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Aqueous Extract of *Phyllanthus amarus* Leaves Restore Sexual Competence in Female Rats Induced with Sexual Dysfunction by Fluoxetine

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Abstract: This study aimed to screen the aqueous extract of *Phyllanthus amarus* leaves for its secondary metabolites and its ability in restoring sexual competence of fluoxetine-induced sexual dysfunction in female Wistar rats. Both the qualitative and quantitative screening of the extract for secondary metabolite constituent was done using standard methods. The extract (20, 40, and 80 mg/kg body weight) and the reference drug, Tadalafil were administered orally to fluoxetine-induced sexually impaired female rats, once daily for 7 days, and their sexual behaviour parameters were monitored and/or computed. The serum progesterone, follicle-stimulating hormone (FSH), luteinizing hormone (LH), estrogen and prolactin were determined at the end of treatment period. The results revealed that the extract contained alkaloids (28.5 mg/L), flavonoids (13.6 mg/L), saponins (11.2 mg/L), steroids (8.4 mg/L), anthraquinones (3.8 mg/L), tannins (2.7 mg/L), and terpenes (2.3 mg/L). Administration of fluoxetine to sexually active female rats significantly ($p<0.05$) lowered the darting frequency (DF), hopping frequency (HF), lordosis frequency (LF), genital grooming (GG) and licking behaviour (LB) by 40.91%, 37.93%, 33.33%, 56.12% and 48.31% respectively, whereas darting latency (DL) and hopping latency (HL) were significantly ($p<0.05$) prolonged by 48.70% and 56.33% respectively. Administration of 20, 40, and 80 mg/kg body weight of the aqueous extract of *P. amarus* leaves significantly ($p<0.05$) reversed the fluoxetine-mediated alterations in DF, HF, LF, DL, HL, GG and LB in dose-dependent manner. The reversal of the female sexual behaviour parameters by the 80 mg/kg body weight of the extract compared well ($p<0.05$) with those of Tadalafil-treated animals. In addition, all the doses of the extract elevated ($p<0.05$) the levels of serum LH, FSH, progesterone, estrogen but decreased prolactin concentrations. Data obtained from this study revealed that the aqueous extract of *P. amarus* leaves restored sexual competence in sexually impaired female rats possibly by increasing sexual drive through enhanced reproductive hormones concentration. The sexual behaviour restorative activity of aqueous extract of *Phyllanthus amarus* leaves could be due to the presence of alkaloids, saponins, flavonoids and other phytoconstituents, thus giving scientific support to the age-long folkloric use of *Phyllanthus amarus* in the management of sexual inadequacies in females.

KEYWORDS: Aphrodisiac; Euphorbiaceae; ; Female sexual dysfunction; Fluoxetine; *Phyllanthus amarus*; Tadalafil

1.0 Introduction

Sexual function results from a complex neurovascular process that is controlled by psychological and hormonal inputs. Like any coordinated physiological response, multiple systems are involved in this function. Thus, the normal female sexual response cycle can be functionally divided into three interrelated events that occur in a defined sequence of desire, arousal and orgasm (Marthol and Hilz, 2004).

With several existing female sexual dysfunction (FSD) definitions, the most descriptive denotes FSD as the persistent/recurring decrease in sexual desire or arousal, the difficulty/inability to achieve an orgasm, and/or the feeling of pain during sexual intercourse (Salonia *et al.*, 2004). FSD is a multifaceted disorder, comprising anatomical, psychological, physiological, as well as social-interpersonal components. The prevalence in women (43%), which is more than in men (31%), has been associated with various psychodemographic characteristics such as age, education, poor physical and emotional health

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(Laumann *et al.*, 1999). Vascular insufficiency arising from atherosclerosis and neurologic diseases like diabetic neuropathy are major causes of FSD (Marthol and Hilz, 2004). Additionally, FSD may be due to changes in hormonal levels, psychological or social factors and/or a long term side-effects associated with certain anti-depressants especially selective serotonin reuptake inhibitors (SSRIs) like fluoxetine, fluvoxamine, paroxetine, citalopram and sertraline) (Segraves, 2007). FSD can lead to reduced quality of life and potentially procreative advancement.

Currently, there are few pharmacological options available in the treatment of FSD, and this includes sildenafil, L-arginine, yohimbine, apomorphine and prostaglandin E1 (Meston and Worcel, 2002; Ito *et al.*, 2006). However, these options are too expensive, not readily available in addition to associated side effects such as adverse effects on blood pressure, genital vasocongestion, vaginal erythema, and transudate volume. These problems made many women to feel reluctant towards the use of exogenous hormone therapy and are turning to botanicals which are readily available, accessible and affordable with little or no side effects. Hence, the choice of *Phyllanthus amarus* leaves, a plant widely implicated to be of immense importance in the management of FSD, but without scientific evidence that either corroborated or refuted the ostensible claim.

