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Identification of Two Phytosterol and a Glycoside in *Sabicea Brevipes* Plant Root Extract

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Abstract

Thin-layer chromatographic (TLC) investigation of the petroleum spirit and ethanol extracts of the roots of *Sabicea brevipes* for steroids and glycosides showed (**1**) Dehydro epi androsterone (DHEA), (**2**) 17-Hydroxyprogesterone steroids and (**3**) one type of glycoside. The name and structures of **1** and **2** were identified by comparison of their R_F values with those reported in the literature.

Keywords: Dehydro epi androsterone; 17-Hydroxyprogesterone; glycosides; *Sabicea brevipes;* Erectile dysfunction.

Introduction

Herbal remedies continue to provide a popular alternative for men and women seeking to improve their sexual life despite the increasing availability of effective conventional and orthodox medical treatment. (David and Wendi, 2003). A man's or a woman's level of the sex hormone testosterone or progesterone respectively after peaking in his or her 20s tend to gradually decrease and continue to further decline throughout the remainder of their years. This decrease is often accompanied by closely related changes in everything from skin tone and lean muscle mass to a gradual erosion of libido and the beginning, in some cases, of erectile dysfunction (Moffat, 2005; Beauchet, 2006).

Firstly, relevant aspects of sexual function are defined on the basis of a modified version of Masters and Johnson's pioneer work (Helgason *et al*., 1996; Masters and Jonson, 1966, 1970). The aspects of sexual function defined as being relevant to the assessment include sexual desire (libido) (Nimbi *et al*., 2018; Warnock *et al.* 2006). Which depends on factors such as drugs that acts on the central nervous system, individual's lifestyle (da Cruz et al., 2017; Kendirci *et al.*2005).), personal attractiveness and biological fitness of one's partner seem to markedly affect libido (Nimbi *et al*., 2020).

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Erection which occurs as a result of complex neuro-psychologic processes that requires the interaction of the brain, nerves, hormones, and blood vessels (Hsu, and Liu, 2018; Müntener and Schurch, 2004). Anything that interferes with this chain of events - either by reducing or increasing the blood flow to the penis, fear, anxiety, anger, or any other strong emotion or an illness or physical condition can interrupt the signal from the brain and can cause erectile dysfunction (Hsu, 2018). Ejaculation is the act of semen coming out through the penis (Walter and Emile, 2005; Bohlen *et al*., 1980). Disorder of Ejaculation are Premature Ejaculation (El-Hamd *et al*., 2019), Delayed Ejaculation (Sante *et al*., 2016) and Retrograde Ejaculation (Mehta and Sigman, 2015) which may be causes by physiological and/or psychological which include excessive masturbation, prostate problems, stress, hypertension, hormonal disturbance and weak musculature. Others are performance anxiety and pressure (Schaffir, 2006). An orgasm (or sexual climax) is the conclusion of the plateau phase of the sexual response cycle, and may be experienced by both males and females. Orgasm is characterized by intense physical pleasure, controlled by the involuntary or autonomic nervous system. Post orgasm is often experienced as relaxing, which is attributed to the release of prolactin which is associated with a temporary reduction in the activity of large parts of the cerebral cortex (Helgason *et. al.*, 1996). Orgasmic disorder which may be caused by either interpersonal and marital distress, psychological distress, psychiatric disorders and use of antidepressants, particularly selective serotonin reuptake inhibitors (SSRIs) (Braverman, 2004; Ho *et al*., 2020) are inability to achieve an orgasm during sexual activity which can be extremely debilitating for the individual causing a great deal of distress at personal and emotional levels.

