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Research Article

Effects of Methanolic Crude Extracts *Inula glomerata* and *Salacia kraussii* on Erectile Dysfunction

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ABSTRACT

Inula glomerata Oliv. & Hiern and *Salacia kraussii* (Harv.) Harv are medicinal plants used by traditional healers in remote areas of Mbazwana, Northern Kwazulu-Natal, South Africa for ameliorating male sexual disorders including erectile dysfunction (ED), low sperm count and early ejaculation. The study aimed at determining the ameliorative effect of the methanolic crude extracts of *Inula glomerata* (I. glomerata) and *Salacia kraussii* (*S. kraussii*) on butanol-induced erectile dysfunction in Sprague Dawley rats. The crude extract was prepared by maceration using methanol. Animal study was conducted whereby thirty-five male Sprague Dawley rats were divided into seven experimental groups: normal group, n-but (10 mg/kg), n-but+ Ig (50 and 250 mg/kg), n-but+ Sk (50 and 250 mg/kg) and n-but+ Cialis (5mg/kg). The experiment lasted for 28 days, after which various biochemical assays (acetylcholinesterase, ACE, arginase, testosterone, and uric acid) was done. The cytotoxicity of the crude extracts was also determined. The results revealed that n-butanol induced erectile dysfunction in the rats by decreasing mounting frequency, testosterone and nitric oxide level and simultaneously elevated the activities of arginase and acetylcholinesterase. The plants however, inhibited arginase and acetylcholinesterase when compared to the untreated. Furthermore, the plants' extracts were able to increase the level of testosterone and nitric oxide. It can be inferred that both plants could be promising natural therapy for erectile dysfunction. Nonetheless, the plants' extracts are toxic hence should be taken with caution.

Keywords: *Erectile dysfunction, arginase, acetylcholinesterase, cytotoxicity, ACE, testosterone, Nitric oxide.*

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INTRODUCTION

Penile erection is a coordinated neurovascular process that involves the nervous, endocrine, vascular system, and the penile histo-architecture (Andersson, 2011; Fraga-Silva *et al*., 2013; Castelló-Porcar and Martínez-Jabaloyas, 2016). Therefore, impediments or changes in the neural, vascular and fibroblast structure of the penile tissues have been implicated in the pathogenesis of erectile dysfunction (Ojewole, 2007; Rew and Heidelbaugh, 2016). Erectile dysfunction affects mostly men above the age of 40 years and becomes more severe with increasing age (Lewis *et al*., 2010; Yassin *et al*., 2016). Previous studies show that erectile dysfunction shares common risk factors with cardiovascular diseases including diabetes, smoking, chronic alcohol consumption and obesity (Feldman *et al*., 2000; Fung *et al*., 2004). Although there are accumulations of evidence that erectile dysfunction is a combination of organic and psychogenic causes, most cases however, are attributed to organic causes (Gareri *et al*., 2014; Bella *et al*., 2015; Olabiyi *et al*., 2017). Several pathological conditions including hyperactivities of arginase and acetylcholinesterase through a common denominator known as endothelial dysfunction lead to erectile dysfunction (Olabiyi *et al*., 2017; Decaluwé *et al*., 2014; Morris, 2009; Oboh *et al*., 2015). Hypogonadism (low testosterone) and increased actions of ACE: an enzyme that degrades bradykinins and promotes angiotensin (II) production also contribute to erectile dysfunction (Fraga-Silva *et al*., 2013;

Castelló-Porcar and Martínez-Jabaloyas, 2016; Jin, 2009; Helo *et al*., 2018; Adefegha *et al*., 2018).

The mainstay treatment and management of erectile dysfunction has been the use of phosphodiesterase 5 inhibitors, but their associated side effects have limited their use (Oboh *et al*., 2015; Whittaker, 2010; Saxena *et al*., 2012). However, the exploration of medicinal plants as an alternative therapy continues to gain much interest in recent times mostly due to their little or no side effect, as well as availability to rural communities (Carlson, 2002; Dey and De, 2015).

