http://dx.doi.org/10.4314/ajtcam.v12i2.5

A COMPARATIVE STUDY ON THE APHRODISIAC ACTIVITY OF FOOD PLANTS *MONDIA WHITEI*, *CHENOPODIUM ALBUM*, *CUCURBITA PEPO* AND *SCLEROCARYA BIRREA* EXTRACTS IN MALE WISTAR RATS.

***Constance R. Sewani-Rusike, Jehu E. Iputo, Eugene J. Ndebia, Mavuto Gondwe, and David R. Kamadyaapa**

Physiology Department, Faculty of Health Sciences, Walter Sisulu University, Mthatha, South Africa.
***Corresponding author: Email**: crusike@wsu.ac.za

Abstract

Background: Any substance that increases erectile function, sexual performance and enjoyment is considered an aphrodisiac. The aim of this study was to compare the effects of food plants *Mondia whitei*, *Chenopodium album*, *Cucurbita pepo* and *Sclerocarya birrea* extracts at a fixed dose of 200mg/kg body weight on sexual behavior, sperm parameters and testosterone levels in adult male rats. These are food plants also traditionally used as aphrodisiacs in South Africa, Zimbabwe and other parts of Africa.
Materials and methods: Sexual behavior parameters assessed in this study included an arousal component (mount latency and intromission latency); sexual potency (mount frequency and intromission frequency), erection (copulatory efficiency) and ejaculations. All treatments were administered orally daily for 28 days. Sexual behavior parameters were quantified 2 hours after a single dose, at 14 days and at 28 days of treatment.
Results: The order of efficacy in stimulating sexual behavior in male rats was *M. whitei* >*S. birrea* > *C. pepo* ≥ *C. album.* Although there was no change in number of ejaculations and sperm count (P>0.05) for all treatment groups compared to controls, all treatments increased (P<0.05) sperm motility. *M. whitei* and *C. pepo* treatments resulted in increased (P<0.05) serum testosterone levels.
Conclusion: Therefore, this study demonstrates varying aphrodisiac activities of food plants used traditionally as aphrodisiacs.

Key words: sexual behavior, aphrodisiac, sperm motility, *S. birrea*, *C. pepo*, *C. album*

Introduction

Masculinity encompasses sexual potency as one of its determinants. Indeed, the humiliation associated with failures of sexual performance is common across various cultures. As a result, many men do not disclose aspects of sexual dysfunction to their physicians. This has therefore maintained the popularity of aphrodisiacs for centuries. The knowledge of aphrodisiacs is passed down from generation to generation. An aphrodisiac is defined as any substance that enhances sex drive or desire. In reality, any substance that increases erectile function, sexual performance and enjoyment has been considered an aphrodisiac (Thakur et al., 2009; Wani B.A, et al., 2011). While there has been skepticism on the biological effects of aphrodisiac plants, advances in scientific research have started to demonstrate the efficacy of traditional aphrodisiacs using animal models to elucidate mechanism of action. In a review by Wani B.A. et al. (2011) a total of 123 plants were reported to exhibit aphrodisiac properties. In South Africa, Abdillahi and Van Staden (2012) reported about twenty five plants used for male reproductive ailments, the majority of which are used as aphrodisiacs and to treat impotency. Gelfand et al. (1985) listed over fifty plants used by Zimbabwean males in treating sexual dysfunction. Accumulating evidence supports the role of the neuroendocrine system as responsible for regulation of male sexual behavior with testosterone playing a major role in promoting libido, erection and sexual performance (Seftel et al., 2004; Carro-Juárez et al., 2006; Zamblé et al., 2008; Prabsattroo et al., 2012). Local effects of phytochemicals on the corpus cavernosum smooth muscle and blood vessel endothelium via enhanced release or action of nitric oxide (NO), the primary mediator of erection, is a possible mechanism of action to enhance erectile action by these plants. Antioxidant activities of medicinal plants have also been attributed to enhanced erectile function (Zhang et al., 2011). Oxidative stress results in reduced NO bioavailability as well as endothelial and cavernosal dysfunction, thus contributing to erectile dysfunction (Agarwal et al., 2006, Tostes et al., 2008). Additionally, aphrodisiac activity has been attributed to nutritional boost by the agent leading to improved well-being and hence boosting sexual performance and libido (Yakubu et al., 2007; Sumalatha et al., 2010). As such, some food plants are associated with improved sexual potency, relieving sexual dysfunction and increasing male fertility. Such food plants as garlic (Malviya et al., 2011), asparagus (Wani J.A. et al., 2011), *Garcinia kola* (Ralebona et al., 2012), *Mondia whitei* (Watcho et al., 2007a; Quasie et al., 2008) have been shown to improve erectile function and sexual performance in experimental animals. *Mondia whitei* (white's ginger), *Sclerocarya birrea* (marula) fruit, *Chenopodium album* (wild leafy vegetable), and *Cucurbita pepo* (pumpkin) seed are food products claimed to possess aphrodisiac activity (Table 1). Several studies have demonstrated the erectile and fertility enhancing effects of *Mondia whitei* (Watcho et al., 2004, 2007a; Quasie et al., 2008). We therefore used it in this study as the reference drug. The present study was undertaken to investigate and compare the ability of the aforementioned food plant extracts in influencing sexual behavior in male Wistar rats.