Phyllanthus amarus (Euphorbiaceae), also known as *eyin olobe* (Yoruba), *geeron tsutsaayee* (Hausa) and *Ngwu ite kwowa nasu* (Igbo) is found in the Philippine, Cuba, India and Nigeria, among others, as a weed in cultivated and waste lands (Kumar *et al.*, 2011). It is a widely distributed, small, erect, tropical, annual herb that grows up to 30-40 cm high. It has slender, leaf-bearing branchlets, distichous leaves which are sub-sessile and elliptic-oblong. *P. amarus* leaves has been acclaimed in traditional medicine and literatures to have diverse therapeutic uses like in the management of urinogenital disorders, jaundice, intermittent fevers, dropsy, dysentery, diarrhoea, gonorrhea, pain, swelling, sores, wounds, scabies, stomach pain, sexual disorders, ulcers, ringworm, colic, snake bite, menorrhagia, leucorrhoea and constipation (Khatoon *et al.*, 2004; Patel *et al.*, 2011; Dhongade and Chandewar, 2013; Sen and Batra, 2013). Preliminary screening of secondary metabolite, mineral contents and amino acid profile of the aqueous extract of the leaves revealed the presence of alkaloids, flavonoids, saponins, steroids, tannins, calcium, potassium, iron, zinc, chromium, copper, glutamine and methionine (Nurudeen and Yakubu, 2015). It has also been reported that the aqueous extract possess immunostimulant (Taiwo *et al.*, 2009), antioxidant (Lim and Murtijaya, 2007), anti-bacterial (Akinjogunla *et al.*, 2010), anti-diarrhoeal (Odetola and Akojenu, 2000), anti-cancer and anti-mutagen (Sripanidkulchai *et al.*, 2002), anti-allodynic and antioedematogenic (Kassuya *et al.*, 2003), anti-nociceptive (Santos *et al.*, 2000), anti-inflammatory (Kassuya *et al.*, 2005), antidiabetic and antilipidemic (Adeneye *et al.*, 2006) and anti-viral (Wang *et al.*, 1995; Ott *et al.*, 1997) activities.

Although, studies abound on the chemical constituents and pharmacological activities of *P. amarus*, scientific literature still lacks experimental data that supported the acclaimed aphrodisiac property of *P. amarus*. Therefore, the present study was an attempt to validate or otherwise the acclaimed use of aqueous extract of *P. amarus* leaves as an aphrodisiac in female rats that have been induced with sexual dysfunction by fluoxetine.

2.0 Materials and Methods

2.1 Materials

2.1.1 Plant Material and Authentication

Fresh *P. amarus* leaves were collected within the premises of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria, and authenticated at the University of Ilorin Herbarium, Ilorin, Nigeria. A voucher sample was deposited under UIH 001/1109 for future reference.

2.1.2 Animals

Sixty, healthy, in-bred, sexually active, female Wistar rats weighing 141.52 ± 6.26 g were obtained from the Animal Holding Unit of the

Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. The animals were contained in their respective plastic cages placed in a well-ventilated Animal House and maintained at a temperature of 22 ± 3°C, 12:12 h light and dark cycle and a relative humidity of 45-50%. The animals were maintained on rat pellets (Premier Feeds, Ibadan, Nigeria) and tap water *ad libitum*. This study was carried out after ethical approval from University of Ilorin Ethical Review Committee by a letter referenced UERC/ASN/2015/210.

2.1.3 Drugs, Assay Kits and Chemicals

Fluoxetine, Tadalafil, progesterone and estradiol benzoate were products of Tillomed Laboratories Limited, Herdfordshire, UK; Tuyil Pharmaceuticals Industries Limited, Ilorin, Nigeria; Ningbo Tisun Medic Biochemic Co., Ltd., Ningbo, Peoples Republic of China and Sigma-Aldrich Inc., St. Louis, USA respectively. The assay kits for progesterone, follicle stimulating hormone (FSH), luteinizing hormone (LH), estrogen and prolactin were factory made by Inteco Diagnostics Ltd., London, United Kingdom. All other reagents used were products of Sigma-Aldrich Inc., St. Louis, USA.

