Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Der Pharmacia Sinica, 2015, 6(12):29-38

Der Pharmacia Sinica
ISSN: 0976-8688
CODEN (USA): PSHIBD

Chromatographic fraction of *Abrus precatorius* leaf extract increases cGMP and nitric oxide concentration in female rats

Ijeoma H. Ogbuehi[1, 2], Omotayo O. Ebong[1, 2], Atuboyedia W. Obianime[1] and Ozadheoghene E. Afieroho[3]

[1]*Department of Pharmacology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt, Nigeria*
[2]*Centre of Malaria Research and Phytomedicine, University of Port Harcourt, Nigeria*
[3]*Department of Pharmacognosy and Toxicology, Faculty of Pharmaceutical Sciences, College of Health Sciences, University of Port Harcourt, Nigeria*

ABSTRACT

Methanolic extract of Abrus precatorius leaf was subjected fractionation using the column chromatography. Six fractions of this plant were eluted, and their fluorescent characteristics, wavelengths and Rf values, noted. The fractions were administered to female wistar rats at 5mg/kg orally for eight days. Fraction 4, sub-fraction 4a caused a significant ($p<0.05$) increase in the index of libido (mount frequency, 2.05 ± 0.16; mount latency, 23.48 ± 0.19; intromission latency, 3.05 ± 0.28 and lordosis quotient, 87%±1.0). The cGMP and Nitric oxide levels in the glans clitoridis of the female wistar rats post treatment with the fractions, arginine, yohimbine and sildenafil were also measured. Treatment with Sildenafil, Arginine, Yohimbine and F4a produced the 114.2%, 87.7%, 57.1% and 71.4% increase respectively, in NO levels; cGMP levels were also markedly increased in the Sildenafil, Arginine, and F4a treated groups. There was also a significant reduction in the systolic blood pressure and heart rate of female rats during the treatment period with f4a, Sildenafil citrate, Arginine and Yohimbine, possibly due to a vasodilatation effect. In conclusion, sub - fraction 4a may be responsible for the libido- enhancing activity of the plant extract.

Keywords: sexual behavior, libido, mounting, lordosis, *glans clitoridis*

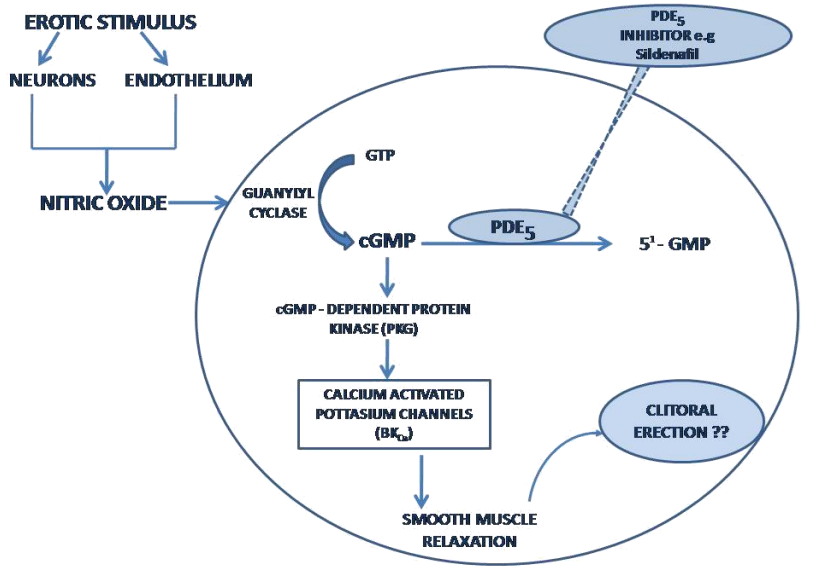
INTRODUCTION

A huge variety of phytochemicals have shown promise as potential sources of novel pharmacologic agents [1]. Quite a number of these plant components have been validated for their effect on sexual behavior and can therefore serve as basis for the identification of new chemical leads useful in sexual dysfunction [2]. Detailed studies of these promising compounds at molecular levels have helped in the identification of the key physiologic factors involved in sexual arousal.

Since the turn of the 21st century, research on female sexual dysfunction (FSD) has gained momentum. While FSD is often assessed in people with ill health, sexual dysfunction is an illness of its own entity and is also prevalent in non-patient populations [3]. Female sexual arousal disorder is characterized by a persistent or recurrent inability to attain sexual arousal or to maintain it until the completion of a sexual activity [4], which may sometimes be as a result of an inadequate lubrication-swelling response. Female sexual dysfunction is a complex condition with both psychological and physiologic components [5]. Therefore, there are basically two therapeutic options for female sexual arousal disorder; psychotherapy and pharmacotherapy [6]. In the quest for an aphrodisiac of choice, several agents have been used in the management of sexual arousal disorder in women [7, 8, 9, 10]. However, there is no Food and Drug Administration (FDA) approved drug for this condition [6]. While some argue that commonly cited prevalence estimates of female sexual dysfunction are exaggerated for the purposes of marketing products [11],

other researchers consider it a poorly described but significant public health condition [3].
The physiology of penile erection has been well researched, thus the relationship of the L-arginine/NO/ Guanylylcyclase/cGMP pathway and male sexual arousal is clearly elucidated in literature [12, 13, 14], but that cannot be said of female sexual dysfunction. Generally, there is limited understanding of local regulatory mechanisms modulating clitoral and vaginal smooth muscle tone. However, studies in female genital tissues have shown that the physiology of vaginal hemodynamics and lubrication responses is highly dependent on tissue structural and functional integrity and involves complex neurovascular processes modulated by various local neurotransmitters [15, 16].

