In vivo aphrodisiac efficacy of aqueous and hydroalcoholic extracts of the leaf and root bark of Citropsis articulata in male Wistar rats

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Abstract

Background: The African cherry orange tree *Citropsis articulata* Swingle & Kellerman (family Rutaceae) is traditionally used to manage erectile dysfunction. This study aimed to determine the aphrodisiac activity of *C. articulata* leaves and root bark in male Wistar rats.

Methods: Aqueous and hydroalcoholic extracts of leaves and root bark were prepared via decoction and soxhlation, respectively. Extracts were administered to the animals at three dose levels, i.e., 100, 500 and 1000 mg/kg. Six male animals (aged 8–12 weeks, weighing 140–200 g) were used in each of the groups. Three animals per group (group B), unlike those immediately sacrificed at the end of 28 days (group A), were left for an extra 14 days without dosing to look out for delayed or sustained efficacy signs. Male sexual behavior, including attraction towards the female, penile erection, mount frequency, and mount latency, was monitored on days 0, 3, 7, 14, 21, 28, 35 and 42. At the end of both 28 days and the extra 14 days, the animals were humanely sacrificed via anesthesia with halothane, and their blood was collected for testosterone level analysis, while the sexual organs harvested were assessed grossly and histologically.

Results: *Citropsis articulata* root bark and leaves increased the attraction towards the female, penile erection, and mount frequency while lowering the mount latency. The plant extracts also increased testosterone levels after 28 days of daily extract administration but greatly lowered the testosterone levels in the animals that were left for an extra 14 days without dosing. Even histologically, there was the first evidence of very active spermatogenesis, which disappeared when the administration of the extract ceased. The administration of *C. articulata* leaf decoction at a dose of 500 mg/kg significantly increased the testosterone level (14.50 ± 2.53 ng/ml) compared with that of the positive control (4.00 ± 2.70 ng/ml), with a p-value of 0.021 (confidence interval: -19.45 to -1.533).

Conclusion: This study revealed that both the root bark and leaf of *Citropsis articulata* have aphrodisiac efficacy in male Wistar rats *in vitro*.

Keywords: *Citropsis articulata,* aphrodisiac, testosterone, male sexual behaviour, mount frequency

1. Background

The management of erectile dysfunction may involve lifestyle modifications, nonsurgical and surgical procedures (Yafi et al., 2016). Conventionally, nonsurgical methods involve the use of mainly phosphodiesterase 5 inhibitor drugs but also vacuum erection devices, intraurethral suppositories, and intracavernosal injections. The use of phosphodiesterase 5 inhibitor drugs has various adverse effects, including headache, heartburn, facial flushing, nasal congestion, visual disturbances, tinnitus, hypotension and priapism (1). Owing to some of the adverse effects of these therapies, there has been a shift toward natural plant-based remedies.

The aphrodisiac potential of medicinal plants has been traditionally known since time immemorial. It refers to the ability to enhance male sexual function by increasing desire, arousal, and performance. Singh *et al.* (2012) listed approximately 198 medicinal plants with aphrodisiac potential. Plants with aphrodisiac potential are traditionally used to treat erectile dysfunction. Erectile dysfunction is the recurring failure to obtain and retain an erection sufficient for sexual intercourse in men. The etiology of erectile dysfunction is multicausal. This could be due to psychogenic, neurogenic, vasculogenic, iatrogenic, and/or endocrine causes (3).

Medicinal plants and herbal products are rapidly increasing alternatives to conventional treatments for erectile dysfunction. Some of the known aphrodisiac medicinal plants include *Citropsis articulata, Kigelia africana, Panax ginseng, Rumex usambarensis, Mondia whitei, Acanthus pubescens, Lactuca inermis, Kalanchoë densiflora, Acalypha villicaulis, Rhus vulgaris, Balanites aegyptiaca, Carica papaya, Tragia benthamii, Rumex abyssinicus, Coffea eugenioides, Capsicum frutescens, Solanum incanum, Zingiber officinale, Cola acuminata, Aframomum melegueta,* and *Garcinia kola* (4–6).

Citropsis articulata is one of the most treasured medicinal plants in southwestern Uganda and is often referred to as a natural "Viagra" (7). It is called "Omuboro" in Runyankore-Rukiga language and "katimboro" in Rutooro (4,8). Traditionally, the plant is wholly uprooted, and the tap root is debarked. This bark can be chewed fresh or shredded, dried and pounded to make a powder (9). The powder can then be infused in hot water or boiled, and the extract can be taken once a day for up to three days to increase sexual performance. The leaves of this plant are also gaining popularity as an alternative to the root bark for the same purpose (7,10). This plant is a vulnerable species according to the Red List of Threatened Species of Uganda (11). It is becoming depleted at an alarming rate from the tropical rainforests in which it favorably grows. This has been attributed to unsustainable methods of harvesting and overexploitation of the plant due to its high demand (12).

Whereas Gakunga et al. (2014) and Vudriko et al. (2014) studied the leaf of *C. articulata*, few studies have investigated the aphrodisiac properties of the more traditionally used part, i.e., the root bark. In this study, we simultaneously determined the aphrodisiac activity of both the root bark and leaves of *C. articulata* in Wistar albino rats. This study not only validated the traditional use of the root bark but also provided insight for comparison with leaves as a viable alternative.

Methods

Plant collection and processing

The plant materials were obtained from Kibale Conservation Area (Latitude – N 0° 26’8” Longitude – E 30° 23’8”, Altitude 1252.7 m) in southwestern Uganda in July and August 2021 following the World Health Organization guidelines on good agricultural and collection practices for medicinal plants (13). Prior approval (reference number: COD/96/05) was obtained from the Uganda Wildlife Authority before plant collection. An herbarium specimen (ARA 004) was prepared and submitted to the Makerere University Herbarium, Department of Botany, identified by Mr. Protase K. Rwaburindore and given the accession number 50965. The plant name was checked at https://wfoplantlist.org/plant-list with the identification code wfo-0000607733.

The roots were washed and debarked, and the leaves were directly rinsed with clean running water to remove any debris. The plant materials were air dried for between 7 to 11 days. The dried plant materials were then ground using an electric grinder and sieved to a moderately coarse powder.

Extraction

The plant materials were extracted using two standard methods: decoction and soxhlation. For decoction, approximately 500 g of herbal powder was boiled in 4 L of distilled water for 60 minutes. For soxhlation, 500 g were placed in a Soxhlet thimble and percolated with 2.5 L of 70% ethanol. The liquid contents were filtered through cotton wool, and the volume was reduced using a rotary evaporator (IKA® RV 10 digital, Germany). Finally, the viscous concentrate was lyophilized to obtain dry extracts. The percentage yield was estimated to be 33.47%, 27.26%, 19.27% and 21.58% for the root bark decoction, root bark Soxhlet, leaf decoction and leaf Soxhlet extracts, respectively. All dry extracts were refrigerated between 2 and 6 °C until use.

Preliminary phytochemical screening

Following standard methods (14), all extracts were screened for the presence of alkaloids, saponins, L-arginine, flavonoids, reducing sugars, coumarins, glycosides, and phytosterols. These phytochemicals are known to contribute to the aphrodisiac potential of plants (10,15). All the tests were performed in triplicate to ensure repeatability. One gram of each extract was first dissolved in 50 ml of distilled water, shaken vigorously and filtered through filter paper. The resulting filtrate was used for phytochemical screening, unless otherwise stated in the test procedure (14). Crude and pure standard reference materials were employed as positive controls (WHO, 2011).

Selection and preparation of animals

Male Wistar albino rats (8–12 weeks old, weighing 140–200 g) were selected for the study. Natural lighting at approximately 12-hour intervals was considered sufficient to provide 12 hours of light and darkness each. The animals were housed in groups of 6 animals in clean plastic cages and allowed an unlimited supply of drinking water and feeds. The animals were allowed to acclimatize to laboratory conditions for 10 days before being used in the study. Only healthy animals (with no signs of morbidity) were selected for the study. Fifteen groups with 6 animals each were used for the 4 extracts (3 dose levels per extract, i.e., 1000, 500 and 100 mg/kg), 2 negative controls (distilled water alone and then distilled water/dimethylsulfoxide containing 0.1% gum arabic) and 1 positive control. This study was conducted in accordance with the Guide for the Care and Use of Laboratory Animals (16). Ethical approval (reference number: MUREC 1/7) was obtained prior to experimentation.

Preparation and administration of extract and control doses

The decoction extracts were dissolved in distilled water to achieve a stock solution of approximately 100 mg/ml. The Soxhlet extracts were dissolved in dimethylsulfoxide and distilled water (1:9 ratio containing 0.1% gum arabic) to achieve a concentration of 50 mg/ml. Two negative control groups, distilled water and distilled water/dimethylsulfoxide containing 0.1% gum arabic, were used. A positive control, i.e., sildenafil citrate at a dose of 5 mg/kg (Viagra®, Pfizer Pharmaceuticals Ltd., Cairo, Egypt), was also used.

Food but not water was withheld overnight for each animal prior to dosing. The fasted body weight of each animal was determined. The actual dose in ml was calculated according to the following formula: volume administered in ml = (dose in mg/kg × body weight in kg) ÷ concentration of extract in mg/ml. The test substance was administered slowly via gastric gavage using an intubation cannula (10).

Induction of estrus in female animals

To render the female rats receptive to the males, they were given a suspension of estradiol valerate (Progynova®, Delpharm, Bayer AG, Berlin, Germany) at a dose of 0.4 mg/kg 48 hours before the experiment. Progesterone (Gestone™, Ferring Pharmaceutical PVT, Ltd., Bhiwandi, India) was subsequently injected subcutaneously at a dose of 5 mg/kg approximately 4–6 hours before the experiment. Only receptive females were considered for the study after they were exposed to male animals other than those used for the actual study.

Mating behavior – noncontact and contact studies

Following the administration of the doses of the extracts or control substances, the male animals were placed in one half of a transparent glass observation chamber measuring about 24 by 12 by 12 (length by width by height) inches. The chamber had a transparent glass separation with at least one perforated hole, which allowed the transfer of auditory, visual and pheromonal cues between the two animals. A receptive female animal was placed in the other half of the chamber 1 hour after drug administration to the male and acclimatized to the other half of the chamber. This model is referred to as the noncontact model (15,17), in which the time spent by the male rat near the perforation in a 30-minute period was recorded as the *attraction towards the female*. In addition, the number of times the male animal bent to lick its erect penis was also recorded as *penile erection* (15).

After 30 minutes, the female rat was introduced into the chamber half with the male rat for another 30 minutes. This was referred to as the contact model (15). The time taken by the male for its first mount and the number of mounts in this period were recorded as the *mount latency* and *mount frequency,* respectively.

Gross and histological investigation

For each animal, the noncontact and contact models were studied on days 0, 3, 7, 14, 21 and 28. At the end of 28 days, 3 animals per group were humanely sacrificed, and their sex organs (penis, seminal vesicles, testes, epididymis and prostate glands) were harvested for gross and histological investigation. The remaining 3 animals per group were further studied on days 35 and 42. This was done to assess the delayed or sustained aphrodisiac effects of the extracts (18).

The weights of the animals were recorded weekly. The relative weights of the organs (particularly the penis and testes) were also noted. During organ harvesting, the penile length was also determined by measuring from the tip to the inner base of the penis. Furthermore, histological investigations of the penis, seminal vesicles, testes, epididymis and prostate gland were performed.

Testosterone analysis

At the end of day 28 and day 42, the animals were anesthetized by administration of halothane followed by cardiac puncture to obtain blood for testosterone analysis. Serum testosterone levels were determined via a dry-type immunofluorescence quantitative analyzer (Fluorecare™, Model MF-T1000, Shenzhen Microprofit Biotech Co. Ltd., Nanshan, China), which is a faster technique that employs immunochromatographic assays and fluorescence detection (19).

