EVALUATION OF THE POTENTIAL APHRODIAC ACTIVITY OF P-VIRIL®, A PHYTOMEDICINE BASED LYOPHILIZED AQUEOUS EXTRACT OF THE ROOT BARK OF PENIANTHUS LONGIFOLIA MIERS (MENISPERMACEAE) IN MALE WISTAR RATS AND ITS ACUTE TOXICITY


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ABSTRACT

In the present study, it was examined the aphrodisiac activity of P-VIRIL®, a phytomedicine based lyophilized aqueous extract (LAE) from Penianthus longifolia or P. longifolius root bark on sexual behaviors in male Wistar rats. Results showed that the extract possesses aphrodisiac properties by its oral administration at respective doses of 100, 200 and 400 mg/kg body weight characterized by significant increase of mount frequency, intromission frequency, ejaculation frequency, copulatory efficiency, number of intromission and penile trust, and serum testosterone as well as significant decrease of of mount frequency, intromission latency, ejaculation latency, mount latency and inter-intromission interval. In addition, the extract provoked the increase of the number of spermatozoa and their mobility, the level of testosterone and spontaneous penile erection at all administered oral doses at different extents. LAE extract of P-VIRIL® also showed good increase in potency parameters such as...
erection, quick flip, long fi-lip and total reflex. All these effects of LAE extract of P-VIRIL® on evaluated sexual behaviors confirm its aphrodisiac properties. The oral dose of 200 mg/kg was found to be more effective than other administered doses. The extract did not show toxic effects and its LD₅₀ was estimated to be greater than 10 g/kg body weight. It was concluded that the phytomedicine P-VIRIL® based lyophilized aqueous extract of P. longifolia root bark functions as a quick acting and aphrodisiac agent. The results support and justify the use the phytomedicine P-VIRIL® as well as the plant part acclaimed aphrodisiac agent and for the management of young, adult men and women sexual disorders.

**KEYWORDS:** P-VIRIL®, Penianthus longifolia, Menispermaceae, root bark, erectile dysfunction, aphrodisiac effects.

**INTRODUCTION**

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.[1] 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.[2] These plant species also produce physical effects inducing physiological action on cavernosum the corpus.[3]

Erectile dysfunction is known clinically as an inability to obtain or to maintain an erection sufficient for naturally satisfactory intercourse[4-7] It is a medical problem affecting in some cases young, men and women without distinction of age. Sexual dysfunction is associated with disorders of desire, ejaculation and orgasm, erectile dysfunction, depression, anxiety, stress, failure of detumsence and traumatic sexual experience in the past.[3,8] In addition, 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.[5] In some cases, the terms erectile dysfunction, aphrodisiac, sterility and infertility are used to mean the same thing since it seems not easy to clearly distinguish using traditional ethnobotanical data.[8] Male sexual dysfunction (MSD) affects not only sexual relationships, but also the overall quality of live and includes erectile dysfunction (ED), ejaculation dysfunction, hypogonadism and represents a serious public health problem.[9]
Although a number of modern treatments became available nowadays including phosphodiesterase type5 (PDES) inhibitors, Viagra (Sildenafil), Cialis (Tadalafil), Levira (Vardenafil), Amyl nitrite, Vitamin 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. These medicines are expensive, not easily available and accessible by people in rural areas and have some serious side effects. 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 as well as to phytomedicines based aphrodisiac plant extracts. Quite number of aphrodisiac natural products are excellent sexual enhancers and those exist and it is difficult to say which one is best since different people respond to different ones.

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 and its aphrodisiac properties were previously also reported.

In Democratic Republic of Congo (DRCongo), 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 speciality used. It is frequently used by young and adults men who found positive response during their sexual relations. Some of these aphrodisiac plant extracts are presented in ameliorated galenic form for their easy use by people and this is the case of P-VIRIL® based lyophilized aqueous extract of P. longifolia root bark. Other ameliorated galenic form based plant extracts exist under specific names such as Passion Rx®, Tongkat ali®, Tribulus® and Mucuna puriens® under the form of capsule formulated by the medical doctor Dr. Ray Sahelian and are used by more than 80% users satisfied by the end and the first week, with additional satisfaction by the end of the second week after their sexual rapport.
Different plant parts such as root and stem bark, leaves gum, corns and latex are used as aphrodisiac, sexual stimulants, to treat impotence, sterility and infertility and erectile dysfunction by young, adult male and females in traditional medicine. These plants may be used singly, but often in combination with herbs from other medicinal plants.[3] On the other hand other medicinal plants such as extracts from Allium tuberosum seeds (Liliaceae)[17], Tichopus zeylanicus leaves (Trichopodaceae)[19], Camelia sinensis tea (Theaceae)[20], Mucuna pruriens seeds (Fabaceae)[21] and Turnea diffusa leaves (Tuneraceae)[22], Phoenix dactylifera (Arecaceae)[23] among other more medicinal plants, were previously investigated in animals et were reported to possess interesting aphrodisiac properties.

