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**Phytochemical and efficacy study on four herbs used in
erectile dysfunction: *Mondia whiteii*, *Cola acuminata*,
Urtica massaica, and *Tarenna graveolens*****Joseph Oloro¹, Tanayen Julius Kihdze^{1,4}, Barbra Katusiime², Lawrence Imanirampa², Paul
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There is a resurgence in the use of herbal medicine in the developed countries, with even much more in low developed Countries and especially for conditions such as erectile dysfunction. Studies thus need to be conducted to scientifically validate claims on certain medicinal plants reported to be efficacious in traditional medicine. This current study was conducted to determine the phytochemical composition and efficacy of four herbs *Mondia whiteii* (Hook. F.) roots locally called (Mulondo), *Cola acuminata* (P. Beauv.) fruits locally called (Engongoli), *Urtica massaica* (Mildbr) leaves, locally called (Engyenyi) and *Tarenna graveolens* (S. Moore) roots, locally called (Munywamaizi) in Runyankole which have been reported as remedies for the management of erectile dysfunction in South-western Uganda. Phytochemical screening was conducted following methods described in Kokate, Trease and Evans. Sexual function was tested using both the contact and non-contact model. Arginine was present in all the extracts. Aqueous extract of *Tarenna graveolens* significantly improved testosterone levels but none of the extracts had significant effects on mounting frequency. Aqueous extract of *Tarenna graveolens* could be useful in management of erectile dysfunction associated with hypogonadism.

Key words: Erectile dysfunction, efficacy, phytochemical, testosterone, *Mondia*, *Cola*, *Urtica*, *Tarenna*.

INTRODUCTION

Medicinal plants are used worldwide as an alternative or complementary medicine to treat various conditions and

as a result, interest in medicinal herbs is increasing as precursors of pharmacological actives. The use of herbal

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medicine to treat sexual dysfunction among males is on the rise in Uganda and studies have documented several plants that have been used, including *Mondia whitei* (Hook. F.) roots locally called (Mulondo), *Cola acuminata* (P. Beauv) fruits locally called (Engongoli), *Urtica massaica* (Mildbr) leaves, locally called (Engenyeni) and *Tarenna graveolens* (S. Moore) roots, locally called (Munywamaizi) in Runyankole (Kamatenesi-Mugisha and Oryem-Origa, 2005). The wide spread use of herbs is compounded by the fact that sildenafil, the standard drug for treatment of erectile dysfunction (ED) is very expensive, only effective for about 70% of those with erectile dysfunction (NIH, 2003; Magoha, 2000) and there is high availability of brands that are not very effective.

The estimated range of men suffering from ED worldwide is from 15 to 30 million (NIH, 2003). According to a survey done in three countries of Nigeria, Egypt and Pakistan by Shaeer et al (2003), the age-adjusted prevalence rates of ED among men attending primary care clinics was 57.4% in Nigeria, 63.6% in Egypt, and 80.8% in Pakistan, while prevalence rate in Uganda is not known.

Although not documented, erectile dysfunction seems problematic among Ugandan men especially as noted by the rate of advertisement and sale of herbs for the condition in Uganda. The NIH Consensus Development Conference on Impotence (NIH; 1992) defined impotence as "male erectile dysfunction", the inability to achieve or maintain an erection sufficient for satisfactory sexual performance. Although reported at 16% (Isis-WICCE; 2011), sexual dysfunction might be contributing more to prevalence of domestic violence in Uganda and indeed the rest of the world. Several medicinal plants including those studied in this current work are sold within Uganda for the treatment of erectile dysfunction.

Mondia Whitei is used traditionally as an antacid and to treat indigestion; as a tonic; to stimulate appetite; and infusions of the root are used in Zimbabwe for treating anorexia and bilharzia. Fits in children and stress and tension in adults are also to be treated with this plant. The roots are used as an aphrodisiac and for the treatment of erectile dysfunction and impotence (<http://www.plantzafrica.com> accessed 6.6.2015) and for appetite and libido, as a galactagogue, fertility medication, and as an antidepressant (Oketch-Rabah, 2012). *Urtica massaica* is reportedly used for treatment of diarrhea in Rwanda and commonly eaten by Mountain Gorillas (Alphonse et al., 2008). *Cola acuminata* is reported to have stimulant action apart from the caffeine content, valuable nervine, heart tonic, and a good general tonic (www.botanical.com) and no other medicinal use of *Tarenna graveolens* was found other than its use in the management of erectile dysfunction in south-western Uganda (Kamatenesi-Mugisha et al., 2005). Earlier studies on the phytochemical compositions indicates that *M. whitei* has reducing sugars, triterpenes, steroids, phenolic compounds and flavonoids (Watcho et al., 2007). Sonibare et al. (2009) reported that aqueous

extract of *Cola acuminata* contains; alkaloids, saponins, tannins, cardenolides and no anthraquinones. Studies on the hydromethanolic extract of *U. massaica* indicated that anthocyanes, flavonoids, saponins and tannins were present while alkaloids, quinines and sterols were absent (Nahayo et al., 2008).