Table 1: Food and medicinal uses of plants used in the present study.

Plant Name	Plant Part	Medicinal and Food Uses
Mondia whitei	root	Aphrodisiac, cure male infertility, gastrointestinal problems, induce labour, appetite stimulant, antihelmintic, antimalarial taken as a tea, prepare energizing drink, used as a spice (Gelfand et al., 1985; Crouch et al., 1998; Gundidza et al., 2009)
Chenopodium album	leaves	Aphrodisiac, improve appetite, antihelmintic, diuretic, laxative, wild leafy vegetable (Pande and Pathak, 2008; Yadav et al., 2007)
Cucurbita pepo	seeds	Aphrodisiac, androgenic properties, antihelmintic, urinary problems, treat prostate hypertrophy, toasted as a snack, added to vegetables for flavour (Caili et al., 2006; Gundidza et al., 2009)
Sclerocarya birrea	Fruit (juice)	Aphrodisiac, eaten fresh or as a jelly, fermented into an alcoholic beverage "mukumbi" (Zimbabwe and South Africa), marula liquer commercially available (Shackleton et al., 2002; Van Wyk et al., 1997)

Material and Methods

Plant materials and extract preparation

M. whitei root was collected from Uganda by one of the authors. Aerial parts were also collected and used for authentication in the Botany Department, WSU. The root was cut into small pieces and air dried. *C. album*, a local leafy vegetable, was collected from its natural habitat around Mthatha where it grows in abundance. The leaves were boiled for 15 minutes to mimic local preparation for consumption, and then dried in a fan oven. *C. pepo* (pumpkin) seeds were purchased from a local health shop with the outer seed coat already removed. Ripe *S. birrea* (marula) fruit was collected from Limpopo Province of South Africa with the assistance of Mr W.

http://dx.doi.org/10.4314/ajtcam.v12i2.5
Ramasila and transported to Mthatha whole. The fruit was peeled and fruit juice and pulp expressed into a clean beaker and dried in a fan oven. The crystalline dry fruit juice was ground into a powder and used for animal treatments. The dry plant materials (*M. whitei* root, *C. album* leaves and *C. pepo* seeds) were pulverized into a crude powder using a household blender. The powders were each mixed with 70% ethanol and incubated overnight with continuous agitation. After vacuum filtration through filter paper (Whatman No. 1), ethanol was removed using a rotary evaporator (Buchi RE111) under reduced pressure at 40^{o} C. Water was removed using a freeze drier (Modulyo Edwards). Resultant powders were used for animal treatments.

Animals

Fourteen week old, sexually mature male and female albino rats of Wistar strain were used for the study. All animals were housed in polypropylene cages and maintained in a 12h:12hr light:dark cycle, at ambient temperature with water and solid pellet food (Epol-SA) *ad libitum*. Ethical care and handling of experimental animals was observed at all times and the study was approved by the Walter Sisulu University ethics committee (clearance number 0023/009). Prior to commencement of the study, male rats were exposed to receptive females once a week for 2 consecutive weeks and those males showing sexual activity within 5 min after exposure to the female were selected for the study. The sexually active animals were randomly divided into 5 oral treatment experimental groups each consisting of 6 rats. The groups were:

Control group (distilled water)
M. whitei root (200 mg/kg body weight; positive control)
C. album leaves (200 mg/kg body weight)
C. pepo seeds (200 mg/kg body weight)
S. birrea fruit (200 mg/kg body weight)

M. whitei was used as the positive control and a dose of 200mg/kg body weight was selected based on published reports (Watcho et al., 2004, 2007a; Quasie et al., 2008). The same treatment dose was used for the other plant materials to allow for comparison.