Several studies has demonstrated that the NO-cGMP pathway plays a key role in the female genital sexual arousal response [15, 16, 17, 18]. Nitric oxide (NO) is produced from oxygen and L-arginine under the control of nitric oxide synthase (NOS). The critical role of arginine as a substrate for both nitric oxide synthase and arginase serves as a potential point of regulation for the NO/cGMP pathway. NO penetrates into the cytoplasm of smooth muscle cells and activates the guanylylcyclase causing a catalytic production of cyclic guanosine monophosphate from guanosine-triphosphate. Increased intracellular cGMP inhibits calcium entry into cells and decreases intracellular calcium. Activation of K^+ channels caused hyperpolarization and smooth muscle relaxation. More so, cGMP activates cGMP-dependent protein kinase (PKG), which in turn activates myosin light chain phosphatase, the enzyme that dephosphorylates myosin light chains. These interactions result in reduced intracellular calcium levels and a consequent relaxation of arterial and trabecular smooth muscle, leading to arterial dilatation, venous constriction, and the rigidity of clitoral erection. When sufficient sexual excitement is attained, the female genital arousal response is evident, leading to genital engorgement, swelling, and lubrication. Thus genital vasocongestion and vaginal lubrication responses result from increased blood flow to the genital tissues and production of lubricating fluid transudate from the vaginal epithelium. The vascular engorgement seen in female genital tissues during the excitement phase has been shown to be mediated by the parasympathetic nervous system and the NO-cGMP pathway. Little wonder then that, relaxation of corporal vascular smooth muscles leads to clitoral tumescence and dilation of the perivaginal arterioles, with seeping of vascular transudate to the vaginal wall culminating in lubrication [19,20].



Mechanism of Smooth Muscle Relaxation via the NO/cGMP pathway

Since the NO/cGMP pathway is an important modulator of vaginal smooth muscle contractility [21] and blood flow in the animal model [22, 23], the present study investigated the effect of fractions from *A. precatorius* leaf extract on NO and cGMP concentrations [24]. Nitric oxide synthases (nNOS&eNOS) have been reported as differentially distributed among the anatomic regions of the rabbit vagina with higher activity seen in the proximal than distal vagina [25]. Also, it is possible that since nitric oxide (NO) synthase isoforms have been identified in the uterine [26] and clitoral tissues [27]; the NO–cyclic guanosine monophosphate (cGMP) pathway, which is involved in penile erection and enhanced by sildenafil [18, 28, 29], may also be involved in female sexual arousal response. Thus, in addition to the extracts, we also studied the effect of sildenafil on heart rate, lordosis quotient and mounting frequency in female rats.

In female rats, libido and other sexual behaviors can be measured using an array of parameters. Mounting is defined as the climbing of one animal by another usually from the posterior end with the intention of introducing one organ into another. Sexually active or artificially 'warmed' females evoke increase in frequency of mounts by the male due to their heightened display of copulatory behaviors (such as postures, darting, wiggling of ears, sounds and hyperactivity).

Mount Frequency (MF) is therefore defined as the number of mounts without intromission from the time of introduction of the female until ejaculation [30]. The Mount latency is reduced by the introduction of a female animal in heat**.** Mount Latency (ML) is defined as the time interval between the introduction of the female and the first mount by the male [30]. The Intromission Latency (IL) is the time interval from the time of introduction of the female to the first intromission by the male. This is usually characterized by pelvic thrusting, and springing dismounts [30].

In an earlier study [31], it was suggested that *A. precatorius* leaf extract is a potent stimulator of sexual arousal in female rats due to its effect on parameters such as lordosis quotient and mounting frequency. However, in a bid to narrow down the exact agent responsible for the said action and determine the possible mechanism of action of the extract, chromatographic fractions of the leaf extract were generated, tested on female rats for possible effect on second messenger, cGMP and neurotransmitter, nitric oxide.

MATERIALS AND METHODS

EQUIPMENT AND REAGENTS

Photo Microscope (Olympus, Japan), Rotary Evaporator (Heildolph Instruments, Germany), tissue homogenizer (Omni International), Mettler – toledo GmbH digital weighing balance (Type BD202, SNR 06653), Uniscope Laboratory Centrifuge (Model M800B, Surgifriend Medicals and Essex, England), Non-invasive Blood Pressure Monitor (UgoBasile) and Photoelectric Colorimeter are the instruments used. Syringes (1 ml, 5 ml), oral cannula, cotton wool, Heparinized and non-heparinized sample bottles, capillary tubes, EDTA bottles, Methanol, n-Hexane, Acetic acid glacial, Silica gel (200 - 400 mesh), Chloroform (Sigma Aldrich, St, Louis, MO, USA) were also used. Drugs and supplements such as Clomiphene citrate (Clomid, Bruno Farmaceutici S.P.A. Roma), Estradiol (Estrace®, Trimel Pharmaceuticals Corporation), Arginine (Argmatrix™, Boresha International Products), Tween- 80, Yohimbine (Yohimbine HCl, A-Z Diet products Inc), Sildenafil (Viagra, Pfizer Sandwich, UK) were purchased from *Litha Pharma Stores*, Cape Town, South Africa. Nitric Oxide Colorimetric Assay Kit (BioVision Research Products, USA) and cGMP Enzyme Immunoassay Kit (Assay Designs Inc., Ann Arbor, MI, USA) were ordered from Joechem Ventures, Nsukka, Nigeria. All reagents used were of analytical grade and were prepared according to specifications using appropriate solvents and distilled water.