The aim of this current study was to determine separately, the effects of these four herbal remedies on sexual function in male rats by specifically; (i) conducting qualitative phytochemical screening on the four plants extract, (ii) evaluating their effects on erection and mounting frequency and to ascertain the effects of the extracts on testosterone levels in male rats.

MATERIALS AND METHODS

Study design

This was a short term prospective experimental study that was conducted in a period of 2 months.

Materials

120 Male and forty female albino Wistar rats, 4 month of age were used in the experiment. Sildenafil citrate (Pfizer), estrogen and progesterone (sigma Aldrich), extract of four different plants that were studied, oral cannulas for drug and extract administration, transparent glass observation cages, ketamine for anesthesia.

Experimental animals

Male Wister Rats 4 months old were secured for use in the experiment, 120 for erectile function test. There were five main groups of 30 animals each for the four plants extracts, and a control group; and the five main groups were divided into five sub-groups of six each for the efficacy study. The animals were kept at standard conditions following the NIH guidelines for animal handling in teaching and research (Guide for care and use of Laboratory Animals, 2011)

Extract preparation

Plant collection

The parts of the various plants used were collected from various villages within Western Uganda. These included *M. whitei* (Mulondo) roots, *C. acuminata* (Engongoli) fruit, *U. massaica* (Engenyeni) leaves, and *T. graveolens* (Munywamaizi) roots. Herbarium specimens were prepared and taken for botanical identification, at Science Laboratory Technology/Biology, at Mbarara University of Science and Technology and voucher specimens were deposited in the herbarium at the Faculty of Science.

Plant processing and extraction

The different plant parts once brought to laboratory were washed under running water, shade dried at room temperature for two weeks then crushed into powder form and extracted by warm maceration. About 4 kg of the various plants powder were extracted

at once for both phytochemical screening and efficacy evaluations using water.

Separation of crude extract from extracting solvent

The crude extract that were obtained was evaporated at controlled temperatures of 60°C to reduce them to semi solid states and then transferred to a desiccator with a hygroscopic substance to absorb the remaining moisture and the final powder were collected and stored ready for the tests.

Phytochemical screening

Phytochemical screening was conducted following the methods described by Trease and Evans (2009) and Kokate et al. (2010). All were conducted using chemicals of analytical grade, secured from reputable companies.

Efficacy study

Non-contact erections

A non-contact erection was conducted following the method described by Sachs et al. (1994). Female rats were placed in behavioural oestrous by the administration of ethinyl oestradiol orally at the dose of 100 µg/animal 48 h prior to the pairing and progesterone injected subcutaneously, at the dose of 1 mg/animal 6 h before the experiment. The receptivity of the female animals was confirmed before the test by exposing them to male animals, other than the control, test and Standard drug group animals. The most receptive females were selected for the study.

On the day of experiment, male rats in group A, B and C for each of the four extracts were treated with 250, 500 and 1000 mg/kg respectively. The control groups D was treated with Sildenafil Citrate at the dose of 10 mg/kg, while Group E was only given distilled water. Groups in 250 mg/kg for all the extracts were tested in one day and likewise for the other dose levels. All male animals in each group were treated with extracts 1 h before pairing with the females. For the non-contact model, Male and female rats were placed in different chambers of the glass observation cages which had a dividing wall with a perforation that separated the two halves. The perforation was to allow the passage of auditory, visual and pheromonal cues between the male and female animals in either side of the observation cage. The males were then observed and recorded for general sexual characteristic behavior for 1 h.

Contact model

In this model, the male rats were initially treated once a day with the extracts orally. On the third day, each male was treated and after one hour they were paired up with the receptive female and the number of successful mounting recorded for 1 h.

For the next four days, each male rat was treated with the same dose up to a total of 7 days of treatment. On day 8, the animals were sacrificed, their blood samples removed and then analyzed for the testosterone levels.

Ethical consideration

The proposal was submitted to Mbarara University of Science and Technology Institutional Review Committee (MUST-IRC), approval number MUIRC 01/02-13 and Uganda Nation Council for Science

and Technology (UNCST), approval number HS 1557. Animals were deeply anesthetized with ketamine at the end of the experiment to prevent pain.