2.2 Methods

2.2.1 Preparation of Extract

Fresh leaves devoid of the stalks were rinsed in distilled water, oven-dried at 40°C for 48 hours and pulverized in a blender (Master Chef Blender, Model MC-BL 1980, China). The powder (50 g) was extracted in 500 ml of distilled water for 48 hours at room temperature with constant shaking. The resulting filtrate was lyophilized to give a yield of 5.25 g (percentage yield of 10.5 %). Calculated amounts were reconstituted in distilled water to give the required doses of 20, 40, and 80 mg/kg body weight that were administered to the female rats. Information from ethnobotanical survey were put together to arrive at the most frequently mentioned dose of 40 mg/kg body weight, while the doses of 20 and 80 mg/kg body weight were half and twice the required dose of 40 mg/kg body weight.

2.2.2 Screening of Secondary Metabolites

The procedures for qualitative determination of alkaloids, steroids, anthraquinones, cardenolides, saponins, phenolics, flavonoids, cardiac glycosides, tannins and terpenoids were adopted from the methods described by Odebiyi and Sofowora (1978), Trease and Evans (1989), Sofowora, (1993) and Edeoga *et al.* (2005), while the detected metabolites were quantified as described for saponins (Obadoni and Ochuko, 2001), alkaloids (Adeniyi *et al.*, 2009), tannins (Makkar *et al.*, 1993), flavonoids (Boham and Kocipai, 1974), anthraquinones (El-Olemy *et al.*, 1994), terpenoids (Luo *et al.*, 2007) and steroids (Gao *et al.*, 2009).

2.2.3 Induction of Sexual Dysfunction and Assessment of Sexual Behaviour Parameters in Female Rats

Fifty female rats were rendered sexually incompetent after daily oral administration of 15 mg/kg of fluoxetine (prepared daily in distilled water) with the aid of a metal oropharyngeal cannula for 14 days (Sarkar *et al.*, 2008). On day 15, the female rats were then introduced into the male rats in their respective rectangular plastic cages with wire mesh top and mating behaviours were observed for 30 minutes. Female rats which showed minimum of 25% reduction in Darting Frequency [DF; number of darts (a short run where the female rat abruptly stops presenting the posterior to the male rat) without intromission of male rat genitals, from the time of introduction of the female rat into the plastic cage], Hopping Frequency [HF; number of hops (a short jump with stiff legs followed by immobility and presentation of posterior of the female rat to the male rat) without intromission of male rat genitals, from the time of introduction of the female rat into the plastic cage], Lordosis Frequency [LF; number of times the female rat assumes mounting position (in which the back is arched downward) when the female rat is ready to mate without intromission, from the time of introduction of the female rat

into the plastic cage], Genital Grooming (GG; number of times the female rat touches its sexual organs when the female rat is ready to mate from the time of introduction of the female rat into the plastic cage) and Licking Behaviour (LB; number of times the female rat moves its tongue across the surface of its genitals as signals to show that it is ready to mate) as well as minimum increase of 25% in Darting Latency (DL; time interval from the time of introduction of the female rat into the plastic cage to the first dart by the female rat) and Hopping Latency (HL; time interval from the time of introduction of the female rat to the first hops by the female rat) were declared as having been induced with sexual dysfunction and were assigned into various groups.

2.2.4 Animal Grouping and Administration of Extract and Reference Drug

A total of 60 female rats that were acclimatized for 2 weeks were assigned into six Groups (A-F) in a complete randomized design, with each group comprising ten animals as follows:

- Group A: Rats that received distilled water only
- Group B: Rats induced with sexual dysfunction and administered distilled water only
- Group C: Rats induced with sexual dysfunction and administered 10 mg/kg body weight of Tadalafil only
- Group D: Rats induced with sexual dysfunction and administered 20 mg/kg body weight of the extract only
- Group E: Rats induced with sexual dysfunction and administered 40 mg/kg body weight of the extract only
- Group F: Rats induced with sexual dysfunction and administered 80 mg/kg body weight of the extract only

The various groups of animals were orally administered 0.5 ml each of distilled water, Tadalafil and the extract, once daily (08:00 - 08:45 h) for 7 days, with the aid of a metal oropharyngeal cannula. The female sexual behaviour parameters (DF, HF, LF, GG, LB, DL and HL) were monitored and results were recorded 30 minutes after dosing on days 1, 3, and 7 (between 22:00 and 03:00 hours) under dim light condition at room temperature (26-28°C).

