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## Phytochemical Screening and Preliminary TLC Characterization of Alkaloids in *Sabicea brevipes* Root

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### ABSTRACT

*Sabicea brevipes* (susu) plant root in Oghe Community medicine is of value in the management of male erectile dysfunction. The objective of the study was to evaluate the phytochemicals and the number of alkaloids present. Phytochemical screening of n-hexane, ethyl acetate and methanol crude extract of *Sabicea brevipes* (susu) root were evaluated. The extracts were subjected to qualitative chemical analysis for identification of various secondary metabolites or active principles. The extracts revealed the presence of steroids, alkaloids, glycosides, saponins, tannins, triterpenoids, anthracene, flavonoids and volatile oils. The Result of this study x-rayed valuable evidence in support of *Sabicea brevipes* plant as an enhancer of sexual function in men. Phyto-chemical investigation resulted in isolation and TLC identification of several alkaloids using five solvent system. Results showed that the yields of the extract were 0.43% and 0.29% for morphine and non-morphine alkaloids, respectively. There were seven spots observed in the chromatogram developed for morphine alkaloids while five spots were seen for the other (non-morphine) alkaloids in the various solvent system used, visualizing with dragendoffs location reagent. The result shows that the solvent systems A chloroform: ethanol (9:1), C methanol Ammonia (99:1) and E chloroform: Methanol: Ammonia (60:60:1) contain one alkaloid each for morphine and non morphine alkaloids with  $R_f$  values 0.64 and 0.55 (solvent system A); 0.55 and 0.27 (solvent system C) and 0.55 and 0.52 (solvent system E)s, respectively. While solvent systems B chloroform: ethanol (85:15) had two spots for morphine alkaloids with  $R_f$  value 0.34 and 0.84 and solvent system D, Methanol: ethyl acetate: ammonia (9.5:81:9.5) also had two spots for morphine alkaloids with  $R_f$  of 0.27 and 0.95 and two spots for non-morphine alkaloid with  $R_f$  0.60 and 0.95, respectively. These results support the claim about the use of this herb in folk medicines.

**Key words:** Phytochemical screening, root extracts, *Sabicea brevipes*, active principles, alkaloids

### INTRODUCTION

Herbal remedies from plants continue to provide a popular alternative for treating known and emerging disease that have defiled many orthodox medical treatments. Herbal medicines are also in great demand in the developing world for primary health care because of their efficiency, safety and lesser side effects. According to World Health Organization (WHO) traditional medicines are relied upon by 60-80% of the world's population for their primary health care needs (WHO, 2002;

Zhang, 2004). Most of these herbal remedies have stood the test of time, particularly for the treatment of allergic, metabolic and cardio-vascular diseases (Igoli *et al.*, 2005). *Sabicea brevipes* plant belongs to the rubiaceae family that has more than 6,500 species out of which 152 are members of the *Sabicea* genus (Davis *et al.*, 2009). It is an erect or climbing shrub, which is usually 0.6-1.2 m in height. The shrub has a mass of red flowers in the month of July. It is normally found in some part of Africa, Madagascar and America.

There is a need for novel erectile dysfunction treatment drugs, as some of the currently available drugs are frequently associated with lots of side effects. A typical example is with Viagra known for abnormal vision, chest pain, diarrhea, dyspepsia, flushing, headache, hypertension, indigestion, nausea, palpitation, photophobia, priapism and temporary rash with possible severe side effects of intraocular pressure, myocardial infarction, severe hypotension, stroke, sudden death and ventricular arrhythmias (Akash *et al.*, 2005). There is some evidence suggesting that *Sabicea brevipes* plant root may help in remedying erectile dysfunction. *Sabicea brevipes* is a herb used for centuries in Oghe community traditional folk medicine practice as a remedy for erectile dysfunction. Indeed, more than 60 species are used for more than 70 medicinal indications which include sexual weakness (Karou *et al.*, 2011). Maca remains one of the most popular natural remedies in Peru for erectile dysfunction and as an overall male sexual stimulant and libido aid (Oshima *et al.*, 2003). Ashwagandtia, tribulus berries, catuaba (Antunes *et al.*, 2001); *Lepidum meyenii* walp, damianana, Yohimbe (*Pausinystalid yohimbe*) (Ernst and Pittler, 1998; Rowland *et al.*, 1997); Muria Puama (*Lirisoma ovata*), Ginkgo Biloba (*Folium ginkgoidis*) all contain different kinds of alkaloids that act as vasodilator and androgen precursor. Medical studies have shown that muria puama and catuaba dilates blood vessels and are strong tonic and nervous system fortifies due to the alkaloids present in them that enhance erectile function (Hollman and Katan, 1999; Di Carlo *et al.*, 1999).

