EFFECTS OF THE LYOPHILIZED AQUEOUS EXTRACT FORM THE ROOT BARK OF *PERIANTHUS LONGIFOLIA* MIERS (MENISPERMACEAE) ON SEXUAL BEHAVIORS OF NORMAL MALE WISTAR RATS AND ITS ACUTE TOXICITY


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ABSTRACT

The present study revealed the effects of the lyophilized aqueous extract from *Perianthus longifolia* or *P. longifolius* root bark on some sexual behavior in male Wistar rats. The overall results showed that the extract possesses aphrodisiac properties by the oral administration of the lyophilized aqueous extract at respective doses of 100, 200 and 400 mg/kg body weight in term of significant increase in dose-dependent manner of mount frequency, intromission frequency, ejaculation frequency, copulatory efficiency, number of intromission and penile trusting, spontaneous penile erection, number of spermatozoa and its mobility, and serum testosterone. On the other hand, it was also observed that the oral administration of the same oral doses induced in dose-dependent manner significant decrease of other sexual parameters behavior such as mount frequency, intromission latency, ejaculation latency, mount latency and inter-intromission interval which are also claimed as evidences for aphrodisiac property. The extract also showed good increase in potency parameters such as erection, quick flip, long flip and total reflex. All these effects of the lyophilized aqueous extract of *P. longifolia* root bark on evaluated sexual behavior more confirm its aphrodisiac properties. The oral dose of 200 mg/kg was found...
to be more effective than other administered oral doses. In acute toxicity, the extract was found non-toxic since no death or other changes in behaviour of treated animals was observed, Its LD₅₀ was estimated to be greater than 5 g/kg body weight. The results more support and justify the use of this plant part to treat erection dysfunction and to enhance sexual behavior of men in traditional medicine as a natural aphrodiac agent.

**KEYWORDS:** *Perianthus longifolia*, Menispermaceae, root bark, aphrodisiac activity, acute toxicity.

**INTRODUCTION**

Despite of the advance in modern medicine, it is well known that traditional medicine still plays an important role in the live and health of people particularly in developing countries. Erectile dysfunction, known clinically as, an inability to obtain or to obtain and maintain an erection sufficient for naturally satisfactory intercourse (Guirguis, 1998, Singh et al., 2013a, 2013b), is a medical problem affecting in some cases men without distinction of age. Sexual dysfunction includes disorders of desire, ejaculation and orgasm, erectile dysfunction and failure of detumescence (Abdillahi and Van Staden, 2012, Porst, 2013). It is a serious medical and social symptom that occurs in 10-52% of men and 25-63% women and is adversely affected by diabetes mellitus, hypertension, antipsychotic and antidepressant agents (Singh et al., 2013a). In some cases, the terms erectile dysfunction, aphrodisiac, sterility, infertility and sexual are used to mean the same thing since it seems not easy to clearly distinguish using traditional ethnobotanical data (Porst, 2013). Male sexual dysfunction (MSD) effects not only sexual relationships, but also the overall quality of live and includes erectile dysfunction, ejaculation dysfunction, hypogonadism and represents a serious public health problem (Pare et al., 2014).

Although a number of modern treatments became available nowadays including phosphodiesterase type5 (PDES) inhibitors, Viagra (Sildenafil), Cialis (Tadalafil), Levira (Vardenafil), Amyl nitrite, Vitamine E and Levodopa (Dopamine), these medicines are considered as the first therapy in the management of erectile dysfunction (ED) unless contra-indicated, but loss and sexual desire in male partners may be common than arousal and orgasm disorder (Potts et al., 2003). These medicines are expensive, not easily available and accessible by people in rural areas and have some serious side effects (Adbillahi and Van Staden, 2012). In response to modern medicine, there has renewed interest in various medicinal plants belonging to different botanical families for the treatment of sexual
dysfunction, which are showed to be effective and safe in the daily practice of traditional healers (Adimoelja, 2000). Quite number of herbs and plant extracts that are excellent sexual enhancers exist (Goundidza et al., 2009 Pallavi et al., 2011; Patel et al., 2011; Semwal et al., 2013; Singh et al., 2013a; Chouhan et al., 2014; Sharma et al., 2014) and it is difficult to say which one is best since different people respond to different ones. Nature has provided us many aphrodisiac herbs and plants which are effective in both men and women, and there are countless sexual enhancing plants in nature that not yet been discovered.

Thus, young and adults males or females turn off to traditional medicine near traditional healers who administered them traditional preparations based mainly of some medicinal plants claimed to possess aphrodisiac properties in their daily practices, and find often satisfaction during sexual act.