Penianthus 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 root bark is chewed alone or with some seeds of Afronumum 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 or root bark is taken with ripe banana in palm oil or food. The root and root bark itself is taken in food for their reputed aphrodisiac properties[24,25] and is in DR Congo put in ameliorated galenic form (capsule) under the name P-VIRIL®. To our knowledge, the aphrodisiac activity of the root bark of P. longifolia or that of the phytomedicine P-VIRIL® based lyophilized aqueous extract of this medicinal plant was not yet investigated. Thus, present study was undertaken to evaluate the effects P-VIRIL® based lyophilized aqueous extract of P. longifolia on sexual behaviors of male Wistar rats.

2. MATERIALS AND METHODS

2.1. Preparation of P-VRIL capsule

Root bark of Penianthus longifolia Miers were collected in Kinshasa in Mai 2013. The plant was identified by Mr. Nlandu Lukebiabo 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. Rook barks were dried at room temperature and reduced to powder keeper in brun bottles. 500 g of the plant part was macerated in 2000 ml distilled water for 24 h. After, the mixture was filtered on Wathman paper (N°4). The filtrate was concentration in vacuum using rotavapor and lyophilized to obtain a dried extract (45.78 g). 30 g of this lyophilized...
aqueous extract was mixed with different excipients to manufacturer tablets of P-VIRIL® with the following composition:

Lyophilized aqueous extract: 350 mg
Excipients: s.q.………………1 capsule
Average weight of capsule: 0.3994 g
Box: 10 capsules

2.2. 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, coumarines 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. + formol) and proanthocyanidins with vanillin 1%/H$_2$SO$_4$ 5% after heating giving a red color.[26]

2.2. Experimental animals
Sexually experienced healthy adult male, body weight (bw): 200-220g and female Wistar rats (bw: 185-215g) 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.

2.3. Mating behavior test
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 (40 cm x 30 cm x 20 cm). The test was conducted after oral administration of different oral doses of the extracts to animals. In this way, twenty four Wistar rats were randomly assigned into four equal groups with six rats for each group and orally administered with LAE extract of P-VIRIL® in the following manner: group I as negative control group only received 5 ml distilled water, group II received Yohimbose as positive control group, groups III, IV and V received 100, 200 and 400 mg/kg body weight of
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LAE extract of P-VIRIL® 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 LAE extract of P-VIRIL®. 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 behaviors 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.\[^{[9,27]}\]

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, mount latency in seconds (ML) is the time interval (h) between the introduction of the female and the first mount by the male, ejaculation latency in seconds (EL) which is the time interval (h) between the first intromission and ejaculation, copulation efficiency (CF), intromission ration (IR) and intercopulatory interval (ICI/) in seconds 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 seconds 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.\[^{[20-23,28]}\]

2.3. Test for libido

Libido was assessed according to the method described by\[^{[27]}\] The number of mountings along with intromission and ejaculation were analyzed according to.\[^{[21]}\]

2.4. Effects on penile erections

Fifteen male rats were randomly divided into three groups of 3 animals each and orally treated with LAE extract of P-VIRIL® in the following way: group I,II and III received 100,
200 and 400 mg/kg respectively while the negative control (2 rats) only received water. Shortly after treatment, these rats were placed individually in plexiglas 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.\textsuperscript{[20]}

2.5. Determination of serum testosterone level

Fifteen male rats were randomly divided into three groups of 5 animals each and orally treated with LAE extract of P-VIRIL\textsuperscript{®} in the following way: group I, II and III received 100, 200 and 400 mg/kg respectively while the negative control (2 rats) only received water daily 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 using an immunocheminisence assay with Elecsis 1010 analyzer (Rocher Diagnostic, Mannheim) as instructions from the manufacturer.\textsuperscript{[20]}

2.6. Histopathological examination

The histopalogical examination was carried out according to the procedure previously described by.\textsuperscript{[29]} Briefly, testes were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer. Tissues were removed and dehydrated through ethanol (HPLC grade), cleared with xylene (HPLC grade) and finally embedded in paraffin. Sectioning was done by using microtone (7 µm thickness) and tissues were counterstained and observed under optic microscrop under 4X, 10X and 40X magnification values for identification of any side effect caused by LAE extract of P-VIRIL\textsuperscript{®}.