It can be said that *Sabicea brevipes* when probably in central nervous system increase blood flow to the penis. Men with penile circulatory problem should typically get the best result with the root while psychological impotence also fare well using the root product (Reid, 2004; Brown 1995). The overall objective of this study is an assessment of the extracts of *Sabicea brevipes* (susu) plant root samples for selected active ingredients responsible for enhancement of erectile functioning in men and to isolate and conduct preliminary TLC characterization of alkaloid extracted from the roots of Nigeria *Sabicea brevipes* using selected solvent systems.

## **MATERIALS AND METHODS**

**Sample collection:** The plant material (*Sabicea brevipes* root) was collected from Oghe in Ezeagu Local Government Area of Enugu State Nigeria on 10th July 2009 by 11.15 a.m. Presented and defended on September 2011. The herbarilum specimen was identified by Prof. J.C. Okafor of Applied Biology and Biotechnology, Enugu State University of Science and Technology, Enugu State, Nigeria.

**Extraction and preparation of plant extract:** The root was sun dried for 14 days and pulverized with mechanical grinder. About 42.7 g the powdered plant material was weighed and extracted successively and exhaustively with n-hexane, ethyl acetate and methanol for 4 h, respectively using soxhlet extractor technique. The extracts were concentrated on a reduced pressure to yield a brown-coloured paste (n hexane extract 24.8 mg, ethyl acetate extract 19.4 mg

and methanol extract 27.3 mg, respectively). The crude root extracts were later screen qualitatively for the phytochemical constituent utilizing standard methods of analysis (Vishnoi, 1979; Sofowora, 1993; Trease and Evans, 2002).

**Phytochemical tests:** To about 2.5 cm<sup>3</sup> of each extract was added to 10 cm<sup>3</sup> of distilled water in a test tube and shaken vigorously with 2 cm<sup>3</sup> of olive oil. Fronting which persisted was taken as an evidence for the presence of saponins (Sofowora, 1993). Exactly 2.5 cm<sup>3</sup> of each extract was added to 2.5 cm<sup>3</sup> of mixed fehling solution A and B in a test tube. The appearance of bluish green precipitate indicates the presence of sugar bonded to saponin as a non-carbohydrate moiety (Sofowora, 1993). Liebermann-Burchard Test for Steroid and Triterpenoids: To 2 cm<sup>3</sup> of each extract was added to acetic acid in a test tube and conc. sulphuric acid (H<sub>2</sub>SO<sub>4</sub>) was carefully added. Colour development from violet to blue-green indicates the presence of a steroidal ring (Sofowora, 1993). Diluted sulphuric acid was added to each extract in a test tube and boiled for 15 min. Then 10% sodium hydroxide and mixed Fehling's solution were added. The formation of brick red precipitate indicates the presence of glycoside (Sofowora, 1993). A drop of ferric chloride was added to each extract in a test tube. Then glacial acetic acid and conc. H<sub>2</sub>SO<sub>4</sub><sup>-</sup> were added. The development or appearance of blue layer indicates the presence of digitalis glycosides (Sofowora, 1993). Born-Traggers Test was used to detect antracenes or anthraquinones. Two 2 cm<sup>3</sup> of chloroform was added to each extract in a test tube and allowed to separate. To the chloroform layer was added 10% ammonia solution and vigorously shaken and kept to separate. The formation of brick-red precipitate indicates the presence of antracenes or anthraquinones (Sofowora, 1993). A mixture of 4 cm<sup>3</sup> of each extract and 4 cm<sup>3</sup> of distilled water was stirred very well in a test tube and 3 drops of ferric chloride added. The occurrence of a blue-black, green or blue-green precipitate indicates the presence of tannins (Trease and Evans, 2002). About 4 cm<sup>3</sup> of 10% ammonia solution was added to 4 cm<sup>3</sup> of each extract in a test tube and shaken very well. The formation of an emulsion indicates the presence of hydrollisable tannins (Sofowora, 1993). A matchstick was dropped into a 3 cm<sup>3</sup> of each extract in a test tube and 2 drops of conc. HCl added. Then the resulting mixture was left undisturbed for 5 min. The development of a dark purple colouration indicates the presence of pseudotannins (Sofowora, 1993). Shinodas test for Flavonoids a small quantity of magnesium chips was dropped into 3 cm<sup>3</sup> of each extract in a test tube and 5 drops of conc. HCl added. A pink, orange, or red to purple colouration indicates the presence of flavonoids (Trease and Evans, 2002). Resins was detected by adding 2 cm<sup>3</sup> of acetic anhydride to 2 cm<sup>3</sup> of each extract in a test tube. Then 2 drops of conc. H<sub>2</sub>SO<sub>4</sub> was added to the mixture. The appearance of violet colouration indicates the presence of resins (Sofowora, 1993). The presence of alkaloids was confirmed thus: 2 cm<sup>3</sup> of each extract was taken individually into 4 test tubes. To the test-tubes, 2 drops of Dragendorff's reagent, Mayer's reagent, Wagners's reagent and 10% (w/v) tannic acid were added, respectively. Occurrence of orange-red precipitate, appearance of blue-coloured precipitate, observation of a deep brown precipitate and development of cream colouration is a positive tests, respectively (Sofowora, 1993; Trease and Evans, 1978, 1989). Six drops of Ferric chloride was added to a mixture of 2 cm<sup>3</sup> of each extract and 2 cm<sup>3</sup> of 90% (v/v) ethanol in a test tube. The observation of green colouration indicates the presence of volatile oil (Sofowora, 1993).