Sexual behavior tests

Sexual behaviour tests were carried out using standard methods (Yakubu et al., 2007; Watcho et al., 2007a). An acute sexual behaviour observation was carried out 2 hours after a single dose of plant extract (acute effect) then on days 14 and 28 of daily treatment of male rats. In these tests, female rats artificially brought to oestrus by treatment with estradiol (500 µg/kg s.c., 48h before tests) and progesterone (5.0 mg/kg s.c., 4h before tests) were introduced into the observation cage of the male animal with 1 female to 1 male ratio. The observation for mating behavior was started immediately after introduction of the female and parameters were recorded as they occurred for 20 minutes. The parameters of male sexual behavior that were monitored were: mount latency (ML= time interval between the introduction of the female and the first mount by the male); intromission latency (IL = time interval from the introduction of the female to the first intromission by the male); mount frequency (MF = number of mounts without intromission from the time of introduction of the female); intromission frequency (IF= number of intromissions from the time of introduction of the female); ejaculation frequency (EF= number of ejaculations from the time of introduction of the female). Copulatory efficiency percent (%) was calculated as IF/MF*100.

Serum collection and Organ weights

On day 30, rats were weighed and deeply anaesthetized with sodium pentobarbital (65mg/kg IP). Blood was collected by cardiac puncture into plain tubes and serum collected by centrifugation (3000 x g for 10 minutes) and stored at -70^{o}C until used to assay for total testosterone. Reproductive organs (testes, epididymis, prostate gland, seminal vesicles) were dissected, excess fat cleaned off and weighed.

Sperm count and motility

Right cauda epididymis was separated and used for sperm count using the method of (Robb et al., 1978) and as we describe previously (Ralebona et al., 2012). Briefly, the isolated cauda epididymis was placed in a petri dish with 10 ml of warmed buffered normal saline, pH 7.2. It was minced using a pair of scissors and the petri dish placed at 37ºC to allow sperm to swim into the warmed normal saline to form a suspension. A tissue-free aliquot was further diluted (6x) and loaded onto the Neubauer haemocytometer (Deep 1/10mm, Labart, Germany). Five squares were counted in triplicate per sample. Sperm count was calculated and reported as millions of sperm/ml. Total motility was assessed by counting motile sperm vs non motile sperm. Motile sperm was expressed as a percentage of total sperm counted to give the value of sperm motility.

Serum total Testosterone

Total testosterone (ng/ml) in serum was measured using competitive inhibition enzyme immunoassay technique (Uscn Life Sciences Inc).

The procedure was performed as described in the manufacturer's manual. Minimal detection dose for the testosterone assay was 0.050 ng/ml, with negligible cross-reactivity between testosterone and its analogues. All samples were run in a single assay. Intraassay coefficient of variation was 10%.

Statistical analysis

Results were expressed as mean ± standard error of mean (SEM). Statistical analysis was done using GraphPad Prism (Version 6). Data was compared using the non-paired ANOVA followed by Dunnett's multiple comparison test, comparing each mean to the untreated control. Values were considered significantly different when $P \leq 0.05$.

Results

Sexual behavior

The effects of the various extracts on sexual behaviour are summarized in Tables 2 to 4. Single dose treatment followed by sexual behavior observations 2 hours after treatment showed an increase ($P<0.05$) in all aspects of sexual activity in the *M. whitei* - treated animals compared to untreated controls. There was no change ($P>0.05$) in sexual behavior parameters for the other treatment groups (Table 2).

http://dx.doi.org/10.4314/ajtcam.v12i2.5

Sexual behavior after daily treatments for 14 days showed an increase (P<0.05) in all aspects of sexual behavior, except for number of ejaculations, for *M. whitei* and *S. birrea* fruit juice extract treated animals. *C. pepo* showed an increased (P<0.05) copulatory efficiency compared to controls, with the other behavior parameters remaining similar to controls. Sexual behaviour parameters for the other treatment groups remained similar to controls (Table 3).