Statistical analysis

The quantitative data are expressed as the means ± SEMs ($n \geq 3$), following descriptive analysis using GraphPad Prism Version 8.0.2.263. This was followed by further comparison with the negative control and positive control using ordinary one-way analysis of variance. Then, the *post hoc* Tukey's multiple comparisons test with a level of significance of 0.05 was performed to obtain the familywise adjusted p-values and 95% confidence intervals.

Results

Preliminary phytochemical screening

All the phytochemical groups (alkaloids, saponins, flavonoids, reducing sugars, coumarins, glycosides, and phytosterols) and L-arginine were present in at least one of the extracts (Table 1).

Table 1 Phytochemical composition of the plant extracts

No	Test	Inference	CABD	CABS	CALD	CALS
1.	Reducing sugars	+++	+	++	++	+++
	Glucose	*Reddish-brown ppt*	Green ppt	Orange ppt	Brown ppt	Red ppt
2.	Alkaloids	+++	+/-	+++	-	-
	Quinine HCL	*Orange ppt*	Bluish black ppt	Orange ppt	None	None
3.	Coumarin	+++	+++	+++	+++	+++
	Warfarin	*Yellow*	Yellow	Yellow	Yellow	Yellow
4.	Glycosides	+++	+++	+++	+++	+++
	Digoxin	*Deep blue*	Deep blue	Deep blue	Deep blue	Deep blue
5.	Flavonoids	+++	+++	+++	+	+++
	Quercetin	*Yellow ppt*	Yellow ppt	Yellow ppt	Cream ppt	Yellowish green ppt
6.	Phytosterols	+++	+++	+++	++	++
	Beta-sitosterol	*Golden yellow*	Golden yellow	Golden yellow	Deep green	Deep green
7.	Saponins	+++	+	+++	++	+++
	Crude saponin	*10.0 mm foam*	0.5 mm foam	11.0 mm foam	1.0 mm foam	7.0 mm foam
8.	L-Arginine	+++	++	++	+++	++
	L-arginine	*Deep red*	Red	Red	Dark red	Red

Extracts: CABD – *Citropsis articulata* root bark decoction, CABS – *Citropsis articulata* root bark Soxhlet, CALD – *Citropsis articulata* leaf decoction, CALS – *Citropsis articulata* leaf Soxhlet, Relative presence/absence: +++ (high), ++ (medium), + (low), - (absent), +/- (undefined). Observations were recorded as changes in the color of the solution or the formation of colored precipitates (ppt). Reference materials (italicized) are placed below the name of the test (in bold), and their observations are recorded and noted as the inference. "None" meant that no significant observable change was noted.

Mating behavior

Attraction towards the female (AF)

The highest AF (833.50±98.00 s) recorded during the observation period was for *C. articulata* leaf decoction at a dose of 100 mg/kg on day 14. On day 7, *C. articulata* root bark Soxhlet extract at a dose of 500 mg/kg produced a statistically significant increased AF of 602.00±79.92 s (p = 0.012: confidence interval (CI) = -604.3 to -66.72 when compared to the positive control. Similarly, on the same day, *C. articulata* leaf decoction extract at a dose of 500 mg/kg produced a statistically significant increase in AF of 630.7±151.10 s (p = 0.016: CI = -865.7 to -67.27) compared with that of the negative control. On varying days of extract administration and different dose levels, there was barely any trend association as AF increased or decreased from one day to another or from one dose level to another, as depicted in Table 2. However, there was a general increase in AF with increasing time, i.e., from day 0 to day 14, which had the highest AF values for most extracts. There was a slight decrease in AF from days 14 to 28. On the nondosing days, day 42 generally had greater AFs than did day 35 for all extracts except for the animals in the group that previously received the *C. articulata* root bark Soxhlet extract at a dose of 500 mg/kg.

Penile Erections

On day 0, *C. articulata* root bark decoction extract at a dose of 100 mg/kg resulted in the greatest number of penile erections (3.33±0.99), which was significantly greater than that of the negative control (p = 0.035: CI = -5.841 to -0.159). On day 7, *C. articulata* root bark Soxhlet extract at a dose of 1000 mg/kg resulted in more penile erections (1.00±0.00) than did the positive control – sildenafil citrate at a dose of 5 mg/kg. This value was significantly higher (p = 0.022: CI = -1.873 to -0.127). *Citropsis articulata* leaf decoction at doses of 500 and 1000 mg/kg on day 21 caused significantly more penile erections than both the negative and positive controls did (Table 3). In general, for all the extracts, the number of penile erections decreased from day 0 to day 7, increased from day 14 and peaked on day 21.

Mount Latency

During the first 28 days of extract administration, with the exception of the mount latency (2.83±0.60 s) elicited by the positive control on day 21, the lowest mount latency (5.50±0.50 s) was elicited by the *C. articulata* root bark Soxhlet extract (500 mg/kg). Following dosing cessation and monitoring for an additional 14 days, the lowest mount latencies (1.00±1.00 and 3.67±3.67 s) were elicited by *C. articulata* leaf Soxhlet extract at doses of 100 and 500 mg/kg, respectively. Although no statistically significant differences were obtained following the conservative familywise analysis, it is evident that the different extracts decreased the mount latency on different days examined at different dose levels (Table 4).

Mount frequency

On day 0, the highest mount frequency recorded was 32.83±11.09 in the group treated with *C. articulata* root bark decoction extract at a dose of 100 mg/kg. Additionally, on the same day, *C. articulata* leaf decoction extract at a dose of 100 mg/kg elicited a statistically significant number of mounts, i.e., 28.67±3.67 (p = 0.008: CI = -32.36 to -3.969), when a familywise comparison (3 dose levels and the positive and negative control) was performed in relation to the negative control.

In general, the number of mounts decreased with increasing dose on most days for all the extracts except on day 28, when the number of mounts increased with increasing dose. Soxhlet extracts evoked fewer mounts than did the decoctions. On day 21, *C. articulata* leaf Soxhlet extracts at all three dose levels evoked statistically significant but lower mounts than the positive control. In general, day 3, followed by days 35 and 28, had the lowest number of mounts for all the extracts. The highest recorded number of mounts (63.00±0.00) for the entire study was recorded on day 42 (2 weeks after cessation of extract dosing). *Citropsis articulata* leaf decoction extract at a dose of 1000 mg/kg elicited a statistically significant number of mounts, i.e., 42.00±8.15 ($p = 0.010$: CI = -60.42 to -8.243 and $p = 0.022$: CI = -56.42 to -4.243), when a familywise comparison (3 dose levels and the positive and negative control) was performed in relation to the negative control and positive control, respectively. Table 5 shows the details of the mount frequency for the extracts across all dose levels on observation days 0, 3, 7, 14, 21, 28, 35 and 42.

Table 2 Attraction towards the female when different doses of the extracts were administered over 42 days