In this context, a series of some medicinal plants belonging to different botanical families claimed to be used in male reproductive healthcare as well as their effectiveness as a mode of treatment was previously reported. Different plant parts such root, bark, leaves gum, corns and latex are used as aphrodisiac, sexual stimulants, to treat impotence, sterility and infertility and erectile dysfunction. These plants may be used singly, but often in combination with herbs from other medicinal plants (Abdillahi and Van Staden, 2012). On the other hand other medicinal plants such as extracts from *Tichopus zeylanius* leaves (Trichopodaceae) (Subramoniam et al. 1997), *Camelia sinensis* tea (Theaceae) (Ratnosooriya and Fernado, 2008), *Allium tuberosum* seeds (Liliaceae) (Guohua et al. 2009), *Mucuna pruriens* seeds (Fabaceae) (Suresh et al. 2009) and *Turnea diffusa* leaves (Turneraceae) (Estrada-Reyes et al. 2013), *Phoenix dactylifera* (Arecaceae) (Abedi et al., 2013) among other medicinal plants, were previously investigated in animals et were reported to possess interesting aphrodisiac properties.

Aphrodisiac is a word derived from Aphrodite, the Greek goddess of sexual, love and beauty, defined as an agent (food or drug) that arouses sexual desire and are then agents used to induce venereal desire and increase pleasure and sexual performance, and to arouse sexual instinct (Malviya et al., 2011). The medicinal plants with aphrodisiac effects produce physiological effects including mental stimulants to combat tiredness and psychotropic actions to produce an abnormal state of reality or sedative effect to allay stress or to have calming effect (Dweck, 2009). These plant species also produce physical effects inducing physiological action on cavernosum the corpus (Abdillahi and Van Staden, 2012).
Perianthus longifolia or P. longifolius is a medicinal plant largely used in traditional medicine. Each plant part is used to treat various ailments. A fresh piece of bark is chewed with some seeds of Afrumomum melegueta as aphrodisiac and for sexual asthenia. The pulverized-golden-yellow roots and some seeds of A. melegueta eaten with a banana or suspended in palm wine and drunk are taken as common aphrodisiac. Against sexual weakness, one teaspoon of pulverized root is taken with ripe banana in palm oil or food. The root and root bark itself is taken in food or not for their reputed aphrodisiac properties (Neuwinger, 2000; Schmelzer and Gurbin-Sakin, 2008) (Schmelzer and Gurbin-Sakin, 2008). Since the advocated sexual stimulant activity of extracts from this plant part is not yet investigated, the present study was undertaken to evaluate the effects of the lyophilized aqueous extract of P. longifolia on male and female Wistar rats sexual behavior at various dosages.

2. MATERIALS AND METHODS

2.1. Plant materials: Root bark of Perianthus longifolia Miers were collected in Kinshasa in Mai 2013. The plant was identified by Mr. Nlandu Lukebiabo, B. of the Institut National d'Etudes et de Recherches en Agronomie, Department of Biology, Faculty of Sciences, University of Kinshasa. A voucher specimen of the plant NL052013PLRB was deposited in the herbarium of this institute. Root barks were dried at room temperature and reduced to powder keeper in brun bottles.

2.2. Preparation of extract: 20 g of powdered plant material was mixed by maceration with 100 ml distilled water for 24 h in a flask of 250 ml. After filtration, the filtrate was concentrated in vacuum to 50 ml and the flask was connected to a vacuum pump and evacuated till drying. Lyophilization enables concentration of plant compounds by evaporation under pressure and low temperature through lyophilizer (Terroni Lyophiliser LS 300, São Carlos, Brasil) and lyophilized resulting in a dried extract denoted as LAE (16.37 g) stored in sterile brun bottle till its use.

2.3. Phytochemical screening: This study was conducted by thin layer chromatography on precoated silica plates (thickness layer 0.25 mm, Merck, Germany) using different mobile phase et reagents described in the literature for the identification of major phytochemical groups such as alkaloids, anthraquinones, flavonoids, coumarins steroids and terpenes. Anthocyanins were detected with HCl 0.2N after heating and extraction of the red color with
isoamylic alcohol, saponins were revealed with froth test while tannins were detected with Stiasny's reagent (HCl conc. + formal) and proanthocyanidins with vanillin 1%/H$_2$SO$_4$ 5% after heating giving a red color (Harborne, 1998).

2.3. Experimental animals: Sexually experienced healthy adult male (body weight (b.w): 145-150 g) and female Wistar rats (b.w: 155-160 g) kept under standardized animal house conditions (temperature 28-30°C, photoperiod: 12h of natural light per day, relative humidity 50-55%) were used in the study. They had free access to pelleted food and tap water at libitum. Twelve rats were selected for the present study.

