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APHRODISIAC ACTIVITY OF ETHANOL ROOT EXTRACT AND FRACTIONS OF *LANDOLPHIA DULCIS* (SABINE) PICHON

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ABSTRACT

Background: Among the traditionally used sex enhancement natural remedies in south eastern Nigeria, *L. dulcis* root is very popular because of its quick onset of action. However, its use has not been scientifically validated. This study therefore investigated the effects of its ethanol extract and fractions on sexual behavior in male albino rats.

Methods: Forty-five male albino rats were randomly divided into nine groups $A_1 – D_1$, $A_2 – D_2$ and E. Animals in groups $A_1 – D_1$ received daily doses of 500mg/kg of ethanol extract and fractions (n-Hexane, Ethylacetate and Methanol) respectively while groups $A_2 – D_2$ received 1000mg/kg. Group E served as the control and receive 1ml of 10% tween 80. Equal numbers of female albino rats (45) were made receptive by hormonal treatment with estradiol benzoate (10µg/100g) and progesterone (0.5mg/100g). Sexual behavior parameters in male rats when paired with receptive females were monitored on days 1, 3 and 5. Male serum testosterone concentration was also determined.

Results: At 500 and 1000mg/kg, the ethanol extract and methanol fraction of *L.dulcis* showed a significant ($p<0.05$) increase in mount, intromission and ejaculation frequencies. This extract and fraction also significantly ($p<0.05$) reduce the mount and intromission latencies and prolonged ejaculation latency compared with the control animals. The ethanol extract and methanol fraction of *L. dulcis* also produced significant ($p<0.05$) increase in serum testosterone concentration.

Conclusion: The results from this study suggest that ethanol extract and methanol fraction of *L. dulcis* enhanced sexual ability in male rats and this could be attributed to relatively high contents of alkaloids, flavonoids and steroids. This study therefore justifies the traditional use of the root of *L. dulcis* in treating impotence and erectile dysfunction.

INTRODUCTION: Historically in all cultures, the sexual potency is considered a significant part of male ego and, anxiety and humiliation is frequently associated with a declining sexual ability [1].

Although erectile dysfunction does not affect life expectancy, it can have a significant negative impact in an individual's well-being and quality of life [2].

Male impotence is a common medical condition that affects the sexual life of millions of men worldwide [3, 4]. It is caused by various factors such as psychological disorders like Anxiety, depression, stress, fear of sex, neurological disorders, diabetes, hypertension, chronic alcohol abuse and aging [5, 6] to mention but a few.

Plants are an important source of medicines and play a key role in the health of the world's population. The use of plant materials to treat sexual disorders have a long history in most countries, and plant materials have been proven effective in improving sexual desire and sexual behavior in male animals [7 - 9]. *L. dulcis* also known as 'utu akwara' in the south-east and south-south, Nigeria is a climber widely dispersed in dense forest from Guinea to south Nigeria and extending to Congo. The leafy twigs and powdered back decoction is use in the treatment of serious wounds [10] while the trunk-bark and root decoction are used as a galactogogue by application to the breast [10]. The root decoction is used traditionally in south eastern Nigeria to enhanece sexual performance.

Pharmacotherapy of erectile dysfunction involves locally acting vasoactive drugs such as papaverine and alprostadin [11]; with phosphodiesterase type-5 (PDE-5) inhibitors such as sildenafil, verdenafil and tadalafil acting as the first line oral therapy. These options however, are too expensive, not easily accessible and characterized with some serious side effects [12, 13]. This problem coupled with the increasing number of men seeking help for the treatment of erectile dysfunction, has necessitated the need for more pharmacological research on cheaper available natural treatment options.

MATERIALS AND METHOD:

Plant material: Root Samples of *L. dulcis* were collected from Nsukka Enugu State, Nigeria and authenticated by Mr Ugwuozo Paulinus of the Department of Botany, Nnamdi Azikiwe University Awka, Anambra State, Nigeria.

Assay Kits: Testosterone Enzyme Immunoassay test kit was obtained from Diagnostic Automation Inc., Calabasas, USA while estradiol benzoate and progesterone were obtained from Sigma Chemicals, USA and Shalina Laboratories Mumbai, India respectively.

Extraction and Fractionation: The root, cut into pieces was air dried under room temperature for one month and pulverized into coarse powder. 1.2 g of the powder was extracted with 12.5 L of 70% ethanol for one week using cold maceration method with constant shaking. The extract was filtered and concentrated in vaccuo at 40^0C using rotary evaporator. 129.78 g was adsorbed in silica gel (70-200 mesh size) and eluted in succession with n-hexane, Ethylacetate and methanol. The resultant yields were stored at -4^0C in a refrigerator till further use.