No	Group	Day 0	Day 3	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
1.	NC-DW	417.8±70.04	224.30±106.40	164.20±73.16	617.80±156.90	296.50±68.50	503.50±94.02	305.30±28.88	516.00±100.10
2.	NC-DS	483.80±124.20	220.70±48.29	601.50±119.50	519.80±74.98	246.30±48.59	242.50±59.73	222.70±36.10	403.30±121.70
3.	PC-SC	297.20 ± 88.87	256.70±145.70	266.50±40.29	788.70±143.00	567.80±37.62	628.50±60.05	418.30±43.18	671.70±260.70
4.	CABD	377.00±63.19	250.50±78.46	117.20±31.18	635.50±54.59	**230.50±16.04***	348.80±61.99	214.30±91.79	478.00±18.00
	100	0.997 (-348.8 to 430.4)	0.999 (-504.6 to 452.3	0.987 (-230.4 to 324.3)	>0.999 (-508.4 to 473.0)	0.732 (-94.70 to 226.7)	0.574 (-148.7 to 458.0)	0.710 (-143.5 to 325.5)	0.999 (-819.3 to 895.3)
		0.974 (-469.4 to 309.8)	>0.99 (-472.3 to 484.6)	0.520 (-128.0 to 426.6)	0.868 (-314.7 to 621.1)	**<0.001 (223.7 to 451.0)**	0.081 (-23.66 to 583.0)	0.097 (-30.54 to 438.5)	0.92 (-663.7 to 1051)
5.	CABD	411.20±138.40	216.20±62.69	124.20±36.93	522.00±52.55	**196.80±20.65***	363.50±55.71	258.70±31.67	319.00±0.00
	500	>0.999 (-382.9 to 396.3)	>0.99 (-419.8 to 436.1)	0.991 (-224.4 to 304.4)	0.978 (-394.9 to 586.5)	0.424 (-70.70 to 270.2)	0.660 (-163.3 to 443.3)	0.962 (-187.9 to 281.2)	0.961 (-887.4 to 1281)
		0.909 (-503.6 to 275.6)	0.999 (-387.5 to 468.5)	0.520 (-122.1 to 406.7)	0.465 (-201.2 to 734.6)	**<0.001 (244.0 to 498.1)**	0.108 (-38.32 to 568.3)	0.241 (-74.87 to 394.2)	0.771 (-731.8 to 1437)
6.	CABD	271.30±89.71	356.30±96.31	372.20±101.70	639.00±134.00	**260.00±23.60***	401.70±85.30	254.00±23.86	639.00±47.72
	1000	0.803 (-243.1 to 536.1)	0.890 (-560.0 to 296.0)	0.174 (-472.4 to 56.42)	>0.999 (-511.9 to 469.5)	0.958 (-124.2 to 197.2)	0.859 (-201.5 to 405.2)	0.947 (-183.2 to 285.9)	0.975 (-889.8 to 643.8)
		0.999 (-363.8 to 415.4)	0.957 (-527.6 to 328.3)	0.764 (-370.1 to 158.7)	0.877 (-318.2 to 617.6)	**<0.001 (194.2 to 421.5)**	0.214 (-76.49 to 530.2)	0.220 (-70.20 to 398.9)	0.999 (-734.1 to 799.5)
7.	CABS	259.50±71.92	346.00±44.80	255.00±45.00	431.20±63.41	**316.5±54.96***	**309.3±77.11***	214.30±9.06	**421.70±40.34***
	100	0.500 (-182.9 to 631.5)	0.764 (-437.9 to 187.3)	0.192 (-119.0 to 812.0)	0.965 (-312.0 to 489.4)	0.911 (-311.7 to 171.3)	0.916 (-356.2 to 222.5)	0.996 (-196.0 to 158.7)	0.999 (-422.6 to 385.9)
		0.999 (-369.5 to 444.9)	0.916 (-401.9 to 223.3)	0.999 (-368.6 to 391.6)	0.097 (-3.20 to 758.2)	**0.039 (9.819 to 492.8)**	**0.027 (29.82 to 608.5)**	0.051 (-0.3646 to 354.4)	**0.044 (-819.6 to -11.07)**
8.	CABS	437.20±85.37	114.20±30.55	**602.00±79.92***	468.20±70.08	339.20±58.51	425.20±90.86	329.00±52.00	285.70±127.70
	500	0.997 (-360.5 to 453.9)	0.853 (-206.1 to 419.1)	>0.999 (-380.6 to 379.6)	0.995 (-349.0 to 452.4)	0.770 (-334.3 to 148.7)	0.318 (-472.0 to 106.7)	0.343 (-283.7 to 71.03)	0.868 (-286.6 to 521.9)
		0.848 (-547.2 to 267.2)	0.670(-170.1 to 455.1)	**0.012 (-604.3 to -66.72)**	0.163 (-80.20 to 721.2)	0.070 (-12.85 to 470.2)	0.233 (-86.01 to 492.7)	0.498 (-88.03 to 266.7)	0.230 (-683.6 to 124.9)
9.	CABS	323.30±110.7	349.80±42.79	555.00±75.00	**358.30±107.5***	**253.0±81.77***		**204.00±36.14***	240.30±70.57
	1000	0.775 (-246.7 to 567.7)	0.775 (-246.7 to 567.7)	0.998 (-419.0 to 512.0)	0.760 (-239.2 to 562.2)	>1000 (-248.2 to 234.8)		0.996 (-158.7 to 196.0)	0.682 (-241.3 to 567.3)
		0.999 (-433.4 to 381.0)	0.999 (-433.4 to 381.0)	0.179 (-668.6 to 91.61)	**0.031 (29.64 to 831.0)**	**0.031 (29.64 to 831.0)**		**0.017 (36.97 to 391.7)**	0.374 (-638.3 to 170.3)
10.	CALD	154.30±40.14	276.30±83.97	257.80±78.07	833.50±98.00	196.20±37.60	424.30±53.64	**262.30±29.41***	698.00±343.10
	100	0.068 (-12.94 to 540.1)	0.996 (-474.9 to 370.9)	0.957 (-492.9 to 305.6)	0.720 (-722.2 to 290.8)	0.979 (-431.7 to 632.3)	0.961 (-271.5 to 430.0)	0.882 (-110.2 to 196.2)	0.965 (-1122 to 758.5)
		0.553 (-133.6 to 419.4)	>0.999 (-442.5 to 403.2)	>0.999 (-390.6 to 407.9)	0.999 (-527.7 to 438.1)	0.062 (-13.38 to 756.6)	0.441 (-146.5 to 555.0)	**0.046 (2.777 to 309.2)**	>0.999 (-966.8 to 914.2)
11.	CALD	**144.20±41.06***	108.00±52.25	**630.7±151.10***	555.20±119.70	734.50±35.37	523.80±81.90	**254.30±29.08***	554.00±35.10
	500	**0.0253 (26.34 to 521.0)**	0.787 (-40.39 to 19.73)	**0.016 (-865.7 to -67.27)**	0.996 (-443.8 to 569.1)	0.1246 (-957.2 to 81.15)	0.999 (-334.1 to 293.4)	0.805 (-102.2 to 204.2)	>0.999 (-978.5 to 902.5)
		0.380 (-94.33 to 400.3)	0.883 (-324.1 to 621.5)	0.086 (-763.4 to 35.06)	0.619 (-249.4 to 716.4)	0.660 (-533.8 to 200.4)	0.859 (-209.1 to 418.4)	**0.035 (10.78 to 317.2)**	0.993 (-822.8 to 105.8)
12.	CALD	**42.00±22.20****	174.00±79.93	315.80±101.40	**287.50±60.81***	653.30±161.80	403.00±74.88	**257.30±31.78***	326.30±85.05
	1000	**0.002 (134.8 to 653.6)**	0.997 (-372.5 to 473.2)	0.797 (-550.9 to 247.6)	0.334 (-176.2 to 836.8)	0.277 (-876.0 to 162.3)	0.876 (-213.3 to 414.3)	0.837 (-105.2 to 201.2)	0.960 (-750.8 to 1130)
		0.035 (14.17 to 533.0)	0.977 (-340.2 to 505.5)	0.996(-448.6 to 349.9)	**0.039 (18.26 to 984.1)**	0.955 (-452.6 to 281.6)	0.244 (-88.25 to 539.3)	**0.039 (7.777 to 314.2)**	0.747 (-595.2 to 1286)
13.	CALS	388.00±105.40	205.50±52.85	500.00±117.50	613.20±45.02	372.00±82.56	388.30±60.37	**240.00±21.39***	**555.00±57.18****
	100	0.958 (-315.6 to 507.2)	>0.999 (-302.2 to 332.5)	0.971 (-403.1 to 606.1)	0.945 (-463.5 to 276.9)	0.579 (-373.5 to 122.2)	0.489 (-407.6 to 115.9)	0.994 (-162.0 to 127.3)	0.736 (-557.8 to 254.4)
		0.965 (-502.2 to 320.6)	0.989 (-266.2 to 368.5)	0.311 (-590.3 to 123.3)	0.638 (-194.7 to 545.7)	0.172 (-52.00 to 443.7)	0.083 (-21.61 to 501.9)	**0.015 (33.68 to 323.0)**	**0.009 (-954.8 to -142.6)**
14.	CALS	291.50±104.50	233.20±33.76	225.00±135.00	**330.50±44.37***	413.20±43.81	**322.30±43.80***	300.00±10.54	297.70±92.99
	500	0.650 (-219.1 to 603.7)	>0.999 (-329.8 to 304.8)	0.378 (-241.6 to 994.6)	0.571 (-180.9 to 559.5)	0.3057 (-414.7 to 81.00)	0.896 (-341.6 to 181.9)	0.444 (-222.0 to 67.32)	0.849 (-282.4 to 529.8)
		>0.999 (-405.7 to 417.1)	0.999(-293.8 to 340.8)	0.999 (-463.1 to 546.1)	**0.010 (87.96 to 828.4)**	0.378 (-93.17 to 402.5)	**0.016 (44.39 to 567.9)**	0.125 (-26.32 to 263.0)	0.249 (-679.4 to 132.8)
15.	CALS	193.00±60.81	114.00±41.31	588.50±76.03	572.70±98.34	**310.80±72.74***	**336.70±84.41***	**228.00±33.06***	**503.30±106.50***
	1000	0.261 (-120.6 to 702.2)	0.859 (-210.7 to 424.0)	>0.999 (-491.6 to 517.6)	0.993 (-423.0 to 317.4)	0.938 (-312.3 to 183.3)	0.827 (-355.9 to 167.6)	>0.999 (-150.0 to 139.3)	0.922 (-506.1 to 306.1)
		0.944 (-307.2 to 515.6)	0.682 (-174.7 to 460.0)	0.088 (-678.8 to 34.83)	0.444 (-154.2 to 586.2)	**0.039 (9.166 to 504.8)**	**0.024 (30.06 to 553.6)**	**0.010 (45.68 to 335.0)**	**0.016 (-903.1 to -90.90)**

Attraction towards the female (in seconds) for animals given extracts of *Citropsis articulata* rootbark decoction extracts at doses of 100 mg/kg (CABD 100), 500 mg/kg (CABD 500) and 1000 mg/kg (CABD 1000); *Citropsis articulata* rootbark Soxhlet extracts at doses of 100 mg/kg (CABS 100), 500 mg/kg (CABS 500) and 1000 mg/kg (CABS 1000); *Citropsis articulata* leaf decoction extracts at doses of 100 mg/kg (CALD 100), 500 mg/kg (CALD 500) and 1000 mg/kg (CALD 1000); and *Citropsis articulata* leaf Soxhlet extracts at doses of 100 mg/kg (CALS 100), 500 mg/kg (CALS 500) and 1000 mg/kg (CALS 1000). Values were The values are expressed as the means ± SEMs and then p-value (95% confidence interval) against the negative control, followed by comparisons against the positive control (below). N = 6 for days 0 to 28, whereas N = 3 for days 35 and 42 (no extracts were subsequently administered). Significant values (p-value ≤ 0.05) are marked in bold and with asterisks, where (*) indicates p ≤ 0.05, (**) indicates p ≤ 0.01, and (***) indicates p ≤ 0.001.