**Isolation and purification of alkaloids:** The roots of the harvested plant sample were cut off, dried and ground using a mechanical grinder. About 180 g of ground plant material was acidified

with alcoholic solution of tartaric acid (15 g tartaric acid dissolved in 900 cm<sup>3</sup> of 95% ethanol) and warmed to 50°C. This was left in 2000 cm<sup>3</sup> conical flask to stand overnight and thereafter filtered. The filtrate was partially evaporated and filtered again to remove fat and protein before the evaporation was completed. The residue was dissolved in boiling 100 cm<sup>3</sup> of 95% (v/v) ethanol and the solution cooled overnight in a refrigerator. The alcoholic extract was filtered and the filtrate evaporated to dryness under reduced pressure. The residue was dissolved in 50 cm<sup>3</sup> of 2% sulphuric acid (v/v) and the fat extracted with light petroleum ether (40-60°C). The light petroleum ether extract was washed with 200 cm<sup>3</sup> of 2% sulphuric acid (v/v) and the washing added to the aqueous solution and later neutralized extract was evaporated to dryness and the resulting residue dissolved in 100 cm<sup>3</sup> sodium carbonate. The neutralized extract was evaporated to dryness and the resulting residue dissolved in 100 cm<sup>3</sup> of absolute ethanol and filtered and the filtrate evaporated to dryness under reduced pressure. The residue was dissolved in 100 cm<sup>3</sup> of 1 Mol dm<sup>-3</sup> acetic acid and 50 cm<sup>3</sup> of 0.25 mol dm<sup>-3</sup> aqueous solution of lead acetate and warmed. The coagulated precipitate was filtered off. The excess lead was removed by cautiously adding in drops dilute sulphuric acid and the lead sulphate filtered off. At this stage non-basic poisons were removed by extracting twice with 30 cm<sup>3</sup> petroleum ether. The aqueous liquid was made alkaline with 2 mol dm<sup>-3</sup> sodium hydroxide and the alkaloids except morphine were extracted three times by shaking with 25 cm<sup>3</sup> of chloroform. The chloroform extract was later evaporated to dryness in a water bath and the alkaloid weighed and the weight recorded.

For the morphine alkaloid analysis the procedure was thus: the aqueous liquid was acidified with 2 mol dm<sup>-3</sup> of H<sub>2</sub>SO<sub>4</sub> and then made alkaline with ammonia and equal volume of methanol (110 cm<sup>3</sup>) added. The morphine alkaloid was extracted three times with 25 cm<sup>3</sup> of chloroform and the extracts evaporated in a water bath and the morphine alkaloid weighed and the weight recorded (Allport, 1951; Dickel *et al.*, 1958; Grinkerich and Safronich, 1983).

**TLC analysis for alkaloids:** All thin-layer chromatographic analysis was performed using TLC plates pre-coated aluminium oxide F-254 (Type E) with layer thickness of 0.25 mm made in Germany by E. Merck, Darmstadt.

TLC plate (F-254, type E) was used to separate the active compounds present in the extracts (Hashmi *et al.*, 1968). The TLC sheets after development are visualized by spraying with Dragendoff's reagent.