Phytochemical Analysis: This was carried out using standared method as described by [14, 15].

Experimental animals: Healthy adult male albino rats weighing 150-200g and females weighing 120-160g were obtained from the animal house of Department of Pharmacology/Toxicology, Faculty of Phrmaceutical Science, Nnamdi Azikiwe University, Agulu Campus Anambra State, Nigeria. The animals were housed in clean aluminum cages and maintained in standard animal conditions. They were allowed free access to food and water *ad libitum*.

Acute toxicity study: This was done using Lorke method [16]. A total of 13 animals were used for the study. The study was divided in two phases. In the first phase nine animals were used they were grouped into three groups of three animals each and were given 1000, 2000 and 4000mg/kg of the extract (p.o). The mice were observed for signs and symptoms of toxicity and mortality over a period of 24 h. Four mice were administered 5000mg/kg of the extract in the second phase and observed for another 24 h post administration.

Experimental Design: A total of 90 animals made up of equal number of male and female rats were used. Forty-five male rats were grouped into nine (A_1 - D_1, A_2 - D_2 and E) consisting of five animals each. Animals in groups A_1 - D_1 were treated once daily with 500mg/kg of the ethanol extract and fractions while animals in groups A_2 - D_2 were treated with 1000mg/kg of the extract and fraction respectively.

Animals in group E (control) were orally administered once daily with 1ml of 10% tween 80 using metal oropharyngeal cannula.

The male rats from each group were monitored for sexual behavior on day 1 (after a single dose), day 3 (after three doses, once daily) and day 5 (after five doses, once daily).

Male rat sexual behavior test procedure: The male rats were trained with sexually receptive females three times according to the method described by Burses *et al*., [17] before the commencement of the experiment. The test was carried out under dim light. The female rats were made receptive following the sequential subcutaneous administration of 10µg/100g of estradiol benzoate, and 0.5mg/100g of progesterone 48 h and 4 h respectively prior to pairing. This treatment assures intense proceptivity and receptivity [18].

The receptive females were introduced to the male after a 30 min adaptation period in a plastic cage. The male and female rats were observed for proceptive and precopulatory behaviours respectively. The male rats from each group were monitored for sexual behavior for 30 min observatory period after their daily doses on days 1, 3 and 5.

Adopting the standard procedures of Agmo [18] and Gauthaman *et al* [19], the following male sexual behaviour indices were recorded or calculated for the observatory period: mount frequency (MF), (the number of times the male assumed copulatory position but failed to achieve intromission-characterized by lifting of the male's forebody over the hindquarter of the female and clasping her flanks with his forepaw); intromission frequency (IF), (the number of vaginal penetration made by the male); ejaculation frequency (EF), (the number of times there was expulsion of semen by the males after vaginal penetration-characterized by rhythmic contraction of the posterior abdomen).

Other standard parameters of sexual behaviour obtained through manual data acquisition using stopwatch included mount latency (ML), (the time from the introduction of the female until first mount by the male); intromission latency (IL), (the time from the introduction of the female until the first intromission by the male-usually characterized by pelvic thrusting and springing dismount; ejaculation latency (EL), (the time from the first intromission until ejaculation-usually characterized by longer, deeper pelvic thrusting and slow dismount followed by a period of reduced activity; and postejaculatory interval (PEI), (the time interval from ejaculation to intromission of the next series).

Determination of Serum Testosterone Concentration: Blood samples were collected from the male rats 3 h after the 5th day of ethanol root extract administration through retro orbital venous plexus. The blood samples were allowed to clot for 10min at room temperature and subsequently centrifuged. The sera were aspirated with Pasteur pipette and used for the determination of testosterone concentration within 12 hours of preparation.

The serum testosterone concentration of the animals was determined using the procedure outlined in the manufacturer's instruction manual. This was based on the principle of competitive binding between testosterone in the test specimen (serum) and testosterone-HRP conjugate for a constant amount of rabbit antitestosterone [20].

Statistical Analysis: Data were expressed as the mean of five replicates ± SD. Means were analyzed using a one-way analysis of variance (ANOVA) and complemented with Student's t-test. All the statistical analyses were done using SPSS, Version 15.0 (SPSS Inc., Chicago, IL, USA). P values < 0.05 were considered significant.