Table 3 Penile erections elicited by extracts

No	Group	Day 0	Day 3	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
1.	NC-DW	0.33±0.33	0.83±0.40	0.00±0.00	2.40±0.87	1.50±0.50	1.67±0.61	0.67±0.67	5.33±1.45
2.	NC-DS	0.00±0.00	0.00±0.00	0.00±0.00	0.83±0.54	0.17±0.17	0.00±0.00	0.67±0.67	0.33±0.33
3.	PC-SC	0.67 ± 0.33	0.00±0.00	0.00±0.00	0.67±0.33	1.33±0.49	2.33±0.42	0.00±0.00	6.33±2.60
4.	CABD	**3.33±0.99***	0.25±0.25	0.00±0.00	0.67±0.49	1.33±0.21	1.33±0.58	0.00±0.00	1.50±1.50
	100	**0.035 (-5.841 to -0.159)**	0.748 (-0.8466 to 2.013)	Std error =0	0.203 (-0.556 to 4.023)	0.999 (-1.825 to 2.158)	0.987(-1.634 to 2.301)	0.525 (-0.8429 to 2.176)	0.618 (-5.609 to 13.28)
		0.073 (-5.507 to 0.1740)	0.985 (-1.680 to 1.180)	Std error =0	>0.999 (-2.183 to 2.183)	>0.999 (-1.408 to 1.408)	0.577 (-0.9677 to 2.968)	>0.999 (-1.510 to 1.510)	0.427 (-4.609 to 14.28)
5.	CABD	0.83±0.48	0.67±0.33	0.00±0.00	0.17±0.17	1.00±0.41	1.17±0.48	0.00±0.00	0.00±0.00
	500	0.985 (-3.341 to 2.341)	0.995 (-1.112 to 1.446)	Std error =0	0.058 (-0.056 to 4.523)	0.951 (-1.612 to 2.612)	0.943 (-1.468 to 2.468)	0.525 (-0.8429 to 2.176)	0.541 (-6.610 to 17.28)
		0.999 (-3.007 to 2.674)	0.548 (-1.946 to 0.6123)	Std error =0	0.960 (-1.683 to 2.683)	0.967 (-1.241 to 1.908)	0.428 (-0.8010 to 3.134)	>0.999 (-1.510 to 1.510)	0.397 (-5.610 to 18.28)
6.	CABD	2.33±0.95	1.00±0.37	0.00±0.00	1.17±0.65	0.67±0.21	0.83±0.17	0.00±0.00	0.67±0.33
	1000	0.265 (-4.841 to 0.8407)	0.995 (-1.446 to 1.112)	Std error =0	0.519 (-1.056 to 3.523)	0.719 (-1.158 to 2.825)	0.727 (-1.134 to 2.801)	0.525 (-0.8429 to 2.176)	0.363 (-3.779 to 13.11)
		0.439 (-4.507 to 1.174)	0.177 (-2.279 to 0.2790)	Std error =0	0.96 (-2.683 to 1.683)	0.621 (-0.7416 to 2.075)	0.199 (-0.4677 to 3.468)	>0.999 (-1.510 to 1.510)	0.220 (-2.779 to 14.11)
7.	CABS	0.33±0.33	0.00±0.00	0.00±0.00	0.50±0.34	**0.17±0.17***	**0.00±0.00*****	0.33±0.33	**0.00±0.00***
	100	0.960(-1.785 to 1.119)	Std error =0	>0.999 (-1.070 to 1.070)	0.963(1.151 to 1.818)	>0.999 (-1.063 to 1.063)	>0.999 (-0.835 to 0.835)	0.972 (-1.502 to 2.169)	0.999 (-5.130 to 5.796)
		0.960 (-1.119 to 1.785)	Std error =0	>0.999 (-0.873 to 0.873)	0.997 (-1.318 to 1.651)	**0.026 (0.103 to 2.230)**	**<0.001 (1.499 to 3.168)**	0.972 (-2.169 to 1.502)	**0.022 (0.8702 to 11.80)**
8.	CABS	1.17±0.60	0.00±0.00	0.50±0.22	0.00±0.00	**0.17±0.17***	**0.00±0.00*****	0.67±0.33	**0.00±0.00***
	500	0.160 (-2.619 to 0.2854)	Std error =0	0.412 (-1.373 to 0.3733)	0.482 (-0.6513 to 2.318)	>0.999 (-1.063 to 1.063)	>0.999 (-0.835 to 0.835)	>0.999 (-1.836 to 1.836)	0.999(-5.130 to 5.796)
		0.848 (-1.952 to 0.952)	Std error =0	0.139 (-1.118 to 0.1175)	0.683 (-0.8180 to 2.151)	**0.027 (0.1032 to 2.230)**	**<0.001 (1.499 to 3.168)**	0.754 (-2.502 to 1.169)	**0.022 (0.8702 to 11.80)**
9.	CABS	0.17±0.17	0.00±0.00	**1.00±0.00***	0.50±0.34	**0.00±0.00****		0.33±0.33	0.00±0.00
	1000	0.848 (-0.952 to 1.952)	Std error =0	0.848 (-0.9520 to 1.952)	0.848 (-0.9520 to 1.952)	0.990 (-0.8968 to 1.230)		0.972 (-1.502 to 2.169)	0.999 (-5.130 to 5.796)
		0.997 (-1.619 to 1.285)	Std error =0	**0.022 (-1.873 to -0.127)**	0.997 (-1.318 to 1.651)	**0.009 (0.2699 to 2.397)**		0.972 (-2.169 to 1.502)	0.972 (-2.169 to 1.502)
10.	CALD	0.50±0.50	0.83±0.31	0.17±0.17	2.00±0.93	0.60±0.24	0.25±0.25	0.00±0.00	2.00±1.16
	100	0.994(-1.404 to 1.070)	>0.999 (-1.328 to 1.328)	0.956 (-0.8726 to 0.539)	0.993 (-2.358 to 3.158)	0.876 (-1.947 to 3.747)	0.306 (-0.6980 to 3.531)	0.920 (-2.020 to 3.354)	0.526 (-3.500 to 10.17)
		0.994 (-1.070 to 1.404)	0.368 (-2.161 to 0.4942)	0.956 (-0.8726 to 0.539)	0.576 (-3.963 to 1.296)	0.822 (-1.327 to 2.794)	0.055 (0.0314 to 4.198)	>0.999 (-2.687 to 2.687)	0.296 (-2.500 to 11.17)
11.	CALD	0.00±0.00	1.25±0.48	0.00±0.00	2.83±0.65	**4.83±0.54*****	0.50±0.50	1.33±0.67	1.33±0.33
	500	0.898 (-0.7731 to 1.440)	0.9186 (-1.901 to 1.068)	>0.999 (-0.706 to 0.706)	0.990 (-3.191 to 2.325)	**0.014 (-6.112 to -0.555)**	0.385 (-0.725 to 3.058)	0.920 (-3.354 to 2.020)	0.364 (-2.833 to 10.83)
		0.408 (-0.4398 to 1.773)	0.128 (-2.734 to 0.2342)	>0.999 (-0.706 to 0.706)	0.142 (-4.796 to 0.4630)	**0.0003 (-5.47 to -1.535)**	0.061 (-0.0581 to 3.725)	0.511 (-4.020 to 1.354)	0.190 (-1.833 to 11.83)
12.	CALD	0.00±0.00	0.50±0.34	0.50±0.34	0.00±0.00	**5.00±0.52***	0.67±0.33	1.67±0.88	0.67±0.67
	1000	0.898 (-0.7731 to 1.440)	0.944 (-0.9942 to 1.661)	0.260 (-1.206 to 0.206)	0.110 (-0.3580 to 5.158)	**0.009 (-6.279 to -0.721)**	0.535 (-0.8914 to 2.891)	0.739 (-3.687 to 1.687)	0.2384 (-2.167 to 11.50)
		0.408 (-0.4398 to 1.773)	0.798 (-1.828 to 0.8276)	0.260 (-1.206 to 0.2059)	0.943 (-1.963 to 3.296)	**0.0002 (-5.63 to -1.702)**	0.103 (-0.2248 to 3.558)	0.315 (-4.354 to 1.020)	0.119 (-1.167 to 12.50)
13.	CALS	1.00±0.68	0.00±0.00	0.00±0.00	0.17±0.17	**0.17±0.17***	**0.17±0.17*****	0.33±0.33	**0.33±0.33***
	100	0.4134 (-2.661 to 0.661)	Std error =0	>0.999 (-1.100 to 1.100)	0.567 (-0.6321 to 1.965)	>0.999 (-1.063 to 1.063)	0.987 (-1.146 to 0.8123)	0.964 (-1.366 to 2.033)	>0.999 (-5.594 to 5.594)
		0.976 (-1.995 to 1.328)	Std error =0	>0.999 (-0.778 to 0.778)	0.789 (-0.7987 to 1.799)	**0.027 (0.1032 to 2.230)**	**<0.001 (1.188 to 3.146)**	0.964 (-2.033 to 1.366)	**0.035 (0.4062 to 11.59)**
14.	CALS	0.67±0.33	0.00±0.00	0.00±0.00	0.17±0.17	**0.00±0.00****	**0.17±0.17*****	0.00±0.00	**0.33±0.33***
	500	0.763 (-2.328 to 0.9947)	Std error =0	>0.999 (-1.347 to 1.347)	0.567 (-0.6321 to 1.965)	0.990 (-0.8968 to 1.230)	0.987 (-1.146 to 0.8123)	0.702 (-1.033 to 2.366)	>0.999 (-5.594 to 5.594)
		>0.999 (-1.661 to 1.661)	Std error =0	>0.999 (-1.100 to 1.100)	0.789 (-0.7987 to 1.799)	**0.009 (0.2699 to 2.397)**	**<0.001 (1.188 to 3.146)**	>0.999 (-1.700 to 1.700)	**0.035 (0.406 to 11.59)**
15.	CALS	0.33±0.33	0.00±0.00	0.67±0.33	0.17±0.17	**0.17±0.17***	**0.33±0.21*****	0.67±0.33	**0.33±0.33***
	1000	0.976 (-1.995 to 1.328)	Std error =0	0.382 (-1.767 to 0.433)	0.567 (-0.6321 to 1.965)	>0.999 (-1.063 to 1.063)	0.853 (-1.312 to 0.6456)	>0.999 (-1.700 to 1.700)	>0.999 (-5.594 to 5.594)
		0.976 (-1.328 to 1.995)	Std error =0	0.113 (-1.444 to 0.1112)	0.789 (-0.7987 to 1.799)	**0.027 (0.1032 to 2.230)**	**<0.001 (1.021 to 2.979)**	0.702 (-2.366 to 1.033)	**0.035 (0.4062 to 11.59)**

Penile erections (in number of time) for animals given extracts of *Citropsis articulata* rootbark decoction extracts at doses of 100 mg/kg (CABD 100), 500 mg/kg (CABD 500) and 1000 mg/kg (CABD 1000); *Citropsis articulata* rootbark Soxhlet extracts at doses of 100 mg/kg (CABS 100), 500 mg/kg (CABS 500) and 1000 mg/kg (CABS 1000); *Citropsis articulata* leaf decoction extracts at doses of 100 mg/kg (CALD 100), 500 mg/kg (CALD 500) and 1000 mg/kg (CALD 1000); and *Citropsis articulata* leaf Soxhlet extracts at doses of 100 mg/kg (CALS 100), 500 mg/kg (CALS 500) and 1000 mg/kg (CALS 1000). PC-SC: positive control–sildenafil citrate (5 mg/kg), NC-DW: negative control–distilled water (CABD and CALD), NC-DS: negative control–dimethyl sulfoxide (CABS and CALS). The values are expressed as the means ± SEMs and then as p values (95% confidence intervals) against the negative control, followed by comparisons against the positive control (below). N = 6 for days 0 to 28, whereas N = 3 for days 35 and 42 (no extracts were subsequently administered). Significant values (p-value ≤ 0.05) are marked in bold and with asterisks, where (*) indicates p ≤ 0.05, (**) indicates p ≤ 0.01, and (***) indicates p ≤ 0.001.