If these capabilities decline as an effect of age or sudden physical degeneration through an accident or disease the result is that sexual dysfunction has set in and all other aspects of life diminishes. However, many factors can interfere with one or more component of the sexual function. The problem shifts from one of external factors to one of internal factors: how to modify one's body chemistry so that the levels of sexual functioning and sexual satisfaction will be optimal. (Chawla and London 2018). Erectile dysfunction (ED, or "male impotence") is a sexual problem characterized by the inability to develop or maintain an erection of the penis sufficient for satisfactory sexual performance (Milenković and Albersen, 2018; Frederick et al., 2018; Cunningham and Rosen, 2018; Chowdhury *et al*., 2017). It is a serious life – altering problem for millions of men. A man's inability to achieve or maintain an erection is inevitably linked to complex feelings of inadequacy, frustration and shaken confidence, which may spill over into other areas of his life. The psychological and quality-of-life consequences of ED must not be underestimated. There are a large number of people in fear of developing erectile dysfunction due to toxicity or poison, life style, disease and old age (Amarasekera *et al*., 2020).

Sabicea brevipes – Warnham is an erect or climbing shrub, which is usually 0.6096-1.2192 meters in height (Plate 1) and belongs to the Rubiaceae family. When in full bloom in July and August, the plant produces a mass of red flowers and small, red and juicy fruit eaten in Sierra Leone and eastern Nigeria mostly (Wernham, 1914; Dalziel, 1937). In Ghana, it is called "Ashananse Ntoroma" (Irvin, 1961) and "susu" in Oghe, Ezeagu L.G.A. of Eastern Nigeria (Ogbuanu *et al*., 2014). It is usually found in some part of Africa, Madgascar and America (http://www.zipcodezoo.com/plants/s/sabicea%5brevipes/). The result of earlier phytochemical analysis of Sabicea brevipes revealed the presence of steroids, alkaloids, glycosides, saponins, tannins, triterpenoids, antracenes, flavonoids, and volatile oils (Ogbuanu *et al*., 2014).

Plate 1: Sabicea Brevipes plant image (Taxonomy ID: 409386).

Due to the side effect of some orthodox drugs such as 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, myocardinal infraction, severe hypotension, stroke, sudden death and ventricular arrahytmias (Akash *et al*., 2005) there is a need for novel drug drugs treatment for erectile dysfunction. More than 60 species are used for more than 70 medicinal indications which include sexual weakness (Karou *et al*., 2011). *Sabicea brevipes* (susu) plant root has been found to contain some of the active principles such asalkaloids, glycosides, steroids, volatile oils and fatty acids known to have positive effect on erectile dysfunction in men (Ogbuanu et al., 2014).Many other plant families and species provide popular natural remedy for erectile dysfunction and as an overall male sexual stimulant and libido aid (Oshima *et al*., 2003; Antunes *et al*., 2001; Ernst and Pittler, 1998; Rowland *et al*., 1997; Hollman and Katan, 1999; Di Carlo *et al*., 1999).

Survey indicates that *Sabica brevipes* plant root has long been in use in Oghe Community traditional medicine in the management of male erectile dysfunction. It is a material of interest among the old men of Oghe community who use it as chewing stick and swallow the fluid or take a local alcohol extract of the root to enhance their penis erection and as an overall male sexual stimulant and libido aid. The overall objective of this study is to extract and determine the type(s) of steroids and number of glycosides present in *Sabice brevipes* (susu) plant root.

Materials and Methods

Collection of samples

A live *sabicea brevipes* (Susu) plant was pulled-off from the soil (Plate 2) and its roots were cut off with the aid of a knife. The root sample were collected on 10th August 2019 by 2.45 p.m. from Oyofo Oghe communities in Ezeagu Local government Area and identified by Prof. J.C. Okafor of Applied Biology and Biotechnology, Enugu State University of Science and Technology, Nigeria. Enugu State, Nigeria.

Extraction of steroids

The powered plant root sample (180.66 g) was soaked in 1 liter of petroleum spirit for 22 hours. The extract was decanted and filtered with a filter paper. The filtrate was evaporated in a water bath to dryness. The crude steroid extract was collected and its weight recorded. (curriculum.toxicology.wikispaces.net/…/p3+L10-11+plant+steroids.ppt).

