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Sexual behavior effect of orally administered crude aqueous extract of *Terminalia schimperiana* Root in male Wistar rat

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Abstract

The Proximate composition, phytochemical composition and the effect of oral administration of aqueous extract of *Terminalia schimperiana* root on male wistar rat sexual behavior were determined. The root was collected and extracted with water, the phytochemical screening was carried out using the standard procedures and revealed the presence of alkaloids, saponins, Phenolics, anthocyanin and Tannins. The Proximate analysis results obtained showed the amount of crude protein, crude fat and total carbohydrates to be 6.31 ± 0.75 , 4.64 ± 0.82 and 17.79 ± 0.53 respectively of the sample while the percentage of crude fiber, ash and moisture content in the root sample were 32.72 ± 0.59 , 0.35 ± 0.12 and 10.35 ± 0.18 respectively. Aqueous extract of *Terminalia schimperiana* root was orally administered to sexually active rats in a daily dose of 1000, 2000 and 3000 mg/Kg body weight for 5 days, the sexual behavior was monitored and testosterone serum level was measured on *Terminalia schimperiana* treated rats, control rats “that received vehicle” and the reference group “ that received Powmax”. Oral administration of *Terminalia schimperiana* significantly increased the mount frequency, intromission frequency and prolonged the ejaculatory latency. In addition, a significant increase in the copulatory efficacy was reported for the test groups and testosterone serum level was significantly increased in *Terminalia schimperiana* treated rats compared to controls and reference. The present study revealed that continuous administration of *Terminalia schimperiana* aqueous extract for a period of 5 days improves the sexual motivation and performance in adult male rats, an effect that may be linked to increased testosterone level, the nutrient composition and the phytochemicals.

Keywords: *Terminalia schimperiana*, sexual behavior, testosterone, wistar rat, phytochemical, proximate composition

1. Introduction

Traditional medicine has been practiced for many centuries by rural people for management and treatment of several diseases because of its availability, low cost and little side effects. Nature has provided a source of medicinal agents for many years and many of modern drugs have been isolated from natural sources, many based on their use in traditional medicine [1]. *Terminalia schimperiana* belong to the order myrtales and family combretaceae, it is known as Idi in Yoruba language. It is a broadleaved small tree that can reach up to 7–14 m, variably deciduous in the dry season to semi-evergreen, depending on the climate. The leaves are alternate, simple, elliptic to obovate, 9–15 cm long and 3–8 cm broad, green above with pale undersides. The flowers are tiny and form pale spikes at the base of the leaves. The fruit is a samara with a single wing 6–9 cm long, that turns brown with age [2]. It can be found in open forest habitats with more than 1300 mm of rainfall per year, when it is found in closed forest, it typically part of the forest canopy and it may be the dominant tree species where it is found [3]. In parts of West Africa, *T. schimperiana* is used as a medicinal plant; the bark being applied to wounds while the twigs may be chewed to promote oral hygiene. In laboratory, experiments on extracts of the plant were found to have *in vitro* antibiotic properties against *Staphylococcus* [4] and the plant extract has been found to also have antifungal properties *in vitro* [5]. The infusion of the plant root is claimed to be used as aphrodisiac by the locals of oko community in Kwara state of Nigeria. Consequently, a scientific study to determine the phytochemical and proximate analysis of the root for the presence of various secondary metabolites was done, the sexual behavior was monitored and testosterone serum level was measured to ascertain its aphrodisiac efficacy and the effective dosage in *Terminalia schimperiana* treated male wistar rats.