PLANT EXTRACTION

Freshly harvested leaves of *A. precatorius* were washed and shade dried to constant weight. The dried samples were milled into coarse powder by a mechanical grinder. Three hundred grams of dried powdered leaves were soaked in 2 litres of Methanol (70%) for 72 hrs. The mixture was allowed overnight, and stirred at regular intervals, thereafter it was filtered with a Whatman No. 1 filter paper. The filtrate was concentrated in a hot water bath at 60^{o}C for 5 hrs to give an extract. The residue (Marc) thus obtained was dried in a dessicator and refrigerated at 5^{o}C until required for use.

The percentage yield of extract was calculated as:

$$\frac{\text{weight of dried extract x 100\%}}{\text{weight of plant material}}$$

PLANT PURIFICATION

Column Chromatography

The extract was further subjected to bioassay guided fractionation, using column chromatography for separation and elucidation of its components. Silica gel 200-400 mesh was used as the stationary phase while varying solvent combinations of increasing polarity were used as the mobile phase.

In the setting up of column chromatography, dry packing technique was used in which 300g of sample was first dissolved in 350ml of methanol solvent and then, 100mg of silica gel was added

to form the slurry. The mixture was swirled continuously until the solvent evaporated and only a dry powder remained. The column was prepared by stocking the lower part of the glass column with cotton wool using a glass rod. The dried slurry was weighed and poured down carefully into the prepared column. The elution sequence is as

follows: a 25% binary gradient (stepwise) of n-hexane and ethyl acetate through methanol, in increasing order of polarity. Each gradient was 500ml. The eluting solvents were forced through the column so that the solvents were at the very top of the silica. At the end of the packing process, the tap was locked and the column, allowed for 24 hours to stabilize, after which, the clear solvent on top of the silica gel was drained down to the silica gel meniscus. The column tap was opened to allow the eluent to flow at the rate of 40 drops per minute. The colored bands travelled down the column as the compound is eluted. As soon as the colored compound begins to elute, the collection beaker was put under the stopcock to collect fractions. The eluted fractions were then collected in aliquots of 10 ml in test tubes.

Finally, eluents having similar constituents were pooled. Some of the column chromatographic eluents of the crude methanol leaf extract were combined and re-designated. Column chromatographic fractions were collected in separate vials and stored in deep freeze.

Thin Layer Chromatography

The eluted fractions obtained from the column chromatography were further subjected to a complementary process of purification using the TLC. Silica gel plates were activated at 120°C for 1h before use as adsorbent on pre-coated 20×20 cm and 0.25 mm thick plates, while iodine vapour was the detecting agent. Column chromatographic elution of crude methanol extract was made using solvent systems including 100% n-hexane (M1), 50% n-hexane/ 50% chloroform (M2), 100% chloroform (M3), 75% chloroform / 25% methanol (M4) , 50% chloroform / 50% methanol (M5), 25% chloroform / 75% methanol (M6) and 100% methanol (M7).

The TLC plates were trimmed to strips and the position of the origin marked by a straight line. Using a micro pipette, 10µl of each fraction collected from column chromatography was loaded to the marked points about 10mm from the bottom of silica plate, and put in a lidded tank containing a solvent system. The plates were developed in hexane: ethyl acetate (90:10, v/v) at

room temperature, left overnight for air drying, and the separated spots were visualized by iodine fume. The procedure was followed with other strips and various solvent-solvent ratios (n-butanol: acetic acid: water in 12:3:5, 12:1:1, 10:1:1, and 4:1:1) until good resolution was noticed.

The solvent travelled up the plate by capillary action till it reached the solvent frontal point which was also marked by a straight line across [32, 33]. The lid was opened and the strip dried and then sprayed with silver nitrate and iodine vapour so as to be visualized under UV light (254nm). Commercially prepared Abrus was used as reference standard.

IN VIVO BIOASSAY SCREENING OF THE FRACTIONS OF *A. PRECATORIUS* LEAF EXTRACT

Libido study

The different pure fractions/eluents obtained from chromatographical separation were individually tested on female animals. The fractions (f1, f2, f3, f4, f5 and f6) were administered to experimental animals *per os* for 8 days (2 estrus cycles). The rats were randomly partitioned into 9 groups of 5 animals each and using distilled water (0.2ml) as control and Arginine (10mg/kg), yohimbine HCl (10mg/kg), sildenafil (50mg/kg), estradiol (10mg/kg), as references. Animals on the same estrous cycle stage were selected for this study. Extracts were administered to experimental animals *per os* for 8 days (2 estrus cycles). The rats were randomly partitioned into 8 groups of 5 animals each and treated as follows: Group 1: PBS (0.2ml); Group 2: yohimbine (10mg/kg); Group 3: sildenafil citrate (50mg/kg); Group 4: estradiol (10mg/kg); Group 5 - 10: APL f1, f2, f3, f4, f5 and f6 (5mg/kg). The following sexual behavioral parameters, as described in a previous study [34], were recorded for 30 minutes by a trained observer who was unaware of the treatment given to each group:

- Mount latency: Time duration (in mins) from the introduction of the male into the cage till the first mount.
- Intromission latency: Time duration (in mins) from the introduction of the male into the cage till the first intromission (vaginal penetration).
- Mount frequency: Total number of mounts preceding ejaculation.
- Lordosis quotient which is simply the ratio of the number of lordosis postures characterized by immobility by the female, arching of the back and hind leg extension that elevates the rump and head, shown by a female in response to a 10 mounts, times 100. These were determined using on-site observation, stopwatch and videotape review and were taken as an index of libido.

Measurement of Blood Pressure

Pre, during and post treatment, blood pressure of the experimental rats were also measured non-invasively using tail

cuff blood pressure monitor. The animals were placed one at a time into the holder, and their noses were properly aligned to achieve a comfortable position. The animals were allowed to relax for minimum of 15 mins and then the cuff was fastened. The monitor was inflated to 300mmHg, and pulses were recorded during deflation at a rate of 3 mmHg. Blood pressure was determined in triplicate for each animal, with the average systolic blood pressure being recorded. Blood pressure was categorized as high when SBP > 150.

Preparation of *Glans clitoridis* and Tissue Homogenization

On the 8th day after treatment and observation, the treated animals were fasted overnight. The fasted animals were anaesthetized under chloroform and then, sacrificed. The clitoris (*glansclitoridis*) was separated from the rest of the genitalia by sharp dissection, cleared of blood and immediately stored in a freezer at −80°C until use.

Determination of Tissue Nitric Oxide ($NO2^-$+$NO3^-$) Levels (NOS Activity)

Glans clitoridis tissue (0.2 g) was homogenized on ice, in 20ml of phosphate buffered saline(PBS), pH 7.4 using a handheld tissue homogenizer. The homogenate was centrifuged at 10,000 x g for 20 minutes. The supernatant was centrifuged at 100,000 x g for 15 minutes and then ultrafiltered using a 10 kDa Molecular Weight micron ultrafiltration chamber. 2 ml of glans clitoridis tissue homogenates were diluted two times with a reaction buffer provided in the assay kit. BioVision's Nitric Oxide Colorimetric Assay Kit was used to measure the total nitrate/nitrite in the samples.

Determination of cyclic Guanosine Mono Phosphate Levels

A weighed amount (0.2 g) of the *glans clitoridis* tissue of each sample was homogenized on ice using a handheld tissue homogenizer. The homogenate was centrifuged at 600 x g for 10 minutes and the supernatant were assayed for cGMP using cGMP Enzyme Immunoassay Kit.

STATISTICAL ANALYSIS

For each parameter, a minimum of three replicates were used and the results were expressed as Mean ± Standard Error of Mean (S.E.M) and analyzed using Student`s t-test to compare values from experimental and control groups. All the data were analyzed with least significant differences at $p<0.05$. Statistical analysis was carried out by using one-way analysis of variance

(ANOVA) followed by Dunnett's test.

ETHICAL CONSIDERATION

Ethical approval for the study was got from the Research Ethics Committee of the College of Health Sciences, University of Port Harcourt, Nigeria. The study was conducted in accordance with the Organization for Economic Development guidelines on good laboratory practice and use of experimental animals [35].

RESULTS

PLANT EXTRACTION

Table 1: Percentage Yield of Extracts

Plant Used	Part	Weight of Plant Sample Used (g)	Solvent Type	Extract yield (%)
Leaves		527	70% Methanol	18.4 g w/w

PLANT PURIFICATION
Column Chromatography

Fractionation of the Methanolic crude extract of *A. precatorius* leaves
A total of six (6) preliminary fractions of *A. precatorius* extract were eluted and identified. The fluorescent characteristics of the fractions including their Rf values and wavelengths are presented in Table 2 below.

Table 2: Characteristics of *A. precatorius*methanolic extract's fractions

Fractions	Quantity (mg)	Rf values	TLC Coloration @ UV 254nm
F1	1.87	0.78	Yellowish brown
F2	1.12	0.12	Light yellow
F3	1.08	0.41	Orange
F4	0.93	0.62	Grey
F5	1.64	0.82	Brown
F6	1.17	0.22	Reddish brown

Eluent: chloroform - hexane - methanol (3:2:1)

IN VIVO BIOASSAY SCREENING OF THE FRACTIONS OF *A. PRECATORIUS* LEAF EXTRACT
Libido study: The values of the sexual behavioural parameters studied, as reported in table 3 below.