## RESULTS AND DISCUSSION

The result of the phytochemical screening tests conducted on the crude extracts of *Sabicea brevipes* roots shows the presence of saponins, tannins, glycosides, alkaloids, steroids and triterpenoids as well as volatile oils as shown in Table 1.

The phytochemical test of the crude n-Hexane, ethyl acetate and methanol extracts of *Sabicea brevipes* roots revealed the presence of steroids, glycosides, alkaloids, saponins, tannins and volatile oil (Table 1). The active principles identified in the n-hexane extract (HE) were steroids, alkaloids (in Wagner's and Dragendorff's reagents). Surprisingly, the Mayers and Tannic Acids tests for alkaloids were negative. This observation for alkaloids also occurred in the Ethyl Acetate Extract (EA) and Methanol Extract (ME).

The active ingredients found in the ME were saponins, saponin glycosides, tannins, pseudo tannins, steroids and volatile oils.

Table 1: Phytochemical constituents of root of *Sabicea brevipes*

Constituents	Test	Inference		
		HE	EEA	ME
Saponins	Frothing Sofowora (1993)	-	-	+
Saponin glycosides	Fehling's Sofowora (1993)	-	-	+
Tannins	Ferric chloride Trease and Evans (2002)	-	-	+
Hydrolysable tannins	Ammonia Sofowora (1993)	-	-	-
Pseudo tannins	Matchstick and HCl Sofowora (1993)	-	-	-
Glycosides	Fehlings Sofowora (1993)	-	-	+
Digitalis glycosides	Ferric chloride, glacial acetic acid Sofowora (1993)	-	-	-
Anthracenes	Borntrager's Sofowora (1993)	-	-	-
Steroids/triterpenoids	Liebermann-Buchard's Sofowora (1993)	+	-	-
Alkaloids	Wagner's Sofowora (1993)	+	+	+
	Mayer's Trease and Evans (2002)	-	-	-
	Dragendorff's Trease and Evans (1989)	+	-	+
	Tannic Acid's Sofowora (1993)	-	+	-
Volatile oils	Sofowora (1993)	-	-	+
Flavonoids	Shinoda's Trease and Evans (2002)	-	-	-

+: Present, -: Absent, HE: N-Hexane extract, EA: Ethyl acetate extract, ME: Methanol extract

The root extracts in the three solvents-HE, EA and ME-show no presence (negative results) for the following active principles: hydrolysable tannins, pseudo tannins, digitalis glycosides, anthracenes, Mayers' test alkaloids, tannins acid test alkaloid, flavonoids and triterpenoids.

This positive observations help in providing chemotaxonomic evidence for the classification of the species since *Sabicea brevipes* belongs to the rubiaceae family, which has been reported to contain these compounds (Stary and Storchova, 1991). The identification of these family of compounds further supports claims of the use of the root as a traditional medicine as these compounds have valuable amoebicidal, anti-fungal and anti-inflammatory properties (Opakunle and Jayicoba, 1988). They have stimulating and tonifying effects on the muscles when consumed and this probably accounts for their use in enhancing male potency. Furthermore, the metabolites: alkaloids, steroids, glycosides, saponins and tannins found in the root extracts are known to have curative activity against several pathogens and therefore the plant root is use traditionally for the treatment of various illnesses (Hassan *et al.*, 2004; Usman and Osuji, 2007).

Probably the constituents of *Sabicea brevipes* from which its male potency enhancing properties emanated are based upon the actions of certain steroidal alkaloid and glycosides. A study has implicated saponin component of plants in enhancing aphrodisiac properties due to its androgen increasing property (Gauthaman *et al.*, 2002). Saponins present in the methanol extract of this plant might have assisted in stimulating an increase in the body natural endogenous testosterone levels by raising the level of Leutinizing Hormones (LH). The LH release normally by the pituitary gland helps to maintain testosterone levels, as LH increases, so does the testosterone (Gauthaman *et al.*, 2002). The increase in testosterone seemed to have translated into the male sexual competence. Furthermore, this study suggests that the aphrodisiac action may be mediated through a change in the blood testosterone level (Yakubu *et al.*, 2005).

