama *et al.,* 2011). The seeds have a fat content of about 60%. The fat is rich in cis-vaccenic acid, an oleic acid isomer that can be used in the industrial production of nylon-11. The approximate fatty acid composition of samples of the oil from Ghana and Nigeria is: palmitic acid 4–6%, palmitoleic acid 11–16%, hexadecadienoic acid 3–5%, stearic acid 10–15%, oleic acid 2–3%, vaccenic acid 32–43%, linoleic acid 11–15% and arachidic acid 1–2%. Tests with tadpoles showed that the seeds contain toxic compounds, probably limonoids (Asiamah, 2000).

E. angolense caused increase in the secretion of malondialdehyde enzyme concentration over treatment period and was most pronounced at the low dose ($p<0.05$). The reduction in MDA concentration which implies reduced lipid peroxidation may be due to the ability of the extract to increase antioxidant activity and also scavenge reactive oxygen radicals. This is justified by the intactness of the gastric mucosa and the hypercellularity and hyperactivity of mucus cells (Oluwole *et al.,* 2007).

Antioxidants are substances that may protect cells from the damage caused by the unstable molecules known as free radicals. Antioxidants interact with and stabilize free radicals and may prevent some of the damage free radicals might otherwise cause. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols (Sies, 1997). Antioxidants are such type of agents which completely stop or delay the process of oxidation. The antioxidant compounds blocked the chain reaction of oxidation. Natural and synthetic are two group of antioxidant but the synthetic antioxidant can be carcinogenic (Papas, 1999). The interest in natural antioxidants has increased considerably, especially to the antioxidant compounds present in plants which are considered nontoxic and environmental friendly. The antioxidants from plants origin are recently used as food additives (Abdou 2011).

This study aim to further investigate the antioxidant potentials of the methanol extracts of the bark and leaves of *E. angolense,* as suggested by the observations of the gastric mucosa by Oluwole *et al.,* using DPPH and Nitric oxide scavenging activity assays methods while also confirming the phytoconstituents in the two extracts.

METHODOLOGY

Collection and preparation of plant materials

The bark and leaves of *Entandrophragma angolense* was collected from Ketu-Epe, Ogun state. They were properly indentified by Mr O.Oyebanji and specimen kept at the herbarium of the Department of Botany, Faculty of science, University of Lagos with voucher specimen number LUH6955. The materials (leaves and bark) were dried for 7-14 days in an oven at temperature 40°C. The samples were later pulverized after drying. The pulverized bark and leaves samples (700g each) were then subjected to cold maceration with 2L methanol each, and filtered with Whatman filter paper after 5 to 7 days. The extracts were concentrated with rotary evaporator at 40°C and further dried completely in the oven at 40°C. The dry

extracts were weighed and stored in sample bottles, ready for phytochemical screening and antioxidant analysis.

Phytochemical screening of the methanol extract

A portion of the bark and leaves extracts of *E. angolense* was screened for the presence of different phytochemical constituents. The constituents tested for includes alkaloids, saponins, flavonoids, reducing sugars, anthraquinones, cardiac glycosides using standard procedures outlined in Sofowora, 2008; Evans 2005; Trease and Evans, 1998.

Test for saponins

About 0.5g of the extract was dissolved with distilled water in a test tube. The test tube was stoppered and shaken vigorously for 30 seconds, frothing which persists on warming indicates the presence of saponins.

Test for tannin

Hydrolysable Tannins

A small portion of the extract was boiled with water and filtered, two drops of ferric chloride was added to the filtrate, formation of a blue-black, green or blue-green precipitate indicates the presence of hydrolysable tannins (Trease and Evans 1998).

Condensed Tannins

Bromine water was added to 5ml of the extract, the presence of orange precipitate indicates presence of condensed tannins

Test for Alkaloids

About 0.5g of each extract was stirred with 5ml of 1% aqueous hydrochloric acid on a water bath and filtered. To 3ml of each filtrate, few drops of Dragendorff's reagents was added and observed for orange to brownish precipitate.

Test for Flavonoids

About 0.5g of the extract was treated with few drops of 10% lead acetate. Yellow gelatinous precipitate was taken to indicate the presence of flavonoids.