Salacia kraussii is a wild fruit with runner underground stems that are commonly distributed in the Kwazulu Natal province of South Africa. It is popularly called Ibhonsi and belongs to the family known as Celastraceae and the genus Hippocrateacea (Raimondo *et al*., 2009). This plant possesses anti-diarrhoea and anti-malaria potential (Bandeira *et al*., 2001). In Mbazwana, a region that is close to Manguzi, located in the Northern region of Kwazulu-Natal, the wild fruits of *S. kraussii* are eaten as food and decoction mixture of the roots with other medicinal plants taken once daily are believed to improve sexual potency (submissions gathered from the responses to the questionnaires given to 20 traditional healers). *Inula glomerata*belongs to the genus Inula and the family Asteraceae. It is locally called Nzeveyatsuro, meaning the hare's ears in Zimbabwe. *Inula glomerata*is 2m tall with a basal rosette leaves of 45 by 20cm in size that has irregularly toothed margin and endemic to Northern South Africa, Zimbabwe, Angola and Tanzania (Burrows and Willis, 2005). Much of this plant is unknown, however, aqueous infusion of the roots and leaves taking twice daily by locals especially the male folks residing in Sodwana Bay, Northern region of Kwazulu-Natal are used for the treatment of hypertension and to enhance sexual performance respectively (personal communication with most of the traditional healers that were interviewed). In addition, a closely related species, Inula racemosa Hook.f. possess several bioactivities and are used traditionally to manage tuberculosis, angina, dyspnoea and improve insulin sensitivity (Tan *et al*., 1998; Arumugam *et al*., 2012). The root of *Salacia kraussii* and leaves of *Inula glomerata*are used by more than eighty-five percent (85 %) of traditional healers interviewed in Kwazulu-Natal for treating erectile dysfunction but have not been validated scientifically.

There appears to be a dearth of information on the medicinal properties of these plants (except for *S. kraussii* with known anti-malaria and anti-diarrhoea properties). Therefore, the present work focuses on evaluating the inhibitory effect of the methanolic extracts of these plants on arginase and acetylcholinesterase, their modulatory capacity on the level of testosterone, nitric oxide, and uric acid as well as their cytotoxicity.

MATERIALS AND METHODS

Chemicals: Analytic grade chemicals and kits used in this study were purchased from Sigma Aldrich Co.Ltd (Steinheim, Germany).

Plant Identification: The roots of *Salacia kraussii* (Harv.) Harv and leaves of *Inula glomerata* Oliv. & Hiern were collected from Mbazwana (27º 15'11.3" S 32º28'14.3" E) KwaZulu Natal, South Africa. The names of the plants were accessed on 27-05-2020 from http://www.the plantlist.org. The plants were authenticated at the Department of Botany, the University of Zululand. The plants with specimen numbers V04 and V06 respectively were deposited at the university's herbarium.

Plant Extraction: The plants were rinsed with tap water and air-dried at room temperature. Pulverized plants samples (200 mg) were macerated with methanol (1:5 w/v) with the aid of a Labcon orbital mechanical shaker (150 rpm; 25ºC) for 72 h. The extracts were filtered (Whatman filter paper no 1) and the filtrate concentrated in vacuo (Heidolph rotary evaporator 40ºC).

Phytochemical screening: The methods of Odebiyi and Sofowora (Odebiyi OO, Sofowora, 1978), as well as Harbone (Harbone, 1973) were used to analyze the pulverized samples for the presence of phytochemicals. The phytochemicals screened for were Tannins, saponins, flavonoids, alkaloids, terpenoids, and steroids.

Cytotoxicity Assay: Cytotoxicity study for the crude extracts was determined against the HEK293 and Hela cell lines using the MTT assay as described by Mosmann, (Mosmann, 1983). The cells were first plated in 96-micro plate well with a cell suspension of 1.8×104 cells/ml. Thereafter, they were seeded with different concentrations of the crude extract (25, 50, 100 and 200 µg/ml). 1 % FBS was then added into the medium and incubated for 48 hours for the cells to attach. Afterwards, tetrazolium salt was added as a cytotoxicity indicator after the old medium had been removed. 100 µl of the medium was then added to each well and incubated for 4 hours at 37 ºC. The Formazan crystals formed from the aspirated media were solubilized in 100 µl of dimethyl sulfoxide. The absorbance was read at 570 nm with a mindray-96A microplate reader. The percentage inhibition of cell viability was calculated using the formula:

$$\% \text{ cell death} = ((Ac-At)/Ac \times 100))$$

where Ac and At represent control and sample absorbance respectively.