Table 3: Effect of the Chromatographic Fractions of *A. Precatorius* Leaf Extract on Index of Libido in Female Rats

Treatment Groups	Mount Latency (min)	Mount Frequency	Intromission Latency (min)	Lordosis Quotient (%)
PBS (0.2mls)	3.83 ± 0.34	16.25 ± 0.67	4.17 ± 0.05	48 ±1.0
Yoh (10mg/kg)	2.19 ± 0.17*	19.46 ± 0.45*	3.30 ± 0.17	65±1.0
SC (50mg/kg)	3.28 ± 0.48	18.00 ± 0.16	2.37 ± 0.09*	66±2.0
E2 (10mg/kg)	3.46 ± 0.10	15.85 ± 0.56	4.25 ± 0.14	62±1.0
Arg (10mg/kg)	2.33 ± 0.30*	17.18 ± 0.28	3.18 ± 0.15	73±1.0
F1 (5mg/kg)	3.57 ± 0.10	17.01 ± 0.10	4.03 ± 0.21*	56±1.0
F2 (5mg/kg)	3.73 ± 0.15	16.20 ± 0.09	4. 13± 0.18	57±1.0
F3 (5mg/kg)	3.39 ± 0.18	16.29 ± 0.12	4.20 ± 0.13	50±1.0
F4 (5mg/kg)	2.43 ± 0.40*	18.14 ± 0.32*	3.26 ± 0.23*	65±2.0
F4a (5mg/kg)	2.05 ± 0.16*	23.48 ± 0.19*	3.05 ± 0.28*	87±1.0*
F4b (5mg/kg)	3.71 ± 0.11	16.23 ± 0.17	3.94 ± 0.30	69±1.0
F5 (5mg/kg)	3.89 ± 0.30	16.12 ± 0.35	4.20 ± 0.13	64±1.0
F6 (5mg/kg)	3.72 ± 0.26	15.97 ± 0.31	3.86 ± 0.19	67±1.0

*Data represents the Mean ± S.E.M for each group of rats, n = 5. *p<0.05 = significant difference*

Determination of the Systolic Blood Pressure and Pulse Rates:

Effect of Chromatographic Fractions of *A. Precatorius* Leaf Extract on mean systolic blood pressure and pulse rates of female rats are shown in Table 4 and Figure 1.

Fig 1: Effect of the Chromatographic Fractions of *A. Precatorius* Leaf Extract on Systolic Blood Pressure in Female Rats

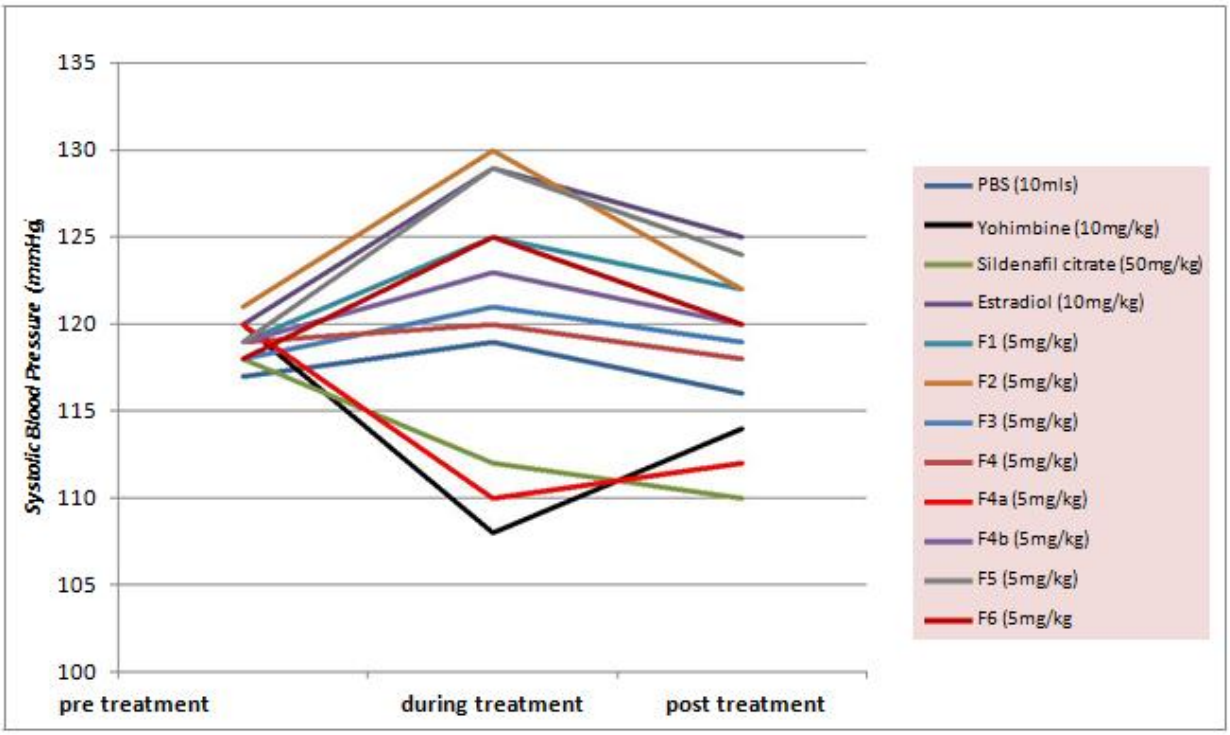


Table 4: Effect of the Chromatographic Fractions of *A. Precatorius* Leaf Extract on pre/post treatment mean pulse rate (beats/min) of Female Rats