RESULTS AND DISCUSSION: The median lethal dose (LD_{50}) of the ethanol extract of *L. dulcis* reveals that the plant is safe up to 5000mg/kg as no death was recorded at this dose [21]. The result of the phytochemical analysis indicates the presence of useful phytocompound (**Table 1**) most of which have been established to possess aphrodisiac activity [22].

Throughout the duration of the experiment, procopulatory sexual behavior (chasing, anogenital sniffing, mounting) was observed most especially in the groups treated with ethanol extract and methanol fraction, an indication of sexual arousal.

Ethanol extract and methanol fraction at 500mg/kg showed significant ($p<0.05$) increase in mount frequency (MF) on days 3 and 5 while at 1000mg/kg, there was a significant ($P<0.05$) increase from the first day of administration and subsequently at all other days (**Table 2**).

Significant (p<0.05) increase in intromission frequency (IF) was observed on days 3 and 5 for the ethanol extract at 500 and 1000mg/kg; and on days 1, 3 and 5 for the methanol fraction at same doses (**Table 3**). Mount Frequency and Intromission Frequency are useful indices of vigour, libido and potency. While the number of mount (MF) reflects sexual motivation, increase in the number of intromission (IF) shows the efficiency of erection, penile orientation and the ease by which ejaculatory reflexes are activated [18].

These effects suggest that ethanol extract and methanol fraction of *L. dulcis* enhance libido. Such enhancement of libido might have arisen from increase in the number of concentrations of several anterior pituitary hormones and serum testosterone, which in turn stimulated dopamine receptor synthesis and sexual behaviour [23]. This is supported by the fact that there was a significant (p<0.05) increase in serum testosterone concentration of ethanol extract and methanol fraction at 500 and 1000mg/kg (**Table 4**).

Furthermore, since intromission is not possible without adequate erection and coordinated activity of penile muscles [18], the increase in IF by the extract and fraction in this study suggests that the mechanism of penile erection was activated. Therefore, ethanolic extract and methanolic fraction of *L. dulcis* root may increase potency by allowing or sustaining erection.

Alkaloids have been shown to have ergogenic properties by inducing vasodilation of the blood vessels which consequently result in erection [18]. Therefore the alkaloid content in the ethanol extract and methanol fraction of *L. dulcis* root may be implicated in the increased aphrodisiac activities through same mechanism. Saponin have been indicated as acting as a nitric oxide donor, inducing the relaxation of smooth muscle corpus cavanosum through the L-arginine/nitric oxide pathway [24].

The enhanced intromission frequency associated with the ethanol extract and methanol fraction may be attributed to their alkaloid and or saponin content. The increase in MF and IF is accompanied by a significant (p<0.05) increase in ejaculation frequency recorded at days 3 & 5 for both ethanol extract and methanol fraction at 500mg/kg and on day 3 & 5 for ethanol extract and days 1, 3 and 5 for methanol fraction at 1000mg/kg.

Mount latency (ML) and intromission latency (IL) are indicators of sexual motivation. ML and IL are inversely proportional to sexual motivation. Therefore, the significant ($P < 0.05$) decrease in the mount and intromission latencies observed at 500 and 1000mg/kg bodyweight of the ethanolic extract and methanolic fraction on days 1, 3 and 5 as compared to the negative control (**Table 6 and 7**) in this study might imply stimulation of sexual motivation and arousability [18].

It may also be an indication of enhanced sexual appetitive behaviour in the male rats. All these further support the sexual function improving effect of the extract and fraction at these doses. Furthermore, the prolonged ejaculation latency by the 500 and 1000mg/kg bodyweight of ethanolic extract, methanolic fraction on days 1, 3 and 5 as compared to the negative control (**Table 8**) is an indication that copulatory performance in the animals was enhanced. It may also imply prolongation in the duration of coitus.

The post ejaculatory interval (PEI) is considered an index of potency, libido and the rate of recovery from exhaustion after first series of mating [25]. A post ejaculatory interval of more than 5400s indicates that the male is sexually exhausted and the intensity of sexual behaviour will be reduced in subsequent mating [18].

Therefore, the significant ($P < 0.05$) decreased in post ejaculatory interval at 500 and 1000mg/kg bodyweight of ethanolic extract and methanolic fraction of *L. dulcis* on days 1, 3 and 5 as compared to the control (**Table 9**) may be attributed to enhanced potency and libido or less exhaustion in the first series of mating or both, more so, since the values of PEI obtained in this study are not up to or in any way close to the 5400s cut-off [25].