Animal experiment: Ethical clearance certificate (UZREC 171110-030 PGM 2018/576) was obtained from the University of Zululand Ethics committee for the use of animal experiments. The standard operating procedures for experimental animals according to Public Health Service (PHS) policy (OLAWPHS, 2002) were adhered to. Thirty-five male Sprague-Dawley rats (250 g) of 10 weeks old were collected from the animal house of the Department of Biochemistry and Microbiology, University of Zululand. The animals were housed in standard cages and kept under conducive environmental conditions (250C; humidity~ 50%; 12:12 light: dark cycle) with free access to safe drinking water and pellet feeds. The animals were acclimatized for 5 days before the commencement of the experiment.

Animal model: The method of Garza *et al* (Garza *et al*., 2015) with slight modification was followed to perform the animal experiment. Thirty-five male Sprague-Dawley rats weighing

between 250 g were collected and randomly divided asymmetrically into seven groups (5 rats/group). Erectile dysfunction was induced with n-butanol which was injected interperitoneally (5 mg/kg b.w) for four days at two days interval. Afterward, each animal was allowed to mate with two estrous female rats (injected with estradiol 7.5 mg/kg b.w for 2 days at 24 h interval) to establish the baseline of their sexual prowess. Group 1, normal control, was not disabled with n-butanol and did not receive any treatment. Group 2, positive control, was treated with Cialis 5 mg/kg b.w. Group 3 and 4 were treated with 50 and 250 mg/kg b.w of *Inula glomerata*respectively. Group 5 was treated with 50 mg and group 6 with 250 mg/kg b.w of *Salacia kraussii*. Group 7 disabled but untreated group. All the animals had free access to feed (commercial ret chow) and water throughout the experiment period. The dosage used for the crude extracts (50 and 250 mg) was chosen due to previous studies by Cele *et al* (Cele *et al*., 2017). The medication was given orally with the aid of a gavage daily for 28 days. At the end of the experiment, the rats were fasted for 18 hr and then euthanized under anesthesia (pentobarbital@ 30 mg/kg body weight, intraperitoneally). Before further procedures was carried out on the rats, complete loss of sensation confirmed by pedal withdrawal reflex was ensured. Blood was immediately collected by open cardiac puncture into a 50 ml centrifugation tube. After the blood had clotted, it was centrifuged at 1200 rpm for 10 min in a Rotofix mini-centrifuge machine. The obtained serum was stored at −80 °C for subsequent biochemical assays.

Biochemical estimation of liver function biomarkers, testosterone, and uric acid: The level of testosterone and uric acid in the serum were analyzed using standard pathology laboratory procedures (Global laboratory & Viral Laboratory, Richards Bay).

Biochemical estimation of arginase and acetyl cholinesterase: The serum activities of arginase and acetylcholinesterase were determined using the respective standard commercial assay kit (Sigma- Aldrich), following the manufacturer's instructions.

Determination of serum nitric oxide level: The accumulation of nitrite in the serum was determined following the method of Lidija *et al* (Lidija *et al*., 2004). Griess reagent (0.1% N-1-napthylethyleneaminediyhdrochoride, 1% sulphanilic acid and 2.5% phosphoric acid, 125 µl) and the serum was mixed and plated in a 96- well microplate. Thereafter, the mixture was incubated at room temperature for 10 min and the absorbance was read with Biotek plate reader (Synergy HT) at 546 nm. The nitrite level in the serum was extrapolated from the standard curve of sodium nitrite and expressed as mg/ml.

Sexual Behaviour Protocol: Estrous female rats were used to investigate the sexual behaviour of the male rats in different groups. Estrous was induced in the female rats with progesterone at 7 mg/kg b.w for two days at a twenty-four hours interval. Before the commencement of the sexual behavioural studies, the behaviours of the female rats were observed for four hours after progesterone administration. In order to monitor the sexual behaviours of the male rats, a male rat was introduced into a cage with two estrous female rats in a separate room for thirty minutes. Mounting number (the number of mounts without intromission from the time of introduction of the female rats to the male rat), mount latency (time from introduction of the female rats until the first mount with pelvic thrusting), intromission number (the number of intromissions from the time of introduction of the female rats until the end of the experiment), intromission latency (time from introduction of the female rats until the first mount with pelvic thrusting and vaginal penetration) were parameters monitored by a video recording with an IPAD, and used to determine the sexual behaviours of the rats.

