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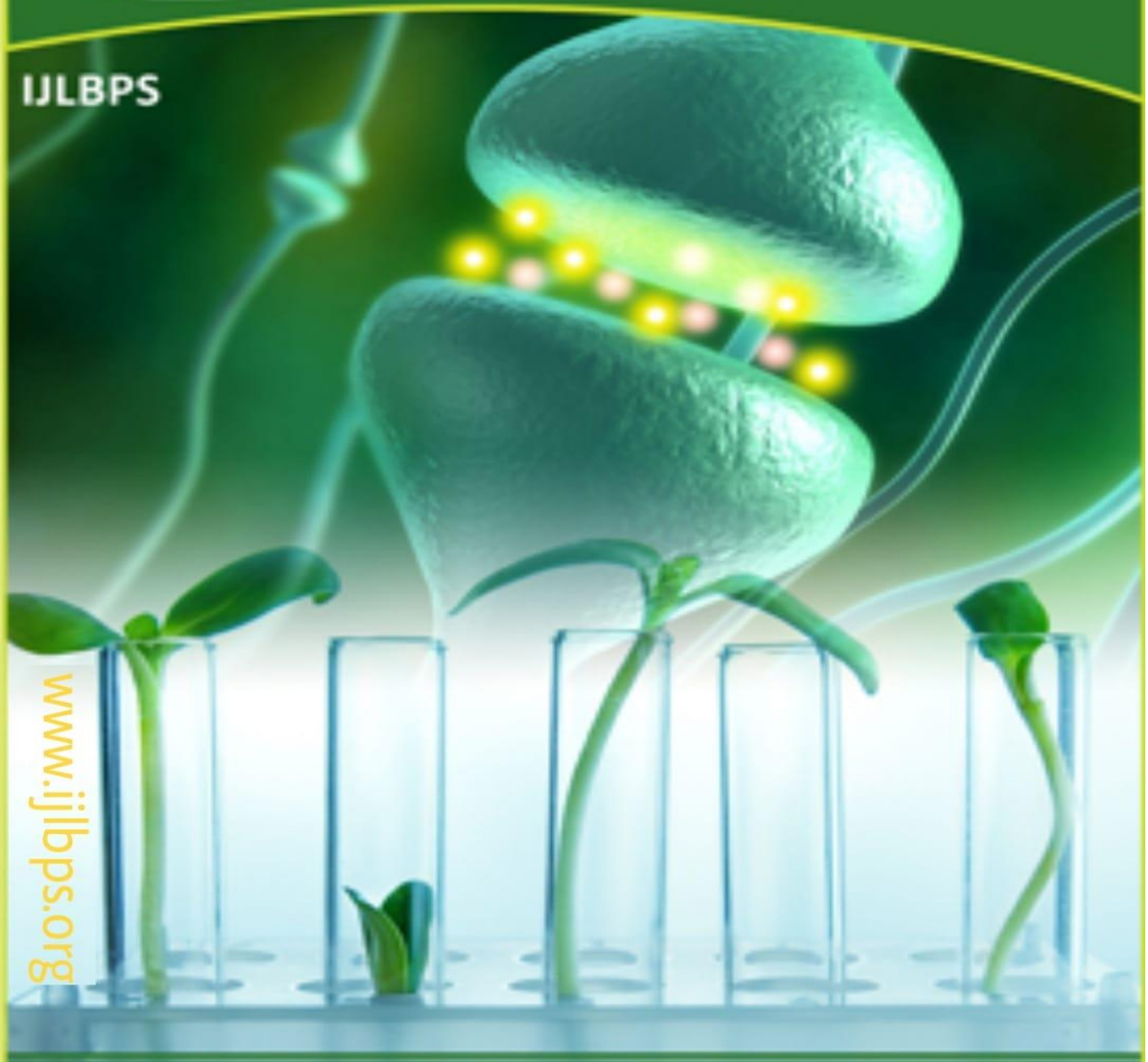