2. Materials and Methods

The roots of *Terminalia schimperiana* was collected from Oko, irepodun local government area of Kwara state, Nigeria. Identification and authentication of plant was carried out at the botany unit of the department of pure and applied biology, Ladoke Akintola College of science and technology, Ogbomosho, Nigeria. The root was cleaned, cut into pieces and oven-dried at 40 °C, the dried pieces were then pulverized into powder using an electric grinder. 200g of the powder was percolated in 2000ml of distilled water with constant shaking for 48hrs at room temperature. The extract was filtered with Whatman no.1 filter paper, the filtrate was lyophilized and the percentage yield calculated and stored until further use. The proximate analysis and phytochemical screening of the plant was carried out on the root and aqueous extract of the plant root of *Terminalia schimperiana* to detect the active components that are present. Adult white albino rats (*Rattus norvegicus*) of both sexes weighing between 140 - 150g were obtained from the Animal Research Laboratory in Ilorin, Nigeria and Makerere University, Kampala, Uganda respectively. The rats were placed in a well-ventilated house of 28- 31 °C temperature, 50-55% humidity, in clean aluminium cages. They were allowed to acclimatize for seven days before the start of the experiment and were maintained on standard mouse pellets (Bendel Feeds and Flour Mills Ltd.,Ewu, Nigeria) and tap water.

2.1 The phytochemical screening

The phytochemical screening of the plant was carried out on the aqueous extract of the plant root of *Terminalia schimperiana* to detect the active components like alkaloids, steroids, terpenoids, tannins, saponins, anthocyanins, coumarins, flavonoids, cardiac glycosides, phenols and betacyanin using the standard procedures described by Sofowora [6].

2.2 The nutritional composition

The nutritional compositions of *Terminalia schimperiana* root like moisture content, ash content, crude fiber, total protein, crude fat, total carbohydrate were determined by the methods described by AOAC (1996) [7] and Indrayan, *et al.*, (2005) [8].

2.3 Sexual behavior test

In order to establish the effect of increasing concentration of the extract on the sexual behavior of the male Wistar rats, lyophilized aqueous extract of *Terminalia schimperiana* roots was reconstituted in the concentrations of 1000, 2000 and 3000mg/kg body weight, was administered orally and their mating behavior observed using the method of [9]. Typically, male rats was individually placed in cages and 30 min after their daily administration; they were paired with receptive females. Their male sexual behavior parameters were monitored for 5 days. The test was performed 24 h after the last administration for each day. Single male rat was placed in the observation chamber to acclimatize for 7 min. Then, a sexually receptive female rat was introduced in the chamber. The following parameters of sexual behavior were measured as described by [9]. The parameters of sexual behavior monitored included: a) Mount Frequency : (the number of mounts without intromission from the time of introduction of the female until ejaculation), b) Intromission Frequency: (the number of intromissions from the time of introduction of female until ejaculation), c) Mount Latency: (the time interval between the introduction of the female and the first mount by the male), d) Intromission Latency: (the time interval between the introductions of female to the first mount by the male), e)

Ejaculation Latency: (the time interval between the first intromission and ejaculation),

2.4 Reproductive Hormone

The serum testosterone concentration, serum follicle stimulating hormone and serum luteinizing hormone were quantitatively determined using the direct human testosterone enzyme immunoassay (EIA) kit of the Manufacturer's protocol version (2001). The principle of testosterone assay (EIA) is based on a direct assay of a limited (competitive) type following the general antibody-antigen reaction based on the enzyme-linked immunosorbent assay (ELISA) as described by Tietz, *et al.*, (1994) using Serozyme I Sero (Diagnostics, Freiburg, Germany), the serum testosterone concentration, serum follicle stimulating hormone and serum luteinizing hormone were then interpolated from a standard calibration curve [10].

2.5 Statistical analysis: Data was expressed as the mean of five replicates \pm SD. Means was analyzed using a one-way analysis of variance (ANOVA) and complimented with student's *t-test*. Post-test analysis was carried out using Turkey's multiple comparison test to determine significant differences in all parameters. All the statistical analysis was done using IBM SPSS statistics 20 while the graphs was plotted with Excel program. Differences with values of $P < 0.05$ was considered statically significant.

3. Results

Table 1: Phytochemical constituent of *Terminalia schimperiana* root.

Phytochemicals	Present
Alkaloids	+
Steroids	-
Anthocyanin	+
Cardenolides	-
Dienolides	-
Saponins	+
Phenolics	+
Flavonoids	-
Cardiac glycosides	-
Tannins	+
Triterpenes	-

Table 2: Nutritional composition of *Terminalia schimperiana* root.