2.2.5 Preparation of Serum

The serum was prepared according to the procedure described by Yakubu *et al.* (2009). Rats were anesthetized in diethyl ether fumes. When they became unconscious, the jugular veins were cut, and 5 ml of the blood was collected into clean, dry centrifuge tubes. The samples were left for 15 minutes at room temperature for the blood to clot. Clear serum was then collected using Pasteur pipette after centrifuging at 503 × *g* for 10 minutes using Uniscope Laboratory Centrifuge (Model SM800B, Surgifriend Medicals, Essex, UK). The sera were kept frozen for 12 hours before being used for the various hormonal assays.

2.2.6 Determination of Reproductive Hormones

The concentrations of progesterone, FSH, LH, estrogen and prolactin in the serum of the female rats 7 days post administration were determined via the tube-based serum enzyme immunoassay. The protocols adopted for the determination of the hormones were as highlighted in the manufacturer's manual (Inteco Diagnostics UK Ltd., London, United Kingdom).

2.2.7 Data Analyses

Data were expressed as the mean ± SEM of ten determinations. Means were analyzed using Duncan Multiple Range Test and complemented with unpaired Student's t-test. Statistical Package for Social Sciences, version 20.0 (SPSS Inc., Chicago, IL, USA) was used for the statistical analyses of the data. Differences were considered statistically significant at $p<0.05$.

3.0 Results

The analysis of secondary metabolite constituents of *P. amarus* leaves revealed that the leaf powder contained alkaloids (28.50 mg/L), flavonoids (13.60 mg/L), saponins (11.20 mg/L), steroids (8.40 mg/L), anthraquinones (3.80 mg/L), tannins (2.70

mg/L) and terpenes (2.30 mg/L), whereas cardenolides, phenolics and cardiac glycosides were not detected (Table 1).

The administration of fluoxetine to sexually active female rats significantly ($p<0.05$) lowered the DF, HF, LF, GG and LB by 40.91%, 37.93%, 33.33%, 56.12% and 48.31% respectively, whereas the DL and HL were significantly prolonged by 48.70% and 56.33% respectively (Table 2).

P. amarus at the various doses (20, 40, and 80 mg/kg body weight) investigated in this study significantly ($p<0.05$) raised the DF, HF, LF, GG and LB of the sexually incompetent animals in a dose- and time-dependent manners (Tables 3 and 4). For instance, on day 1, when compared with the sexually impaired female rats that received distilled water, there was no significant ($p>0.05$) difference on DF, HF and LF between the fluoxetine-treated female rats that received distilled water and the other treatment groups (tadalafil- and 20, 40 and 80 mg/kg body weight of the extract-treated sexually impaired female rats). However, on days 3 and 7, the administration of the aqueous extract of *P. amarus* leaves at 20 and 40 mg/kg body weight significantly ($p<0.05$) raised the fluoxetine-treatment related reductions in the frequencies of darting, hopping and lordosis of the female rats. In addition, the reversal made on the DF, HF, LF, GG and LB by the 80 mg/kg body weight of the aqueous extract of *P. amarus* leaves was the most pronounced and compared favourably well ($p>0.05$) with the female rats treated with fluoxetine that received Tadalafil (Table 3). Also, it was on day 7 that the administration of the aqueous extract of *P. amarus* leaves, most especially the highest dose (80 mg/kg body weight of the extract) produced a recovery on GG and LB and these female sexual behaviour parameters compared favourably well ($p>0.05$) with those of non-sexually impaired female rats that received distilled water (Table 4).

Furthermore, administration of 20, 40, and 80 mg/kg body weight of aqueous extract of *P. amarus* significantly ($p<0.05$) reduced the fluoxetine-treatment related prolonged latencies of darting and hopping in the female rats (Table 5). These effects were observed throughout the experimental period. As the days progressed, the highest dose (80 mg/kg body weight) reversed the fluoxetine-treatment related prolonged latencies of darting and hopping. All these changes compared favourably well ($p>0.05$) with Tadalafil (Tables 3, 4 and 5).

Administration of fluoxetine to sexually active female rats significantly ($p<0.05$) reduced the levels of progesterone, FSH, LH and estrogen by 28.95%, 47.89%, 28.62% and 31.14% respectively, whereas the levels of prolactin increased significantly by 53.67% when compared with the distilled water treated control animals (Table 6). The reduced levels of progesterone, FSH, LH and estrogen in the fluoxetine-treated animals were reversed after the administration of the aqueous extract of *P. amarus*, but the reversals were most pronounced ($P<0.05$) on progesterone, FSH, LH, estrogen and prolactin by 95.29%, 96.13%, 97.80%, 100% and 114.14% respectively in the fluoxetine-induced sexually impaired animals that received 80 mg/kg body weight of the extract; these reversals on progesterone, FSH, LH, estrogen and prolactin compared favourably ($P>0.05$) with those of sexually dysfunction female rats that received tadalafil and those of the non-sexually impaired female rats that received distilled water (Table 6).