Thin – Layer Chromatographic Analysis of Steroids

The ascending technique was used. About 150 cm^3 of the running solvent (mobile phase) methanol, benzene, water was constituted in the ratio 1:2:1 and labelled (A) and was used to develop the chromatogram for 4hrs. Zimmermann location reagent was sprayed to detect keto-methylene groups in the steroids.

Another chromatogram was developed with methanol, petroleum ether ($80\text{-}100^0$) and water (4:5:1) labelled (B) and a third chromatogram was developed with methanol, petroleum ether, benzene and water (7:5:5:3) labeled (C). The second (B) and third (C) chromatograms were separately visualized with m-Dinitrobenzene (2%) in ethanol mixed with freshly prepared 2.5 mol dm^{-3} KOH and heated gently. The colour and position of the sports were noted and recorded. The third chromatogram (C) was visualized with tetrazolium reagent. The colour and position of the spots noted after dipping and warming gently. (Callow *et al*., 2013; Bhawani *et al*., 2010; Long, 1971)

Isolation of glycoside

The powdered *sabicea brevipes* (200 g) root was soaked with ethanol for one and half hour. The mixture was sieved and the liquid filtered off with filter paper and funnel. The filtrate was concentrated by evaporating the ethanol in a water bath. The brown extract was partitioned between chloroform- water (1:1) mixtures. The chloroform extract was concentrated and allowed to evaporate to dryness, to a constant weight at room temperature and the weight recorded (Bălăşoiu *et al*., 2013).

The glycoside in the aqueous fraction was extracted with $50cm^3$ of ethyl acetate (SG 0.906) was used to extract the glycoside. This extraction

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was repeated three times using 50cm^3 ethyl acetate. The various ethyl acetate extracts were combined and evaporated to dryness and the weight recorded.

Detection of Glycosides by the Thin Layer Chromatography Using Anthrone Reagent

The isolated glycosides were applied with a capillary tube. After completion of sample application, the plates were developed in a TLC chromatographic tank presaturated with benzene and ethyl – acetate (9:1); and methanol and chloroform (3:7) respectively for two hours. The TLC runs were performed under laboratory conditions. After development, the plates were taken off and dried. The spots were detected using anthrone reagent. The distance travelled by solvent front and compound was measured and retention factors (R_F) calculated. The R_F is defined as the distance travelled by the compound divided by the distance traveled by the solvent front (Chelyn *et al*., 2014).

Results and Discussion

The results of the various tests, determination and analysis are presented subsequently using tables and charts. The chromatograph of steroids was performed on all the three extracts using various solvent systems and two location reagents. The results are presented in Tables 1. The following conclusions were drawn from the results.

- No steroid was identified with tetrazolum location reagent for the three solvent systems on the three extracts.
- In the case of Zimmermann location reagent, no steroid was identified with solvent systems A and C for the three extracts.
- Two steroids were identified and they are Dehydro-epi-androsterone (R_F value 0.42) on n-hexane and ethyl acetate extract, and 17-hydroxy–progesterone (R_F value 0.54) on methanol extract for the solvent system B.

Fig.1: Structures of Dehydro-epi-androsterone (DHEA) and Fig.2: 17- Hydroxyorigesterone

Fig. 1:

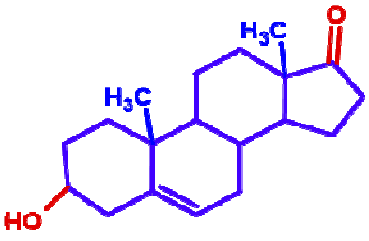


Fig. 2:

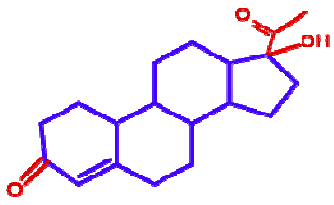


Table 1: Steroids and their R_f Values Using Zimmermann and Tetrazolium Location Reagent