The 14-day sexual behavior trend was maintained to day 28 of daily treatment for *M. whitei, S. birrea* fruit juice extract and *C. pepo* treated animals. The *C. album* treated group also showed an increase in copulatory efficiency compared to untreated controls (Table 4).

Table 2. Single dose effects of treatment with extracts on male sexual behavior.

	Control	*M.whitei*	*C.album*	*C.pepo*	*S.birrea*
ML (sec)	71.2±4.3	49.2±4.5*	62.0±4.6	61.3±3.4	60.7±5.1
IL (sec)	75.7±2.3	58.3±5.4*	77.0±4.4	73.7±2.9	73.2±4.9
MF	20.7±1.1	32.5±3.4*	25.8±3.7	26.0±2.0	26.0±2.5
IF	14.7±1.6	23.0±2.5*	16.8±2.3	16.3±1.8	17.3±1.8
CE (%)	60.7±2.7	70.7±1.5*	65.8±3.6	62.3±2.8	67.0±2.9
Ejac	0.5±0.2	1.3±0.2*	0.5±0.2	0.7±0.2	0.7±0.2

Data are presented as mean ±SEM. N=6/group. Significant difference compared to control *P<0.05.

Table 3: Effects of treatment with extracts for 14 days on male sexual behavior.

	Control	*M. whitei*	*C. album*	*C. pepo*	*S. birrea*
ML (sec)	51.0±2.2	40.3±2.7*	56.2±3.0	48.2±3.3	43.5±4.4*
IL (sec)	65.7±2.4	50.7±3.1*	64.7±3.3	57.2±4.1	52.5±4.3*
MF	28.7±3.2	38.8±3.0*	30.7±1.8	34.2±2.1	38.3±2.8*
IF	19.8±2.6	34.0±2.4**	21.8±1.5	26.7±2.0	31.2±2.5**
CE (%)	68.5±1.9	87.7±1.6**	71.3±2.2	78.0±2.7*	81.3±1.8**
Ejac	1.0±0.4	1.5±0.2	1.2±0.4	1.0±0.3	1.5±0.2

Data are presented as mean ±SEM. N=6/group. Significant difference compared to control *P<0.05; **P<0.01.

Table 4: Effects of treatment with extracts for 28 days on male sexual behavior.

	Control	*M.whitei*	*C.album*	*C.pepo*	*S.birrea*
ML (sec)	50.7±2.4	35.3±1.7*	47.7±3.1	40.3±3.3	41.3±4.5
IL (sec)	62.7±3.3	47.3±2.6*	56.7±2.9	52.3±3.2	48.7±4.3*
MF	31.7±2.3	41.8±2.4**	34.7±1.6	34.7±1.9	44.7±3.0**
IF	22.3±1.9	38.2±2.2**	27.2±1.5	27.2±2.2	39.3±3.0**
CE (%)	70.3±1.2	91.3±1.0**	78.0±1.5*	78.0±2.7*	87.8±1.3**
Ejac	1.2±0.2	1.7±0.2	1.3±0.3	1.5±0.2	1.7±0.2

Data are presented as mean ±SEM. N=6/group. Significant difference compared to control *P<0.05; **P<0.01.

Reproductive organ weights

The reproductive organ weights for all treatment groups remained similar compared to untreated controls (Table 5).

Table 5: Effects of treatment with extracts for 28 days on reproductive organ weights.

	Reproductive organ weights (grams)			
	Testes	Epididymis	Prostate	Seminal vesicles
Control	3.12±0.15	1.31±0.10	0.82±0.08	0.98±0.05
M.whitei	3.38±0.13	1.29±0.08	0.68±0.03	1.29±0.11
C.album	3.21±0.09	1.34±0.07	0.76±0.11	1.27±0.07
C.pepo	3.14±0.12	1.31±0.07	0.89±0.06	1.07±0.14
S.birrea	3.13±0.13	1.16±0.05	0.66±0.04	0.98±0.03

Data are presented as mean ±SEM. N=6/group.

Sperm parameters and testosterone levels

Treatment had no effect on sperm counts for all treatment groups. However, all treatment groups showed an increase (P<0.05) in sperm motility compared to untreated controls. *M. whitei* and *C.pepo* treated animals showed an increase in serum testosterone levels compared to controls whereas *C. album* and *S. birrea* treatment groups remained similar to controls (Figure 1).