2.4. Effects and observation of sexual behavior: Sexual behavior was examined in non-competitive copulation test performed during the dark phase of the natural light-dark cycle (18.00-19.00h) with subdued light in a quiet room with adequate ventilation. Each receptive female rat was introduced to a male rat for 30 min (adaption period) in metabolic cage (45 cm x 30 cmx 20 cm). The test was conducted after oral administration of different oral doses of the extracts and controls. In this way, twelve male Wistar rats were randomly assigned into four equal groups (n=3) and orally administered with PLAE extract in the following manner: negative control only received water, group I , group II received Yohimbine as positive control group, groups III, IV and V received 100, 200 and 400 mg/kg body weight of PLASE respectively exposing to sexually receptive females one daily for 4 consecutive days. The treated animal were placed individually in cages for 2 h following administration of the vehicle or PLS extract. Following a 30 min. adaptation period, a female that had been brought into estrous (oestradiol benzoate 12 µg in olive oil 8h prior to pairing) was placed in the cage. The pre-coital behavior was observed for the first 15 minutes until ejaculation. During this period, the mount latency (the time from the introduction of the receptive female to the first mount), intromission latency (the time from the introduction of the respective female to first intromission) and ejaculation latency (the time from the introduction of the receptive female to ejaculation) and the number of mounts and number of intromission displayed by rats were recorded. When there is no intromission and ejaculation during the observation period, their latency were taken as 15 minutes. Using these data, the following parameters were calculated and computed: intromission frequency in seconds (IF) is the number of intromission from the time of introduction of the female until ejaculation, mounts latency in seconds (ML) is the time interval (h) between the introduction of the female and the first mount by the male, ejaculation latency (EL) which is the time interval (h) between the first intromission and
ejaculation, copulation efficiency (CF), intromission ration (IR) and intercopulatory interval in seconds (ICI) is the average time between intromission, ejaculation frequency is the number of ejaculation from the time of introduction of the male rats to the female within a given time interval (30 min.), post-ejaculation interval (PEI) in hour is the time from the first ejaculation up to next intromission by the male, penile erection determined by multiplying the percentage of rats exhibiting at least one episode of penile erection during 30 min of the observation period with the mean number of penile erections and inter-intromission interval in seconds (III) is the time between two adjacent intromission (Ratnosooriya and Yayakody, 2006; Ratnosooriya and Fernando, 2008; Suresh et al. 2009; Abedi et al., 2013; Fouche et al., 2015; Ondele et al., 2015). In the present study PEI in treated animals significantly increase compared to negative control group as shown in figure 3.

2.5. Test for libido: Libido was assessed according to the method described by Davidson (1982) as modified by Suresh et al. (2009). The number of mountings along with intromission and ejaculation were analyzed according to Suresh et al. (2009).

2.6. Effects on penile erections
Fifteen male rats were randomly divided into three groups of 3 animals each and orally treated with PLAE extract in the following way: group I, II and III received 100, 200 and 400 mg/kg respectively while the negative control only received water. Shortly after treatment, these rats were placed individually in plexiglass cages and the number of penile erections (characterized by penile body elongation and dorsiflexion, reddening and engorgement of glands and its flaring) were recorded for 30 minutes (Ratnosooriya and Fernando, 2008).

2.7. Determination of serum testosterone level
Fifteen male rats were randomly divided into four groups of 5 animals each. Group I received distilled water as negative control group. Groups II, III and IV received 100, 200 and 400 mg/kg respectively of the lyophilized aqueous extract of P. longifolia (LAE) for 3 and 7 consecutive days. On days 1, post-treatment these rats were anesthetized with ether and 1 ml blood was obtained from the tail using aseptic conditions. The level of testosterone was measured after the treatment time using an immunocheminisence assay with Elecsis 1010 analyser (Rocher diagnostic, Mannheim) (Ratnosooriya and Fernando, 2008)

2.8. Acute Toxicity: The acute toxicity of the lyophilized aqueous extract of P. longifolia root bark (LAE) was evaluated in Wistar rats according to the procedure described by the
Organization for Economic Co-operation and Development (OECD) guideline for testing chemicals: 420 (2001). Wistar rats (130-140 g b.w ) were divided into three groups as followed: Group I orally received by gavage 5 ml distilled water and constituted the negative control group. Groups II, III and IV received 0.5, 1 and the 5 g/kg body weight respectively of LAE extract in the same way. The animals were observed for toxic symptoms continuously for the first 4 h dosing and were daily weighed. Finally, all animals were then maintained in daily observation and the number of survivors was recorded for further 28 days.

3. RESULTS AND DISCUSSION

Many medicinal plants are reported to be effective as aphrodisiac agents through mechanisms such as vasodilatation, generation of nitrite oxide, gonatropins and elevation of androgens (Yakubu et al., 2010, Singh et al, 2013a, 2013b). In addition, the administration of an aqueous medicinal plant extract at tested doses can modify the rat copulatory behavior as well as the orientation activity considered as the main determinant for measuring male sexual behavior (Abedi et al., 2013).

