

Crude ethanolic leaf extracts of *Citropsis articulata*: a potential phytomedicine for treatment of male erectile dysfunction associated with testosterone deficiency

Patrick Vudriko, Martin K. Baru, John Kateregga, James G. Ndukui*

Department of Veterinary Pharmacy, Clinics and Comparative Medicine, School of Veterinary Medicine and Animal Resources, College of Veterinary Medicine, Animal Resources and Biosecurity, Makerere University, Kampala, Uganda

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***Correspondence to:**

James G. Ndukui,

Email: ndukuiga@gmail.com

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ABSTRACT

Background: Erectile dysfunction is the inability to sustain erection of the penis firm enough for sexual intercourse in males. *Citropsis articulata* is used locally by communities in Uganda for the management of erectile dysfunction. The current study evaluated the effect of ethanolic leaf extract of *C. articulata* on the serum level of testosterone and mounting frequency in Male albino rats.

Methods: The study animals were divided into four groups and the extract groups dosed daily orally with 400 mg/kg and 600 mg/kg of extract for three weeks. Sildenafil citrate (1mg/kg) and distilled water (10 ml/kg) were used as positive and negative control respectively. The mounting frequencies of the males exposed to estrus induced females rats on day (7 and 21), testosterone levels (day 21) and phytochemicals present in the extract were determined.

Results: At 400 mg/kg and 600 mg/kg, both testosterone level and mounting frequency increased significantly ($p < 0.05$) by third week. The mean testosterone level at 600 mg/kg (2.678 ng/ml) of the extract was higher than that of group treated with 1mg/kg sildenafil citrate (2.18 ng/ml). The aphrodisiac activity of this extract may be associated with presence of saponins and basic alkaloids in the plant extract.

Conclusions: Taken together, the crude leaf extracts of *C. articulata* could be used as a cheap alternative for the treatment of low libido due to testosterone deficiency. However, further studies are needed on the safety profile and the identification of the molecules responsible for the biological activity of the plant extract.

Keywords: *Citropsis articulata*, Aphrodisiac, Testosterone

INTRODUCTION

Citropsis articulata, also locally known as the *Omuboro* is a popular plant in Central and Western Uganda for its claimed aphrodisiac properties. The plant naturally grows in forests and locals uproot it to obtain the roots for treatment of erectile dysfunctions. The root of this plant has been cherished as one of the most valuable herbal Viagra by most rural communities in Uganda.¹ The fast disappearance of these culturally important medicinal

plants due to poor utilization practices certainly pose major threat towards a looming extinction. Conservationists in the country are concerned that demand for the roots of the plant threatens the survival of this species' genetic diversity in conservation areas like Mabira forest.² The roots are either chewed while fresh/dried or pounded into powder form and then mixed with water to form a concoction. Other plants like *Warburgia ugandensis*, *Cola acuminata*, *Prunus americana* and *Ekebergia capensis* have been used in the

management of erectile dysfunction.^{2,3} The use of medicinal plants for boosting male sexual performance is also popular in China.⁴ The increase in testosterone level has been reported as one of the mechanisms through which medicinal plants boost sexual performance in male.⁵ Testosterone is a steroid hormone that is responsible for the development and maintenance of the masculine characteristics. Low testosterone levels cause countless problems for both sexes.⁶ According to Caruthers *et al.*,⁷ 20-50% of healthy men between the ages of 50 and 70 have sub-normal levels of testosterone and up to 5% of all men are at risk of low testosterone states. Low levels of testosterone may be caused by aging (andropause) cells of the testes, diseases affecting the hypothalamus and pituitary glands, drugs and genetic diseases.⁸ The current study was designed to verify the traditional claims on the aphrodisiac properties of the leaves of *C. articulata*.

METHODS

Study design

This was an experimental study in which the effects of ethanolic leaves extract of *C. articulata* on mounting frequency and serum testosterone levels was determined and compared to reference drugs (Sildenafil citrate) used for the treatment of erectile dysfunction. The method by Trease *et al.*⁹ was used to determine the phytochemical composition of the extract.

Collection of the plant materials and identification

The leaves of *C. articulata* were obtained from Mabira forest which is 25 kilometers from Kampala to the east of Uganda. They were cut using a knife and packed into a nylon sack and transported to the Pharmacology and Toxicology Research Laboratory, (COVAB). Authentication of the plant was done by a Botanist in the Herbarium, College of Natural Sciences - Makerere University and voucher specimen No: 41658 was deposited.