Discussion

Assessment of sexual behavior in male rats encompasses components of arousal, erection and ejaculation. In the presence of a receptive female, male rats undergo a predictive series of mounts and intromissions culminating in ejaculation. Male rat mating behavior involves interaction of olfactory stimulation by female pheromones that augment actions of androgens centrally with possible stimulation of prosexual central neurotransmitters such as dopamine (Leonelli et al., 2011; Cavalcante et al., 2006). Sexual behavior parameters assessed in this study included an arousal component (mount and intromission latencies); libido and erectile response also referred to as potency (mount and intromission frequency) and ejaculations (Vyawahare et al., 2012; Sach and Barfield., 1970). Mount frequency (MF) is an indication of libido or sexual motivation while intromission frequency (IF) gives a measure of erectile efficiency and penile orientation (Agmo, 1997). Copulatory efficiency is the proportion of mounts that gain penile insertion. Since penile erection is required for penile insertion into the vagina – copulatory efficiency gives a measure of erectile function in the male rat. In addition to testosterone playing a role in erection, local factors especially nitric oxide (NO), are responsible for penile smooth muscle vasodilation and erection (Sharma et al, 2012).

Results of this study demonstrated that *M. whitei* at 200mg/kg was most effective in enhancing sexual activity in male rats compared to the other extracts tested. Prosexual effects were evident throughout the study, including after a single dose. Erectile effects of *M. whitei* on corpus cavernosal tissue have been demonstrated and attributed to an increase in NO (Quasie et al., 2010; Watcho et al., 2007b). Effects of *M. whitei* in increasing sperm motility and testosterone levels

http://dx.doi.org/10.4314/ajtcam.v12i2.5

have also been demonstrated (Watcho et al, 2007a; Lampiao et al., 2008; Gundidza et al., 2009). The sustained stimulatory effects of *M. whitei* on sexual behavior over the 28 day study period may be attributed to the observed increased testosterone levels, in addition to possible local cavernosal effects (Watcho et al, 2007a). *M. whitei* sexual behavior stimulatory effects were maintained throughout the study period, serving well as a positive control. *S. birrea* (marula) fruit juice and pulp extract was the next most effective in stimulating sexual performance in male rats. The prosexual effects were evident when behavior was tested after 14 days of daily treatment, with a dramatic increase in arousal (ML and IL), sexual potency (MF and IF) and erectile function (IF and copulatory efficiency), similar to *M. whitei*. However, there was no effect on ejaculatory function in both groups. This is the first time that the aphrodisiac effects of this marula fruit have been demonstrated, validating the traditional claims in traditional culture. However, these effects may not be testosterone dependent since testosterone levels remained similar to controls. Further investigations into androgen receptor numbers, inducible NO synthase and central dopamine levels may elucidate mechanisms of action of *S. birrea* fruit extract. Suffice to say, prosexual effects were maintained through day 28 of treatment. *C. pepo* treatment resulted in increased erectile function after 14 days of treatment as shown by increased copulatory efficiency. This effect was accompanied by increased testosterone levels – also validating traditional pumpkin seed use for increasing "vitality" through increased testosterone levels. A previous study showed positive aphrodisiac effects of *C.pepo* mixed with other plant extracts not on its own (Gundidza et al, 2009). Thus *C.pepo* may augment aphrodisiac effects of other plants via its androgenic effects. After 28 days of treatment, *C. album* increased erectile function as shown by the increased copulatory efficiency with no change on arousal, libido and ejaculation. Unlike our findings with *C. album*, previous findings reported that *C. album* treatments in male mice showed an increase MF, IF, IL and ejaculations (Pande and Pathak, 2008).