Composition	(%)
Ash content	0.35 \pm 0.12
Moisture content	10.35 \pm 0.18
Total carbohydrate	17.79 \pm 0.53
Total Protein	6.31 \pm 0.75
Crude Fat	4.64 \pm 0.82
Crude Fibre	32.72 \pm 0.59

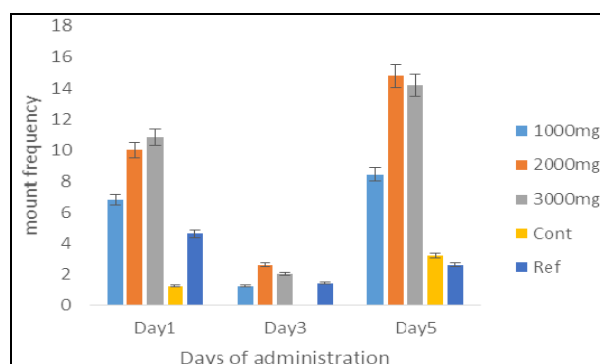


Fig 1: Effect of administration of aqueous extract *Terminalia schimperiana* root on male rat mount frequency.

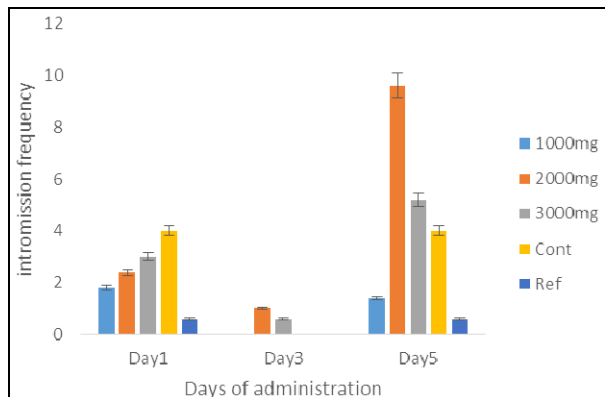


Fig 2: Effect of administration of aqueous extract *Terminalia schimperiana* root on male rat intromission frequency.

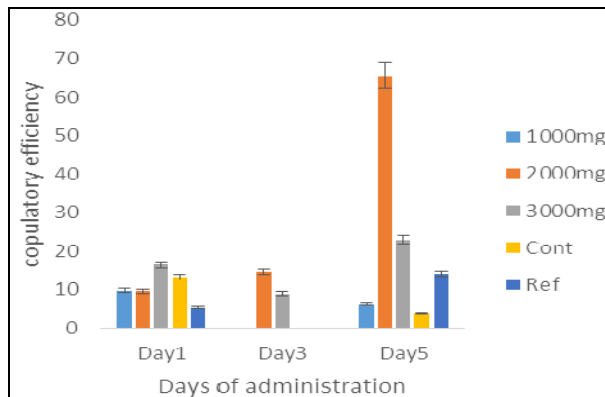


Fig 6: Effect of administration of aqueous extract *Terminalia schimperiana* root on male rat copulatory efficiency.

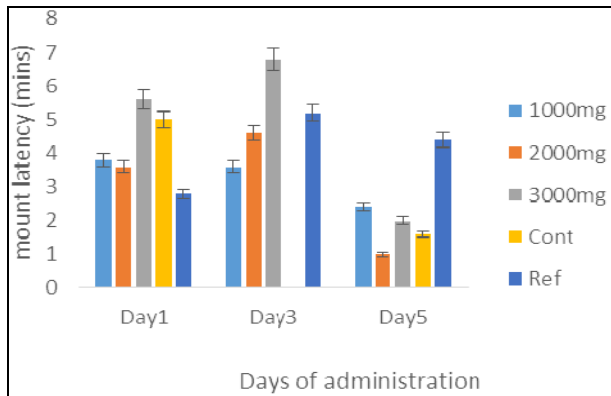


Fig 3: Effect of administration of aqueous extract *Terminalia schimperiana* root on male rat mount latency.