It was previously reported the increase or decrease of some sexual parameters provoked by some plant extracts indicating the less robust sexual behavior of the test samples and are evidence for aphrodisiac properties of the tested plant extract (Suresh et al. 2009; Estrada-Reyes et al. 2013). In male rats, latency for mount and intromission are considered as indicators of the sexual motivation while intromission and ejaculation are seen as behavioral indications of sexual performance (Dabhadkar and Zade, 2013).

The present study was undertaken to study the effects of the lyophilized aqueous extract of P. longifolia root bark (AE) on sexual behavior of normal rats to confirm its claimed aphrodisiac action by traditional healers. The computed sexual behavior parameters of the treated male rats are shown in Tables 1 and 2. Results obtained indicated that the oral administration of AE extract at doses of 100, 200 and 400 mg significantly modified all behavior parameters.

The mount frequency (MF) was recoded at all administered of all oral doses of the lyophilized aqueous extract of P. longifolia (LAE) and results indicated that the administered oral doses induced significant increase of mount frequency in dose-dependent manner. At the oral dose of 200 mg/kg body weight, the mount frequency passed to 21.7±1.1, higher compared that of 100 and 400 mg/kg reaching 13.4 ± 0.7 and 14.8±0.3 sexual mounts respectively and negative control (11.7 ± 1.1), and showed a statistically significant
difference \( p < 0.05 \) (Table 1). This parameter is an indice of vigour, libido and potency which are increased in male animals after the administration of the AE extract. The increase of mounts frequency in dose-dependent manner after the oral administration of the lyophilized aqueous extract (LAE) of *P. longifolia* root bark is well illustrated in the figure 1.

![Figure 1. Effects of the lyophilized aqueous extract (LAE) of *P. longifolia* root bark on sexual mounts frequency in untreated and treated male Wistar rats. 0: negative control group.](image)

Mount latency (ML) and intromission latency (IL) decreased significantly \( p < 0.05 \) with the increase oral doses administered of the extract compared to negative control group. This effect was also reported by Pare et al. (2014) concerning the effect of extracts of *Gloriosa superba* in male albino rat. From the three oral doses, 400 mg/kg showed the least mount latency and intromission latency (Table 1). The decrease of this behavioral parameter might imply stimulation of sexual motivation and arousal, and also may be an indication of enhanced sexual appetitive behavior in treated male animals which, further supports the sexual improvement effect of the administered extract (Fouche et al., 2015). The same effect was also previously reported by Chaturapanich et al., (2008); Wani et al., 2011; Dabhdkar and Zade, 2013; Fouche et al. (2015) in other medicinal plant extracts found with aphrodisiac effects.

Intromission frequency (IF), inter-intromission interval (III), number of trusting (NT) and number of penile trusting (NPT) significantly increased at all administered doses in dose-dependent manner. While the number of MF reflects sexual motivation, increase in the number of IF and other behavior parameters mentioned above showed the efficiency of reception, penile orientation and the ease by which ejaculatory reflexes are activated as
evidences of aphrodisiac properties (Wani et al., 2011; Abedi et al., 2013; Fouche et al., 2015).

Penile erection is important for evaluating the effect of administered sample on erectile function. The potency test showed that the lyophilized aqueous extract of *P. longifolia* root bark (LAE) would be able to favor the blood flux towards the cavernous bodies of the penis and significantly increased the frequency of penile erection in dose-dependent manner compared to negative control, but to a lesser degree than Yohimbine used as a reference aphrodisiac product in treated animals. At all oral doses, all treated rats provoked significant difference (p <0.05) in spontaneous penile erection in dose-dependent manner compared to negative control group as illustrated in figure 2. This was characterized by the elongation and hardening of penis seen in treated groups compared to untreated groups. This observation indicated that the administration of LAE extract facilitated the flow of blood into the penis and is in good agreement with Rakuambo et al. (2006), Dabhadkar and Zade (2013) and Toma et al., 2015. With the administration of oral dose of 200 mg/kg of LAE extract, the erection number passed of 64.3 ±0.7 at Day 5 to 68.5 ± 0.8 in treated animals, and is significantly higher compared to the effect produced by the administration of 100 and 400 mg/kg and that produced by negative control (Tables 1 and 2). Similar effect was also recovered with the roots of *Rauwolfia obscura* (Pare et al., 2014) and peels extract of *Buccholzia coriacea* (Ondele et al., 2015). The penile erection increased by LAE extract could be due to its androgenic effect (Bahmanpour et al., 2006; Dabhadkar and Zade, 2013). This results suggests that LAE extract would act on the laxity of the muscle smooth péniens as also proposed by Ondele et al., (2015) for the aqueous extract of *Buchholzia coriacea.*

![Figure 2](image-url)