Table 4 Mount Latency

No	Group	Day 0	Day 3	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
1.	NC-DW	80.67±41.75	29.67±17.95	25.33±6.88	32.40±17.16	41.50±14.76	54.33±28.82	30.00±21.73	27.67±13.91
2.	NC-DS	75.00±22.80	85.33±49.10	12.50±8.50	205.00±182.40	10.33±2.32	55.83±31.95	15.00±2.52	29.67±19.34
3.	PC-SC	126.70 ± 82.79	93.00±87.71	21.00±10.28	7.83±2.59	2.83±0.60	67.17±37.48	9.33±6.36	138.30±109.90
4.	CABD	22.33±12.66	7.50±4.63	64.00±30.82	47.67±20.70	52.50±25.58	50.00±34.92	126.70±60.06	450.50±406.50
	100	0.944 (-171.6 to 288.3)	0.999 (-385.1 to 429.5)	0.664 (-123.1 to 45.77)	0.987 (-105.8 to 75.26)	0.998 (-114.9 to 92.90)	>0.999 (-105.4 to 114.1)	0.562 (-303.3 to 109.9)	0.387 (-1210 to 364.4)
		0.674 (-125.6 to 334.3)	0.970 (-321.8 to 492.8)	0.572 (-127.4 to 41.44)	0.658 (-126.1 to 46.48)	0.631 (-153.6 to 54.23)	0.990 (-92.58 to 126.9)	0.390 (-323.9 to 89.26)	0.636 (-1099 to 475.1)
5.	CABD	158.80±80.12	67.00±50.48	69.00±30.59	45.67±17.30	76.17±37.16	13.00±4.87	81.00±71.53	63.00±0.00
	500	0.854 (-308.1 to 151.8)	0.998 (-401.6 to 327.0)	0.513 (-124.2 to 36.84)	0.992 (-103.8 to 77.26)	0.862 (-138.6 to 69.23)	0.802 (-68.41 to 151.1)	0.921 (-257.6 to 155.6)	>0.999 (-1031 to 960.5)
		0.994 (-262.1 to 197.8)	0.999 (-338.3 to 390.3)	0.421 (-128.5 to 32.51)	0.699 (-124.1 to 48.48)	0.263 (-177.2 to 30.57)	0.603 (-55.58 to 163.9)	0.782 (-278.3 to 134.9)	0.999 (-920.5 to 1071)
6.	CABD	26.83±12.22	268.30±156.10	16.67±8.37	49.33±33.60	70.00±29.59	9.17±3.54	64.00±24.79	44.67±20.58
	1000	0.957 (-176.1 to 283.8)	0.327 (-603.0 to 125.6)	0.998 (-71.84 to 89.18)	0.981 (-107.5 to 73.59)	0.926 (-132.4 to 75.40)	0.747 (-64.58 to 154.9)	0.981 (-240.6 to 172.6)	>0.999 (-721.1 to 687.1)
		0.708 (-130.1 to 329.8)	0.620 (-539.6 to 189.0)	0.999 (-76.18 to 84.84)	0.623 (-127.8 to 44.81)	0.344 (-171.1 to 36.73)	0.540 (-51.75 to 167.7)	0.901 (-261.3 to 151.9)	0.987 (-610.5 to 797.8)
7.	CABS	69.67±30.41	31.33±11.93	5.50±0.50	49.17±29.31	150.80±83.86	262.70±150.4	251.30±219.40	92.33±60.99
	100	>0.999 (-187.6 to 198.2)	0.928 (-143.9 to 251.9)	0.999 (-81.01 to 95.01)	0.882 (-335.3 to 646.9)	0.133 (-308.3 to 27.26)	0.761 (-795.7 to 382.0)	0.543 (-730.4 to 257.7)	0.962 (-377.4 to 252.1)
		0.906 (-135.9 to 249.9)	0.888(-136.3 to 259.6)	0.958 (-56.36 to 87.36)	0.999 (532.4 to 449.8)	0.102 (-315.8 to 19.76)	0.790 (-784.4 to 393.4)	0.522 (-736.0 to 252.0)	0.987 (-268.7 to 360.7)
8.	CABS	63.83±44.17	35.00±16.04	10.17±2.75	191.50±183.50	16.17±4.13	304.30±252.0	74.00±52.09	8.00±5.69
	500	0.999 (-181.7 to 204.1)	0.943 (-147.6 to 248.3)	>0.999 (-69.53 to 74.19)	>0.999 (-477.6 to 504.6)	>0.999 (-173.6 to 161.9)	0.645 (-837.4 to 340.4)	0.994 (-553.0 to 435.0)	0.999 (-293.1 to 336.4)
		0.872 (-130.1 to 255.7)	0.909 (-139.9 to 255.9)	0.959 (-39.98 to 61.65)	0.806 (-674.8 to 307.4)	0.999 (-181.1 to 154.4)	0.678 (-826.0 to 351.7)	0.992 (-558.7 to 429.4)	0.662 (-184.4 to 445.1)
9.	CABS	70.67±23.11	41.33±29.19	64.50±57.50	110.20±45.71	58.83±33.19		103.30±73.85	137.30±81.60
	1000	>0.999 (-188.6 to 197.2)	0.965 (-153.9 to 241.9)	0.383 (-140.0 to 36.01)	0.979 (-396.3 to 585.9)	0.912 (-216.3 to 119.3)		0.974 (-582.4 to 405.7)	0.974 (-582.4 to 405.7)
		0.911(-136.9 to 248.9)	0.911 (-136.9 to 248.9)	0.361 (-115.4 to 28.36)	0.972 (-593.4 to 388.8)	0.862 (-223.8 to 111.8)		0.967 (-588.0 to 400.0)	>0.999 (-313.7 to 315.7)
10.	CALD	35.00±19.32	30.17±12.94	19.00±8.93	50.83±28.27	54.80±25.40	41.50±23.57	27.00±19.60	15.67±13.25
	100	0.970 (-169.8 to 261.1)	>0.999(-245.3 to 244.3)	0.996 (-46.16 to 58.83)	0.999 (-273.1 to 236.2)	0.979 (-82.80 to 56.20)	0.999 (-125.3 to 151.0)	>0.999 (-76.39 to 82.39)	>0.999 (-470.2 to 494.2)
		0.723 (-123.8 to 307.1)	0.940(-182.0 to 307.7)	>0.999 (-50.49 to 54.49)	0.984 (-285.8 to 199.8)	0.213 (-121.5 to 17.54)	0.980 (-112.5 to 163.8)	0.944 (-97.06 to 61.73)	0.913 (-359.6 to 604.9)
11.	CALD	34.00±6.78	250.80±133.70	28.50±13.85	129.80±84.32	16.83±3.64	108.00±105.00	26.00±22.07	71.33±20.63
	500	0.968 (-168.8 to 262.1)	0.155 (-494.8 to 52.65)	0.999 (-55.66 to 49.33)	0.791 (-352.1 to 157.2)	0.807 (-41.60 to 90.94)	0.884 (-228.4 to 121.1)	0.999 (-75.39 to 83.39)	0.998 (-525.9 to 438.6)
		0.715 (-122.8 to 308.1)	0.451 (-431.5 to 116.0)	0.993 (-59.99 to 44.99)	0.993 (-59.99 to 44.99)	0.97 (-80.27 to 52.27)	0.953 (-215.6 to 133.9)	0.954 (-96.06 to 62.73)	0.990 (-415.2 to 549.2)
12.	CALD	91.83±66.64	44.33±21.15	27.67±19.34	102.20±90.49	52.00±23.51	12.67±3.89	14.67±8.41	203.00±202.00
	1000	0.999 (-226.6 to 204.3)	0.999 (-259.5 to 230.2)	>0.999(-54.83 to 50.16)	>0.999 (-54.83 to 50.16)	0.990 (-76.77 to 55.77)	0.846 (-81.89 to 165.2)	0.966 (-64.06 to 94.73)	0.754 (-657.6 to 306.9)
		0.989 (-180.6 to 250.3)	0.976 (-196.2 to 293.5)	0.996 (-59.16 to 45.83)	0.782 (-337.1 to 148.4)	0.219 (-115.4 to 17.10)	0.679 (-69.06 to 178.1)	0.999 (-84.73 to 74.06)	0.991 (-546.9 to 417.6)
13.	CALS	65.17±10.18	67.50±25.88	39.33±13.77	68.83±28.58	11.00±3.89	145.20±76.58	1.00±1.00	52.00±10.69
	100	>0.999 (-209.6 to 229.3)	0.999 (-348.3 to 384.0)	0.998 (-299.8 to 246.2)	0.786 (-215.5 to 487.9)	>0.999 (-287.3 to 285.9)	0.852 (-351.4 to 172.7)	0.999 (-212.8 to 240.8)	>0.999 (-795.0 to 750.3)
		0.921 (-157.9 to 280.9)	0.999 (-340.6 to 391.6)	0.998 (-211.4 to 174.7)	0.986 (-412.7 to 290.7)	>0.999 (-294.8 to 278.4)	0.904 (-340.0 to 184.0)	>0.999 (-218.4 to 235.1)	0.995 (-686.3 to 859.0)
14.	CALS	128.20±77.99	43.00±18.55	**309.0±309.0***	43.83±19.17	171.00±100.50	92.33±46.59	3.67±3.67	400.70±353.70
	500	0.952 (-272.6 to 166.3)	0.997 (-323.8 to 408.5)	0.096 (-630.8 to 37.84)	0.666 (-190.5 to 512.9)	0.483 (-447.3 to 125.9)	0.994 (-298.5 to 225.5)	0.999 (-215.4 to 238.1)	0.540 (-1144 to 401.7)
		>0.999 (-220.9 to 217.9)	0.994 (-316.1 to 416.1)	**0.036 (-561.0 to -15.01)**	0.998 (-387.7 to 315.7)	0.439 (-454.8 to 118.4)	0.999 (-287.2 to 236.9)	>0.999 (-221.1 to 232.4)	0.794 (-1035 to 510.3)
15.	CALS	55.50±19.89	335.30±166.50	40.00±13.33	58.83±37.21	168.80±117.00	119.50±97.16	145.00±108.70	12.67±11.20
	1000	0.999 (-199.9 to 238.9)	0.293 (-616.1 to 116.1)	0.998 (-300.5 to 245.5)	0.740 (-205.5 to 497.9)	0.497 (-445.1 to 128.1)	0.951 (-325.7 to 198.4)	0.382 (-356.8 to 96.77)	>0.999 (-755.7 to 789.7)
		0.873 (-148.3 to 290.6)	0.322 (-608.5 to 123.8)	0.998 (-212.0 to 174.0)	0.993 (-402.7 to 300.7)	0.451 (-452.6 to 120.6)	0.976 (-314.4 to 209.7)	0.345 (-362.4 to 91.10)	0.981 (-647.0 to 898.3)

Mount latency (in seconds) for animals given extracts of *Citropsis articulata* rootbark decoction extracts at doses of 100 mg/kg (CABD 100), 500 mg/kg (CABD 500) and 1000 mg/kg (CABD 1000); *Citropsis articulata* rootbark Soxhlet extracts at doses of 100 mg/kg (CABS 100), 500 mg/kg (CABS 500) and 1000 mg/kg (CABS 1000); *Citropsis articulata* leaf decoction extracts at doses of 100 mg/kg (CALD 100), 500 mg/kg (CALD 500) and 1000 mg/kg (CALD 1000); and *Citropsis articulata* leaf Soxhlet extracts at doses of 100 mg/kg (CALS 100), 500 mg/kg (CALS 500) and 1000 mg/kg (CALS 1000). PC-SC: positive control–sildenafil citrate (5 mg/kg), NC-DW: negative control–distilled water (CABD and CALD), NC-DS: negative control–dimethyl sulfoxide (CABS and CALS). The values are expressed as the means ± SEMs and then p-value (95% confidence intervals) against the negative control, followed by comparisons against the positive control (below). N = 6 for days 0 to 28, whereas N = 3 for days 35 and 42 (no extracts were subsequently administered). Significant values (p-value ≤ 0.05) are marked in bold and with asterisks, where (*) indicates p ≤ 0.05, (**) indicates p ≤ 0.01, and (***) indicates p ≤ 0.001.