Treatment Groups	Mean Pulse Rates		
	Treatment Periods		
	Pre treatment	During treatment	Post treatment
PBS (10mls)	346 ± 1.0	358 ± 1.0	346 ± 2.0
Yohimbine (10mg/kg)	347 ± 3.0	323 ± 1.0	335 ± 2.0
Sildenafil citrate (50mg/kg)	347 ± 1.0	316 ± 3.0*	339 ± 2.0
Arg(10mg/kg)	345 ± 2.0	314 ± 1.0*	343 ± 2.0
Estradiol (10mg/kg)	345 ± 2.0	350 ± 1.0	351 ± 3.0
F1 (5mg/kg)	345 ± 1.0	356 ± 2.0	349± 2.0
F2 (5mg/kg)	345 ± 3.0	358 ± 3.0	354 ± 3.0
F3 (5mg/kg)	346 ± 3.0	350 ± 1.0	341 ± 1.0
F4 (5mg/kg)	347 ± 1.0	318 ± 1.0	307 ± 1.0*
F4a (5mg/kg)	348 ± 1.0	312 ± 1.0*	343 ± 4.0
F4b (5mg/kg)	348 ± 2.0	315 ± 3.0*	341 ± 2.0
F5 (5mg/kg)	347 ± 2.0	334 ± 2.0	343 ± 1.0
F6 (5mg/kg)	346 ± 1.0	349 ± 3.0	345 ± 1.0

Data represents the Mean ± S.E.M for each group of rats, n = 5.
**p<0.05 = significant difference*

Determination of Tissue cGMP Level and Tissue Nitric oxide Level

The nitric oxide and cGMP levels in the glans clitoridis of the female wistar rats post treatment with the fractions, arginine, yohimbine and sildenafil is as shown in the table 5 below.

Table 5: Effect of the Chromatographic Fractions of *A. Precatorius* Leaf Extract on Tissue Nitric oxide and cGMP levels in Female Rats

Treatment Groups	NO (mmol)	cGMP (µm)
PBS (10mls)	0.07±0.01	0.97±0.03
Yohimbine (10mg/kg)	0.11±0.02*	1.35±0.01*
Sildenafil citrate (50mg/kg)	0.15±0.01	1.58±0.01*
Arg(10mg/kg)	0.13±0.01	1.22±0.04
F1 (5mg/kg)	0.06±0.001	1.02±0.01
F2 (5mg/kg)	0.08±0.002	1.11±0.02
F3 (5mg/kg)	0.10±0.01	1.27±0.01
F4 (5mg/kg)	0.09±0.02	1.05±0.02
F4a (5mg/kg)	0.12±0.01*	2.04±0.03*
F4b (5mg/kg)	0.08±0.001	1.23±0.01
F5 (5mg/kg)	0.10±0.02	0.89±0.02
F6 (5mg/kg)	0.07±0.004	0.96±0.01

Data represents the Mean ± S.E.M for each group of rats, n = 5.
**p<0.05 = significant differen*

DISCUSSION

From the preliminary phytochemical evaluations, it was noted that 70% methanol had a higher percentage yield than the water, acetone and petroleum ether (Table 1). It may be due to the combination of the attributes of water as a universal solvent and the increase in the polarity of methanol by addition of water to the absolute methanol up to 30%. Thus, in the present study, the percent yield of the extract was 18.4% w/w.

Results obtained from preliminary phytochemical, toxicity, hemato-biochemical and reproductive studies on *A. precatorius* leaves gave indication favoring further pharmacological screening [36, 37, 38]. Following fractionation of the extract, the appearance of F4 necessitated added fractionation, yielding two sub-fractions (F4a and F4b). Sub-fraction 4a had Rf value of 0.16 while that of sub-fraction 4b was 0.70.

Research works on female sexual arousal has shown that biochemical factors and intracellular mechanisms responsible for smooth muscle contraction and relaxation are implicated in Female sexual dysfunction. Nitric Oxide Synthase (NOS), a calcium/calmodulin dependent enzyme is

responsible for the biosynthesis of nitric oxide (NO) from L-arginine. Nitric oxide is responsible for the relaxation of smooth muscles of the cavernosum which eventually lead to in-flow of blood into the male organ. The increase in tissue NO levels in the Sildenafil, Arginine and F4a treated groups lend credence to the hypothesis that female clitoral erection works via the same mechanism as the male. From the present investigation, treatment with Sildenafil citrate, Arginine, Yohimbine and F4a produced the 114.2%, 87.7%, 57.1% and 71.4% increase respectively, in NO level amongst the treatment groups. Literature review shows that nitric oxide synthase isoforms

and PDE5 are clearly expressed in the vagina and clitoris [39, 40]. Furthermore, several studies have implicated nitric oxide in the regulation of female sexual behavior [15, 16, 17, 18, 41]. A study on female rats established the relationship between the expression of lordosis and nitric oxide [42]. Data from the study shows that in the presence of vaginal stimulation, nitric oxide release triggered by the release of adrenergic hormones is necessary for the expression of lordosis. Inhibiting the release of nitric oxide resulted in no expression of lordosis by any of the test rats [42].

In the present study, the levels of the second messenger, cGMP were increased as were the Nitric oxide levels in the PDE5 inhibitor, Arginine, and F4a treated groups. Therefore it is not surprising that there was a significant reduction in the systolic blood pressure and heart rate of female rats during the treatment period with Sildenafil citrate, Arginine and Yohimbine, since these agents cause vasodilation [43, 44, 45].