Method/Solvent system		Extracts		
		n-Hexane	Ethyl acetate	Methanol
Zimmermann	A	-	-	-
(Steroids /R_F)	B	**Dehydro-epi androsterone (0.42)**	**Dehydro-epi androsterone (0.42)**	**17-Hydroxy—Progesterone** (0.54)
	C	-	-	-
Tetrazolium	A	-	-	-
(Steroids /R_F)	B	-	-	-
	C	-	-	-

Key: A→ Methanol Petroleum ether and Benzene; B→ Methanol and Petroleum ether; C→ Methanol and Benzene

Result on the percentage yield of glycosides for the various plant extracts is presented in Table 2.

Table 2: Percentage Yield of Glycosides from the Various Plant Root Extracts

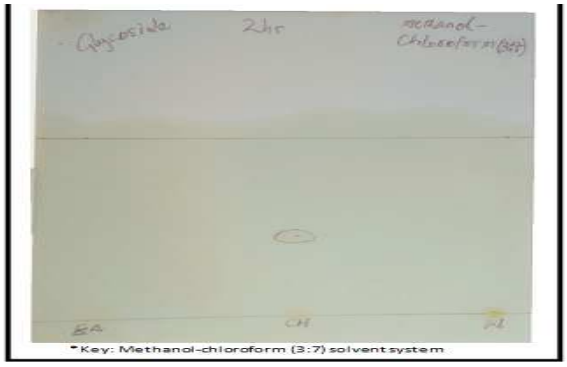
Extract	Weight of sample used (g)	Weight of extract (g)	Yield (%)
Water	200	3.82	1.91
Ethyl acetate	200	1.35	0.68
Chloroform	200	2.31	1.16

Key: Running solvent B using Zimmermann location reagent

Plate 2: TLC photoprint of Steroids identified in the roots of *sabicea brevipes*

*Key: Benzene-ethyl acetate (9:1) solvent system

Plate 3: TLC photo print of glycoside identified in the root of sabicea brevipes.



*Key: Methanol-chloroform (3:7) solvent system

Plate 4: TLC photo print of glycoside identified in the root of sabicea brevipes.

From the results of the thin-layer chromatographic analysis (Table 3), it was only chloroform extract that gave one spot each for the two solvent systems used in developing the chromatograms with R_F values of 0.95 and 0.45 for Benzene/ ethyl acetate, and methanol/ chloroform solvent systems respectively. No spot was observed in the water and ethyl acetate extracts. Consequently, there are no glycoside in them.

Table 3: R_f Values and Colour of Glycoside Extracts from *Sabicea Brevipes* Root

Solvent system	Colour of spot and R_F value of extracts		
	Water	Ethyl acetate	Chloroform
Benzene: ethyl acetate (9:1)	-	-	0.95 (Brown)
Methanol: chloroform (3:7)	-	-	0.45 (Brown)

The results of the glycoside analysis (Tables 2 and 3) showed that only the chloroform extract gave one spot each for the two solvent systems used in developing the chromatograms. These are 0.95 (Brown) and 0.45 (Brown) for the benzene ethyl acetate and methanol-chloroform solvent respectively. This indicates that only one type of glycoside was identified in the root sample.

The crude steroids extract (petroleum ether extract) yielded 2.3% of the dry powdered seeds sample. In this experiment, it was observed that two steroidal compounds were present in the crude steroids extract (Table 1). They are 17-Hydroxy-progestrone (SHP) with R_F value of 0.54 and Dehydro-epi-androsterone (DHEA) with R_F value of 0.42 (plate 2). DHEA is an endogenous steroid (www.nlm.Nih/Gov/ medicineplus/druginfo/naturalpattine-dhea-tml).