2.7. Acute Toxicity

The acute toxicity of the LAE extract of P-VIRIL\textsuperscript{®} was evaluated in Wistar rats according to the procedure described by.\textsuperscript{[30]}

Thirty two Wistar rats of either sex (weight: 140–150 g, aged 8–10 weeks) were divided in three groups of 10 rats each. The first group (2 rats) orally received by gavage 5 ml distilled water and constituted the negative control group. The second to fourth group (10 rats each) received 0.5, 5 and 10 g/kg body weight of the extract respectively 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 survivor was recorded for further 14 days.
3. RESULTS AND DISCUSSION

Extracts of 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.\[31\] In addition, the administration of a 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 behaviors.\[23\]

It was previously reported that the increase or decrease of some sexual behaviors provoked by some plant extracts indicated the robust sexual behaviors of the test samples.\[21,22\] In male, 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 and facilitation.\[27\]

The present study was undertaken to study the effects P-VIRIL® based lyophilized aqueous extract of \textit{P. longifolia} root bark (LAE) on sexual behaviors of normal rats to confirm its claimed aphrodisiac salesmen. The computed sexual behavior parameters of the treated male rats are shown in Tables 1 and 2. Results indicated that the oral administration of LAE extract of P-VIRIL® at oral doses of 100, 200 and 400 mg/kg bw significantly reached significant modification of all parameters compared to control group.

The oral administration of LAE extract of P-VIRIL® in male rats significantly increased the copulatory efficiency in dose dependent manner compared to negative control and a statistically significant difference was observed (\(p < 0.05\)). This effect was dose-dependent (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.\[32\] In addition, the copulatory performance of treated male rats enhanced by the administration LAE extract of P-VIRIL® 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.\[28,33\]

The mount frequency (MF) was recorded at all administered oral doses and results indicated that all doses induced significant increase of mount frequency. At the oral dose of 200 mg/kg bw, the mount frequency was 21.7±1.1, higher compared that of 100 and 400 mg/kg (13.4 ± 0.7 and 14.8±0.3 and negative control (11.7 ± 1.1) at Day 15. A statistically significant
difference (p < 0.05) was deduced between negative groups and treated groups and specially between groups treated at oral dose of 200 mg/kg compared to those treated with 100 and 400 mg/kg respectively (Table 1). This parameter is an indice of vigor, libido and potency which are increased in male animals after treatment with LAE extract of P-VIRIL®.

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. The high decrease of these parameters were observed with the treatment of 200 mg/kg bw (5.8 ±0.8) compared to 100 and 400 mg/kg bw (12.3 ±0.3and 14.8 ±0.1 respectively) as well as to negative control group (11.7 ±1.1). These effects were also reported by [9,27] concerning the effect of extracts of Psoralea coylifolia and Gloriosa superba respectively in male albino rats. The decrease of these behavioral parameters might imply the stimulation of sexual motivation and arousal, and also may be an indication of enhanced sexual appetitive behavior in male animal which, further supports the sexual improvement effect of the administered extract. The same effect was also previously reported by [27,28,34] while the number of MF reflects sexual motivation, increase in the number of IF showed the efficiency of reception, penile orientation and the ease by which ejaculatory reflexes are activated [28].

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 (Tables 1 and 2). The increase of IF and MF suggested improved sexual vigor and libido. Similar finding was also recorded by [28,35].