Yohimbine increased mount frequency while reducing mount latency in the test animals, in a manner that varied significantly from the control. On the other hand, Arginine decreased mount latency from 3.83±0.34 minutes to 2.33±0.30 minutes and caused a significant increase in the lordosis quotient when compared to control. These observations are in agreement with several other studies that have reported the role of Arginase and Yohimbine in the female sexual response [39, 45, 46]. However, it was interesting to note that F4a produced mean mount latency of 2.05±0.16 minutes, causing the Mount frequency to shoot up to a staggering figure of 23.48±0.19 mounts. Consequently, test animals receiving fraction 4a showed LQ value of 87% higher than control and other test groups. This *in vivo* test result reveals that sub - fraction 4a may be responsible for the libido activity of the plant extract.

Also, the result of this study raises the possibility of different parts of a plant exhibiting opposed pharmacological property [31]. This is with reference to the well published anti fertility effect of *A. precatorius*seeds [47, 48, 49, 50, 51].

CONCLUSION

Sexual function is an important contributor to the quality of life. Sexual dysfunction in all sexes is widespread and adversely affects mood, well-being, and interpersonal functioning. Thus, successful treatment of sexual dysfunction may improve not only sexual relationships, but also the overall quality of life. This present study is one of a series of ongoing studies on a plant that has shown concrete evidence of being useful in the management of sexual arousal disorder. In summary, the present study provides evidence that *A. precatorius* leaf is a potent stimulator of sexual behavior, particularly of sexual arousal in female rats. On this basis, fraction 4a from the methanolic extract of *A. precatorius* can be considered to possess libido enhancing properties.

Moreover, detailed studies are still underway with regards to the molecular identification and comprehensive safety profile of the fraction.

Acknowledgement
The authors are thankful to the staff of Pharmacognosy Laboratory, Pharmacology Laboratory and Centre for Malaria Research Laboratory, University of Port Harcourt, for their kind help during the bench work for this study.

REFERENCES

[1] Fabricant DS, Farnsworth NR, *Environ. Health Perspect*. **2001**,109 (suppl 1):69–75.
[2] Chauhan NS, Sharma V, Dixit VK,Thakur M, *BioMed Research International*. **2014**, Article ID 868062, http://dx.doi.org/10.1155/2014/868062
[3] McCool ME,Theurich MA,ApfelbacherC, *Systematic Reviews* **2014**, 3:75. doi:10.1186/2046-4053-3-75.
[4] Phillips NA, *Am Fam Physician*. Jul 1; **2000**, 62(1):127-136.
[5] Bachmann GA, Phillips NA, "Sexual dysfunction". In: Steege JF, Metzger DA, Levy BS, eds. Chronic pelvic pain: an integrated approach. Philadelphia: Saunders, **1998**:77–90.
[6] AkhtariE,Raisi F, KeshavarzM, HosseiniH, SohrabvandF, BioosS, KamalinejadM, GhobadiA, *DARU Journal of Pharmaceutical Sciences* **2014**, 22:40. doi:10.1186/2008-2231-22-40
[7] Berman JR, Berman LA, WerbinTJ, Goldstein I, *CurrOpinUrol* **1999**, 9(6):563-568.
[8] SegravesRT, Clayton A, Croft H, Wolf A,Wamock J, *J ClinPsychopharmacol*,**2004**; 24(3):339-342.
[9] Davis SR, van der MoorenMJ, van LunsenRH, Lopes P, RibotJ,MoufaregeA, RodenbergC, BuchA, Purdie DW, *Menopause*,**2006**; 13(3):387-396.
[10] KashaniL, RaisiF, SaroukhaniS, SohrabiH, ModabberniaA,Nasehi AA, JamshidiAM, Ashrafi, GhaeliPMP,AkhondzadehS, *Hum Psychopharmacol* **2013**, 28(1):54-60.
[11] Moynihan R,*BMJ* **2003**; 326:45 doi: http://dx.doi.org/10.1136/bmj.326.7379.45