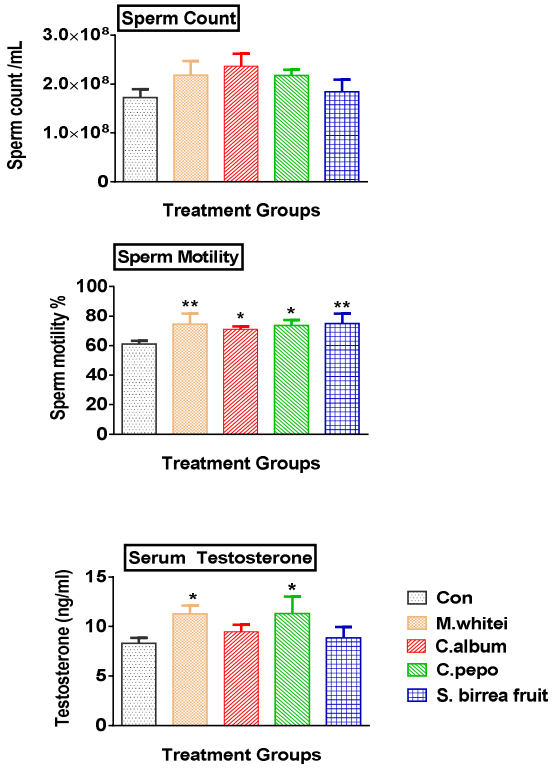

Figure 1: Comparison of effects of treatment on sperm counts, sperm motility and serum testosterone levels. Data are presented as mean ± SEM. *P<0.05; **P<0.01

In all treatment groups in this study, no effects on sperm count were observed. One complete spermatogenic cycle takes 58 days in the rat (Franca et al. 1998). Since our study was less than 58 days, spermatogonia exposed to treatment had not been deposited into the epididymis for observation. However, these findings imply extracts had no deleterious effects on the maturing and already formed sperm since counts remained similar to untreated controls over the 28 day treatment period.

All treatment groups showed increased sperm motility. An increase in oxidative stress can cause metabolic or functional disorders of spermatozoa reducing sperm motility (Li et al., 2009). *S. birrea* fruit and *S. pepo* seed have been shown to possess polyphenols and high antioxidant activity (Borochov-Neori et al., 2008; Steinberg, 1991; Kunyanga et al, 2012). Thus plant extracts in this study may increase sperm motility via a reduction in oxidative stress. It would be useful to measure oxidative parameters in testis homogenates of extract treated animals to clarify this hypothesis.

We have demonstrated varying degrees of aphrodisiac activity in food plants claimed to possess aphrodisiac activity. Our study validates traditional use of these plants in increasing aspects of sexual potency. Indeed, traditionally, aphrodisiacs are used as formulations, combinations of two or more plants. The preferential effects of different plants on testosterone production, libido and erection may augment sexual function when taken in combination. Further studies are warranted to further elucidate effects of various doses, longer treatment periods and effects of plant combinations.

Acknowledgments

We acknowledge the Medical Research Council of South Africa for financial support.

References

1. Abdillahi, H.S. and Van Staden, J. (2012). African plants and male reproductive healthcare: Conception and contraception. J. Ethnopharmacol., 143:475-480.
2. Agarwal, A., Nandipati, K.C., Sharma, K.R., Zippe, C.D. and Raina, R. (2006). Role of oxidative stress in the review pathophysiological mechanism of erectile dysfunction. J. Androl., 7(3):335-347.
3. Agmo, A. (1997). Male rat sexual behavior. Brain Res Protoc., 1(2):203-209.