Testosterone analysis

Testosterone analysis was performed using the AXSYM Testosterone reagent by Abbott AXSYM system. The system is based on Microparticle Enzyme Immunoassay (MEIA) technology for the quantitative determination of testosterone in human serum and plasma. The AXSYM Testosterone assay displaces bound testosterone from the protein and measures total testosterone. Measurement of results by the AXSYM system is based on the Beer's Law. The unit of measurement that was used in this case was ng/ml. (Abbott AxSYM system Operation Manual, 1996).

Data analysis

Data obtained from the study were analyzed using graph pad prism software version 5.0. The testosterone levels in the five rat groups was compared using one way analysis of variance (ANOVA) followed by Turkey multiple comparison test. In all, results were considered statistically significant if the p value is less than 0.05.

RESULTS

Phytochemical screening test

Phytochemical screening test Indicated the presence of saponins, Tannins, Arginine, Reducing sugars and phenolic compounds in all the four plant extracts. Glycosides was present in all extracts except *C. acuminata*, protein and free amino acids was present in all extracts except *M. whiteii*, terpenoids was present in all except *T. graveolens* and *U. massaica*, triterpenoids was present in all except *C. acuminata*, flavonoids was present in *M. whiteii*, *C. acuminata* and absent in *U. massaica* and *T. graveolens*, alkaloids was only present in *U. massaica* extract and absent in all the other three extracts and finally steroids and phlobatannins which were absent in all the four plants extracts (Table 1).

Effects on mounting frequency

None of the four plants extracts showed any positive increase in mounting frequency in the male rats except the standard drug sildenafil at 10 mg/kg with a $p=0.0002$ (Table 2).

Effects on testosterone levels

Of the four plants extracts, only *T. graveolens* caused a significant increase in testosterone levels with maximum effects achieved at 250 mg/kg ($p=0.0038$) while 500 mg/kg though significant indicated decrease in testosterone levels ($p=0.0385$) (Table 3).

Table 1. Phytochemical test results for the four plants extracts studied.

Phytochemical	<i>M. whitei</i> (Omulondo)	<i>C. acuminata</i> (cola nut)	<i>U. massaica</i> (Engenyeni)	<i>T. graveolens</i> (Munywamaizi)
Alkaloids	-	-	+	-
Flavonoids	+	+	-	-
Saponins	+	+	+	+
Tannins	+	+	+	+
Phlobatanins	-	-	-	-
Steroids	-	-	-	-
Terpenoids	+	+	-	-
Triterpenoid	+	-	+	+
Protein	-	+	+	+
Amino acids	+	+	+	+
Arginine	+	+	+	+
Glycosides	+	-	+	+
Reducing sugars	+	+	+	+
Phenolic compounds	+	+	+	+
Anthraquinones	-	-	-	-

+ = Preset, - = absent.

Table 2. Effects of the four plants extracts on mounting frequency.

Extract and dose	Mean mount in 1 h	Std. dev	P-Value	95% CI
Mondia 250	8.33	20.41	0.5808	-13.09 to 29.75
Mondia 500	13.33	13.81	0.3452	-1.16 to 27.82
Mondia 1000	5.33	8.29	0.7970	-3.36 to 14.03
Cola 250	20	15.88	0.1271	3.33 to 36.67
Cola 500	10	11.08	0.5199	-1.63 to 21.63
Cola 1000	9.5	5.32	0.5627	3.92 to 15.08
Urtica 250	0	0	0	0
Urtica 500	6.2	10.67	0.7357	-5.03 to 17.36
Urtica 1000	8.83	6.71	0.6064	1.79 to 15.87
Tarenna 250	5.83	7.81	0.7778	-2.36 to 14.03
Tarenna 500	5.5	8.17	0.7905	-3.07 to 14.07
Tarenna 1000	7.83	9.35	0.6564	-1.98 to 17.64
Sildenafil 10mg	36	2.37	0.0002***	33.52 to 38.48
Distilled water	10.33	11.43		-1.66 to 22.33

*= Significance levels, Mondia = *M. whitei*, Cola = *C. acuminata*, U.= *U. massaica* and Tarenna = *T. graveolens*. The table above indicates that none of the extracts improved mounting frequency in the male rats.