4.0 Discussion

P. amarus leaves have been acclaimed traditionally to be used in the management of FSD without any scientific study that has authenticated or refuted this claim. Therefore, the present study examined the ameliorative effects of *P. amarus* leaves in fluoxetine-induced sexually impaired female Wistar rats as experimental models.

Fluoxetine, an anti-depressant of the SSRI used for the treatment of major depressive disorder, obsessive-compulsive disorder, bulimia nervosa, panic disorder and premenstrual disorder, is commonly associated with high incidence of adverse sexual effects such as loss of libido, anorgasmia and/or lack of vaginal lubrication (Clark *et al.*, 2013). Fluoxetine affects neurotransmitters, the chemicals that nerves within the brain use to communicate with each other. An imbalance among

Table 1: Secondary metabolite constituents of *Phyllanthus amarus* leaves

Secondary Metabolites	Concentration (mg/L)
Alkaloids	28.50 ± 1.18
Tannins	2.70 ± 0.11
Saponins	11.20 ± 0.54
Flavonoids	13.60 ± 0.61
Anthraquinones	3.80 ± 0.19
Steroids	8.40 ± 0.46
Terpenes	2.30 ± 0.08
Phenolics	Not Detected
Cardenolides	Not Detected
Cardiac glycosides	Not Detected

n =3 ± SEM

Table 2: Effect of administration of fluoxetine on the sexual behaviour parameters of female rats

Parameters	**Control**	**Fluoxetine + Distilled Water**	***Difference (%)**
Darting frequency (DF)	8.80 ± 0.35^{a}	5.20 ± 0.11^{b}	$40.91^{\downarrow}$
Hopping frequency (HF)	2.90 ± 0.06^{a}	1.80 ± 0.03^{b}	$37.93^{\downarrow}$
Lordosis frequency (LF)	1.50 ± 0.02^{a}	1.00 ± 0.01^{b}	$33.33^{\downarrow}$
Darting latency (DL) (seconds)	785.40 ± 53.51^{a}	1167.90 ± 82.05^{b}	$48.70^{\uparrow}$
Hopping latency (HL) (seconds)	945.50 ± 77.24^{a}	1478.10 ± 101.01^{b}	$56.33^{\uparrow}$
Genital grooming	15.50 ± 0.81^{a}	6.80 ± 0.23^{b}	$56.12^{\downarrow}$
Licking behavior	8.90 ± 0.21^{a}	4.60 ± 0.11^{b}	$48.31^{\downarrow}$

Data are mean of ten determinations ± SEM; Test values with superscripts different from the control for each parameter are significantly different ($P<0.05$); $^{\downarrow}$means percentage reduction in parameter; $^{\uparrow}$means percentage increase in parameters; Difference was expressed as a function of the control

Table 3: Darting, hopping and lordosis frequencies of fluoxetine-induced female rats after oral administration of aqueous extract of *P. amarus* leaves

Treatments	Darting frequency			Hopping frequency			Lordosis frequency		
	Days								
	1	3	7	1	3	7	1	3	7
Distilled Water	8.20 ± 0.35^{a}	8.80 ± 0.43^{a}	8.40 ± 0.34^{a}	3.00 ± 0.14^{a}	2.90 ± 0.12^{a}	3.10 ± 0.15^{a}	1.40 ± 0.02^{a}	1.50 ± 0.05^{a}	1.40 ± 0.04^{a}
Fluoxetine + Distilled Water	5.10 ± 0.11^{b}	5.70 ± 0.18^{b}	5.40 ± 0.20^{b}	1.90 ± 0.06^{b}	1.80 ± 0.04^{b}	1.90 ± 0.08^{b}	1.10 ± 0.03^{b}	1.00 ± 0.03^{b}	1.00 ± 0.02^{b}
Fluoxetine+ 10mg/kg body weight of Tadalafil	5.50 ± 0.22^{b}	6.10 ± 0.31^{c}	7.60 ± 0.35^{c}	1.80 ± 0.06^{b}	2.20 ± 0.05^{c}	2.70 ± 0.04^{c}	1.00 ± 0.02^{b}	1.20 ± 0.03^{c}	1.40 ± 0.05^{a}
Fluoxetine + 20 mg/kg body weight of extract	5.20 ± 0.18^{b}	6.00 ± 0.23^{c}	6.50 ± 0.26^{d}	1.70 ± 0.04^{b}	2.10 ± 0.05^{c}	2.20 ± 0.08^{d}	1.10 ± 0.06^{b}	1.00 ± 0.02^{b}	1.10 ± 0.06^{b}
Fluoxetine + 40 mg/kg body weight of extract	5.30 ± 0.21^{b}	5.80 ± 0.19^{b}	6.60 ± 0.36^{d}	1.90 ± 0.04^{b}	2.20 ± 0.06^{c}	2.30 ± 0.08^{d}	1.20 ± 0.03^{b}	1.20 ± 0.05^{c}	1.30 ± 0.07^{c}
Fluoxetine + 80 mg/kg body weight of extract	5.20 ± 0.27^{b}	6.40 ± 0.37^{d}	8.00 ± 0.41^{a}	2.00 ± 0.04^{b}	2.30 ± 0.05^{c}	2.80 ± 0.09^{c}	1.10 ± 0.04^{b}	1.20 ± 0.05^{c}	1.50 ± 0.08^{a}