Table 5 Mount frequency

No	Group	Day 0	Day 3	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
1.	NC-DW	10.50±2.72	15.67±8.87	48.17±8.91	34.80±12.11	24.83±2.20	14.83±3.74	7.67±1.45	17.67±9.60
2.	NC-DS	22.50±5.17	8.83±5.12	6.50±2.50	14.67±3.13	7.17±0.75	6.33±3.58	10.33±2.33	14.67±5.46
3.	PC-SC	15.33±4.32	2.17±1.64	55.67±22.88	31.00±8.38	32.33±4.81	13.00±4.08	11.67±3.18	7.33±2.60
4.	CABD	32.83±11.09	6.50±5.55	27.40±11.47	28.50±6.52	37.67±8.81	13.00±3.96	5.00±2.52	8.00±2.00
	100	0.277 (-54.47 to 9.806)	0.806 (-15.51 to 33.85)	0.833 (-37.85 to 79.39)	0.989 (-33.12 to 45.72)	0.500 (-36.13 to 10.46)	0.999 (-28.41 to 32.08)	0.313 (-249.6 to 58.31)	0.784 (-20.76 to 40.09)
		0.512 (-49.64 to 14.64)	0.985 (-29.01 to 20.35)	0.621 (-30.35 to 86.89)	0.999 (-35.08 to 40.08)	0.961 (-28.63 to 17.96)	>0.999 (-30.24 to 30.24)	0.349 (-245.6 to 62.31)	>0.99 (-31.09 to 29.76)
5.	CABD	9.17±4.25	1.50±0.62	20.17±7.71	30.67±7.47	35.83±4.14	16.00±7.72	10.00±1.53	63.00±0.00
	500	>0.999 (-30.81 to 33.47)	0.347 (-7.908 to 36.24)	0.587 (-27.89 to 83.89)	0.998 (-35.28 to 43.55)	0.642 (-34.30 to 12.30)	>0.999 (-31.41 to 29.08)	>0.999 (-156.3 to 151.6)	0.616 (-22.82 to 54.15)
		0.979 (-25.97 to 38.31)	>0.999 (-21.41 to 22.74)	0.359 (-20.39 to 91.39)	>0.999 (-37.25 to 37.92)	0.992 (-26.80 to 19.80)	0.998 (-33.24 to 27.24)	>0.999 (-152.3 to 155.6)	0.985 (-33.15 to 43.82)
6.	CABD	27.67±11.50	13.33±5.85	36.00±10.67	37.33±11.15	18.50±5.88	38.33±12.62	10.67±2.03	7.00±1.00
	1000	0.530 (-49.31 to 14.97)	0.998 (-19.74 to 24.41)	0.967 (-43.73 to 68.06)	0.999 (-41.95 to 36.88)	0.929 (-16.96 to 29.63)	0.184 (-53.74 to 6.745)	>0.999 (-157.0 to 151.0)	0.645 (-16.55 to 37.88)
		0.791 (-44.47 to 19.81)	0.576 (-33.24 to 10.91)	0.836 (-36.23 to 75.56)	0.987 (-43.92 to 31.25)	0.427 (-9.465 to 37.13)	0.133 (-55.58 to 4.912)	>0.999 (-153.0 to 155.0)	>0.99 (-26.88 to 27.55)
7.	CABS	26.00±11.27	5.33±2.47	16.00±7.00	10.17±4.73	**6.50±1.77***	2.17±0.91	6.67±2.19	14.33±5.36
	100	0.997(-34.04 to 27.04)	0.938 (-9.891 to 16.89)	0.999 (-121.8 to 102.8)	0.984 (-20.74 to 29.74)	0.984 (-20.74 to 29.74)	0.724 (-6.972 to 15.30)	0.870 (-9.013 to 16.35)	>0.99 (-20.64 to 21.31)
		0.841(-41.21 to 19.87)	0.956 (-16.56 to 10.22)	0.956 (-16.56 to 10.22)	0.142 (-4.407 to 46.07)	**0.012(4.524 to 47.14)**	0.058 (-0.3049 to 21.97)	0.699 (-7.680 to 17.68)	0.804 (-27.98 to 13.98)
8.	CABS	31.83±8.20	4.17±1.66	19.33±4.76	22.00±7.86	**7.33±1.93***	3.00±1.18	11.33±2.73	8.67±4.33
	500	0.895 (-39.87 to 21.21)	0.842 (-8.725 to 18.06)	0.991 (-104.6 to 78.90)	0.911 (-32.57 to 17.91)	>0.999 (-21.48 to 21.14)	0.836 (-7.805 to 14.47)	0.836 (-7.805 to 14.47)	0.874 (-14.98 to 26.98)
		0.519 (-47.04 to 14.04)	0.992 (-15.39 to 11.39)	0.433 (-28.53 to 101.2)	0.831 (-16.24 to 34.24)	**0.016 (3.691 to 46.31)**	0.088 (-1.138 to 21.14)	>0.999(-12.35 to 13.01)	0.999 (-22.31 to 19.64)
9.	CABS	21.00±5.55	9.50±3.77	33.00±5.00	16.33±4.52	19.67±10.06		5.00±3.06	6.67±4.18
	1000	0.999 (-29.04 to 32.04)	0.999 (-14.06 to 12.72)	0.999 (-14.06 to 12.72)	0.999 (-26.91 to 23.57)	0.439 (-33.81 to 8.809)		0.650 (-7.347 to 18.01)	0.722 (-12.98 to 28.98)
		0.982 (-36.21 to 24.87)	0.506 (-20.72 to 6.058)	0.933 (-69.07 to 114.4)	0.448 (-10.57 to 39.91)	0.426 (-8.642 to 33.98)		0.459 (-6.013 to 19.35)	>0.99 (-20.31 to 21.64)
10.	CALD	**28.67±3.67****	12.33±4.59	37.00±13.15	41.00±5.83	26.00±6.35	25.75±8.27	19.33±7.69	17.67±9.08
	100	**0.008 (-32.36 to -3.969)**	0.988 (-16.96 to 23.63)	0.972 (-42.39 to 64.72)	0.972 (-36.21 to 23.81)	0.999(-25.83 to 23.50)	0.822 (-41.74 to 19.91)	0.601 (-37.76 to 14.42)	0.99 (-30.06 to 30.06)
		0.073 (-27.53 to 0.8646)	0.584 (-30.46 to 10.13)	0.842 (-34.89 to 72.22)	0.839 (-38.62 to 18.62)	0.941 (-18.33 to 31.00)	0.727 (-43.57 to 18.07)	0.864 (-33.76 to 18.42)	0.787 (-40.39 to 19.73)
11.	CALD	17.33±3.86	4.25±1.49	26.33±5.60	7.67±3.52	40.50±8.72	5.00±2.00	23.67±4.41	13.67±3.28
	500	0.625 (-21.03 to 7.365)	0.5804 (-11.27 to 34.10)	0.753 (-31.72 to 75.39)	0.09 (-2.879 to 57.15)	0.3135 (-39.18 to 7.848)	0.939 (-29.15 to 48.82)	0.324 (-42.09 to 10.09)	0.991 (-26.06 to 34.06)
		0.995 (-16.20 to 12.20)	0.999 (-24.77 to 20.60)	0.506 (-24.22 to 82.89)	0.149 (-5.282 to 51.95)	0.842 (-31.68 to 15.35)	0.971 (-30.99 to 46.99)	0.577 (-38.09 to 14.09)	0.953 (-36.39 to 23.73)
12.	CALD	4.67±2.01	5.50±2.25	13.17±4.92	6.83±3.46	23.67±4.70	37.17±10.07	**42.00±8.15***	6.33±4.10
	1000	0.748 (-8.365 to 20.03)	0.584 (-10.13 to 30.46)	0.334 (-18.56 to 88.56)	0.076 (-2.045 to 57.98)	0.999 (-22.35 to 24.68)	0.148 (-49.90 to 5.235)	**0.010 (-60.42 to -8.243)**	0.730 (-18.73 to 41.39)
		0.210 (-3.531 to 24.86)	0.988 (-23.63 to 16.96)	0.168 (-11.06 to 96.06)	0.127 (-4.449 to 52.78)	0.812 (-14.85 to 32.18)	0.103 (-51.74 to 3.402)	**0.022 (-56.42 to -4.243)**	>0.99 (-29.06 to 31.06)
13.	CALS	27.00±5.94	10.67±4.39	7.33±2.01	20.67±4.15	**6.33±1.59*****	4.83±3.10	5.67±5.67	6.33±2.33
	100	0.976 (-27.15 to 18.15)	0.994 (-14.99 to 11.32)	>0.999 (77.22 to 75.55)	0.906 (-26.32 to 14.32)	0.999 (-13.69 to 15.36)	0.999 (-14.69 to 17.69)	0.990 (-29.46 to 38.79)	0.990 (-29.46 to 38.79)
		0.564 (-34.31 to 10.98)	0.345 (-21.66 to 4.657)	0.092 (-5.679 to 102.3)	0.576 (-9.988 to 30.65)	**<0.001 (11.48 to 40.52)**	0.584 (-8.027 to 24.36)	0.975 (-28.12 to 40.12)	>0.99 (-43.89 to 45.89)
14.	CALS	16.17±6.53	1.17±0.48	6.00±6.00	**10.17±2.65***	**14.67±5.90***	4.17±2.68	1.00±1.00	4.67±0.88
	500	0.922 (-16.31 to 28.98)	0.445 (-5.490 to 20.82)	>0.999 (-93.05 to 94.05)	0.965 (-15.82 to 24.82)	0.562 (-22.02 to 7.024)	0.995 (-14.03 to 18.36)	0.891 (-24.79 to 43.46)	0.944 (-34.89 to 54.89)
		>0.999 (-23.48 to 21.81)	0.999 (-12.16 to 14.16)	0.317(-26.72 to 126.1)	**0.043 (0.5120 to 41.15)**	**0.012 (3.142 to 32.19)**	0.510 (-7.361 to 25.03)	0.837 (-23.46 to 44.79)	0.999 (-42.22 to 47.56)
15.	CALS	19.00±05.04	2.67±1.33	11.00±2.28	20.33±3.93	**2.50±0.43*****	12.67±5.48	18.33±14.84	23.00±20.55
	1000	0.991(-19.15 to 26.15)	0.648 (-6.990 to 19.32)	0.999 (-80.88 to 71.88)	0.922 (-25.99 to 14.65)	0.877 (-9.858 to 19.19)	0.780 (-22.53 to 9.861)	0.933 (-42.12 to 26.12)	0.97 (-53.22 to 36.56)
		0.989 (-26.31 to 18.98)	>0.999 (-13.66 to 12.66)	0.133 (-9.346 to 98.68)	0.547 (-9.655 to 30.99)	**<0.001 (15.31 to 44.36)**	>0.999 (-15.86 to 16.53)	0.964 (-40.79 to 27.46)	0.779 (-60.56 to 29.22)

Mount frequency (in number of time) for animals given extracts of *Citropsis articulata* rootbark decoction extracts at doses of 100 mg/kg (CABD 100), 500 mg/kg (CABD 500) and 1000 mg/kg (CABD 1000); *Citropsis articulata* rootbark Soxhlet extracts at doses of 100 mg/kg (CABS 100), 500 mg/kg (CABS 500) and 1000 mg/kg (CABS 1000); *Citropsis articulata* leaf decoction extracts at doses of 100 mg/kg (CALD 100), 500 mg/kg (CALD 500) and 1000 mg/kg (CALD 1000); and *Citropsis articulata* leaf Soxhlet extracts at doses of 100 mg/kg (CALS 100), 500 mg/kg (CALS 500) and 1000 mg/kg (CALS 1000). PC-SC: positive control–sildenafil citrate (5 mg/kg), NC-DW: negative control–distilled water (CABD and CALD), NC-DS: negative control–dimethyl sulfoxide (CABS and CALS). The values are expressed as the means ± SEMs and then p-value (95% confidence intervals) against the negative control, followed by comparisons against the positive control (below). N = 6 for days 0 to 28, whereas N = 3 for days 35 and 42 (no extracts were subsequently administered). Significant values (p-value ≤ 0.05) are marked in bold and with asterisks, where (*) indicates p ≤ 0.05, (**) indicates p ≤ 0.01, and (***) indicates p ≤ 0.001.

Relative sexual organ weight and penile length of the animals

Generally, the penile lengths increased (although slightly) with time as extract dosing continued. However, with increasing extract dose, the penile length increased (Soxhlet extracts) or decreased (decoctions). The highest penile length in the entire study was 13.17 ± 0.75 mm for the leaf decoction extract. Compared with the positive control (8.22 ± 0.48 mm), the animals treated with the root bark Soxhlet extract at a dose of 1000 mg/kg had an average penile length of 12.84 ± 0.35 mm, which was greater ($p = 0.021$: CI = -8.563 to -0.669).

The sexual organs (testes and penis) collected after 42 days of the study weighed more than those collected after only 28 days of the study. With increasing dose, the weight of the sexual organs decreased for the root bark extracts but generally increased for the leaf extracts (Table 6).

Testosterone levels of the animals

All the plant extracts at different doses resulted in more than twice the testosterone level of the positive control, – sildenafil citrate after 28 days of administration. *Citropsis articulata* leaf decoction extract at a dose of 500 mg/kg elicited a statistically significant increase in testosterone, i.e., 14.50 ± 2.53 ng/ml ($p = 0.021$: CI = -19.45 to -1.553), as shown in Fig. 1.

After two additional weeks of extract administration, the testosterone levels of the extract groups were less than half of those of the negative control, the distilled water group (Fig. 2). These findings revealed a marked reduction in testosterone levels after sudden cessation of extract treatment. *Citropsis articulata* root bark decoction extract at doses of 500 mg/kg and 1000 mg/kg resulted in significantly lower testosterone levels of 0.65 ± 0.13 and 0.96 ± 0.17 ($p = 0.040$: CI = 0.4607 - 21.00 and $p = 0.046$: CI = 0.1540 to 20.69), respectively.

Histological analysis of sexual organs

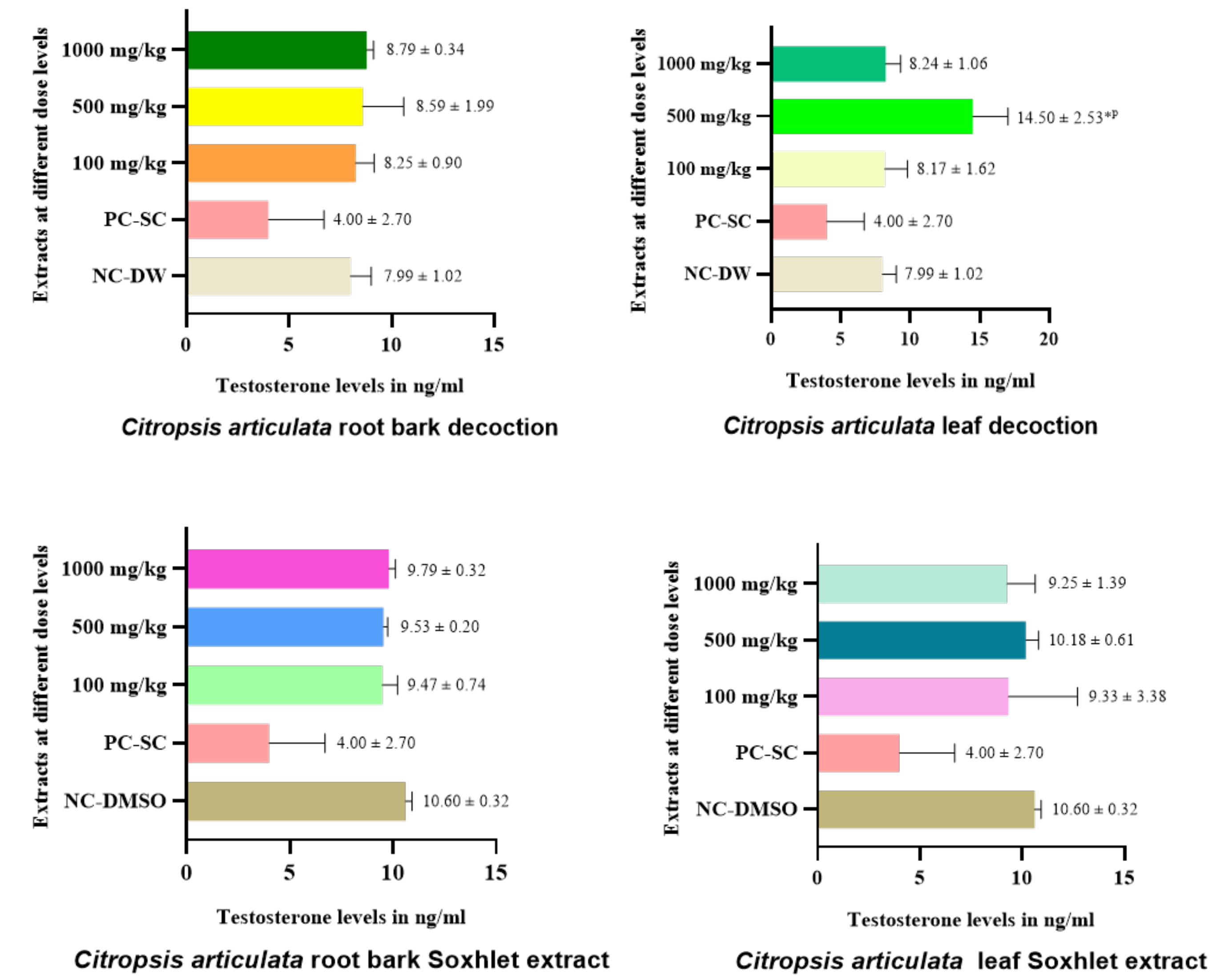
Following harvesting and microscopic analysis of the animals dosed with the test extracts for 28 days, the sexual organs generally appeared normal, with evidence of increased spermatogenesis (dense concentration of spermatozoa), as noted in the epididymis and seminiferous tubules of the testes (Fig. 3 – A and C). The penis also appeared normal with prominent cavernous sinuses (Fig. 3 – B).