**Figure 2:** Effect of the lyophilized aqueous extract (LAE) from *P. longifolia* root bark on penile erection in untreated and treated male Wistar rats. 0: untreated Wistar rats (negative control group).
The prolongation ejaculation time, the decrease of mount and intromission latency among other sexual behavior parameters are generally seen as positive signs of aphrodisiac effect (Ratnasooriya and Yayakody, 2006). In the present study, the administration of LAE extract at all oral doses prolonged the ejaculatory latency in dose related manner (Table 2). Such effect could result from the desensitization (Scuka and Marzymas, 1991). This effect was neither due to sensory motor incoordination’s nor eruption of penile tactile sensitivity (Yeh et al. 2008). It could be attributed at least partly to the anxiolytic property of AE since anxiolytic agents are well reported to prolong the ejaculation time (Jamini et al., 2005). The significant increase in EL suggested that the extract and positive control drug prolonged the duration of coitus as an indicator of sexual motivation as also reported by Dabhakar and Zade (2103) on the effect of extracts of *Psoralea corylifolia* seeds in male albinos rats. In the present study, these effects were profound with 200 mg/kg b.w. According to Suresh et al. (2009) and Estrada-Reyes, (2013), these results provide experimental supports to justify and confirm the folk reputation use of LAE extract as sexual stimulant or as an aphrodisiac agent in men in traditional medicine. Ejaculation was present in control and experimental animal groups and may inferred that the test drug produced a striking increase in pure libido. Ours results are only qualitatively in good agreement with others authors who had previously studied the sexual behavior of some plant extracts in treated normal rats on the prolongation time of ejaculation (Tajuddin et al., 2005; Guochua et al, 2009; Suresh et al. 2009; Dabhdkar and Zade, 2013). In addition the post-ejaculation interval significantly increased in treated animals compared to negative control as show in figure below.

Figure 3: Effects of the lyophilized aqueous extract (LAE) from *P. longifolia* root bark in untreated and treated male Wistar rats. 0: untreated Wistar rats (negative control group)
LAE extract significantly increased of the copulatory behavior at all tested oral doses in treated animals compared to negative control was observed (p < 0.05) (Tables 1 and 2). This action may be due to the dopaminergic system which regulates prolactin secretion and may be involved in the facilitatory effects of the tested extract (Yeh et al. 2008). In addition the copulatory performance of treated male rats enhanced by LAE extract may be attributed to the increase in serum testosterone concentration as demonstrated in the present study. The copulatory behavior is regulated by complex mechanisms in which the hormone testosterone is considered to contribute to the improvement in sexual function, libido and penile erection (Yakubu and Afolayan, 2009; Fouche et al., 2015).

Table 1. Mating behavior of male Wistar rats receiving PL lyophilized aqueous extract (1)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I (negative control group)</th>
<th>Group II (100 mg/kg of LAE extract)</th>
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<tbody>
<tr>
<td></td>
<td>Day 5</td>
<td>Day 10</td>
</tr>
<tr>
<td>MF</td>
<td>13.2±0.2</td>
<td>12.3±0.8</td>
</tr>
<tr>
<td>ML</td>
<td>10.1±0.6</td>
<td>9.3±0.2</td>
</tr>
<tr>
<td>IL</td>
<td>11.7±1.1</td>
<td>10.9±0.8</td>
</tr>
<tr>
<td>IF</td>
<td>73.2±1.2</td>
<td>71.2±1.4</td>
</tr>
<tr>
<td>III</td>
<td>18.0±0.2</td>
<td>19.1±0.7</td>
</tr>
<tr>
<td>NT</td>
<td>4.3±0.3</td>
<td>5.1±0.8</td>
</tr>
<tr>
<td>EL</td>
<td>247.1±0.1</td>
<td>253.4±0.2</td>
</tr>
<tr>
<td>PE</td>
<td>42.3±0.5</td>
<td>44.3±0.1</td>
</tr>
<tr>
<td>PEI</td>
<td>402.3±0.4</td>
<td>398.1±0.8</td>
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<tr>
<td>NPT</td>
<td>41.3±0.4</td>
<td>49.6±0.2</td>
</tr>
<tr>
<td>CE</td>
<td>1.2±0.6</td>
<td>1.4±0.5</td>
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</tbody>
</table>

**Legend:** NM: number of mounts, ML: mounting latency, IL: intromission latency, IF: intromission frequency, III: inter-intromission interval, NI: number of intromission, NT: number of trusting; EL: ejaculation latency, PE: penile erection, PEI: post ejaculating interval, NPT: number of penile trusting, CE: copulatory efficiency