Data are mean of ten determinations ± SEM; Test values with superscripts different from the control down the group within the same parameter are significantly different ($P<0.05$).

Table 4: Genital grooming and licking behaviour of fluoxetine-induced sexual dysfunction in female rats after oral administration of aqueous extract of *P. amarus* leaves

Treatments	Genital grooming			Licking behaviour		
	Days					
	1	3	7	1	3	7
Distilled Water (control)	15.90 ± 0.79^{a}	14.60 ± 0.84^{a}	16.10 ± 1.10^{a}	8.30 ± 0.64^{a}	8.80 ± 0.56^{a}	8.20 ± 0.72^{a}
Fluoxetine + Distilled Water	6.90 ± 0.25^{b}	6.50 ± 0.42^{b}	6.80 ± 0.29^{b}	4.10 ± 0.24^{b}	4.90 ± 0.31^{b}	4.70 ± 0.33^{b}
Fluoxetine+ 10mg/kg body weight of Tadalafil	6.70 ± 0.48^{b}	8.90 ± 0.56^{c}	13.30 ± 0.75^{c}	4.60 ± 0.26^{b}	5.70 ± 0.39^{c}	7.80 ± 0.56^{a}
Fluoxetine + 20 mg/kg of extract	7.20 ± 0.51^{c}	7.90 ± 0.54^{d}	10.20 ± 0.62^{d}	4.90 ± 0.24^{c}	5.20 ± 0.41^{c}	6.50 ± 0.48^{c}
Fluoxetine + 40 mg/kg body weight of extract	7.10 ± 0.61^{c}	8.30 ± 0.62^{c}	12.20 ± 0.71^{c}	4.60 ± 0.32^{b}	6.40 ± 0.41^{d}	6.80 ± 0.46^{c}
Fluoxetine + 80 mg/kg body weight of extract	8.40 ± 0.63^{d}	12.70 ± 0.89^{c}	15.80 ± 1.03^{a}	5.10 ± 0.32^{c}	6.40 ± 0.43^{d}	8.10 ± 0.69^{a}

Data are mean of ten determinations ± SEM; Test values with superscripts different from the control down the group within each parameter are significantly different ($P<0.05$). Genital grooming and Licking behaviour are expressed in numbers

Table 5: Darting and hopping latencies of fluoxetine-induced sexual dysfunction female rats after oral administration of aqueous extract of *P. amaraus* leaves

Treatments	Darting latency (seconds)			Hopping latency (seconds)		
	Days					
	1	3	7	1	3	7
Distilled Water (control)	768.90 ± 64.59^{a}	792.40 ± 59.46^{a}	776.90 ± 62.40^{a}	934.30 ± 87.24^{a}	962.50 ± 81.36^{a}	955.30 ± 75.02^{a}
Fluoxetine + Distilled Water	1120.90 ± 82.05^{b}	1108.50 ± 84.42^{b}	1079.10 ± 92.29^{b}	1492.10 ± 94.01^{b}	1427.90 ± 79.05^{b}	1385.20 ± 90.83^{b}
Fluoxetine+ 10mg/kg body weight of Tadalafil	1074.70 ± 75.84^{b}	956.90 ± 82.56^{c}	844.30 ± 72.75^{c}	1363.60 ± 88.38^{c}	1127.60 ± 95.12^{c}	981.10 ± 73.06^{a}
Fluoxetine + 20 mg/kg body weight of extract	1014.20 ± 88.31^{b}	954.10 ± 78.84^{c}	920.20 ± 81.12^{d}	1385.90 ± 92.44^{c}	1297.20 ± 103.23^{c}	1168.30 ± 98.68^{c}
Fluoxetine + 40 mg/kg body weight of extract	1116.80 ± 92.61^{b}	980.30 ± 80.42^{c}	896.10 ± 72.21^{d}	1468.30 ± 111.28^{b}	1270.40 ± 101.31^{c}	1056.20 ± 99.36^{c}
Fluoxetine + 80 mg/kg body weight of extract	967.40 ± 83.13^{c}	902.70 ± 74.49^{d}	814.60 ± 75.34^{c}	1361.50 ± 104.82^{c}	1061.40 ± 92.89^{d}	980.50 ± 76.89^{a}