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Identification of Two Phytosterol and a Glycoside in *Sabicea Brevipes* Plant RootExtract

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Abstract

Sabicea brevipes root extracts were analyzed by thin-layer chromatography (TLC) for the presence of steroids and glycosides, and the results revealed the presence of (1) Dehydro epi androsterone (DHEA), (2) 17- Hydroxyprogesterone steroids, and (3) one kind of glycoside. The RF values were compared to those found in the published literature, which allowed for the identification of the names and structures of compounds 1 and 2

Erectile dysfunction; dehydroepiandrosterone; 17-hydroxyprogesterone; glycosides; Sabicea brevipes.

Introduction

Despite the growing availability of efficient conventional and orthodox medical therapy, herbal treatments continue to offer a popular option for men and women wanting to better their sexual lives. Wendi and David (2003). After reaching a high in one's twenties, testosterone levels in men and progesterone levels in women often fall further and continue to do so for the rest of their lives. In many cases, this decline is followed by correlated The ground-breaking research of Masters and Johnson (Helgason et al., 1996; Masters and Johnson, 1966, 1970). Sexual desire (libido) is one of the elements of sexual function identified as significant to the evaluation (Nimbi et al., 2018; Warnock et al., 2006). Personal attractiveness and the biological fitness of one's partner seem to have a considerable effect on libido (Nimbi et al., 2020), although this may be due to a combination of variables (medication that operate on the central nrvous system, lifestyle, etc.).changes in body composition, weight, andmuscular mass to a decline in desire and the onset of erection difficulties in certain men (Moffat, 2005; Beauchet, 2006).First, a revised version of is used to describe key concepts related to sexual function. Erection which occurs as a result of complex neuro-psychologic processes that requires the interaction of the brain, nerves, hormones,

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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 outthrough 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 functioningand sexual satisfaction will be optimal. (Chawla and London 2018). Erectile dysfunction (ED, or "male impotence") is a sexual problemcharacterized 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 orclimbing shrub, which is usually 0.6096-1.2192meters in height (Plate 1) and belongs to theRubiaceae family. When in full bloom in July and August, the plant produces a mass of red flowersand small, red and juicy fruit eaten in SierraLeone 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 somepart of Africa, Madgascar and America (http://www.zipcodezoo.com/plants/s/sabicea%5brevipes/). The result of earlier phytochemical analysis of Sabicea brevipes revealed the presenceof 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 useit 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 bathto 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 (80-100^0) 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 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 tablesand 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 solventsystems 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

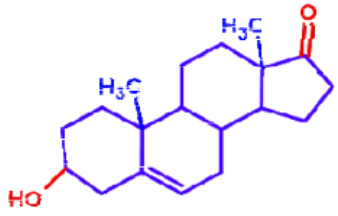
Fig. 1: 

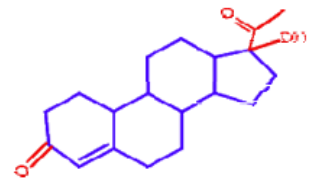
Fig. 2: 

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*

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

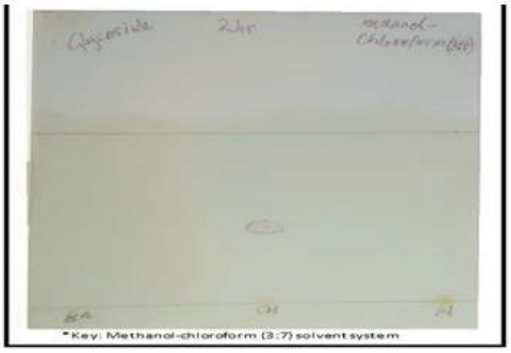


Plate 4: TLC photo print of glycoside identified in the root of sabicea brevipes.