Many plants with medicinal properties are effective as aphrodisiac through mechanisms such as vasodilation, generation of nitric oxide, elevation of androgens and gonadotropins [26]. It has also been documented that sexual behaviour and erection are dependent on androgen which may act through central and peripheral mechanisms [26]. Treatments that alter the concentration of circulating sex hormones may also modify sexual behaviour.

TABLE 1: EFFECT OF *LANDOLPHIA DULCIS* ROOT EXTRACT AND FRACTIONS ON SERUM TESTOSTERONE CONCENTRATIONS OF MALE RATS

Dose (mg/kg)	Concentration (nmol/L)				
	Ethanol extract	N-Hexane F.	Ethyl-acetate F.	Methanol F.	Negative control
500	*3.47±0.08	1.39±0.10	1.39±0.14	*5.55±0.11	-
1000	*5.55±0.14	2.08±0.12	1.39±0.16	*13.89±0.15	-
10% Tween 80	-	-	-	-	1.39±0.09

n =5± SD, *=P‹ 0.05

TABLE 2: EFFECT OF *LANDOLPHIA DULCIS* ROOT EXTRACT AND FRACTIONS ON MOUNT FREQUENCY OF MALE RATS

Dose	Ethanol extract			Methanol F.			N-Hexane F.			Ethyl-acetate F.			Negative control		
(mg/kg)	Day 1	Day 3	Day 5	Day1	Day 3	Day 5	Day 1	Day 3	Day 5	Day 1	Day 3	Day 5	Day 1	Day 3	Day 5
500	7±0.8	* 8.6±0.6	*10±0.9	7±0.6	*11.6±0.2	*14±0.7	6±0.3	6.4±0.4	6.9±0.3	6±0.5	6±0.4	6.8±0.5	-	-	-
1000	*8.9±0.9	*12±0.9	*15±0.8	*10±0.6	*16±0.8	*18.6±0.3	6.6±0.7	7.2±0.9	7.4±0.9	6.6±0.6	6.4±0.7	6.8±0.9	-	-	-
10% Tween 80	-	-	-	-	-	-	-	-	-	-	-	-	6±0.7	5±0.3	6±0.8

n =5± SD, *=P‹ 0.05

TABLE 3: EFFECT OF *LANDOLPHIA DULCIS* ROOT EXTRACT AND FRACTIONS ON INTROMISSION FREQUENCY OF MALE RATS

Dose	Ethanol extract			Methanol F.			N-Hexane F.			Ethyl-acetate F.			Negative control		
(mg/kg)	Day1	Day3	Day5	Day1	Day3	Day5	Day1	Day3	Day5	Day1	Day3	Day5	Day1	Day3	Day5
500	4± 0.2	*6.5±0.9	*7±0.6	*6±0.4	*8.8±0.1	*9.4±0.4	4±0.2	4±0.1	4.6±0.2	4±0.2	4±0.4	4.8±0.2	-	-	-
1000	5.6±0.9	*8±0.1	*9±0.4	*7±0.7	*10±0.9	*11±0.1	4±0.4	4.5±0.2	5±0.7	4.6±0.3	4.8±0.1	5±0.2	-	-	-
10% Tween 80	-	-	-	-	-	-	-	-	-	-	-	-	4±0.3	4±0.1	4.6±0.3

n =5± SD, *=P‹ 0.05

TABLE 5: EFFECT OF *LANDOLPHIA DULCIS* ROOT EXTRACT AND FRACTIONS ON EJACULATION FREQUENCY OF MALE RATS

Dose	Ethanol extract			Methanol F.			N-Hexane F.			Ethyl-acetate F.			Negative control		
(mg/kg)	Day1	Day3	Day5	Day1	Day3	Day5	Day1	Day3	Day5	Day1	Day3	Day5	Day1	Day3	Day5
500	1.4±0.2	*2.8±0.2	*3±0.4	1.4±0.3	*4±0.5	*5.2±0.2	1±0.2	1.6±0.1	2±0.1	1±0.1	1.2±0.1	1.8±0.2	-	-	-
1000	1.8±0.1	*4.4±0.6	*5.8±0.9	*4±0.7	*6.6±0.9	*7.6±0.4	1.4±0.1	1.7±0.2	2.3±0.1	1.2±0.4	1.8±0.1	2±0.2	-	-	-
10% Tween	-	-	-	-	-	-	-	-	-	-	-	-	1±0.0	1.2±0.1	1.8±0.1