Test for phenolic compounds

Ferric chloride test

About 0.5g of the extract was treated with about 3-4 drops of Ferric chloride solution. The formation of bluish-black colour indicated the presence of phenols.

Test for reducing sugars

About 0.5g of extract was dissolved in distilled water and filtered. The filtrate was heated with 5ml of equal volumes of Fehling's solution A and B. Formation of a red precipitate of cuprous oxide indicates the presence of reducing sugars

Test for Cardiac glycosides

Keller killani's test

About 0.5g of the extract was dissolved in 2ml glacial acetic acid containing few drops of ferric chloride solution. This was then underplayed with 1ml of sulphuric acid. A brown ring obtained at the interface indicates the presence of deoxy sugar characteristics of cardenolides.

Salkowski's test

About 0.5gof the extract was admixed in 2ml of chloroform, sulphuric acid was added and the appearance of a reddish-brown colour at the interface indicates the presence of steroidal ring.

Test for Anthraquinone

About 0.5g was extracted with hot water for 5 minutes, filtered hot, cooled and extracted with chloroform. 5ml of 10% ammonia solution was added to the separated chloroform. A pink colouration in ammonical phase indicated the presence of free anthraquinones

Antioxidant analysis

Two methods are employed in the determination of the antioxidant activities of the extracts of *E. angolense.*

Determination of free radical scavenging activity

The free radical scavenging activity of the two extracts, based on the scavenging of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical was estimated according to the procedure described by (Cuendet *et al.,* 1997 and Buritis *et al.,* 2000). An aliquot of 0.5 ml of extract in methanol (95%) at different concentrations (25, 50, 75, 100 µg/ 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:

$$\% \text{ inhibition} = [A_0-A_1]/A_0 \times 100$$

Where A_0 is the absorption of the blank sample and A_1 is the absorption of the extract

This procedure was done for both bark and leaves extract.

Nitric oxide scavenging activity assay

A 4 ml sample of plant extract 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 (Alisi *et al.*, 2008). Ascorbic acid was used as standard. The percentage (%) inhibition activity was calculated from the following equation:

$$\% \text{ inhibition} = [(A_0 - A_1)/A_0] \times 100.$$

Where, A_0 is the absorbance of the Control and A_1 is the absorbance of the extract or standard.

RESULTS

Phytochemical Screening

The results of the phytochemical screening carried out reveals the presence of saponin, hydrolysable tannin, condensed tannin, steroidal nucleus and flavonoid in both extracts and as well the absence of cardiac glycosides as shown in Table 1 below.

Antioxidant Assay

The antioxidant assay methods using DPPH and Nitric oxide shows good antioxidant capacities of the extracts as express in the respective IC_{50} of the two extracts alongside the IC_{50} of the two standards used (Ascorbic acid and Gallic acid).

Scavenging Activity by DPPH

Figure 1 shows the plots of mean percentage inhibition against the concentration of DPPH free radical for the bark, leaves, Ascorbic acid and Gallic acid.

Radical scavenging activity using Nitric oxide

Figure 3 shows the plots of mean percentage inhibition against the concentration of Nitric oxide free radical for the bark, leaves, Ascorbic acid and Gallic acid.

Table 1: Phytochemicals Screening Results

Phytoconstituents	Bark extract	Leaf extract
Alkaloid	Orange precipitate formed (Positive)	No precipitate formed (Negative)
Reducing sugar	Red precipitate formed (Positive)	No precipitate formed (Negative)
Saponin	Formation of persistent froth (Positive)	Formation of persistent froth (Positive)
Hydrolysable Tannins	Blue-black precipitate formed (Positive)	Blue-black precipitate formed (Positive)
Condensed Tannins	Orange precipitate formed (Positive)	Orange precipitate formed (Positive)
Flavonoids	Yellow gelatinous precipitate produced (Positive)	Yellow gelatinous precipitate produced (Positive)
Cardiac glycoside	No colouration at interface (Negative)	No colouration at interface (Negative)
Steroidal nucleus	Red-brown colour at interface (Positive)	Red-brown colour at interface (Positive)
Anthraquinone	Pink colouration in ammoniacal layer (Positive)	No colouration in the ammoniacal layer (Negative)