Nonetheless, following extract cessation for an additional 2 weeks, some of the harvested sexual organs, such as the testes, seemed abnormal, with evidence of degenerate seminiferous tubules and Leydig cells with extremely low levels of spermatozoa (Fig. 3 – D).

Table 6 Relative sexual organ weight and penile length of the animals

Organ	NC-DW	PC-SC	***Citropsis articulata* root bark decoction extract**		
			100 mg/kg	**500 mg/kg**	**1000 mg/kg**
Harvested after 28 Days of Drug Administration					
1. Right testis	1.49 ± 0.14	1.44 ± 0.04	1.45 ± 0.08	1.30 ± 0.03	1.34 ± 0.09
2. Left testis	1.45 ± 0.18	1.49 ± 0.04	1.48 ± 0.12	1.40 ± 0.05	1.28 ± 0.24
3. Penile weight	0.14 ± 0.01	0.16 ± 0.01	0.14 ± 0.01	0.13 ± 0.02	0.13 ± 0.02
4. Penile length	8.15 ± 0.42	8.22 ± 0.48	8.51 ± 0.20	8.47 ± 0.29	8.09 ± 0.13
Harvested after Additional 14 Days of No Drug Administration					
5. Right testis	1.48 ± 0.07	1.38 ± 0.06	1.54 ± 0.12	1.64 ± 0.06	1.63 ± 0.09
6. Left testis	1.53 ± 0.09	1.59 ± 0.06	1.32 ± 0.26	1.69 ± 0.05	1.67 ± 0.19
7. Penile weight	0.15 ± 0.00	0.19 ± 0.03	0.19 ± 0.01	0.16 ± 0.03	0.14 ± 0.02
8. Penile length	10.74 ± 0.31	12.44 ± 2.13	12.01 ± 1.16	11.41 ± 0.47	10.59 ± 1.58
Organ	**NC-DS**	**PC-SC**	***Citropsis articulata* root bark Soxhlet extract**		
			100 mg/kg	**500 mg/kg**	**1000 mg/kg**
Harvested after 28 Days of Drug Administration					
1. Right testis	1.49 ± 0.08	1.44 ± 0.04	1.41 ± 0.09	1.46 ± 0.07	1.35 ± 0.01
2. Left testis	1.62 ± 0.07	1.49 ± 0.04	1.59 ± 0.06	1.49 ± 0.06	1.39 ± 0.05
3. Penile weight	0.17 ± 0.01	0.16 ± 0.01	0.18 ± 0.01	0.18 ± 0.02	0.21 ± 0.01
4. Penile length	11.14 ± 0.97	8.22 ± 0.48	10.66 ± 1.00	11.71 ± 1.14	**12.84 ± 0.35**^{*p}
Harvested after Additional 14 Days of No Drug Administration					
5. Right testis	1.56 ± 0.09	1.38 ± 0.06	1.36 ± 0.06	1.30 ± 0.06	1.40 ± 0.03
6. Left testis	1.55 ± 0.12	1.59 ± 0.06	1.25 ± 0.09	1.38 ± 0.08	1.49 ± 0.05
7. Penile weight	0.19 ± 0.02	0.19 ± 0.03	0.20 ± 0.01	0.19 ± 0.01	0.19 ± 0.00
8. Penile length	13.01 ± 1.09	12.44 ± 2.13	11.89 ± 0.43	12.16 ± 0.86	11.38 ± 0.85
Organ	**NC-DW**	**PC-SC**	***Citropsis articulata* leaf decoction extract**		
			100 mg/kg	**500 mg/kg**	**1000 mg/kg**
Harvested after 28 Days of Drug Administration					
1. Right testis	1.49 ± 0.14	1.44 ± 0.04	1.37 ± 0.09	1.41 ± 0.20	1.44 ± 0.02
2. Left testis	1.45 ± 0.18	1.49 ± 0.04	1.61 ± 0.10	1.50 ± 0.13	1.48 ± 0.01
3. Penile weight	0.14 ± 0.01	0.16 ± 0.01	0.15 ± 0.02	0.12 ± 0.01	0.11 ± 0.00
4. Penile length	8.15 ± 0.42	8.22 ± 0.48	8.88 ± 0.90	8.84 ± 0.92	8.43 ± 0.33
Harvested after Additional 14 Days of No Drug Administration					
5. Right testis	1.48 ± 0.07	1.38 ± 0.06	1.50 ± 0.01	1.43 ± 0.08	1.61 ± 0.11
6. Left testis	1.53 ± 0.09	1.59 ± 0.06	1.51 ± 0.02	1.54 ± 0.04	1.65 ± 0.08
7. Penile weight	0.15 ± 0.00	0.19 ± 0.03	0.19 ± 0.01	0.15 ± 0.01	0.17 ± 0.01
8. Penile length	10.74 ± 0.31	12.44 ± 2.13	13.17 ± 0.75	10.33 ± 0.72	11.08 ± 0.96
Organ	**NC-DS**	**PC-SC**	***Citropsis articulata* leaf Soxhlet extract**		
			100 mg/kg	**500 mg/kg**	**1000 mg/kg**
Harvested after 28 Days of Drug Administration					
1. Right testis	1.49 ± 0.08	1.44 ± 0.04	1.51 ± 0.11	1.54 ± 0.08	1.51 ± 0.03
2. Left testis	1.62 ± 0.07	1.49 ± 0.04	1.45 ± 0.06	1.64 ± 0.04	1.62 ± 0.06
3. Penile weight	0.17 ± 0.01	0.16 ± 0.01	0.18 ± 0.02	0.16 ± 0.01	0.18 ± 0.02
4. Penile length	11.14 ± 0.97	8.22 ± 0.48	10.69 ± 0.67	11.17 ± 0.82	11.03 ± 0.65
Harvested after Additional 14 Days of No Drug Administration					
5. Right testis	1.56 ± 0.09	1.38 ± 0.06	1.31 ± 0.10	1.56 ± 0.12	1.49 ± 0.05
6. Left testis	1.55 ± 0.12	1.59 ± 0.06	1.39 ± 0.08	1.67 ± 0.15	1.51 ± 0.05
7. Penile weight	0.19 ± 0.02	0.19 ± 0.03	0.16 ± 0.00	0.17 ± 0.01	0.17 ± 0.00
8. Penile length	13.01 ± 1.09	12.44 ± 2.13	11.18 ± 0.16	11.08 ± 0.86	11.79 ± 0.41

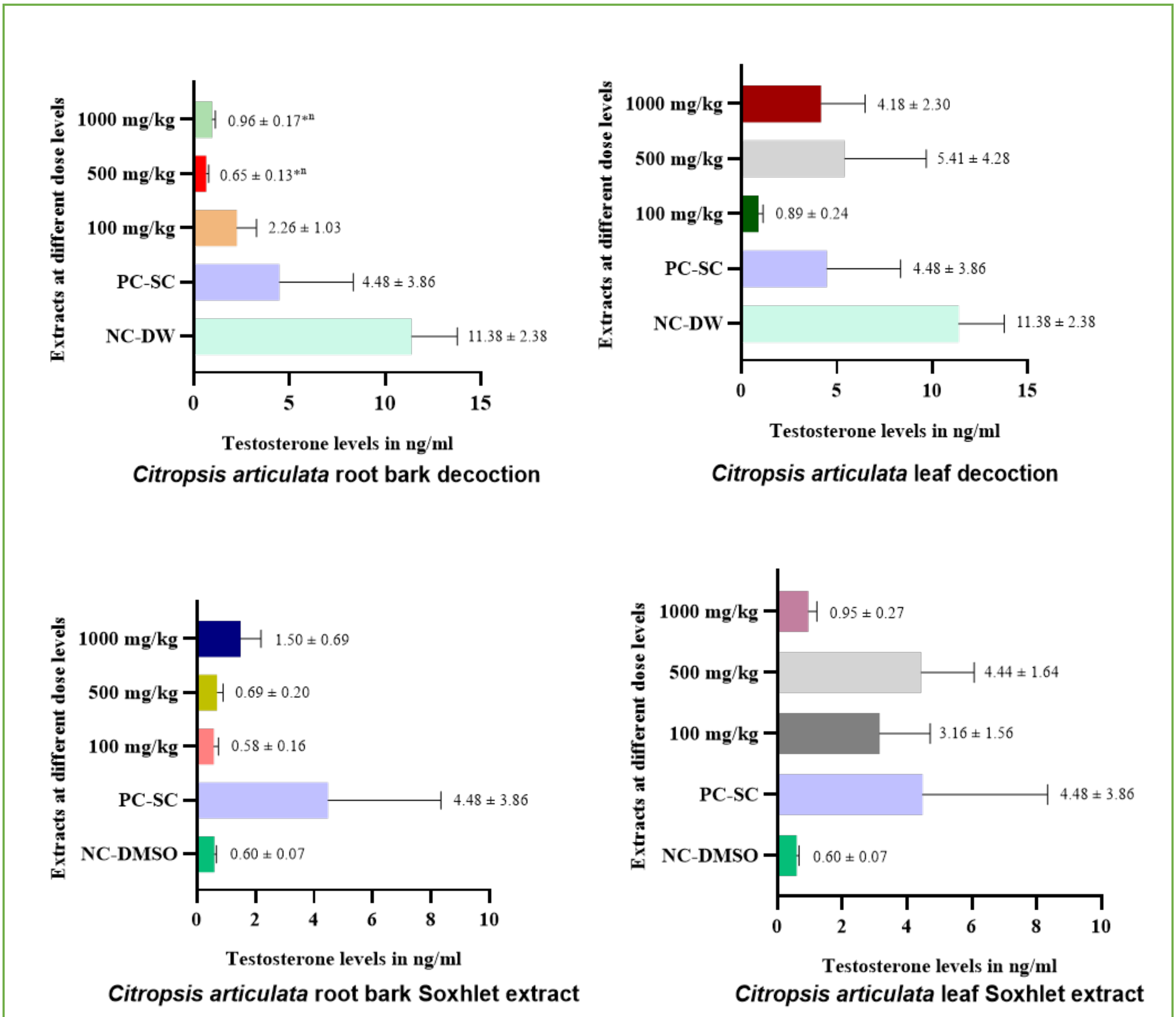
NC-DW – Negative control-distilled water, NC-DS – Negative control-dimethylsulfoxide, PC-SC – Positive control-sildenafil citrate. Penile length was measured in mm. The values are expressed as the means ± SEMs, and the p-value was compared with that of the positive control. Significant values (p-value ≤ 0.05) are marked in bold, and asterisks (^{*p})



Key: PC-SC: positive control – sildenafil citrate at a dose of 5 mg/kg; **NC-DW:** negative control – distilled water; **NC-DMSO:** negative control, dimethylsulfoxide (containing 0.1% gum Arabic).