Table 2. Mating behavior of male Wistar rats receiving PL lyophilized aqueous extract (2)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group III (200 mg/kg of PLA$$\text{E}$$)</th>
<th>Group IV (400 mg/kg of LAE extract)</th>
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<tbody>
<tr>
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<td>Day 5</td>
<td>Day 10</td>
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<tr>
<td>MF</td>
<td>17.2±0.2</td>
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</tr>
<tr>
<td>ML</td>
<td>7.8±0.6</td>
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<tr>
<td>IL</td>
<td>10.3±1.1</td>
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<tr>
<td>IF</td>
<td>86.2±1.2</td>
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<tr>
<td>III</td>
<td>20.0±0.2</td>
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</tr>
<tr>
<td>NI</td>
<td>7.6±0.3</td>
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The oral administration of LAE extract at all oral doses, did not affect the sperm’s progressive motility and fertility probably due to the fact that, these parameters are already high in control animals (Chaturapanich et al., 2008). The epididymal sperm parameters revealed a significant increase in the number of spermatozoa in all the experimental groups (205, 248, 230 million/ml respectively) and at 3, 5 and 4 grade in group I, II and III respectively. This number of spermatozoa is comparable to that reported by Suresh et al. (2009) concerning the effect of ethanolic extract of Mucuna pruriens on sexual behavior in treated normal rats. In the present study, the increase of sperm production was dose-dependent. In some cases, other plant extracts did not affect the sperm’s mobility and their level mainly at low tested doses. In other cases, other herbal extracts inhibited the mobility of spermatozoa suggesting spermicidal action as the case of Hypericum perforatum, Serenoa repens, Sabal serrulatum and Gingko biloba extracts when tested at dose of 0.6 mg/kg leading to the non-viability of spermatozoa (Rakuambo et al. 2006). Thus, these kinds of plant extracts may be employed as contraceptive and aphrodisiac agents, and both activities can be evaluated in the same test. For example, this is the case of the chloroform extract of Securidaca longipedunculata seeds (Palgrave, 1977) and Typha capensis rhizome and leaf (Henkel et al. 2011) when tested at doses from 1 to 10 mg/kg, and found to possess aphrodisiac properties by their positive effects on other sexual behavior parameters such as sexual motivation, reusability and organs, libido, penile erection and ability which remained uninhibited (Ratnasooriya and Yayakody, 2006). Although the mechanism involved in the inhibition of sperm motility is unknown, it seems clear that with no or poor sperm motility, vitality and membrane integrity, and fertilization will be impaired (Rakuambo et al. 2006). On the other hand, it has been also shown other medicinal plant extracts improved sperm production and sperm mobility (Gonzales et al., 2003).

Results from the libido test showed that the pre-coital sexual behaviors, such as chasing, nosing and an genital sniffing were well performed in all treated groups with the administration of LAE extract at all tested oral doses. This was also characterized by the

<table>
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<th>NT</th>
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<th>PE</th>
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<td>66.2±0.1</td>
<td>58.2±0.7</td>
<td>60.3±1.1</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>66.2±0.1</td>
<td>59.6±0.4</td>
<td>61.3±0.3</td>
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<tr>
<td></td>
<td>476.3±0.4</td>
<td>488.1±0.8</td>
<td>470.2±0.7</td>
<td>449.2±0.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>498.1±0.8</td>
<td>494.4±0.7</td>
<td>457.7±0.1</td>
<td>449.2±0.7</td>
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</tr>
<tr>
<td></td>
<td>67.8±0.4</td>
<td>73.2±0.5</td>
<td>62.7±0.2</td>
<td>60.3±1.1</td>
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<tr>
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<td>73.2±0.5</td>
<td>68.5±0.8</td>
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<tr>
<td></td>
<td>470.2±0.7</td>
<td>457.7±0.1</td>
<td>449.2±0.7</td>
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</tr>
<tr>
<td></td>
<td>1.2±0.6</td>
<td>2.1±0.5</td>
<td>2.5±0.3</td>
<td>2.3±0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.1±0.5</td>
<td>2.5±0.3</td>
<td>2.3±0.5</td>
<td>2.3±0.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Legend: see Table 1
increase of mounting and intromission frequency (Dbhakar and Zade, 2013) as already mentioned above.

Table 3. Test of potency of the lyophilized aqueous extract of *P. longifolia* (LAE) in male Wistar rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Negative control group</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erection (E)</td>
<td>6.3 ± 0.4</td>
<td>14.9 ± 2.3</td>
<td>17.7 ± 2.4</td>
<td>15.3 ± 2.1</td>
</tr>
<tr>
<td>Quick flip (QF)</td>
<td>5.4 ± 1.2</td>
<td>8.2 ± 1.6</td>
<td>12.7 ± 2.3</td>
<td>10.3 ± 0.8</td>
</tr>
<tr>
<td>Long flip (LF)</td>
<td>3.6 ± 0.6</td>
<td>6.4 ± 0.2</td>
<td>9.4 ± 1.7</td>
<td>9.6 ± 0.8</td>
</tr>
<tr>
<td>Total reflex (TR)</td>
<td>16.8 ± 1.7</td>
<td>24.4 ± 1.3</td>
<td>34.4 ± 2.7</td>
<td>27.4 ± 2.8</td>
</tr>
</tbody>
</table>

Legend: Groups I, II and III: rats treated with 100, 200 and 400 mg/kg b.w of LAE extract respectively.