DISCUSSION

This current study was conducted to determine the phytochemical constituents in the extracts from four plants *M. whitei*, *C. acuminata*, *T. graveolens* and *U. massaica*. Screening test revealed the presence of a number of phytochemicals, some of which have been known to have effects on erection. Of particular importance is the presence of arginine in all the extracts and sugar. Arginine has been reported as a regulator of

penile erection and those disorders that reduces its synthesis or release in its penile tissues results in erectile dysfunction (Burnett., 1997, 2004). Saponins were also found present in all the four extracts. Available information implicates steroidal saponins as possible causes of increase in testosterone levels in animals (Walid et al., 2007). Studies by Gauthaman et al. (2002; 2008) also reported increase in testosterone levels caused by steroids, specifically Protodioscin isolated from *Tribulus Terrestris* extract. However, this was a

Table 3. Effect of the four plants extracts on testosterone levels.

Extract and dose	Mean testosterone levels (ng/ml)	Std. dev	P-Value	95% CI
Mondia 250	1.882	1.454	0.2370	0.356 to 3.408
Mondia 500	0.370	0.368	0.9997	-0.016 to 0.756
Mondia 1000	1.423	0.469	0.5013	0.215 to 2.631
Cola 250	4.117	5.202	0.1175	-1.343 to 9.576
Cola 500	3.460	3.524	0.0950	-0.238 to 7.158
Cola 1000	2.508	3.975	0.2607	-1.664 to 6.680
Urtica 500	1.270	0.788	0.6631	0.443 to 2.097
Urtica 1000	2.013	1.581	0.1983	0.354 to 3.673
Tarenna 250	4.428	2.180	0.0038**	2.141 to 6.761
Tarenna 500	3.100	2.042	0.0385*	0.957 to 5.243
Tarenna 1000	3.197	3.305	0.1107	0.272 to 6.666
Sildenafil 10 mg	1.492	0.463	0.3957	-0.48 to 1.72
Distilled water	1.425	0.383		1.199 1.717

*= Significance levels, Mondia = *M. whitei*, Cola = *C. acuminata*, U = *U. massaica* and Tarenna = *T. graveolens*. The table above indicates that only *T. graveolens* significantly improved testosterone levels.

qualitative study, making it not possible to estimate how much of each was present in the extract. It was noted that the extract of *Mondia whitei* had reducing sugars, triterpenes, phenolic compounds and flavonoids. This was in agreement with studies conducted by Watcho et al. (2007). It was also noted in this current study that the dried and grounded powder extract of *Mondia whitei* displayed a hygroscopic characteristic when left in the open uncovered. This current study also found similar constituents in aqueous extracts of *Cola acuminata* as in Sonibare et al. (2009) where; alkaloids, saponins tannins were found present. On *U. massaica*, only saponins and taninns occurred in the extracts tested as in the case of Nahayo et al. (2008) and the extracts in this current study. No study was however found conducted on the aqueous extract of the root bark of *Tareena graveolens*, making this probably the first of its kind. Common to all this extracts was however the presence of free amino acids and specifically arginine, which has been reported to have aphrodisiac effects as it is converted into nitric oxide which is an important vasodilator in the penile cavernosal tissue (Burnett, 2004).

The extract did not induce erection in the animals, indicating that all are not inducers of erection. Although mounting was noted in some of the animal groups treated, the results were not statistically significant in comparison to those of the control groups (Table 2). Despite the presence of arginine and saponins in the extract and their being reported as substance known to regulate erectile function and increase in testosterone levels respectively

T. graveolens at the dose of 250 mg/kg significantly increased testosterone levels in males rats (Table 3) more than that reported for *Citropsis articulata* leaf extract (Vudriko et al., 2014) and *Citropsis articulata* root bark extract (Oloro et al., 2014). Although not

quantitatively determined, the increase in testosterone levels could suggest that there was a higher level of saponins in the extract of *T. graveolens* (Koumanov et al., 1982). This result thus indicates that the aqueous extract of *T. graveolens* could be useful in treatment of those with erectile dysfunction resulting from hypogonadism. More so, the increase in testosterone levels indicated by the aqueous extract of *T. graveolens* did not correspond to the mounting frequency, contrary to many reports including (Vudriko et al., 2014; Oloro et al., 2014). The extract of *T. graveolens* as indicated in this study could be improving sexual activity in men with suspected erectile dysfunction by increasing their testosterone level. The experiment however could not show how this is achieved, whether by displacement of bound testosterone from the sex hormone binding proteins, inducing its synthesis or inhibiting its conversion to other metabolites.

Conclusion

The result of this study indicated that aqueous extract of *T. graveolens* improves testosterone levels in male rats and that none of the four herbs studied, *T. graveolens*, *M. whiteii* roots, *C. acuminata* fruit and *U. massaica* leaves, neither induce erection nor improved mounting frequency in male Wistar rats.

Conflict of Interests

The authors have not declared any conflict of interests.

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