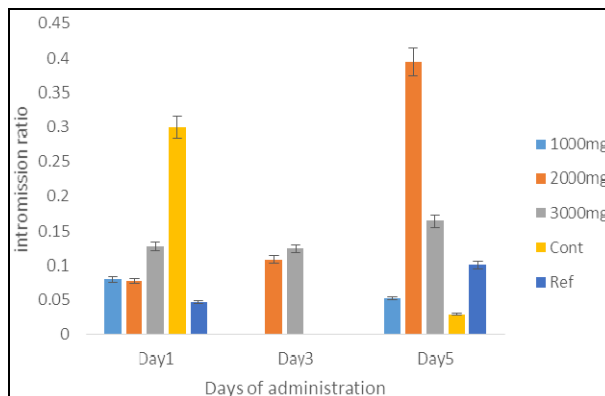


Fig 7: Effect of administration of aqueous extract *Terminalia schimperiana* root on male rat intromission ratio.

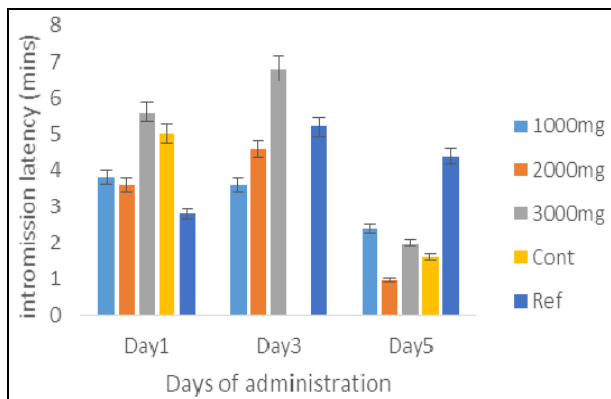


Fig 4: Effect of administration of aqueous extract *Terminalia schimperiana* root on male rat intromission latency.

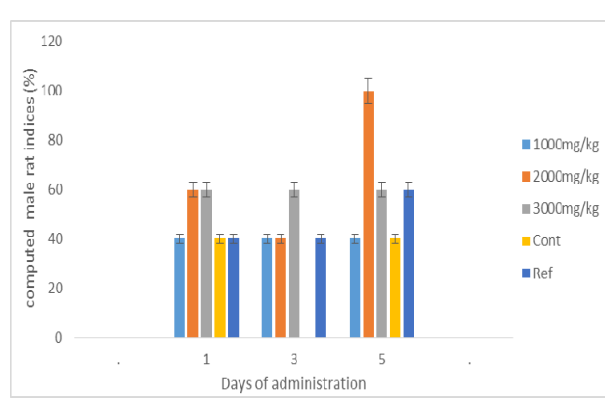


Fig 8: Effect of administration of aqueous extract *Terminalia schimperiana* root on male rat computed % mounted.

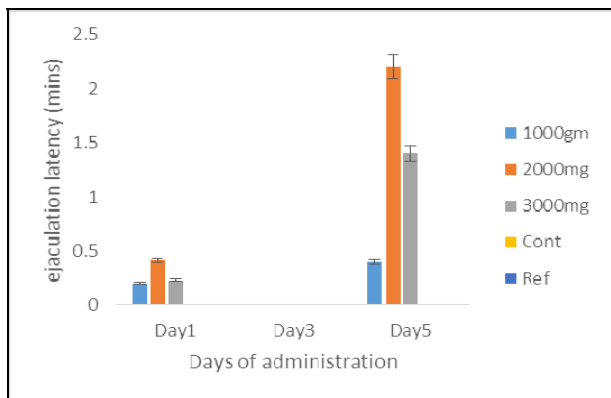


Fig 5: Effect of administration of aqueous extract *Terminalia schimperiana* root on male rat ejaculation latency.