Steroids have been implicated in a broad range of biological activities in human and animals. It acts on the androgen receptor directly and through metabolites (El-Sakka *et al*., 2018). DHEA is also a potent sigma 1 agent (Romicu *et al.* 2003) and considered a neuro-steroid. DHEA influences the in vitro growth of human aorta vascular smooth muscle cells (hASMC). This probably implies that *Sabicea brevipes* widens the blood vessels resulting from relaxation of smooth muscle cells within the vessel walls, particularly in the large arteries, smaller arterioles and large veins. This widening of blood vessels is called vasodilation (http://en.wikipedia.org/wiki/vasodilation). Just as viagra, a phosphodiesterase inhibitor, works to increase blood flow in the penis through vasodilation. It significantly stimulates the mitogenesis of hASMC in the serum–free cultural (Life Science (USA), 1997). This, probably, is why it is used in the management of erectile dysfunction coupled with the fact some small placebo – controlled randomized clinical trial studies on the plant have found long term supplementation to improve mood and relieve depression (Wolkowitz *et al.* 1999; Schmidt, *et al.* 2005) or to decrease insulin resistance and also useful in patients with systemic lupuserythematosus. Furthermore, it is used by athletes to enhance performance/muscle building (Kawans *et al.* 2003).

DHEA treatment was suggested to benefit hypertension or organic etiology patients with erectile dysfunction (ED) but have no benefit on patients with diabetes mellitus and neurological disorder (Reiter *et al*., 2001).

Another steroid, found in the plant root is 17-Hydroxyprogesterone. It is a substance used by the body to make cortisol (Hamilton *et al*., 2008). Cortisol is a hormone that helps to break down protein, glucose and lipids, maintains blood pressure, and regulates the immune system (http://www/labtestsonline.org/ understanding/analytes/6_17hydroxy/saruple.html). This also is likely to have a regulatory effect on the pressure of the blood trapped in the penis and as such can enhance erection of penis.

The crude glycosides extracts (water, ethyl acetate and chloroform) yielded 1.91, 0.68 and 1.96% of the dry powdered root sample. Only the chloroform extract gave one spot each for the two solvent systems used in developing the chromatograms with R_F values and colours as in (Tables 3. These are 0.95 (Brown) and 0.45 (Brown) for the benzene-ethyl acetate (9:1) and methanol-chloroform (3:7) solvent systems respectively. This indicates that only one type of glycoside was identified in the root sample.

Glycosides are of immense importance to man which includes supportive, economic and most importantly, pharmaceutical importance. Glycosides are known to dilate the coronary arteries as well as block calcium channels and also decrease capillary fragility. From recent studies (Brito-Arias Marco, 2007), it may be inferred that glycosides could be a contributory factor to healing and growth of muscle tissues and healing of wounds.

Glycosides isolated from *sabicea brevipes* roots probably have a supporting effect on the action of the steroids and alkaloids present in *sabicea brevipes* plant thereby causing sexual enhancement.

This supports the use of *sabicea brevipes* root as sex enhancer since antiquity in Oghe, Enugu Nigeria by men with erectile problem (erectile dysfunction) and also by men and women with low libido.

Conclusion

It could therefore be concluded that *Sabicea brevipes* root have stimulating and tonifying effects on the muscles which probably makes it appropriate for use in enhancing male potency.

Dehydro-epi-androsterone influences the in-vitro growth of vascular smooth muscle cells and consequently, it can be used in management of erectile dysfunction. 17-Hydroxy progesterone helps, among others, in maintaining blood pressure and it is likely to have a regulatory effect on the pressure of the blood trapped in the penis and as such can enhance erection of penis.

The glycosides identified in *sabicea brevipes* have a supporting effect on the action of the steroids present in the plant. These active ingredients cause sexual enhancement because they are known to dilate the coronary arteries as well as block calcium channels and also cause decreased capillary fragility. This supports the use of *sabicea brevipes* root as sex enhancer since antiquity in Oghe, Enugu Nigeria by men with erectile problem (erectile dysfunction).

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