Drying of plant materials

The leaves of *C. articulata* were first washed using clean tap water to remove visible dirt and dried under the shade in the Pharmacology and Toxicology Research Laboratory unit to a constant weight.

Extraction process

The air dried plant leaves were pounded using a mortar and pestle to increase the surface area for extraction of the active ingredients and solvent interaction. The pounded leaves of *C. articulata* were weighed and 500g was soaked in 2000 ml of 70% ethanol in a 2.5 liters Amber bottles. The mixture was left for three days with intermittent shaking, twice daily to optimize extraction of the chemical constituents from the leaves. The extract was allowed to decant, filtered using cotton wool and

further finely filtered using Whatman no: 1 filter paper. The filtrate was concentrated with a rotary evaporator at a temperature of 50°C. The resultant residue concentrate (Crude extract) was dried in hot air oven at temperature of 40°C until a semi-dry residue was obtained. The dry extract was weighed and stored in a refrigerator at a temperature of 4°C prior to administration to the experimental animals and phytochemical analysis.

Animal selection

Healthy, adult male albino rats weighing 170-180g, aged 8 weeks were used in this study. The rats were given standard commercial rodent diet (Uga-Chick Ltd), water *ad libitum* and kept in a room at temperature of 25±1°C, relative humidity 65± 2% and 12hrs light /dark in the Pharmacology and Toxicology Research Laboratory.

Grouping of experimental animals

The rats were randomly divided into four different groups (n=6) and kept in cages labeled as group I, II, III and IV. Each albino rat in the different groups was labeled on the tail using marker pen and their weight determined using weighing balance (OHAUS NVT 1601/1). Groups I and II contained male albino rats treated with 400 and 600 mg/kg of extract orally respectively. Whereas groups III and IV contained rats that were treated with 1 mg/kg sildenafil citrate (Kamagra, B.No:0561H, India) and 10ml/kg distilled water as positive and negative controls respectively. Rats in each experimental group were treated once every day for twenty one days.

Estrus induction in females

Twelve female rats were treated with first dose of estradiol benzoate (0.5 mg/kg) and progesterone (10 µg/100g) intraperitoneally to induce estrus. On day 3 post estrus inductions, a second dose of the above hormones were administered four hrs prior to the exposure of the estrus female to the treated males in various groups. At the time of observation, males from each group were kept in individual cages and exposed to an estrus female rat. The combined animals were allowed to acclimatize for 25mins before determination of the mounting frequency (attempt to mount). The observations were done on days 7 and 21 of the experiment.

Testosterone assay

Blood was collected on day twenty second of the experiment after last administration of the plant extract to determine the level of serum testosterone. The rats were anesthetized using diethyl ether in a rectangular glass and a cardiac puncture was performed. Two milliliters of blood was collected in clean sterile non-EDTA coated vacutainers for testosterone assay. The whole blood composition was separated by centrifugation at 33.5 revolutions per second for 15 minutes using a Uniscope laboratory centrifuge (Model SM800B, Surgifriend Medicals, Essex, UK). The serum was collected using a

Pasteur pipettes into a clean, dry, vials which was loaded in the Cobas E911 Immunolyser machine for the analysis of testosterone levels by the use of indirect immune assay techniques.

Phytochemical analysis

Methods by Trease *et al.*⁹ were used to qualitatively determine the phytochemical constituents in the ethanolic crude leaves extract of *C. articulata*.

Data analysis

The data of serum testosterone level and mounting frequencies was expressed as Mean±SEM. Graph pad prism vs 5.1 (USA. Inc) was used for ANOVA and post hoc multiple comparison test (Dunnet test) was used, with $p < 0.05$ considered statistically significant.

RESULTS

Qualitative phytochemical analysis revealed strong presence of catechol tannins, moderate presence of saponins, alkaloid salts, reducing compounds and coumarins; quaternary bases and oxides amines, steroid

glycosides and cardiac glycosides aglycones were weakly present (Table 1).

Table 1: Phytochemical profiles of the ethanolic leave extracts of *C. articulata*.

Phytochemicals analysed	Inference
Saponin	++
Catechol tannins	+++
Reducing compounds	++
Alkaloid salts	++
Quaternary bases and oxides amines	+
Coumarins	++
Steroid glycosides	+
Cardiac glycosides aglycones	+
Key: +++ = strongly present, ++ = moderately present, + = weakly present, - = absent	

Effect of extract on mounting frequency

There was a significant increase ($p < 0.05$) in the mounting frequency of the male rats treated with 600 mg/kg on day 21 which was comparable to that of rats treated with 1 mg/kg sildenafil citrate (Table 2).