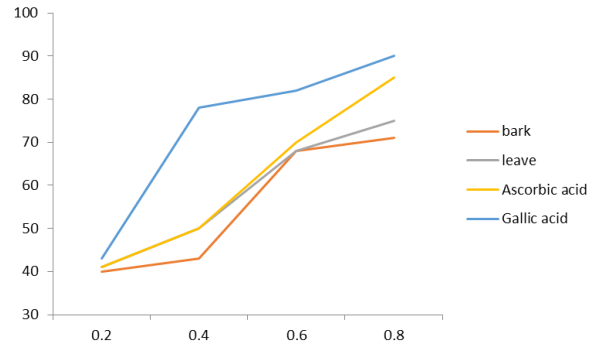

Figure 1: A graph of mean [percentage inhibition] against concentration of DPPH free radical. Bark IC_{50}= 0.45 µg/ml, Leave IC_{50} = 0.41, Ascorbic acid IC_{50}= 0.40, Gallic acid IC_{50} = 0.25

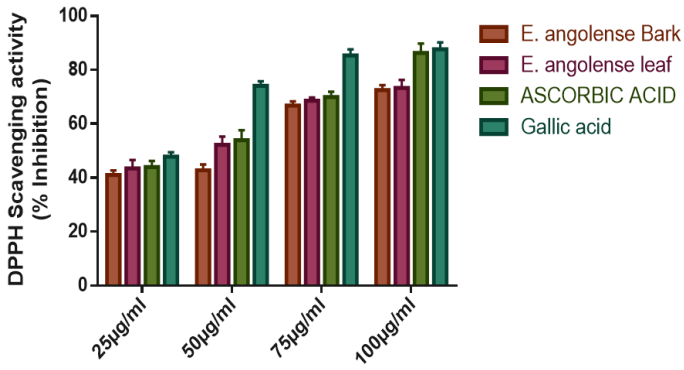

Figure 2: Graphical representation of % inhibition of DPPH against concentration of Extracts

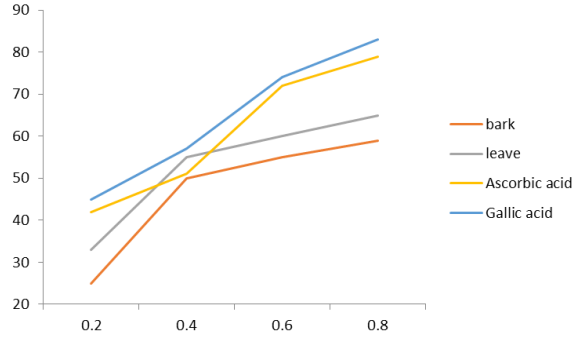

Figure 3: Graph of percentage inhibition against concentration of Nitric oxide free radical scavenging. Bark IC50= 0.42 µg/ml, Leave IC50 = 0.38, Ascorbic acid IC50= 0.40, Gallic acid IC50 = 0.35

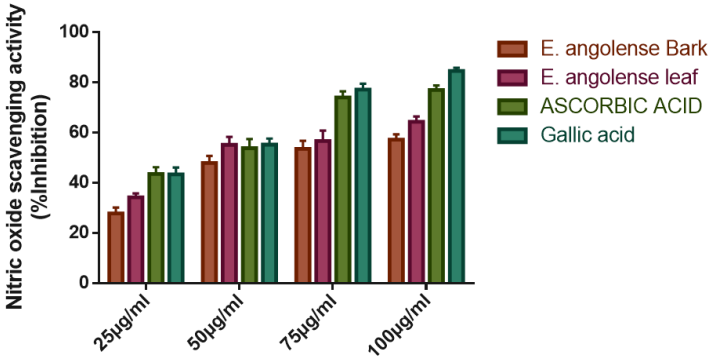

Figure 4: Graphical representation of % inhibition of nitric oxide against concentration of samples

DISCUSSION

Phytochemical screening of the methanolic extract of the bark and leaves of *Entandrophragma angolense* showed the presence of the following bioactive chemical constituents in both the bark and leaves extracts; saponins, tannins, flavonoids. Moreso, the bark extracts also, has the following bioactive chemical constituents; anthraquinone, alkaloids, and reducing sugar which were absent in the leaf extract. However, both extracts were devoid of cardiac glycoside. Several of these components are known to possess potent antioxidant activity especially the flavonoid which may explain the reported anti-ulcer properties (Lee *et al.*, 2004).