Penile erection is important for evaluating the effect of administered samples on erectile function. The potency test showed that the extract of P. longifolia root bark (AE) would be able to favored 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 Yohimbin treated animals used as an aphrodisiac reference product. At all oral doses, all treated rats provoked significant difference (p <0.05) in spontaneous penile erection compared to negative control group. This was characterized by the elongation and hardening of penis seen in treated groups than in untreated groups. This observation indicated that the administration of LAE extract of P-VIRIL® facilitated the flow of blood into the penis as also reported by [36]. The penile erection number in relation to the rats witness passed to 62.2 ± 0.3, 77.4 ±0.5and 64.4 ±0.1 with the oral doses of 100, 200 and 400 mg/kg bw respectively and 81.2 ± 0.5 for Yohimbine as a reference aphrodisiac product.
against 42.0 ±0.5 for negative control group. This results suggested that this extract would act on the laxity of the muscle smooth pennies and by this capacity to loosen the smooth muscular cells, would be able to favored the blood flux toward the cavernous bodies of the penis as also proposed by\textsuperscript{[38]} for the aqueous extract of \textit{Buchholzia coriacea}. It could thus, mislead the sexual pleasure. At all oral doses, significant difference (p <0.05) in spontaneous penile erection compared to negative control group was observed. This was characterized by the elongation and hardening of penis seen in treated groups than in untreated groups. This observation indicated that the administration of LAE extract of P-VIRIL\textsuperscript{®} facilitated the flow of blood into the penis as also reported by\textsuperscript{[36]}

The penile erection index (PEI) is important for evaluating the effect of drug administration on erectile function.\textsuperscript{[37]} With the administration of oral dose of 200 mg/kg of LAE extract of P-VIRIL\textsuperscript{®}, the erection number passed of 64.3 ±0.7 at Day 5 to 68.5 ± 0.8 at Day 15, significantly higher compared to the effect produced by the administration of 100 and 400 mg/kg bw respectively of the same extract and negative control (Tables 1 and 2). Similar effect was also recovered with the roots of \textit{Rauwolfia obscura}.\textsuperscript{[9,38]} The results revealed significant increase in penile erection and genital grooming and showed more frequent and vigorous licking and anogenital sniffing towards the receptive females, increased grooming of the genitals induced by LAE extract of P-VIRIL\textsuperscript{®} compared to negative control group. The penile erection number was found to be increased by LAE extract of P-VIRIL\textsuperscript{®} administration as showed in figure 1 and could be due to its androgenic effect.\textsuperscript{[27, 39]}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Effects of LAE extract of P-ViIRIL\textsuperscript{®} on of penile erection number of male Wistar rats}
\end{figure}
All these effects resulted into significant increase in sexual motivation and vigor produced by the administration of the studied extract.\[40\]

The prolongation ejaculation time, the decrease of mount and intromission latency among other sexual behaviors parameters are generally seen as positive signs of aphrodisiac effect.\[41\]

Table: 1. Mating behaviors of male Wistar rats receiving LAE extract of P-VIRIL®

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I (Control group)</th>
<th>Group II (100 mg/kg of LAE)</th>
</tr>
</thead>
<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>475.3±0.4</td>
<td>462.1±0.8</td>
</tr>
<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>
</tr>
</tbody>
</table>


In the present study, LAE extract of P-VIRIL® prolonged the ejaculatory latency in dose related manner where the oral dose of 200 mg/kg from Day 5 to Day 15 produced the highest activity compared to other administered oral doses and negative control group (Table 2). Such effect could result from the desensitization.\[42\] This effect was neither due to sensory motor incoordination’s nor eruption of penile tactile sensitivity.\[32, 43\] It could be attributed at least partly to the anxiolytic property of LAE since anxiolytic agents are well reported to prolong the ejaculation time.\[20,44\]

The significant increase in EL suggested that the extract and positive control drug prolonged the duration of coitus as an indicator of sexual motivation and sustained increase in sexual activity and aphrodisiac properties in the administered extract as also reported by\[27\] on the
effect of extracts of *Psoralea corylifola* seeds in male albinos rats. In the present study, these effects were profound with the oral dose of 200 mg/kg bw.

Ejaculation was present in treated and untreated animals and may inferred that the test drug produced a striking increase in pure libido. Similar finding was also recorded by.[27,35] In addition, our results are in good agreement with others authors who had previously studied the sexual behaviors of some plant extracts in normal rats.[34,45,46]