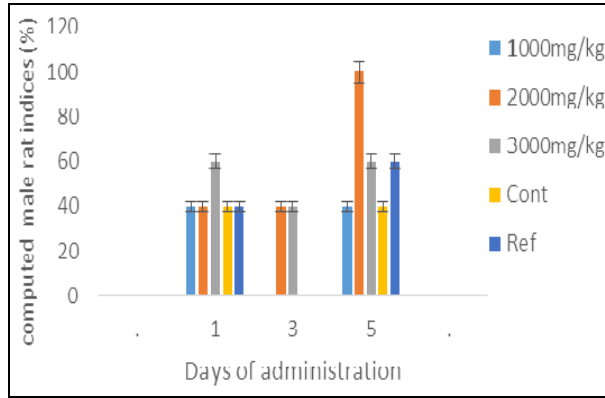


Fig 9: Effect of administration of aqueous extract *Terminalia schimperiana* root on male rat computed % intromitted

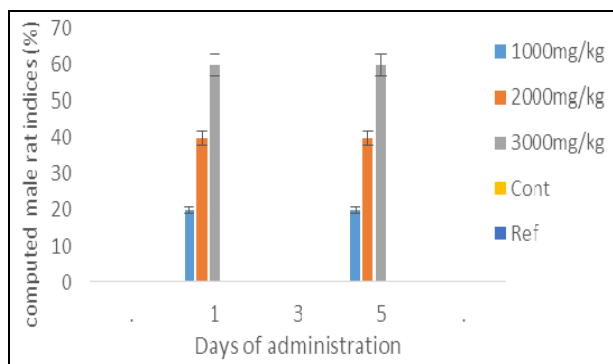


Fig 10: Effect of administration of aqueous extract *Terminalia schimperiana* root on male rat computed % ejaculated.

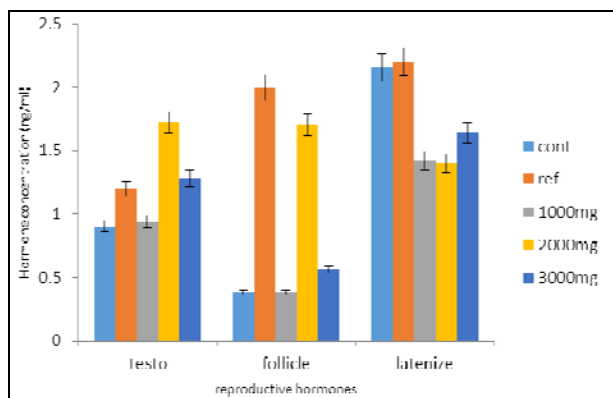


Fig 11: Effect of administration of aqueous extract *Terminalia schimperiana* root on male rat reproductive hormones.

4. Discussion

Phytochemical screening of aqueous extract of *Terminalia schimperiana* root revealed the presence of bioactive agents such as alkaloids, saponins, Phenolics, anthocyanin and Tannins while others like steroids, Flavonoid, Cardiac glucoside, cardenolides, dienolides, and triterpenes were not detected (Table 1). The phytochemicals present in the aqueous extract of *Terminalia schimperiana* root (Phenolics, Alkaloids, Tannins and Saponins) are good candidates that can be used to explain the various pattern of results obtained in the sexual behavior effect study of the plant. Saponins and alkaloids have been shown by previous authors to be responsible for enhancing male sexual behaviour in animals [11]. Saponins act by increasing the biosynthesis of androgens, alkaloids dilate blood while tannins possesses anti-inflammatory, anti-bacterial and wound healing effect vessels [12, 13]. Similarly, the present bioactive agents may also be responsible for the toxicity observed in the other parts of this study. The proximate analysis of *Terminalia schimperiana* root is shown in table 2. The amount of crude protein, crude fat and total carbohydrates were found to be 6.31 ± 0.75 , 4.64 ± 0.82 and 17.79 ± 0.53 respectively of the sample while the percentage of crude fiber, ash and moisture content in the root sample was 32.72 ± 0.59 , 0.35 ± 0.12 and 10.35 ± 0.18 respectively. The plant composition shows that the consumption of the plant can improve general health and well-being and can lead to a burst of energy and translate into an increased sexual appetite. Administration of crude aqueous extract of *Terminalia schimperiana* root for 5 days at the dosages of 1000, 2000 and 3000 mg/kg body weight produced significant increase ($P < 0.05$) in mount frequency (figure 1) with 2000 and 3000mg/kg dosages having the greatest effects, also the administration of the extract at all the dosages produced significant increase ($P < 0.05$) in