The results of the phytochemical screening for the bark extract confirms the presence of alkaloids, tannins, flavonoids, saponins, and steroidal ring in similarity to previous work done (Lagou et al.,2016), differing in the presence of reducing sugar and also in the presence of anthraquinones as well as absence of cardiac glycosides which their work did not test for.

DPPH free radical scavenging and Nitric oxide radical scavenging are commonly used in the determining the free radical scavenging power of antioxidants (Change *et al.*, 2002). The Scavenging activity of DPHH free radical was determined by the decrease in its absorbance at 517nm as well as by degree of colour change from deep violet to yellow.

Ascorbic acid, Gallic acid, the leaf and bark extracts of *E. angolense* showed dose dependent activity, from the results obtained, both extracts and the standards displayed an increase in percentage inhibition as the concentration increased (Fig 2 and 4).

The free radical scavenging activity of *E. angolense* was tested by its ability to bleach the stable DPPH radical. The readings of the DPPH radical scavenging activity was expressed in percentage inhibition and IC_{50} obtained from the plot of percentage inhibition against concentration (Fig 1).

The results obtained shows that the leaf and bark extracts have a high percentage inhibition values

which were within the range of that of the standard which therefore suggests that the methanolic leaf and bark extract of *E. angolense* exhibits free radical scavenging activity.

The IC_{50} is the concentration that causes 50% inhibition of DPPH radical. An increase in the IC_{50} value denotes a decrease in antioxidants activity that is, the higher the IC_{50} value, the lower the antioxidant activity and vice versa. According to the results obtained, the IC_{50} values extrapolated from the graph for the standards ascorbic acid and Gallic acid are 0.40 and 0.25 µg/ml respectively and that of the methanolic leaf and bark extracts are 0.41 and 0.45 µg/ml. This shows a close relationship between antioxidant activities of the *E. angolense* and ascorbic acid as the values are within the same range.

The antioxidant activity was also measured using the nitric oxide scavenging activity assay. The extracts, ascorbic acid and Gallic acid demonstrated noticeable antioxidant effects at all tested concentration. The readings of the nitric oxide radical scavenging activity was expressed in percentage inhibition which IC_{50} values obtained from the graph (Fig 3).

The IC_{50} values extrapolated from the graph for the standards ascorbic acid and Gallic acid are 0.40 and 0.35 µg/ml respectively and that of the methanolic leaf and bark extracts are 0.38 and 0.42 µg/ml. This shows a close relationship between antioxidant activities of the *E. angolense* and ascorbic acid as the values are within close range.

The finding of this present study suggests that *Entandrophragma angolense* could be a potential source of natural antioxidant that could have greater importance as therapeutic agent in preventing or slowing accumulation of lipid peroxidation, and thereby oxidative stress related degenerative diseases.

CONCLUSION

The extract of *E.angolense* bark and leaf showed the presence of wide range of phytoconstituents including alkaloids, saponins, tannins, flavonoids, reducing sugars, and anthraquinone which are implicated in the wide uses of the plant. The extracts also demonstrated good antioxidants activities in-vitro which is comparable to ascorbic acid used as first standard, but less than that of gallic acid used as the second standard. The antioxidant effects may be due to its modified triterpenoids (limonoids) and flavonoid contents with free radical scavenging activity.

REFERENCES

Abdou, H.M. (2011). Comparative Antioxidant Activity study of some Edible Plants Used Spices in Egypt. *J. American Sci.;* **7**:1118- 1122.

Alisi, C.S. and Onyeze, G.C. (2008). Nitric oxide scavenging ability of ethyl acetate fraction of methanolic leaf extracts of *Chromolaena odorata* (Linn.). *African Journal of Biochemistry Research;* **2:** 145−150.

Asiamah, I., (2000). Chemical constituents of the seeds of *Entandrophragma angolense*, B.Sc. Chemistry degree thesis, Department of Chemistry, Faculty of Science, University of Cape Coast, Cape Coast, Ghana, 36.