Analysis of *Sabicea brevipes* root using aluminium oxide TLC, eluted with chloroform/ethanol (A) solvent system followed by staining with Dragendorff reagent, showed one orange brown sport

Table 2: Yield, solvent system composition and R<sub>F</sub> values of the various alkaloids

Solvent system code	Composition/ratio	R <sub>F</sub> values for Morphine	R <sub>F</sub> Values for Non-Morphine
		Alkaloids (0.77 g)	Alkaloids (0.52 g)
A	Chloroform/Ethanol (9:1)	0.64	0.55
B	Chloroform/Ethanol (85:15)	0.84; 0.34	NF
C	Methanol/Ammonia (99:1)	0.55	0.27
D	Methanol/ethylacetate/ammonia (9.5:81:9.5)	0.27; 0.95	0.60; 0.95
E	Chloroform/methanol/Ammonia (60:60:1)	0.55	0.52

NF: Not found

with R<sub>F</sub> values 0.64 and 0.55 each for morphine alkaloids and non-morphine alkaloids, respectively. Organ brown spot (R<sub>F</sub> value of 0.84 and 0.34) were observed only for morphine alkaloids using the chloroform/ethanol (B) solvent system. The “solvent system” methanol/ammonia (C) solvent system showed one spot for morphine alkaloids (R<sub>F</sub> value 0.55) and also one spot for morphine alkaloids (R<sub>F</sub> value 0.55) and also one spot each for non-morphine alkaloids (R<sub>F</sub> value 0.27). Solvent system D (methanol/ethyl acetate/ammonia) show two spots for morphine alkaloids (R<sub>F</sub> values 0.27 and 0.95) and also two spots for the non-morphine alkaloids (R<sub>F</sub> value 0.60 and 0.95). Solvent system E (chloroform/methanol/ammonia) gave one spot each for the morphine and non-morphine alkaloids with R<sub>F</sub> value of 0.55 and 0.52, respectively. Their R<sub>F</sub> values were quite close compared with other solvent systems (Table 2).

Results show that morphine alkaloids contain highest number of spots and percent yield which are 7 spots and 0.433%, respectively; while the non morphine (other) alkaloids had 5 spots and 0.25% yield (Table 2). These findings supported the view that alkaloids are commonly present in *Sabicea brevipes* (Stary and Storchova, 1991). Therefore further studies are needed to isolate these active compounds and elucidate their chemical structures and highlight their bioefficacies.

## CONCLUSION

The results of this study shows that the three extracts of the root of *Sabicea brevipes* indicates the presence of alkaloids in all the extracts and the presence of saponins, saponin glycosides, tannins and volatile oils only in methanol extracts. While steroids/triterpenoids are only present in n hexane extract.

The results also revealed that morphine alkaloids is the main alkaloids (0.43%) in *Sabicea brevipes* than non-morphine alkaloids.

The plant parts studied here has also been seen as a potential source of useful drugs. Further studies are going on in order to isolate, identify characteristics and elucidate the structure of the bioactive compounds. Male potency enhancing activities of the plant root for the treatment of erectile dysfunction as claimed by traditional healers are also being comprehensively investigated.

## REFERENCES

- Akash, R., D. Hrishikesh, P. Amith and S. Sabah, 2005. Case report: Association of combined nonarteritic anterior ischemic optic neuropathy (NAION) and Obstruction of cilioretinal artery with overdose of viagra. J. Ocul. Pharmacol. Ther., 21: 315-317.
- Allport, N.L., 1951. Colorimetric Analysis. 3rd Edn., Chapman and Hall Ltd., London, pp: 287-289.
- Antunes, E., W.M. Gordo, J.F. de Oliveira, C.E. Teixeira, S. Hyslop and G. de Nucci, 2001. The relaxation of isolated rabbit corpus cavernosum by the herbal medicine catuama (B) (and its constituents). Phytoters Res., 15: 416-421.