Data analysis: All experiments were carried out in triplicates and data was expressed as mean ± standard error of the mean. The data were analyzed with a one-way analysis of variance (ANOVA) and IC50 values were determined using the graph pad prism.

RESULTS

Percentage Yield: The crude extracts' yield of *Inula glomerata*was 8.5 % while that of Salacia krausii was 5.97 %.

Cytotoxicity test: The cytotoxicity of the extracts on HEK293 and Hela cell lines is indicated in Table 1. The result showed that both plants' extracts were cytotoxic but *Salacia kraussii* exhibited stronger cytotoxicity.

Sexual behaviour outcome: The mounting frequency was lowered in the n-butanol induced rats (untreated) compared to normal as illustrated in table 2. Both plants significantly increased the mounting frequency compared to the untreated and normal rats. However, *Inula glomerata*was more effective in boosting the mounting frequency of both plants.

Table 1:
The outcome (IC_{50} values µg/ml) of the crude extracts on HEK293 and Hela cells viability

Crude extract	HEK 293 (µg/ml)	Hela (µg/ml)
Inula glomerata	23.3 ± 0.036[a]	0.000
Salacia kraussii	18.7 ± 0.042[a]	19.3 ± 0.054[b]

Data are presented as mean ± SD, n= 3, The same alphabets denotes no significant different P>0.05

Table 2:
Effect of *Inula glomerata* and *Salacia krausii* on the sexual behaviour of n-butanol induced erectile dysfunction rats.

Groups	Mounting frequency
Normal control	20.00 ± 0.00[a]
n-butanol + Cialis	35.67 ± 0.33[b]
n-butanol + *Inula glomerata* 50 mg b.w	26.33 ± 0.33[c]
n-butanol + *Salacia kraussii* 50 mg b.w	23.33 ± 0.33[d]
n-butanol + *Inula glomerata* 250 mg b.w	0.00
n-butanol + *Salacia kraussii* 250 mg b.w	0.00
n-butanol (Untreated)	10.33 ± 0.33[e]

The values represent mean ± SD (n = 5). b.w body weight, different alphabets show a significant difference, P<0.05.

The effect of the crude extracts on arginase activity: Increased arginase activity was observed in the n-butanol induced rats as depicted in figure 1. *Inula glomerata*significantly lowered arginase action in a dose-dependent manner and *Salacia kraussii* only at high concentration diminished arginase activity. Although both plants inhibited arginase activities better than Cialis, *Salacia kraussii* exhibited the best inhibitory efficacy against arginase.

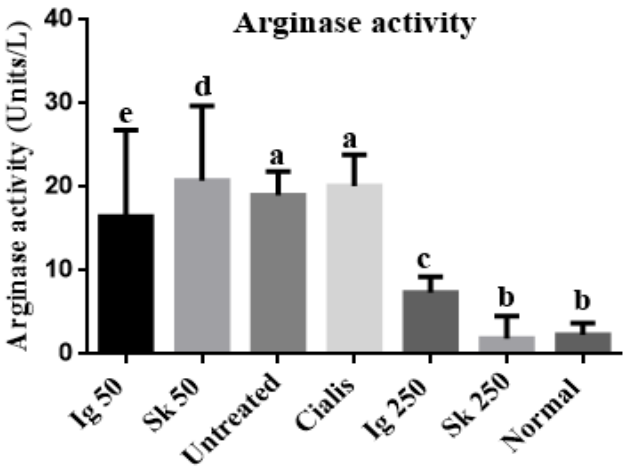

Figure 1
Effect of crude extracts on arginase activity. Data expressed as mean ± SD, n=5. The same alphabets show no significant difference (P>0.05) while different alphabets show a significant difference (P<0.05).

The Effect of The Crude Extracts on Acetylcholinesterase: Acetylcholinesterase inhibitory effects of the plant's extracts are presented in figure 2. Crude extracts of both plants significantly reduced the action of acetylcholinesterase in a concentration-dependent manner. However, acetylcholinesterase inhibitory efficacy of *Inula glomerata*was more effective at lower concentration as shown in figure 2.