Fig. 1 Testosterone levels of animals given extracts for 28 days

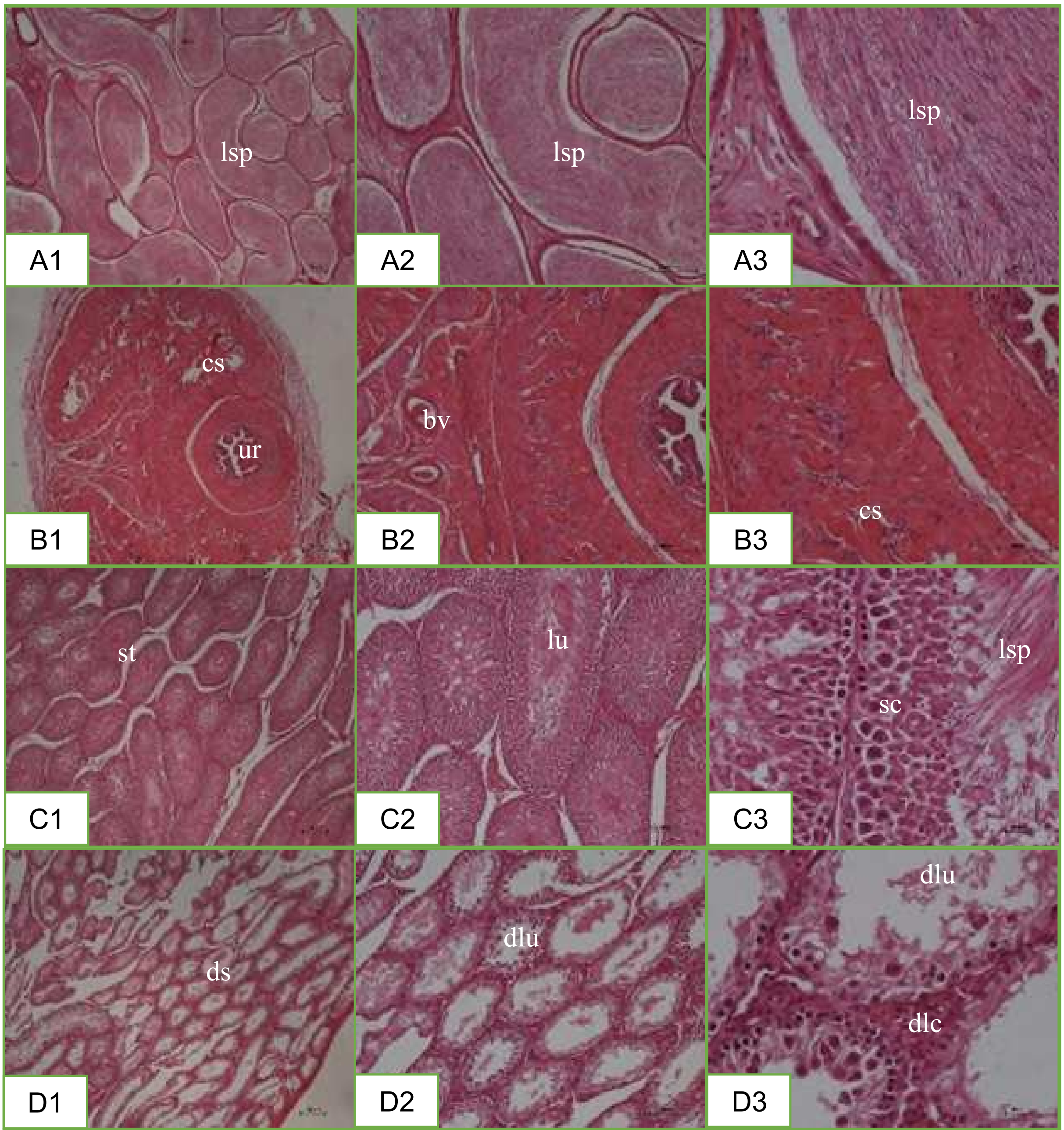
The values are expressed as the means ± SEMs. Significant values (p-value ≤ 0.05) are marked in bold, and asterisks (*) with *p refer to comparisons with the positive control.



Key: PC-SC: positive control – sildenafil citrate at a dose of 5 mg/kg; **NC-DW:** negative control – distilled water; **NC-DMSO:** negative control, dimethylsulfoxide (containing 0.1% gum Arabic).

Fig. 2 Testosterone levels of animals left for an extra 14 days without dosing

The values are expressed as the means ± SEMs. Significant values (p-value ≤ 0.05) are marked in bold, and asterisks (*) with ^{*n} refer to comparisons with the negative control.



Key: **1**, **2** and **3** represent magnifications of 40×, 100× and 400×, respectively. **A** – epididymis tail (lsp – high dense/large numbers of spermatozoa), **B** – penis (cs – prominent cavernous sinuses, ur – urethra, bv – blood vessels), **C** – normal testis (st – numerous seminiferous tubules, lu – enlarged lumen reach in spermatozoa, sc – dense number of Sertoli cells, lsp – highly dense concentration of spermatozoa in lumen), **D** – degenerate testis (ds – degenerate seminiferous tubules, dlu – degenerate lumen of seminiferous tubules, dlc – degenerate Leydig cells)

Fig. 3 Histological analysis of sexual organs

Discussion

The validation of the traditional use of medicinal plants for sexual function is an area of interest for research in the fields of ethnopharmacology and pharmacognosy. *Citropsis articulata* is a prominent medicinal plant in Uganda and other parts of Africa that is used traditionally for managing erectile dysfunction. In this study, we were able to validate the presence of the following phytochemicals: alkaloids, saponins, L-arginine, flavonoids, reducing sugars, coumarins, glycosides, and phytosterols, which were present in varying amounts in the different extracts. Our findings are in agreement with those of Gakunga et al. (2014), Oloro et al. (2016) and Vudriko et al. (2014).

Aphrodisiac parameters such as attraction towards the female (AF) in seconds, penile erections (PEs), mount latency (ML) and mount frequency (MF) were studied for the different extracts at 3 dose levels for 28 days with daily dosing and for an additional 14 days without dosing to establish the sustained effects. High values of AF, PE and MF and low values of ML indicate that the animals have considerable libido and sexual energy. This finding highlights the role of psychic pathways in sexual function (20).

Citropsis articulata root bark and leaf decoction and Soxhlet extracts at different doses of 100, 50 and 1000 mg/kg presented varying AF values. This finding highlights the interest of the male rats in the female rats as influenced by extract ingestion. All the extracts caused PEs on different days during the study, including on days 35 and 42 (the last 14 days of no dosing). *Citropsis articulata* root bark decoction elicited the highest value of PE. It is rich in L-arginine, a known precursor of nitric oxide that promotes erections (21). This may explain why the plant is sometimes referred to as a natural “Viagra.” The general decrease in ML over time meant that the extracts reduced the time required to start sexual activity. The decrease in ML still occurred even after cessation of extract dosing, i.e., on day 35. The increasing MF with time could be attributed to the accumulation of androgens, especially testosterone, whose production is influenced by saponins, flavonoids and other phytosterols present in the extracts (10).

Although the relative sexual organ weights did not significantly differ, a slight increase was noted compared with that of the positive and negative controls. The plant extracts increased penile length, as observed for rats given *C. articulata* root bark Soxhlet extract at a dose of 1000 mg/kg. This may explain why some aphrodisiac medicinal plants are also used for penile enlargement (4,22).

Upon further histological analysis of the sexual organs, a dense concentration of spermatozoa was observed, which showed that the plant extracts enhanced spermatogenesis. This is in agreement with Gakunga et al. (2014). Spermatogenesis is an important indicator of male fertility and sexual function. Nonetheless, upon abrupt extract cessation and subsequent analysis after 14 days, there was a low concentration of spermatozoa in the sexual organs. This may indicate that prolonged extract administration and consequent abrupt cessation could cause toxic reciprocal withdrawal effects on sexual organs. This is further noted by the very low testosterone levels of the same group (23).

Although there were barely any statistically significant differences when the mean testosterone levels of the extracts at different doses were compared with those of the negative and positive controls, there was a noticeable increase in testosterone levels with increasing dose, except for the *C. articulata* leaf decoction and *C. articulata* leaf Soxhlet extracts at a dose of 500 mg/kg. In addition, the *C. articulata* leaf decoction at a dose of 500 mg/kg caused the highest testosterone level. This generally showed that the extracts may increase testosterone levels due to the presence of phytochemicals such as saponins, phytosterols, steroids, flavonoids and amino acids such as L-arginine (10,24). The lowest testosterone levels were recorded for the positive control (sildenafil citrate). This finding contradicts other reports that showed that sildenafil citrate enhanced testosterone production (7,10,25). This could be due to differences in the type and age of the animals used, their feeding habits, and their general housing conditions (26).

During the 28-day dosing period, the rats presented increased testosterone levels compared with those remaining for an additional 14 days without dosing. This finding revealed the beneficial effects of the extracts during drug administration and their untoward effects after 14 days. This may be due to delayed effects and negative feedback mechanisms in the body, similar to the effects of exogenous steroid withdrawal (23,27). This could also be related to cases of degenerate testes when examined grossly and microscopically. This may explain why traditionalists only recommend 3-day dosing for the use of *C. articulata* as an aphrodisiac (11).

Conclusion

The root bark and leaves of *C. articulata* likely possess aphrodisiac efficacy, owing to positive alterations in male sexual behavior, testosterone analysis and histological assessment. However, cessation of consumption after long-term intake can cause delayed toxic effects on the testes, as shown histologically, and lower testosterone levels. Herbal consumers should not use extracts of African cherry orange for long periods. Cessation of consumption should be tapered down gradually.

This study also revealed that both the root bark and the leaf of *C. articulata* possess comparable aphrodisiac efficacy. Herbal consumers may confidently use the plant's leaf instead of the root bark, whose use predisposes the plant to endangerment and extinction.

Abbreviations

AF: Attraction towards the female; CABD: *Citropsis articulata* root bark decoction; CABS: *Citropsis articulata* root bark Soxhlet; CALD: *Citropsis articulata* leaf decoction; CALS:

Citropsis articulata leaf Soxhlet; CI: Confidence interval; MF: Mount frequency; ML: Mount latency; n: Number of observations; NC-DW: Negative control – distilled water; NC-DMSO: Negative control – dimethylsulfoxide (containing 0.1% gum Arabic); p-value: Probability value; PC-SC: positive control – sildenafil citrate at a dose of 5 mg/kg; PE: Penile erection; SEM: Standard error of the mean

Declarations

Ethics approval and consent to participate

Ethical approval was sought from the Research Ethics Committee of Mbarara University of Science and Technology under the reference number – MUREC 1/7. This was followed by further approval from the Uganda Wildlife Authority (Reference number: COD/96/05) in order to collect plant samples. Final approval (Reference number: HS683ES) was obtained from the Uganda National Council for Science and Technology for government recognition of the research study and approval from the Office of the President.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

ARA conceptualized and designed the study. He participated wholly in data collection and wrote the original draft of the manuscript. TAA assisted in data collection and analysis. JT assisted in supervision, mentorship and interpretation of the study findings. VSA also participated fully in data collection and reviewing the draft manuscript. COA supported in guiding experimental handling of the laboratory animals and data analysis. JRA reviewed the draft manuscript and offered guidance in data collection. AAA supported in conceptualization of the study methodology and later reviewed of the manuscript draft. AG reviewed and edited the final manuscript draft while offering mentorship and research related resources. POE was the overall supervisor, revised the manuscript and supported in funding acquisition. All authors read and approved the final manuscript.

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