The effect on potency was assessed by testing the action of LAE extract on the frequency of penile reflexes such as penile erection (PE), quick flip (QF), long flip (LF) and total reflex (TR). Results indicated that the oral administration of LAE extract produced significant increase of the frequency of these parameters in treated animals compared to negative controls demonstrating its potency effect in treated animals (Dabhdkar and Zade, 2013).

Hormonal analysis revealed that the level of testosterone increased in all the experimental groups from Day 5 to Day 15 in dose-dependent manner compared to negative control groups (Fig.1). The high level of testosterone in treated animals could result in improving total sexual behavior (Thakur et al., 2009, Ondele et al., 2015). In addition, this elevation of this hormone in treated animals provoked by the oral administration of LAE extract may be due to the implication of serotonergic action of the extract since it is well known that serotonergic agents increase testosterone synthesis or mediated by the prevention of its metabolic degradation (Yang et al, 2004), and also, testosterone supplementation has previously been shown to improve sexual function and libido (Dabhadkar and Zade, 2013; Toma et al., 2015). Other plant extracts such as that from *Camellia sinensis* (Ratnasooriya and Fernando, 2008), *Bulbine natalensis* (Yakubu and Afolayan, 2009) *Mucuna puriens* (Suresh et al. 2009), among others medicinal plants extracts had been reported to increase testosterone level in animal models.
In the present study, it was observed that the oral dose of 200 mg/kg body weight of LAE extract was found to be the most effective since it produced high effect on evaluated behavior parameters compared to the administered oral doses of 100 and 400 mg/kg body weight. With regards to the efficacy of LAE extract to treat erectile dysfunction and sexual dysfunction as demonstrated in the present study, it is difficult to interpret the exact mechanisms of this extract on potentiation of sexual function. On this way, it was reputed that some plant extracts act as aphrodiac by their effects in dopaminergic nervous and pathway controlling sexual activities due to the presence of high level of L-DOPA content in plant material (Nagashayana et al. 2000). In addition, in a complex mechanism that regulates copulatory behavior, elevated testosterone is considered to have contributed to the improvement in sexual function, libido and penile erection (Yakubu and Afolayan, 2009). Moreover, the treated animals of LAE extract expressed immediately a rhythmic motor pattern of ejaculation. In addition, both serotonergic and dopaminergic system play a principal role in mechanisms (Jamini et al. 2005). In a recent study, Sing et al. (2013a) have proposed three mechanisms of action involved in aphrodisiac potential medicinal plants including the nutritional value improving the immediate health or well sexual performance and libido, the purported aphrodisiac having more specific physiological effects but are not psychologically active affecting blood flow or increasing duration of sexual activity by numbing the genital area and aphrodisiac acting by their psychopharmalogical effects.

Regarding the effects of the reference aphrodisiac product, it was observed that from Day 5 to Day 15, Yohimbine used as reference aphrodisiac product induced significant increase of EL (270-286), NM (19-26), CE (1.5-3.1), NI (7.2-8.1) and IF (89-95) and other sexual
parameters. It also produced significant decrease of IL (8.0-8.5) and PEI (390-315) compared to the effects of negative control groups and PLAE extract respectively. These effects indicated that Yohimbine had a higher aphrodisiac action than LAE extract. The increase or decrease of computed parameters in treated male rats according to the case is an indication of significant and sustained increase in the sexual activity and aphrodisiac property inherent in the plant extract. Thus, above evidences prove that the lyophilized aqueous extract of *P. longifolia* (LAE) root bark possesses aphrodiac properties.

In general, our results reported in the present study on the effects of the lyophilized aqueous extract of *P. longifolia* root bark (LAE) on some sexual behavior parameters evaluated in the present study, are only qualitatively in good agreement with those previously described by other authors in the evaluation of the aphrodisiac activity of others medicinal plant extract claimed to possess this action in traditional medicine of some countries in the world (Subramoniam et al. 1997; Ratnasooriya and Yayakody, 2006; El-Tantawy et al. 2007; Ratnasooriya and Fernando, 2008; Guochua et el., 2009; Suresh et al. 2009; Estrada-Reyes et al. 2009, 2013; Wani et al., 2011; Pare et al., 2014; Dabhakar and Zade, 2013; Ondele et al., 2015;Toma et al., 2015).