Data are mean of ten determinations ± SEM; Test values with superscripts different from the control down the group within each parameter are significantly different ($P<0.05$).

Table 6: Reproductive hormones of fluoxetine-induced sexual dysfunction female rats after oral administration of *P. amarus* leaves

Treatment	Progesterone (nmol/L)	Follicle Stimulating Hormone (mIU/mL)	Luteinizing Hormone (mIU/mL)	Estrogen (pg/mL)	Prolactin (ng/mL)
Distilled water (control)	23.56 ± 1.68^{a}	2.84 ± 0.17^{a}	3.18 ± 0.22^{a}	28.45 ± 2.52^{a}	9.26 ± 0.53^{a}
Fluoxetine + Distilled Water	16.74 ± 1.03^{b} (28.95%)	1.48 ± 0.09^{b} (47.89%)	2.27 ± 0.17^{b} (28.62%)	19.59 ± 1.48^{b} (31.14%)	14.23 ± 0.98^{b} (53.67%)
Fluoxetine+ 10mg/kg body weight of Tadalafil	22.87 ± 1.62^{a}	2.51 ± 0.12^{a}	2.87 ± 0.19^{c}	27.04 ± 1.69^{a}	10.97 ± 0.72^{c}
Fluoxetine + 20 mg/kg body weight of extract	19.40 ± 1.04^{c}	1.98 ± 0.06^{c}	2.49 ± 0.16^{b}	21.40 ± 1.31^{c}	12.18 ± 0.84^{d}
Fluoxetine + 40 mg/kg body weight of extract	20.83 ± 1.62^{d}	2.26 ± 0.14^{ac}	2.71 ± 0.21^{c}	23.09 ± 1.73^{d}	11.64 ± 0.76^{d}
Fluoxetine + 80 mg/kg body weight of extract	22.45 ± 1.38^{a}	2.73 ± 0.14^{a}	3.11 ± 0.27^{a}	28.57 ± 2.04^{a}	10.61 ± 0.85^{c}

Data are mean of ten determinations ± SEM; Test values with superscripts different from the control down the group and for each hormone are significantly different ($P<0.05$).

neurotransmitters is the cause of depression. Fluoxetine acts by preventing the reuptake of one neurotransmitter, serotonin, by nerve cells after it has been released. Since uptake is an important mechanism for removing released neurotransmitters and terminating their actions on adjacent nerves, the reduced uptake caused by fluoxetine increases free serotonin that stimulates nerve cells in the brain (Brunkhorst *et al.*, 2015).

Sexual dysfunction is a common side-effect of fluoxetine and other SSRIs, and it has been reported that over a third of women using SSRIs therapeutically experience sexual dysfunction (Balon, 2006). The mechanisms responsible for such drug-induced sexual dysfunction in females have been difficult to identify, perhaps due to the intricacy of the female reproductive cycle and the number of neural and endocrine loops involved (Frohlich and Meston, 2000; Basson, 2002). Serotonin acts as a neurotransmitter in the central nervous system, and as a vasoconstrictor and vasodilator in the periphery. Since, in the periphery, the principal component of sexual arousal is vasocongestion of the genital tissue, it is likely that serotonin participates in producing normal sexual arousal. It is possible that sexual side effects seen with these drugs may result, at least in part, from their action on peripheral mechanisms (Frohlich and Meston, 2000).

Female rats must show a minimum of 25% reduction in sexual behaviours before they can be declared as having sexual dysfunction. Therefore, the lowered DF, HF, LF, GG and LB, as well the prolonged darting latency (DL) and hopping latency (HL) might be an indication that the animals have been induced with sexual dysfunction.