[12] IgnarroLJ, Bush PA,Buga GM, Wood KS, FukutoJM, Rajfer J, *Biochem. Biophys. Res. Commun*. 170:843-850, **1990**.
[13] Cartledge J, MinhasS, Eardley I, *ExpertOpin. Pharmacother*. 2:95-107, **2001**.
[14] Corbin JD,*Int J Impot Res.* **2004**. Jun; 16 Suppl 1:S4-7.
[15] GiulianoF, Allard J, CompagnieS, AlexandreL, DroupyS,BernabeJ,*Am. J. Physiol*. 281:R140-R149, **2001**.
[16] GiulianoF, RampinO,Allard J,*J. Sex Marital*. Ther. 28:101-121, **2002**.
[17] Kim NN, Christianson DW,TraishAM, *J. Nutr:***2004**; vol. 134 no. 10 2873S-2879S.
[18] GragasinFS, EvangelosD,Michelakis A, Hogan R, Moudgil K, Hashimoto X, Wu S, Bonnet A,Haromy, Archer SL, The *FASEB* Journal vol. 18 **2004**; no. 12 1382-1391. doi:10.1096/fj.04-1978.
[19] Kaplan SA, Reis RB, Kohn IJ,IkeguchiEF, Laor E, Ae TE,MartinsAC,*Urology*. **1999**; 53: 481-486.
[20] ShenWW, UrosevichZ,Clayton DO, *J Reprod Med;***1999**;44:535-542.
[21] GiraldiA, PerssonK, WerkstromV, AlmP, Wagner G, AnderssonKE,*Int. J. Impot. Res.***2001**; 13:58-66.
[22] Min K, O'Connell L,Munarriz R, Huang YH, Choi S, Kim N, Goldstein I,TraishA, *Int. J. Impot. Res*. 13:151-156, **2001**.
[23] Kim SW, JeongSJ, MunarrizR, Kim NN, Goldstein I,Traish AM, *Int. J. Impot. Res.*15:355-361, **2003**.
[24] YakubuMT, AkanjiMA, OladijiAT, *PharmacognosyReviews*,Vol 1, Issue 1, Jan-May, **2007**.
[25] TraishAM, Kim NN, Huang YH, Min K, MunarrizR,Goldstein I,*Int. J. Impot. Res.* 15:397-404, **2003**.
[26] SherG,Fisch JD, *Hum Reprod*. 15:806-809, **2000**.
[27] Burnett AL, Calvin DC, Silver RI, PeppasDS,DocimoSG,*J Urol*. **1997**; 158:75-78.
[28] BoolellM, Gepi-AtteeS,Gingell JC,Allen MJ, *Br J* ***Urol*1996**; 78:257-261.
[29] Goldstein I, LueTF, Padma-Nathan H, Rosen RC, Steers WD,Wicker PA,*N Engl J Med*. **1998**; 338:1397-1404
[30] GauthamanK, AdaikanPG,Prasad RNV,*Life Sciences*, vol. 71, no. 12, pp. 1385–1396, **2002**.
[31] OgbuehiI, EbongO,ObianimeA, *Basic & Clinical Pharmacology & Toxicology*. Volume 115, Issue Supplement S1, Pages 1–374, July **2014**.
[32] Wagner G, *Erection: Physiology and Endocrinology*. In: Wagner, G. and Green, R. (eds) Impotence: Physiological, Psychological, Surgical Diagnosis and Treatment. Plenum Press, NY, (**1981**) pp. 25–36. 13.
[33] Kumar A, Chaudhary P, VermaNK, LalitPK,*Int. Res J Pharm. App Sci.*, **2013**; 3(2):6-12.
[34] Lopez HH, WurzelG,RagenB, PharmacolBiochemBehav. **2007**; 87: 369-79.
[35] Organization for Economic Development, "Acute oral toxicity". Acute oral toxic class method guideline 423 adopted 23.03.1996, In: Eleventh Addendum to the, OECD, guidelines for the testing of chemicals organization for economic co-operation and development, Paris, June, **2000**.
[36] OgbuehiIH, EbongOO,ObianimeAW,*European Journal of Medicinal Plants* 7(3): 156-166, Article no.EJMP.**2015**.078 ISSN: 2231-0894.
[37] Ogbuehi IH, Ebong OO,Obianime AW, *European Journal of Experimental Biology*, 5(1):18-25.
[38] Ogbuehi IH, Obianime AW, Ebong OO,*J Phys Pharm Adv* **2015**, 5(4): DOI: 10.5455/jppa.20150406085357
[39] Burnett AL, *Eur Urol*. **2004**; 46:9-14.
[40] G. d'Amati CRT, Di Gioia L, Proietti-Pannunzi et al., J Endocrinol Invest;**2003**; 26 (Suppl 3): 92–6.
[41] Mani SK, Allen JMC, RettoritV, MccannoSM, O'malleyBW, and Clark JH, *Proc. Natl. Acad. Sci.* USA Vol. 91, pp. 6468-6472, July **1994** Physiology.
[42] Gonzalez-Flores O, Beyer C, Lima-Hernandez FJ,Gomora-Arrati P, Gomez-Camarillo MA, Hoffman K, EtgenAM, Behavioural Brain Research.**2007**; 176(2):237-43, Jan 25.
[43] TwortCH, van BreemenC,*Circ. res.* **1988**; 62: 961- 964.
[44] Katz SD, BalidemajK, Homma S, Wu H, Wang J, MaybaumS, *Journal of the American College of Cardiology.* Vol. 36, No. 3, **2000**.
[45] DishyV, Harris PA, Pierce R, Prasad HC, SofoworaG, Bonar HL, Wood AJ,Stein CM, *Br J ClinPharmacol;*2004; 57(2): 209–212. doi: 10.1046/j.1365-2125.**2003**.01974.x.
[46] MestonCM, WorcelM,*Arch Sexual Behav*. **2002**; 31:323–32.
[47] Rao MV,*ActaEurFertil.* **1987**; 18:217–20.
[48] Sinha R, *J. Ethnopharmacol.* **1990**; 28(2):17381.
[49] RatnasooriyaWD,Amarasekera AS,Perera NS,PremakumaraGA,*J Ethnopharmacol.* **1991**; 33:85–90.
[50] HannanAM, Hossain MA, Islam MT,Islam EH, *J. Bangladesh Soc. Agric. Sci. Technol.* **2006***; 3(3&4):185-188.*
[51] Asha A, *Int. Res. J. Biological Sci.* **2013**; 2(6):27-30. http://www.isca.in/IJBS/Archive/v 2/i6/5.ISCA-IRJBS-201