In acute toxicity, animals were treated once time with oral doses of 0.5, 1 and 5 g/kg body weight of LAE extract respectively. Results from this test revealed that no sign of toxicity such as alteration of the locomotion activity and gastrointestinal disturbances were observed, it doesn’t modify the general behaviour of the treated animals. But rats received all tested oral doses significantly gained body weight compared to negative control group as shown in figure 5. It is evident from the analysis of this figure that the.

![Figure 5.: Effects of the lyophilized aqueous extract (LAE) from *P. longifolia* on the ponderal evolution of Wistar rats](image)

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lyophilized aqueous extract (LAE) of *P. longifolia* root bark (0.5 to 5 g/kg b.w per os) provoked significant increase of body weight in treated Wistar rats compared to untreated control group (*p* < 0.05) from Day 7 to Day-28, the gain ponderal being more important with the oral administration of 5g/kg b.w of the extract. According to Pieme et al. (2006), the progressive increase in body weight during the period of treatment may indicate the improvement of the nutritional state of animals, The growth response effect could be considered as a result of increased food and water intake. The death of animal was not observed after 28 days of observation. Therefore, the LD$_{50}$ of the extract was estimated to be greater than 10 g/kg body weight. According to Kennedy et al. (1986), substances that present LD$_{50}$ higher than 5.0 g/kg body weight via oral route, may be considered as practically non-toxic. Therefore, it may be suggested that acute toxicity of *P. longifolia* root bark aqueous extract is practically null via oral route.

The histopathological examination revealed no particular abnormalities in the vital organs such as lung, heart, kidney, brain, spleen and large intestine in treated animals in comparison to the untreated groups. No necrosis, infiltration, oedema and conjunction which are the signs of hepatotoxicity were found in different treated rat groups at all administered oral doses. In general, histopathological examination of selected organs recovered from treated and control animals showed normal architecture of organs suggesting that no detrimental changes and morphological disturbances were caused by the oral administration of the extract once time for 28 days of observation. In conclusion, this study presented strong evidence of the non-toxic effect of LAE extract. At the oral doses tested, the extract can be considered safe and well tolerated as it did not cause either mortality, any lethality, adverse changes or side effects in the general behavior in the acute toxicity in rats. These reported results also showed the margin safety and tolerability of the extract and can at least, explain its extensive use in traditional medicine without significant visible side effects in patients.

Unfortunately, the literature falls to report the chemical composition of *P. longifolia* root bark. Results from our phytochemical screening revealed the presence of tannins, saponins, sugars, terpenes, steroids and alkaloids. Anthocyanins, flavonoids and anthraquinones were not detected in our experimental conditions. Based these phytochemical results, we could speculated that the observed aphrodisiac activity of AE extract may be due to the presence of saponins, terpenes, steroids and alkaloids since some compounds belonging to these phytochemical groups isolated or found to be present in other plant species had been
previously reported to possess aphrodisiac properties at different extents (Clark et al., 1985; Taha et al., 1995; Abedi et al., 2013; Pare et al., 2014). Other natural products reported with aphrodisiac action included xanthone (Rakuambo et al., 2004, Meyer and Rakuambo, 2008), flavonoids such as pyrano-flavones (Drewes et al., 2002), flavones (Estrada –Reyes et al., 2013), and benzoflavones (Dhawan and Sharma, 2003).

Although several medicinal plants are available and used to treat various ailments including impotence, there is now a great interest in the evaluation of their aphrodisiac properties. However, it is well known that the plant such *Ginseng panax* has been used in treatment of erectile dysfunction for thousands of years in China, nowadays, it knows a large use in many countries in the world for its aphrodisiac effects. *Allium tuberosum* seeds have been reputedly used as a Chinese medicine for the treatment both impotence and nocturnal emissions (Jiangu New Medical College, 1979) and its aphrodisiac properties were previously also reported (Ghuocha et al., 2009). Other medicinal plants mainly those from Africa are concerned. In DR Congo, the lyophilized aqueous extract of *P. longifolia* root bark is now commercialized in an ameliorated galenic form as capsules under the name P-VIRIL (350 mg of extract/capsule) as a pharmaceutical specialty. It is frequently used by young and adults men who found positive response during their sexual relations.

**CONCLUSION**

Sexual behavior parameters were studied in normal rats to understand the role of the lyophilized aqueous *Perianthus longifolia* root bark extract as an aphrodisiac agent. Results from the present study indicated that there was overall in increase sexual behaviors parameters as reflected by MF, IF, CE, NL NPT and EF or in decrease of others parameters such as ML, IL, EL, PEF, III and PEF confirming the aphrodisiac properties of the studied extract.

Thus, this study scientifically demonstrate, for the first time, the aphrodisiac potential of the lyophilized aqueous extract of *P. longifolia* root bark supporting and justifying the claimed used by traditional healers in DR Congo to increase sexual action.

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