intromission frequency with the greatest effect still produced by the 2000 and 3000mg/kg body weight dosages when compared with the reference and control with values of 0.6 ± 0.40 and 0.4 ± 0.39 intromissions (Figure 2). The frequencies of mount (MF) and intromission (IF) are useful indices of sexual vigour, libido and potency [14]. However it should be noted that it was not every mount by the male rat that resulted in intromission. The mount latency for the different dosages showed disparity in activity of the extract because the mount time reduced for some dosages and got prolonged for some other dosages throughout the period of administration when compared with the reference drug and the control (Figure 3) meanwhile the intromission latency was not significantly different from the reference and the control at all the dosages investigated (Figure 4). The observed mount latency and intromission latency showed that the hesitation time of the male rats towards the receptive females due to the effect of the plant extract changed and had varying effects when compared with the control and reference and this indicates enhanced sexual appetitive and behavior which could also be considered as increased sexual motivation or desire. The administration of the extract at the dosages of 1000, 2000 and 3000 mg/kg body weight of the plant extract significantly prolonged the ejaculatory latency throughout the period of observation with the 2000 and 3000mg/kg dosage groups having the most prolonged time of intromission before ejaculation when compared with the reference and control (Figure 5). The significantly prolonged ejaculatory latencies (Figure 5) shown by the extract are indications of vigour and improved sexual performance. The computed male rat sexual behaviour parameters of intromission ratio, % mount, % intromitted, % ejaculated and copulatory efficiency were all increased at all the dosages with the highest effect shown by the dosages of 2000 and 3000mg/kg body weight throughout the period of administration when compared with the control and reference (Figure 6-10). The changes in the male rat reproductive hormones following the administration of aqueous extract of *Terminalia schimperiana* root for 5 days are represented by figure 11. Compared with the control and reference with values of 0.9ng/ml and 1.2 ng/ml respectively, administration of the plant extract at the dosage of 2000 mg/kg resulted in significant increase in testosterone with the value of 1.7ng/ml while the 3000mg/kg compared favourably with the reference drug but the 1000mg/kg was not different from the control value. Administration of the extract at the dosage of 2000 mg/kg resulted in significant increase in follicle stimulating hormone with the value of 1.7 ng/ml when compared with the control value of 0.38 ng/ml and it compares favorably with the reference drug with value of 2.0 ng/ml while the levels of 1000 and 3000 mg/kg body weigh were not raised. However, luteinizing hormone was decreased at all the dosages when compared with the control and reference. Testosterone plays a key role in sexual function such as enhanced libido, increased energy and frequency of erection or clitoral engorgement [15], therefore apart from desire that is essential for initiation of sex: penile tumescence, accessory muscles and ejaculation are also dependent on testosterone for a normal sexual activity [16]. The enhanced sexual function observed in this study above may be attributed to the raised levels of testosterone which might be due to enhancement in the GnRH-(Gonadropine releasing hormone) signaling as the serum follicle stimulating hormone concentrations was also increased.

5. Conclusion

From the sexual behavior study it is evident that the significant increase in the mount and intromission frequencies are indications of stimulation of sexual arousability, performance, motivation and vigour in the rats produced by the extract and which might be as well be mediated by the increase in testosterone level. The phytochemicals present in *Terminalia schimperiana* root which are phenolics, tannins, saponins, anthocyanin and alkaloids may be responsible for the enhanced sexual function seen in this study while the proximate composition shows that the consumption of the plant can improve general health, can lead to a burst of energy and translate into an increased sexual appetite. Therefore, aqueous extract of *Terminalia schimperiana* root increased sexual behavior activities and possess aphrodisiac property. However, further studies using different solvents fractions obtained from the *Terminalia schimperiana* root are required and are in progress to isolate and identify the active ingredient (s) responsible for this effect.

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