Table 2: Table 2: Effect of *C. articulata* on mounting frequency in male albino rats.

Grouping	Treatment	Mouning Frequency at day 7		Mounting Frequency at day 21	
		Mean±SEM	95%CI	Mean±SEM	95%CI
Group I	400 mg/kg E	2.75±0.48	2-4	3.75±0.25 ^d	3-4
Group II	600 mg/kg E	3.5± 0.47	2-5	5.0±0.41 ^e	4-6
Group III	1mg/kg SC	4.25±0.48 ^c	3-5	6.250±0.48 ^f	5-7
Group IV	10 ml/ kg DW	2.0±0.41	1-3	2.25±0.48	1-3

Value presented as Mean±SEM (n=4); E- extract; SC- Sildenafil citrate; DW- distilled water; Comparison with negative control: p value (c = 0.0117; d = 0.0321; e = 0.0047 and f = 0.001); n=4

Effect of extract of *C. articulata* on serum testosterone level

There was dose dependent increase in serum testosterone among male rats in the groups treated with the extract. At a dose rate of 400 mg/kg and 600 mg/kg, the serum

testosterone levels were 1.2975 ng/ml and 2.6775 ng/ml respectively. The above testosterone levels were statistically significant ($p < 0.05$) when compared to the negative control. At 600 mg/kg the testosterone level (2.6775 ng/ml) was greater than that of 1 mg/kg Sildenafil citrate (2.18 ng/ml) (Table 3).

Table 3: Effect of *C. articulata* on testosterone level.

Group	Treatment	Mean±SEM (ng/ml)	95%CI
Group I	400mg/kg Extract	1.345±0.08986 ⁱ	1.13-1.57
Group II	600mg/kg Extract	2.678±0.1160 ^j	2.34-2.87
Group III	1mg/kg Sildanfil citrate	2.180±0.09354 ^k	2.02-2.45
Group IV	1ml Dist.water	0.7500±0.2345	0.25-1.15

Value expressed as Mean± SEM (n=6); Comparison with negative control: p values (I = 0.0762; j = 0.001; k = 0.001)

DISCUSSION

The increased mounting frequency on day 21 could be attributed to the increased level of free serum testosterone level in the blood. The increase in mounting frequency due to testosterone was also reported by Kesler *et al.*¹⁰ Indeed, the rats that received the extract at the dose rate of 600 mg/kg had three fold increase in the level of testosterone compared to the group that was given only distilled water. This result indicates that the extract had the ability to influence synthesis and release of testosterone from the testis into blood circulation. Such effect could be attributed to the presence of phytochemicals such as saponins, and coumarins that have been previously reported¹¹ to increase testosterone level. Similar studies⁴ reported that the increase in testosterone level following treatment with Fenugreek seeds was attributed to the saponins which have the ability to cause release of Luteinizing hormone (LH) that leads to the boost in testosterone levels. It also stimulates the leydig cells of the testes to directly increase testosterone production.¹¹ In the current study we found moderate presence of alkaloids in the extract. Previous study¹² has shown that alkaloids increase blood flow in the sexual organs due to vasodilatation thus sustaining male erection leading to enhanced sexual performance. Similar mechanism is responsible for the enhanced sexual performance associated with Sildenafil citrate. However, there was more pronounced sexual activity in the male rats treated with 600 mg/kg of the test extract compared to 1mg/kg of Sildenafil citrate. Although the local communities prefer using the root bark which has predisposed the plant to extinction, our study provides a scientific evidence for the adoption of the leaves as an alternative plant part for traditional management of erectile dysfunction. This approach presents a timely opportunity for sustainable utilization of the plant biodiversity and its conservation for the future generation.

CONCLUSION

The increase in sexual performance and serum testosterone level in the extract treated rats validates the traditional use of *C. articulata* in the management of erectile dysfunction in Central and Western parts of Uganda. However, further investigation is needed on bioassay guided structural elucidation of the phytoactive agents and the safety profile of *C. articulata*.

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Conflict of interest: None declared

Ethical approval: Ethical clearance was obtained from the College of Veterinary Medicine, Animal Resources and Biosecurity, Makerere University; guidelines for appropriate experimental animal care and handling were also followed.

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