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)

Tables 2 and 3 show the findings of the glycoside analysis, which revealed that only the chloroform extract yielded a single spot for both solvent systems used in the creation of the chromatograms. For benzene ethyl acetate and methanol-chloroform, these values are 0.95 (Brown) and 0.45 (Brown), respectively. This means that there is just one known glycoside in the root material.

2.3% of the powdered seeds were found to contain crude steroids when the extract was made using petroleum ether. It was shown in this study that the crude steroid extract included two steroidal chemicals (Table 1). 17-Hydroxyprogesterone (SHP) and dehydroepiandrosterone (DHEA) both have RF values of 0.42 on plate 2, whereas 17-Hydroxyprogesterone (SHP) is at 0.54. The hormone dehydroepiandrosterone (DHEA) is produced naturally in the body.

Steroids have been linked to several functions in both human and animal biology. (El-Sakka et al., 2018) It has been shown to have an effect on the androgen receptor both directly and via metabolites. DHEA, which is classified as a neuro-steroid, is also a powerful sigma 1 agent (Romicu et al., 2003). The development of arterial smooth muscle cells from human aorta (hASMC) is affected by DHEA when cultured in vitro. This suggests that Sabicea brevipes causes smooth muscle cells inside the artery walls to relax, hence increasing vessel diameter. This effect is most pronounced in the big arteries, smaller arterioles, and large veins. Vasodilation (http://en.wikipedia.org/wiki/vasodilation) refers to the dilation of blood vessels. Similar to how the phosphodiesterase inhibitor Viagra

increases penile blood flow. In serum-free cultures, it greatly enhances hASMC mitogenesis (Life Science (USA), 1997). Some small placebo-controlled randomized clinical trial studies on the plant have found that long-term supplementation improves mood and relieves depression (Wolkowitz et al., 1999; Schmidt et al., 2005), and this is likely why it is used in the management of erectile dysfunction. Long-term supplementation has also been shown to decrease insulin resistance and to be useful in patients with systemic lupus erythematosus. Athletes also use it to boost performance and muscular growth (Kawans et al., 2003).

Patients with hypertension or an organic cause were thought to benefit from DHEA medication.

ED, however they do not help those with diabetes mellitus or neurological disorders (Reiter et a., 2001).

17-Hydroxyprogesterone is another steroid that may be detected in the plant's root. Cortisol is a hormone produced by the body from this molecule (Hamilton et al., 2008). Cortisol is a hormone that aids in the metabolism of protein, carbohydrates, and fats; it also controls the immune system and keeps blood pressure steady.). As a result, this is likely to have a moderating influence on the pressure of the blood trapped in the penis, which in turn may improve penile erection.Water, ethyl acetate, and chloroform crude glycosides extracts respectively produced 1.91, 0.68, and 1.96% of the dry powdered root sample. For both solvent systems utilized to create the chromatograms, only the chloroform extract produced a single spot with RF values and colors as shown in (Tables 3). For the 9:1 benzene-to-ethylacetate and 3:7 methanol-to-chloroform solvent systems, these values are 0.95 and 0.45, respectively (Brown). This means that there is just one known glycoside in the root material.Glycosides have vital supporting, economic, and most crucially medicinal significance for humanity. Capillary fragility is reduced, calcium channels are blocked, and glycosides widen coronary arteries. Recent research (Brito-Arias Marco, 2007) suggests that glycosides may aid in the repair and regeneration of muscle tissue and the closure of wounds.Root glycosides discovered from sabicea brevipes likely aid the steroid and alkaloid activity responsible for the plant's sexual enhancing properties.This provides evidence for the traditional usage of sabicea brevipes root as a sex enhancer by males with erectile trouble (erectile dysfunction) and by men and women with low libido in Oghe, Enugu Nigeria.