Table: 2 Mating behaviors of male Wistar rats receiving LAE extract of P-VIRIL®

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group III (200 mg/kg of LAE)</th>
<th>Group IV (400 mg/kg of LAE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 5</td>
<td>Day 10</td>
</tr>
<tr>
<td>MF</td>
<td>17.2±0.2</td>
<td>19.3±0.8</td>
</tr>
<tr>
<td>ML</td>
<td>7.8±0.6</td>
<td>6.3±0.2</td>
</tr>
<tr>
<td>IL</td>
<td>10.3±1.1</td>
<td>9.7±0.8</td>
</tr>
<tr>
<td>IF</td>
<td>86.2±1.2</td>
<td>88.2±1.4</td>
</tr>
<tr>
<td>III</td>
<td>20.0±0.2</td>
<td>17.1±0.7</td>
</tr>
<tr>
<td>NI</td>
<td>7.6±0.3</td>
<td>8.2±1.2</td>
</tr>
<tr>
<td>NT</td>
<td>5.1±0.3</td>
<td>5.7±0.5</td>
</tr>
<tr>
<td>EI</td>
<td>251.1±0.2</td>
<td>263±0.3</td>
</tr>
<tr>
<td>PE</td>
<td>64.3±0.7</td>
<td>66.2±0.3</td>
</tr>
<tr>
<td>PEI</td>
<td>402.3±0.4</td>
<td>398.1±0.8</td>
</tr>
<tr>
<td>NPT</td>
<td>67.8±0.4</td>
<td>73.2±0.5</td>
</tr>
<tr>
<td>CE</td>
<td>1.2±0.6</td>
<td>2.1±0.5</td>
</tr>
</tbody>
</table>

Legend: see table

The oral administration of LAE extract of P-VIRIL® 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.[47] The epididymal sperm parameters revealed a significant increase in the number of spermatozoa in all the experimental groups (215, 245, 238 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[21] concerning the effect of ethanolic extract of *Mucuna pruriens* on sexual behaviors in 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 aphrodisiac herbal extracts inhibited the motility 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 their non-viability.[36] 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. On the other hand, it has been also shown other medicinal plant extracts improved sperm production and sperm mobility. Thus, these kinds of plant extracts may be employed as contraceptive and aphrodisiac agents.

Results from the libido test showed that the pre-coital sexual behaviors, such as chasing, nosing and genital sniffing were well performed in all treated groups with the administration of LAE extract of P-VIRIL® at all tested oral doses. This was mainly profoundly seen in group II receiving orally 200 mg/kg of the extract than in group I and III receiving orally 100 and 400 mg/kg bw respectively.

Table: 3. Test of potency of LAE of P-VIRIL® in male Wistar rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Negative control</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 bw of LAE extract of P-VIRIL® respectively

In addition, the effect on potency was assessed by testing the action of LAE extract of P-VIRIL® 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 the extract produced significant increase of the frequency of these parameters in dose-dependent manner in treated animals compared to negative controls (p < 0.05) (Table 3). These effects were pronounced in group II receiving 200 mg/kg bw of LAE extract of P-VIRIL® (Table 3). This indicated that the extract increased potency.

Hormonal analysis revealed that the level of testosterone increased in all the experimental groups from Day 5 to Day 15 and was lower in the control groups (Fig.2). The high level of testosterone in treated animals could result in improving total sexual behavior. In addition, this elevation of this hormone in treated animals provoked by the oral administration of LAE extract of P-VIRIL® may be due to the implication of serotoninergic action of the extract since it is well known that serotoninergic agents increase testosterone synthesis or mediated by the prevention of its metabolic degradation, and also
testosterone supplementation has previously been shown to improve sexual function and libido\textsuperscript{[27, 40]}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{Effect of administration of LAE extract of P-VIRIL\textsuperscript{®} at different oral doses (100, 200 and 400 mg/kg bw) on serum testosterone level in male Wistar rats in days post treatment. N.C. negative control.}
\end{figure}

Other plant extracts such as that from \textit{Camellia sinensis}\textsuperscript{[20]}, \textit{Mucuna pruriens}\textsuperscript{[21]}, \textit{Bulbine natalensis}\textsuperscript{[33]} among others had been reported to increase testosterone level in animal models.

Moreover, the treated animals with LAE extract of P-VIRIL\textsuperscript{®} expressed immediately a rhythmic motor pattern of ejaculation. In addition, both serotonergic and dopaminergic system play a principal role in mechanisms\textsuperscript{[44]} As seen in this study, LAE of P-VIRIL\textsuperscript{®} significantly increased or decreased these sexual parameters according to the case, compared to negative control groups, and constitute additional arguments to confirm its aphrodisiac potential effect. In the case of a decrease was observed, the effects of LAE of P-VIRIL\textsuperscript{®} at all administered oral doses remained often significantly higher compared negative control groups (p < 0.05).