http://dx.doi.org/10.4314/ajtcam.v12i2.5

4. Borochov-Neori, H., Judeinstein, S., Greenberg, A., Fuhrman, B., Attias, J., Volkova, N., Hayek, T. and Aviram, M. (2008). Phenolic antioxidants and antiatherogenic effects of marula (*Sclerocarrya birrea* subsp. caffra) fruit juice on healthy humans. J. Agri. Food Chem., 6:9884-9891.
5. Caili, F., Huan, S. and Quanhong, L. (2006). A review on pharmacological activities and utilization technologies of pumpkin. Plant. Foods. Hum. Nutr., 61:73-80.
6. Carro-Juárez, M., Lobatón, I., Benítez, O. and Espíritu, A. (2006). Pro-ejaculatory of aqueous crude extract of cihuapatli (Montanoa tomentosa) in spinal male rats. J. Ethnophamacol., 106:111-116.
7. Cavalcante, J.C., Bittencourt, J.C. and Elias, C.F. (2006). Female odors stimulate CART neurons in the ventral premammillary nucleus of male rats. Physiol. Behav., 88:160-166.
8. Crouch, N.R., Symmonds, R., Nichols, G. and Crouch, T. (1998). Propagation techniques and cultivation ideas for *Mondia whitei* (Periplocaceae), a Zulu muthi of distinction. PlantLife, 18:26–27.
9. Franca, L.R., Ogawa, T., Avarbork, M.R., Brinster, R.L. and Russel, L.D. (1998). Germ cell genotype controls cell cycle during spermatogenesis in the rat. Biol. Reprod., 59(6):1371-1377.
10. Gelfand, M.S., Mavi, R.B., Drummond, N. and Ndemera, B.B. (1985). The traditional medicinal practitioner in Zimbabwe. Mambo Press, Gweru.
11. Gundidza, G.M., Mmbengwa, V.M., Magwa, M.L., Ramalivhana, N.J., Mukwevho, N.T., Ndaradzi, W. and Samie, A. (2009). Aphrodisiac properties of some Zimbabwean medicinal plants formulations. Afr. J. Biotechnol., 8 (22):6402-6407.
12. Kunyanga, C.N., Imungi, J.K., Okoth, M.W., Biesalki, H.K. and Vadivel, V. (2012). Total phenolic content, antioxidant and antidiabetic properties of methanolic extract of raw and traditionally processed Kenyan indigenous food ingredients. Food Sci. Technol., 45:269-276.
13. Lampiao, F., Krom, D. and du Plessis, S.S. (2008). The in vitro effects of *Mondia whitei* on human sperm motility parameters. Phytother Res., 22(9):1272-1273.
14. Leonelli, C., Garcia, P.C. and Pereira, O.C. (2011). Copulatory efficiency and fertility in male rats exposed perinatally to flutamide. Reprod. Toxicol., 31:10-16.
15. Li, P., Hulak, M. and Linhart, O. (2009). Sperm proteins in teleostean and chondrostean (sturgeon) fishes. Fish. Physiol. Biochem., 35:567-581.
16. Malviya, N., Jain, S., Gupta, V.B. and Vyas, S. (2011). Effect of garlic bulb on paroxetine-induced sexual dysfunction in male rats. Asian. J. Pharm. Biol. Res., 1(2):218-221.
17. Ndhlala, A.R., Stafford, G.I., Finnie, J.F. and Van Staden, J. (2011). Commercial herbal preparations in KwaZulu-Natal, South Africa: The urban face of traditional medicine. S. Afr. J. Bot., 77(4):830-843.
18. Pande, M. and Pathak, A. (2008). Sexual function improving effect of *Chenopodium album* (Bathua sag) in normal male mice. Biomed. Pharmacol. J. 1:325-332.
19. Prabsattroo, T., Wattanathorn, J., Iamsa-ard, S., Muchimapura, S. and Thukhammee, W. (2012). *Moringa oleifera* leaves extract attenuates male sexual dysfunction. Am. J. Neuroscience, 3(1):17-24.
20. Quasie, O., Martey, O.N.K., Nyarko, A.K., Gbewonyo, W.S.K. and Okine, L.K.N. (2010). Modulation of penile erection in rabbits by *Mondia whitei*: Possible mechanism of action. Afr. J. Trad. CAM., 7(3): 241–252.
21. Ralebona, N., Sewani-Rusike, C.R., and Nkeh-Chungag, B.N. (2012). Effects of an ethanolic extract of *Garcinia kola* on sexual behaviour and sperm parameters in male Wistar rats. Afr. J. Pharm. Pharmacol., 6(14):1077–1082.
22. Robb, G.W., Amann, R.P. and Killian, G.J. (1978). Daily sperm production and epididymal sperm reserves of pubertal and adult rats. J. Reprod. Fertil., 54:103-107.
23. Sachs, B.D. and Barfield, R.J. (1970). Temporal patterning of sexual behavior in the male rat. J. Comp. Physiol. Psychol., 73:359-364.