Conclusion

It's reasonable to assume that the stimulating and tonifying properties of Sabicea brevipes root on the muscles justify its potential usefulness in increasing male potency. Since dehydro-epi-androsterone promotes the growth of vascular smooth muscle cells in vitro, it could be useful in the treatment of erectile dysfunction. 17-hydroxyprogesterone may enhance erections because, in addition to helping maintain a healthy blood pressure, it has a moderating effect on the pressure of blood trapped in the penis.Glycosides found in Sabicea brevipes help the plant's own hormones execute their jobs. These active ingredients are responsible for increased libido because they reduce arterial tension, block calcium channel activity, and strengthen blood vessel walls. This provides support for the hypothesis that sabicea brevipes root may restore virility in males with erectile dysfunction.

References

Rowland D. L., Tai W. (2003). A review of plant-derived and herbal approaches to the treatment of sexual dysfunctions. Journal of Sex & Marital Therapy. 29(3):185–205.

Moffat, S.D. (2005). Effects of testosterone on cognitive and brain aging in elderly men. Ann NY Acad Sci. 1055: 80-92.

Beauchet, O. (2006). Testosterone and cognitive function: current clinical evidence of a relationship. Eur J Endocrinol. 155(6): 773-81.

Helgason, A.R., Adolfsson, J., Dickman, P., Arver, S., Fredrikson, M., Gothberg,

M. and Steineck, G. (1996). Sexual desire, erection, orgasm and ejaculatory functions and their importance to elderly Swedish men: A population based study.Age and Ageing. 25: 285-291.

Masters, W.H. & Johnson, V.E. (1966). Human Sexual Response. New York: Bantam Books.

Masters, W.H. & Johnson, V.E. (1970). Human Sexual Inadequacy. New York: Bantam Books.

Warnock JK, Clayton A. Coroft H, Segraves R, and Biggs FC (2006). Comparison of androgens in women with hypoactive sexual desire disorder. Those on combined oral contraceptive COCs Vsthose not on COCs. J Sex Medicine; 3: 878-883.

F.M. Nimbi, F. Tripodi, R. Rossi and Simonelli, C. (2018). Testing a conceptual model for men's sexual desire referring to automatic thoughts, emotions, sexual function, and sexism. J Sex Med, 15: 1518-1526

da Cruz, A. C., Guerra, N.G., de Souza, K.E.B.P., de Castro Eleutério, I., da Silva1, L.C., Otoni, E.G., Alves, M.R.A. and Regis, W.C.B. (2017). The action of herbal medicine on the libido: aspects of nutritional intervention in increasing sexual desire. Nutrire 42(29): 1-8

Kendirci M, Nowfar S and Hellstorm WJ (2005). "The impact of vascular risk factors on erectile function". Drugs Today (Barc) 41(1); 65-74.

Nimbi, F.M., Tripodi, F., Rossi, R., Navarro-Cremades, F. and Simonelli, C.(2020). Male Sexual Desire: An Overview of Biological, Psychological, Sexual, Relational, and Cultural Factors Influencing Desire. Sexual Medicine Reviews. 8(1): 59-91.

Hsu, Geng-Long; Liu, Shih-Ping (2018). "Penis Structure". Encyclopedia of Reproduction. Academic Press. Pp. 357–366.

Hsu, Geng-Long (2018). "Erection Abnormality". Encyclopedia ofReproduction. Academic Press. Pp. 382–390.

Walter, F.B. and Emile, L.B. (2005). Medical Physiology: A Cellular and Molecular Approach. Philadelphia,Pa.: Elsevier/Saunders. .

Bohlen, J.G., Held, J.P. and Sanderson,

M.O. (1980). "The Male Orgasm: Pelvic Contractions Measured by Anal Probe". Archives of Sexual Behavior. 9 (6): 503–521.

Sante, S., Mollaioli, D., Gravina, G.L., Ciocca, G., Limoncin, E., Carosa, E., Lenzi, A., Jannini, E.A. (2016). Epidemiology of delayed ejaculation. Translational Andrology and Urology. 5(4): 541-548.