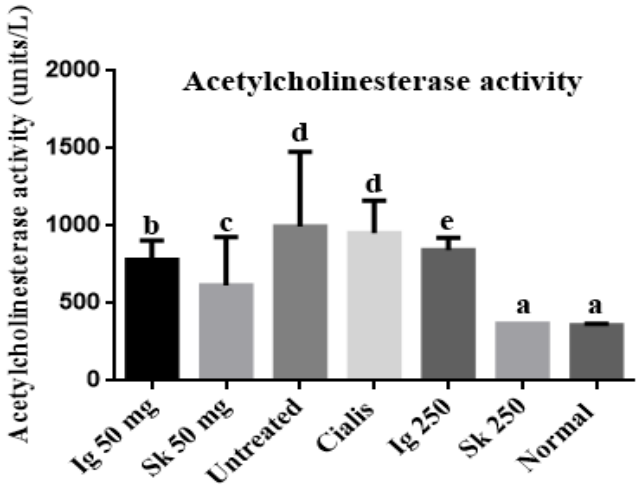

Figure 2:
Inhibitory effect of the crude extracts on acetylcholinesterase activity. Data expressed as mean ± SD, n=5. The same alphabets show no significant difference (P>0.05) while different alphabets show a significant difference (P<0.05).

The Effect of The Extracts on Angiotensin (1) Converting Enzymes (ACE) Activity: Angiotensin (1) converting enzymes (ACE) activities were increased in the n-butanol induced rats but were inadvertently reduced by the dose of the crude extract independently as revealed in figure 3. In addition, at low concentrations (50 mg/ml), both crude extracts reversed ACE activity to normal but there was no significant difference with Cialis.

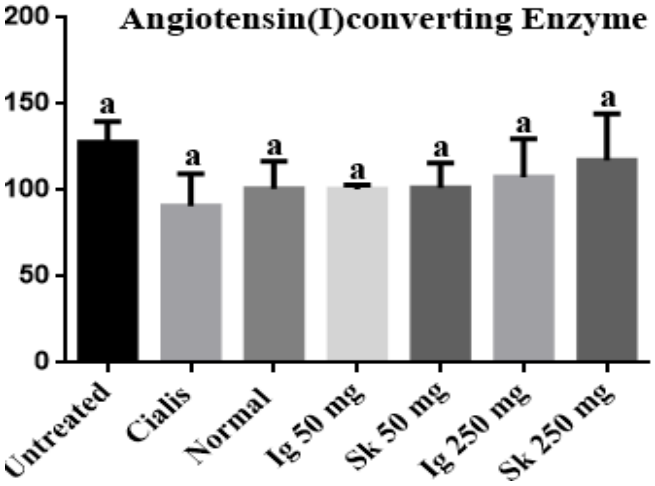

Figure 3:
The inhibitory effect of the crude extracts on the activity of ACE. Data expressed as mean ± SD, n=5. The same alphabets show no significant difference (P>0.05).

The Boosting Capacity of The Crude Extracts on Testosterone level: Testosterone levels dropped in the n-butanol induced (untreated) rats compared to the normal as depicted in Figure 4. However, *Inula glomerata*and *Salacia kraussii* 's crude extracts dose-dependently and independently increased testosterone level respectively. In addition, *Salacia kraussii* at both concentrations (50 and 250 mg/kg b.w) reversed the level of testosterone to normal, hence exhibited better testosterone boosting effect when compared to Inula glomerata.

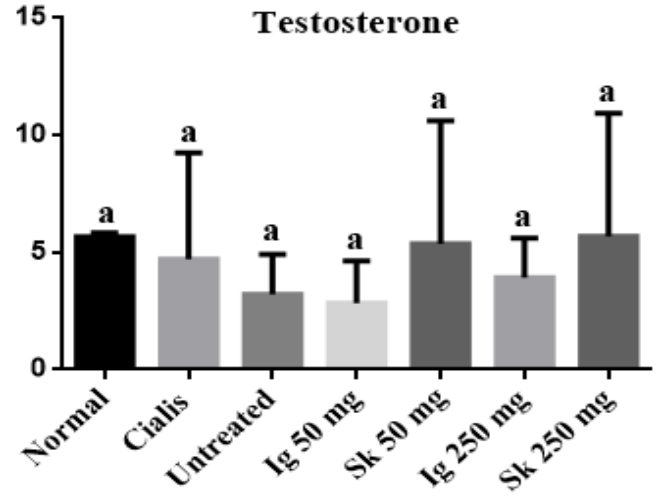

Figure 4:
The boosting effect of the crude extracts on testosterone level. Data expressed as mean ± SD, n=5. The same alphabets show no significant difference (P>0.05).