According to\textsuperscript{[21,22]}, our results provide experimental supports to justify and confirm the reputation use of the phytomedicine P-VIRIL\textsuperscript{®} and in some extents that of the \textit{P. longifolia} root bark as sexual stimulant or as natural aphrodisiac agent by young, adult men and women used in their conjugal live.
With regards to the efficacy of LAE extract of P-VIRIL® 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 aphrodisiac by their effects in dopaminergic nervous and dopaminergic pathway controlling sexual activities due to the presence of high level of L-DOPA content in plant material.\cite{52} In addition, extracts of many medicinal plants are reported to be effective as aphrodisiac agents trough mechanisms such as vasodilatation, generation of nitrite oxide, gonatropins and elevation of androgens.\cite{36}

From Day 5 to Day 15, Yohimbine used as reference aphrodisiac product induced significant increase of MF (22-25), ML (8-11) IL (12- 16), EL (270-286), NM (19-26), CE (1.5-3.1), NI (7.2-8.1), III (23-26), IF (89-95) and other sexual behaviors. On the other hand this aphrodisiac product produced significant decrease of IL (8.0-8.5) and PEI (390-315) compared to the effects of negative control groups and LAE extract of P-VIRIL® administered all tested doses. These effects indicated that Yohimbine had a higher aphrodisiac action than LAE extract of P-VIRIL®. Its high activity was also previously reported by\cite{22} compared to the activity of the aqueous extract from *Turnea diffusa* leaves collected in the State of Hidalgo, Mexico.

In general, our results reported in the present study on the effects of the LAE extract of P-VIRIL® on sexual behaviors evaluated in the present study, are in good agreement with those previously described by other authors after the evaluation of the aphrodisiac activity of others medicinal plant extracts claimed to possess this action in traditional medicine of some countries in the world.\cite{19,20,21,22,47,53,54}

In acute toxicity, animals were treated once time with oral doses of 0.5, 5 and 10 g/kg body weight respectively. Results from this test revealed that no sign of toxicity such as alteration of the locomotion activity and gastrointestinal disturbances were observed. But rats received all tested oral doses significantly gained body weight compared to negative control groups. According to\cite{55}, 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 at all administered oral doses. Therefore, the LD$_{50}$ of LAE extract of p-VIRIL® was estimated to be greater than 10 g/kg body weight. Substances that present LD$_{50}$ higher than 5.0 g/kg body weight via oral route, may be considered as...
practically non-toxic.\textsuperscript{[56]} Interestingly, for OCDE, substance with \( \text{LD}_{50} \) higher than 2 g/kg bw is considered as non-toxic.\textsuperscript{[30]} Therefore, it may be suggested that acute toxicity of LAE extract of P-VIRIL\textsuperscript{®} is practically null via oral route. This observation is also automatically valid for the extract of \textit{P. longifolia}.

The histopatological 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. 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 behaviors of rats in the acute toxicity test. These reported results also showed the margin safety and tolerability of the extract of P-VIRIL\textsuperscript{®} and can at least, explain the extensive use of the phytomedicine P-VIRIL\textsuperscript{®} in Democratic Republic of Congo and other African countries as natural aphrodisiac by young, adult men and women without significant visible side effects and found large satisfaction during their sexual rapports. In conclusion, these results presented strong evidence of the non-toxic effects of LAE extract of P-VIRIL\textsuperscript{®} as well as that of \textit{P. longifolia} extract used in traditional medicine.

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 on these phytochemical results, we could speculated that the observed aphrodisiac activity of LAE extract of P-VIRIL\textsuperscript{®} may be due to the presence of terpenes, steroids and alkaloids since some compounds of the chemical groups isolated in other plant species were been previously reported to possess aphrodisiac properties.\textsuperscript{[57,58]} Other natural products with aphrodisiac action included flavones\textsuperscript{[22]}, xanthone\textsuperscript{[59]}, flavonoids such as pyrano-flavones\textsuperscript{[60]}, and benzoflavones\textsuperscript{[61]} among other natural phytochemicals.

**CONCLUSION**

Sexual behavior parameters were studied in normal rats to understand the role of the lyophilized aqueous extract of P-VIRIL\textsuperscript{®} as well as that of \textit{Penianthus longifolia} root bark as
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 P-VIRIL® based lyophilized aqueous extract of *P. longifolia* root bark supporting and justifying the claimed use by young, adult men and women in their conjugal live as well as the use of *P. longifolia* root extract in traditional medicine in DR Congo and other African countries as natural aphrodisiac agent to increase sexual action.

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