- Brown, D., 1995. Encyclopedia of Herbs and their Uses. University of Chicago Press, Chicago, pp: 249-282.
- Davis, A.P., R. Govaerts, D.M. Bridson, M. Ruhsam, J. Moat and N.A. Brummitt, 2009. A global assessment of distribution, diversity, endemism and taxonomic effort in the rubiaceae. Ann. Missouri Bot. Garden, 96: 68-78.
- Di Carlo, G., N. Mascolo, A.A. Izzo and F. Capasso, 1999. Flavonoids: Old and new aspects of class of natural therapeutic drugs. Life Sci., 65: 337-353.
- Dickel, D.F., C.L. Holden, R.C. Maxfield, L.E. Paszek and W.I. Taylor, 1958. The alkaloids of Tabernanthe iboga. Part II. Isolation studies. J. Amer. Chem. Soc., 80: 123-125.
- Ernst, E. and M.H. Pittler, 1998. Yohimbine for erectile dysfunction: A systematic review and meta-analysis of randomized clinical trials. J. Urol., 159: 433-436.
- Gauthaman, K., P.G. Adaikan and R.N. Prasad, 2002. Aphrodisiac properties of *Tribulus terrestris* extract (Protodioscin) in normal and castrated rates. Life Sci., 71: 1385-1396.
- Grinkerich, N. and L.N. Safronich, 1983. The chemical analysis of medicinal plants. Bioorganic Med. Chem. Lett., 21: 132-136.
- Hashmi, M.H., S. Parveen and N.A. Chughial, 1968. Semiquantitative determination of alkaloids by circulars thin-layer chromatography. Mikrochimica Acta, 57: 449-495.
- Hassan, M.M., A.O. Oyewale, J.O. Amupitan, M.S. Abdullahi and B. Okonkwo, 2004. Preliminary phytochemical and antibacterial investigation of crude extracts of the root bark of *Detarium microcapum*. J. Chem. Soc. Nig., 29: 26-29.
- Hollman, P.C.H. and M.B. Katan, 1999. Dietary flavonoids: Intake, health effects and bioavailability. Food Chem. Toxicol., 37: 937-942.
- Igoli J.O., O.G. Ogaji, T.A. Tor-Anyiin and N.P. Igoli, 2005. Traditional medicine practices amongst the igede people of Nigeria. Afr. J. Trad. CAM, 2: 134-152.
- Karou, S.D., T. Tchacondo, D.P. Ilboudo and J. Simporé, 2011. Sub-saharan rubiaceae: A review of their traditional uses, phytochemistry and biological activities. Pak. J. Biol. Sci., 14: 149-169.
- Opakunle, W.O. and K.T. Jaiyeoba, 1988. Pharmaceutical raw materials sourcing in Nigeria. Proceedings of the 1st National Conference of the Environmental Health Society of Nigeria, August 1991, Zaria, Nigeria, pp: 4.
- Oshima, M., Y. Gu and S. Tsukada, 2003. Effect of *Lepidium meyenii* Walp and *Jatropha macrantha* on blood levels of estradiol 17  $\beta$ , progesterone, testosterone and the rate of embryo implantation in a mice. J. Vet. Med. Sci., 65: 1145-1146.
- Reid, J., 2004. Chinese Herbal Medicine: Material Medica. 3rd Edn., University of Hong Kong, China, pp: 148-150.
- Rowland, D.L., K. Kallan and A.K. Slob, 1997. Yohimbine, erectile capacity and sexual response in men. Arch. Sex. Behav., 26: 49-62.
- Sofowora, A., 1993. Screening Plants for Bioactive Agents: Medicinal Plants and Traditional Medicine in Africa. 2nd Edn., Spectrum Books Ltd., Sunshine House, Ibadan, Nigeria, ISBN-13: 978-0471103677, pp: 134-156.
- Sary, F. and H. Storchova, 1991. A Mutual Guide to Medicinal Herbs and Plants. Tiger Books International, UK., Pages: 44.
- Trease G.E. and W.C. Evans, 1978. A Text Book of Pharmacognosy. 11th Edn., Bailliere Tindall, London, pp: 60-75.
- Trease, G.E. and W.C. Evans, 1989. Pharmacognosy. 13th Edn., ELBS/Bailliere Tindall, London, UK., pp: 345-346, 535-536, 772-773.

- Trease, G.E. and W.C. Evans, 2002. Pharmacognosy. 15th Edn., WB Saunders, London, ISBN: 8131200876, pages: 406.
- Usman, H. and J.C. Osuji, 2007. Phytochemical and *in vitro* antimicrobial assay of the leaf extract of *Newbouldia laevis*. Afr. J. Trad. Compl. Alt Med., 42: 476-480.
- Vishnoi N.R., 1979. Advanced Practical Chemistry. Yikas Publication House, India, pp: 447-449.
- WHO, 2002. The World Health Report: Reducing Risks, Promoting Healthy Life. World Health Organization, Geneva.
- Yakubu, M.T., M.A. Akanji and A.T. Oladiji, 2005. Aphrodisiac potentials of the aqueous extract of *Fadogia agrestis* (Schweinf. Ex Hiern) stem in male albino rats. Asian J. Androl., 7: 399-404.
- Zhang, X., 2004. Traditional Medicine: Its Importance and Protection. In: Protecting and Promoting Traditional Knowledge: Systems, National Experiences and International Dimensions. Part 1. The Role of Traditional Knowledge in Healthcare and Agriculture, Twarog, S. and P. Kapoor (Eds.). United Nations Publications, New York, pp: 3-6.