Bodeker, C., Bodeker, G., Ong, C. K., Grundy, C. K., Burford, G. and Shein, K. (2005). WHO Global Atlas of Traditional, Complementary and Alternative Medicine. Geneva, Switzerland: World Health Organization

Burits, M., and Bucar, F. (2000). Antioxidant activity of *Nigella sativa* essential oil. *Phytother Research;* **14**: 323−328.

Chang, C., Yang, M., Wen, H. and Chern, J. (2002). Estimation of total flavonoids content in propolis by two complementary colorimetric methods. *Journal of Food and Drug Analysis*; **10**:178-182.

Cuendet, M., Hostettmann, K., and Potterat, O. (1997), 'Iridoid glucosides with free radical scavenging properties from *Fagraea blumei. Helvetica Chirurgica ACTA;* **80**: 73−83.

Evans, W.C. (2005), Biologically active compound from marine organisms. Trease and Evans Pharmacognosy. W.B Saunder publisher 15th edition, revised with assistance of Evans, Daphne, 7:31-34.

Lagou, S.M., Lebri, M., Tilaoui, M., Achibat, H., Kone, W.M., Tra Bi, H.F., Ait Mousse, H., Ouchetto, H., Hafid, A., Zyad, A. and Khouili, M. (2016). Phytochemical analysis and in vitro antioxidant activity of extracts of *Entandrophragma angolense* (Welw.) C.DC. (Meliaceae) a medicinal plant used in the treatment of obstetric fistula in Ivory Coast. *Der Pharma Chemica;* **8**(10): 192-197.

Lee, J., Koo, N. and Min, D.B. (2004). Reactive oxygen species, ageing & antioxidative neutraceuticals', *Comp Rev Food Sci Food Safety*; **3**:21-33.

Mohamed, I., Shuid, A., Borhanuddin, B. and Fozi, N. (2012). The Application of Phytomedicine in Modern Drug Development. T*he International Journal of Herbal and Plant Medicine*; **1** (2): 1-9.

Nsiama, T. K., Okamura, H., Hamada, T., Morimoto, Y., Doe, M., Iwagawa, T., and Nakatani, M. (2011). Rings D-seco and B, D-seco tetranortriterpenoids from root bark of *Entandrophragma angolense*, *Phytochemistry*; **72**(14): 1854-1858.

Oluwole, F.S., Omolaso, B.O and Ayo, J.A. (2007). Methanolic Extract of *Entandrophragma angolense* induces Gastric mucus cell counts and gastric mucus secretion. *Journal of biological sciences*; **7**(8): 1531-1534

Orisadipe, A.T., Adesomoju, A.A., D'Ambrosio, M., Guerriero, A. and Okogun, J.I. (2005). Tirucallane triterpenes from the leaf extract of *Entandrophragma angolense*. *Phytochemistry*; **66** (19):2324-2328.

Papas, A.M. (1999). Diet and antioxidant status. *Food Chem Toxicol.;* **37**:999–1007.

Prota (2008). Plant resources of Tropical Africa: Timber I. Eds: Loupper, D., Otng-Amoako, A.A., Brink, M. Prota Foundation/ Backhuys Publishers, Wageningen, Netherland; **7**(1):232

Rates, S.M.K. (2001). Plants as source of drugs, *Toxicon*; **39**: 603-613.

Seigler, D.S. (1998). Plant Secondary Metabolism', Springer Science + business Media. New York, 477.

Sies, H. (1997). Oxidative stress: Oxidants and antioxidants. *Exp. Physiol.;* **82**(2): 291-295.

Soetan, K.O. and Aiyelaagbe, O.O. (2009). The need for bioactivity- safety evaluation and conservation of medicinal plants – A review. *Journal of medicinal plants Research*; **3**(5): 324-328.

Sofowora, E.A. (1982). Screening plants for bioactive agents in medicinal plants and traditional medicine in Africa, John Wiley and sons Ltd New York.

Tiwari, P., Ahirwar D., and Dwivadi S. (2014). Impact of plants having potential action on CNS: An overview. *Asian Journal of Pharmacy and Technology*; **4**(2):100-105.

Trease, G.E. and Evans, W.C. (1998), Pharmacognosy 13th edition. Bialliere Tinalli Ltd, London.

WHO (2008). Trdaitional medicine. In fact sheet 134. Available at http://www.who.int/mediacentre/factsheets/fs134/en/. Accessed: 23th May, 2018

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