The proceptive phase (darting and hopping), which is an initial behaviour proposed by a female to initiate and maintain a sexual interaction and the receptive phase (lordosis: willingness to accept male mounting attempts) are useful indicators in the assessment of libido, sexual vigor, arousability, performance and motivation (Cotton *et al.*, 2006). The significant lowering of DF, HF and LF suggests an impaired sexual desire (libido). This reduction could be due to the capability of selective serotonin reuptake inhibitors (such as fluoxetine) to reduce mesolimbic dopaminergic activity as a result of inhibitory serotonergic midbrain raphe nuclei projections or inactivatory role of 5-HT1A receptor-mediated norepinephrine neurotransmission and decrease libido and arousal (Prabhakar and Richard, 2010). The reversal of these sexual behaviour indices after the administration of the aqueous extract of *P. amarus* leaves with days from the pattern observed in the fluoxetine-treated animals towards the distilled water control animals suggests progressive ameliorative effects on the sexual behaviour by the extract. This could be due to the ability of the extract to increase the levels of dopamine in the mesolimbic dopaminergic system either by blocking reuptake or antagonizing the serotonin subtype-2 receptor and facilitating disinhibition of decreased dopamine downstream (Burghardt and Gardner, 2013).

The prolongation of DL and HL, indicators of sexual arousability in female rats after the administration of fluoxetine could have resulted from decreased genital sensation due to inhibition of the synthesis of nitric oxide in the female genital tissue leading to poor vaginal smooth muscle relaxation in the fluoxetine-treated rats (Burghardt and Gardner, 2013). The ability of the aquous extract of *P. amarus* leaves to reverse the fluoxetine-treatment related prolongation in the DL and HL of female rats re suggestive of stimulation of sexual arousability, excitement, vigor and receptivity in the female rats (Phillips, 2000). These sexual behaviours were preceded by proceptive behaviours in the female rats. The ability of the extract to reverse the lowered numbers of genital grooming and licking behaviours of the sexually impaired female rats in this study implied intense restoration of the proceptivity and receptivity of the animals. It is important to note that the reversal of sexual impairment in the fluoxetine-treated female rats was more pronounced with the highest dose, 80 mg/kg body weight and the result compared favorably with the reference drug (Tadalafil) used in the present study.

Since the medicinal properties of plants are mainly attributed to the presence of secondary plant metabolites (bioactive agents and other nutrients), it is possible that the component(s) of the extract such as saponins, flavonoids and

alkaloids that might have acted by stimulating the clitoral cavernosal smooth muscle and increasing clitoral blood flow which results in genital engorgement and ultimately led to the enhanced sexual behavior in the present study (Lightner, 2002). Quercetin reported to be present in this plant might have also contributed to the ability of the plant extract to ameliorate sexual dysfunction in the female rats.

Progesterone, LH, FSH, estrogen and prolactin are hormonal markers of estrogenicity (Faccio *et al.*, 2013). The reduction in the levels of reproductive hormones (progesterone, luteinizing hormone, follicle-stimulating hormone and estrogen) in fluoxetine-induced sexual dysfunction in female rats could be due to the direct toxic effect of the drug on the gonads or an indirect effect on the pituitary gland (Maclean and Lee, 1999). The reversal in the levels of serum progesterone and estrogen in sexually impaired female rats treated with the extract might have decreased the contractility of the uterine smooth muscle (Patel *et al.*, 2014). The recovery in the levels of estrogen may have facilitated the ameliorative effects on the female sexual behaviour by promoting the formation of female secondary sex characteristics, increasing vaginal lubrication, promoting sexual receptivity and inducing lordosis behaviour (Christensen *et al.*, 2011; Handa *et al.*, 2012). In the same vein, the increase in the concentration of LH and FSH of sexually impaired animals after the treatment of the aqueous extract of *P. amarus* leaves might not only lead to the release of egg from the follicle, but could also initiate the conversion of the residual follicle into a corpus luteum that, in turn, produces progesterone to prepare the endometrium for a possible implantation (Quinn *et al.*, 2007). However, elevated levels of prolactin have been reported to decrease the concentrations of reproductive hormones by counteracting the effect of dopamine, which is responsible for sexual arousal (Grattan *et al.*, 2007). This may be attributed to be the likely cause of the rise in the levels of prolactin in fluoxetine-induced sexually impaired female rats. The trend of reversal in the levels of prolactin after administration of the aqueous extract of *P. amarus* leaves may have promoted the sexual behaviour of the female rats by disinhibiting gonadotropin releasing hormone to stimulate the pituitary gland to increase the levels of sex hormones.

Conclusion

Considering all the findings in the present research work, aqueous extract of *P. amarus* leaves restored sexual competence in fluoxetine-induced sexually impaired female rats and the best activity was observed with the highest dose of 80 mg/kg body weight. This study has thus given scientific support to the popular use of *Phyllanthus amarus* in the management of sexual inadequacies in females.

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