n =5± SD, *=P‹ 0.05

Dose	Ethanol extract			Methanol F.			N-Hexane F.			Ethyl-acetate F.			Negative control		
(mg/kg)	Day1	Day3	Day5	Day1	Day3	Day5	Day1	Day3	Day5	Day1	Day3	Day5	Day1	Day3	Day5
500	*646±1.1	*612±2.8	*598±2.1	*590±4.0	*551±2.6	*514±2.4	720±3.6	714±2.2	706±1.4	726±1.2	712±3.5	700±3.3	-	-	-
1000	*600±3.3	*580±3.5	*509±1.9	*502±1.2	*470±2.9	*406±3.1	696±3.0	684±1.4	678±1.9	720±2.8	710±3.4	708±3.1	-	-	-
10% Tween 80	-	-	-	-	-	-	-	-	-	-	-	-	730±4.7	720±1.6	712±2.3

n =5± SD, *=P‹ 0.05

Dose	Ethanol extract			Methanol F.			N-Hexane F.			Ethyl-acetate F.			Negative control		
(mg/kg)	Day1	Day3	Day5	Day1	Day3	Day5	Day1	Day3	Day5	Day1	Day3	Day5	Day1	Day3	Day5
500	*679±1.6	*640±3.2	*611±1.0	*613±3.5	*567±2.9	*502±2.1	732±3.1	720±1.4	710±0.9	740±1.8	721±3.5	712±1.4	-	-	-
1000	*614±3.1	*593±1.4	*520±3.3	*524±1.9	*482±1.6	*412±4.3	691±3.2	686±1.1	673±2.9	729±1.4	715±2.5	710±3.9	-	-	-
10% Tween 80	-	-	-	-	-	-	-	-	-	-	-	-	750±2.1	726±1.3	719±1.9

n =5± SD, *=P‹ 0.05

TABLE 8: EFFECT OF *LANDOLPHIA DULCIS* ROOT EXTRACT AND FRACTIONS ON EJACULATION LATENCY (SEC) OF MALE RATS

Dose	Ethanol extract			Methanol F.			N-Hexane F.			Ethyl-acetate F.			Negative control		
(mg/kg)	Day1	Day3	Day5	Day1	Day3	Day5	Day1	Day3	Day5	Day1	Day3	Day5	Day1	Day3	Day5
500	*245±3.2	*260±1.5	*318±3.3	*260±3.3	*304±2.8	*380±3.5	190±1.2	195±2.1	200±2.8	198±1.5	200±3.7	198±3.4	-	-	-
1000	*250±3.4	*310±3.6	*417±1.5	*286±2.5	*340±2.1	*450±0.9	212±3.2	220±3.3	226±1.1	198±2.9	198±3.2	202±3.2	-	-	-
10% Tween 80	-	-	-	-	-	-	-	-	-	-	-	-	200±1.6	200±2.2	198±2.9

n =5± SD, *=P‹ 0.05

TABLE 9: EFFECT OF *LANDOLPHIA DULCIS* ROOT EXTRACT AND FRACTIONS ON POST-EJACULATION LATENCY (SEC) OF MALE RATS

Dose (mg/kg)	Ethanol extract			Methanol F.			N-Hexane F.			Ethyl-acetate F.			Negative control		
	Day1	Day3	Day5	Day1	Day3	Day5	Day1	Day3	Day5	Day1	Day3	Day5	Day1	Day3	Day5
500	*300±3.4	*261±1.4	*150±3.3	*216±1.9	*144±1.6	*130±4.3	380±3.2	378±1.1	370±2.9	378±3.4	378±2.5	371±3.9	-	-	-
1000	*210±2.1	*140±2.8	*128±2.1	*146±3.0	*115±2.6	*92±2.4	405±3.6	368±2.2	350±1.4	378±1.2	378±3.5	375±3.3	-	-	-
10% Tween 80	-	-	-	-	-	-	-	-	-	-	-	-	376±1.7	374±1.6	375±3.4

n =5± SD, *=P‹ 0.05

Clinical data on testosterone also suggest that a slight increase in the levels of the hormone in adult males results in a moderate but significant increase in sexual desire and libido [27]. Therefore, the increase in serum testosterone concentration by the ethanol extract and methanol fraction of *L. dulcis* root at 500 and 1000mg/kg might be responsible for the enhanced sexual behaviour in the animals.

CONCLUSION: The ethanol root extract of *L. dulcis* have a significant aphrodisiac activity with more effect residing on its methanol fraction. This justifies the folkloric use of the root of this plant in enhancing sexual activity in south eastern Nigeria. Work on assessment of its safety is on-going.

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