24. Seftel, A.D., Mack, R.J., Secrest, A.R. and Smith, T.M. (2004). Restorative increases in serum testosterone levels are significantly correlated to improvements in sexual functioning. J. Androl., 25:963-972.
25. Shackleton, S.E., Shackleton, C.M., Cunningham, A.B., Lombard, C., Sullivan, C.A. and Netshiluvhi, T.R. (2002). A summary of knowledge on *Sclerocarya birrea* subsp. *caffra* with emphasis on its importance as a non-timber forest product in South and southern Africa. Part 1: Taxonomy, ecology, traditional uses and role in rural livelihoods. S. Afr. For. J., 194:27–41.
26. Sharma, V., Thakur, M. and Dixit, V.K. (2012). A comparative study of ethanolic extracts of *Pedalium murex* Linn. fruits and sildenafil citrate on sexual behaviors and serum testosterone level in male rats during and after treatment. J. Ethnopharmacol., 143: 201-206.
27. Steinberg, D. (1991). Antioxidants and atherosclerosis. A current assessment. Circulation, 84:1420-1425.
28. Sumalatha, K., Kumar, A.S. and Lashmi, S.M. (2010). Review of natural aphrodisiac potentials to treat sexual dysfunction. Int. J. Pharm. Ther., 1(1):6-14.
29. Thakur, M., Chauhan, N.S., Bhargava, S. and Dixit, V.K. (2009). A comparative study on aphrodisiac activity of some Ayurvedic herbs in male Albino rats. Arch. Sex. Behav., 38:1009-1015.
30. Tostes, R.C., Carneiro, F.S., Lee, A.J., Giachini, F.R.C., Leite, R., Osawa Y. and Webb, R.C. (2008). Cigarette smoking and erectile dysfunction: focus on NO bioavailability and ROS generation. J. Sex. Med. 5(6):1284-1295.
31. Van Wyk, B.E., Van Oudtshoorn, B. and Gericke, N. (1997). Medicinal plants of South Africa. Briza Publications, Pretoria.
32. Vyawahare, N.S., Hadambar, A.A., Chothe, A.S., Jalnapurkar, R.R., Bhandare, A.M. and Kathiravan, M.K. (2012). Effect of novel synthetic evodiamine analogue on sexual behavior in male rats. J. Chem. Biol., 5:35-42.
33. Wani, B.A., Ganai, B.A., Ganaie, A.H., Bodha, R.H. and Mohiddin, F.A. (2011). Plants as repository of aphrodisiacs. J. Pharm. Res., 4(11):3882-3887.
34. Wani, J.A., Rajeshwara, N.A. and Nema, R.K. (2011). Phytochemical screening and aphrodisiac activity of *Asparagus racemosus*. IJPSDR, 3(2):112-115.
35. Watcho, P., Zelefack, F., Nguelefack, T.B., Ngouela, S., Bo Telefo, P., Kamtchouing, P., Tsamo, E. and Kamanyi, A. (2007a). Effects of aqueous and hexane extracts of Mondia whitei on the sexual behavior and some fertility parameters of sexually inexperienced male rats. Afr. J. Trad. CAM., 4(1):37-46.
36. Watcho, P., Djoukeng, C.N., Zelefack, F., Nguelefack, T.B., Ngouela, S., Kamtchouing, P., Tsamo, E. and Kamanyi, A. (2007b). Relaxant effect of *Mondia whitei* extracts on isolated guinea pig corpus cavernosum. Pharmacologyonline 2:44-52.
37. Watcho, P., Kamtchouing, P., Sokeng, S.D., Moundipa, P.F., Tantchou, J., Essame, J.L. and Koueta, N. (2004). Androgenic effect of *Mondia whitei* roots in male rats. Asian J Androl., 6:269-272.
38. Yadav, N., Vasudeva, N., Singh, S. and Sharma, K.S. (2007). Medicinal properties of genus *Chenopodium* Linn. IJNPR, 6(2):131-134.
39. Yakubu, M.T., Akanji, A.M. and Oladiji, A.T. (2005). Aphrodisiac potentials of the aqueous extract of *Fadogia agrestis* (Schweinf. Ex Hiern) stem in male albino rats. Asian J. Androl., 7:399-404.
40. Yakubu, M.T., Akanji, M.A. and Oladiji, A.T. (2007). Male sexual dysfunction and methods used in assessing medicinal plants with aphrodisiac potentials. PHCOG. Rev., 1(1):49-52.
41. Zamblé, A., Sahpaz, S., Brunet, C. and Bailleul, F. (2008). Effects of *Microdesmis keayana* roots on sexual behavior of male rats. Phytomed., 15:625-629.
42. Zhang, Q., Radisavljevic, Z.M., Siroky, M.B. and Azadzoi, K.M. (2011). Dietary antioxidants improve arteriogenic erectile dysfunction. Int. J. Androl., 34: 225-235.