The Effect of The Extracts on Nitric Oxide Level: The level of nitric oxide was drastically reduced in the n-butanol induced rats in comparison to the normal rats but the crude extracts non-significantly elevated nitric oxide level (figure 5). Although the crude extracts could not restore nitric oxide to normal, there was no significant difference in the nitric oxide level among the normal, Cialis and extracts treated rats. Furthermore, both extracts at low concentrations displayed equal nitric oxide boosting efficacy. However, *Salacia kraussii* at high concentration 250 mg/kg b.w) had no effect on nitric oxide as depicted in figure 5.

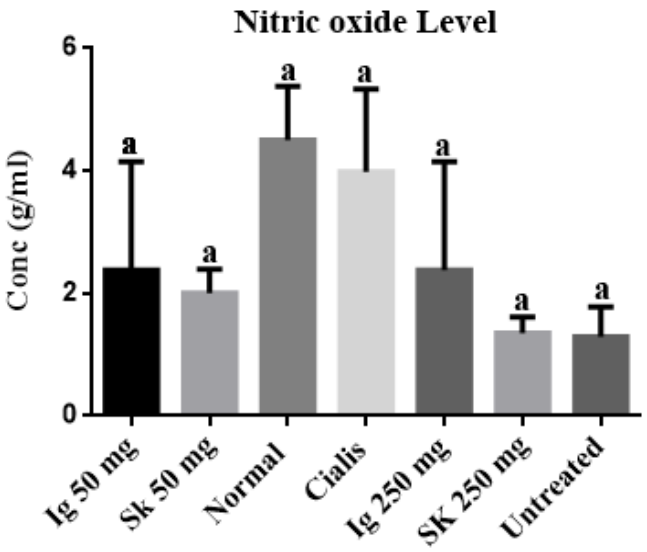

Figure 5
Boosting effect of the crude extracts on nitric oxide level. Data expressed as mean ± SEM, n=5. The same alphabets show no significant difference (P>0.05).

The Effect of The Plants' Extract on Uric Acid Level: The result as illustrated in figure 6 showed that uric acid was elevated in untreated rats compared to the normal. The extracts had no effects on uric acid at low concentrations (50 mg/kg b.w), however, at high concentrations uric acid level was significantly and non-significantly raised by *Inula glomerata*and *Salacia kraussii* respectively using normal rats as a yardstick.

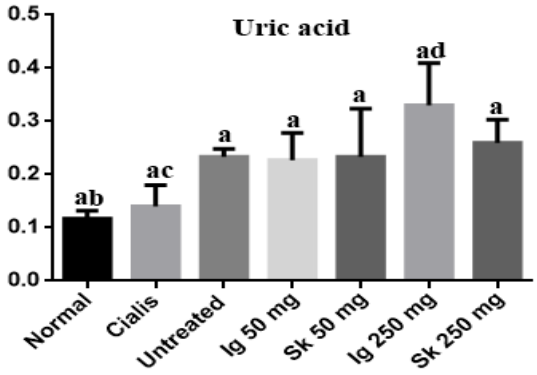

Figure 6
Effect of crude extracts on the uric acid level. Data expressed as mean ± SD, n=5. The same alphabets show no significant difference (P>0.05) while different alphabets denote significance difference (P<0.05).

The Effect of the crude extracts on Liver function enzymes:
Figure 7 illustrates the modulatory effect of the crude extracts on liver function enzymes which are aspartate (AST) and alanine transaminases (ALT). The crude extracts had no effect on the enzymes at high concentrations (250 mg/kg b.w) rather increased the level of AST and ALT in the serum at low concentrations (50 mg/kg b.w) compared to the normal.

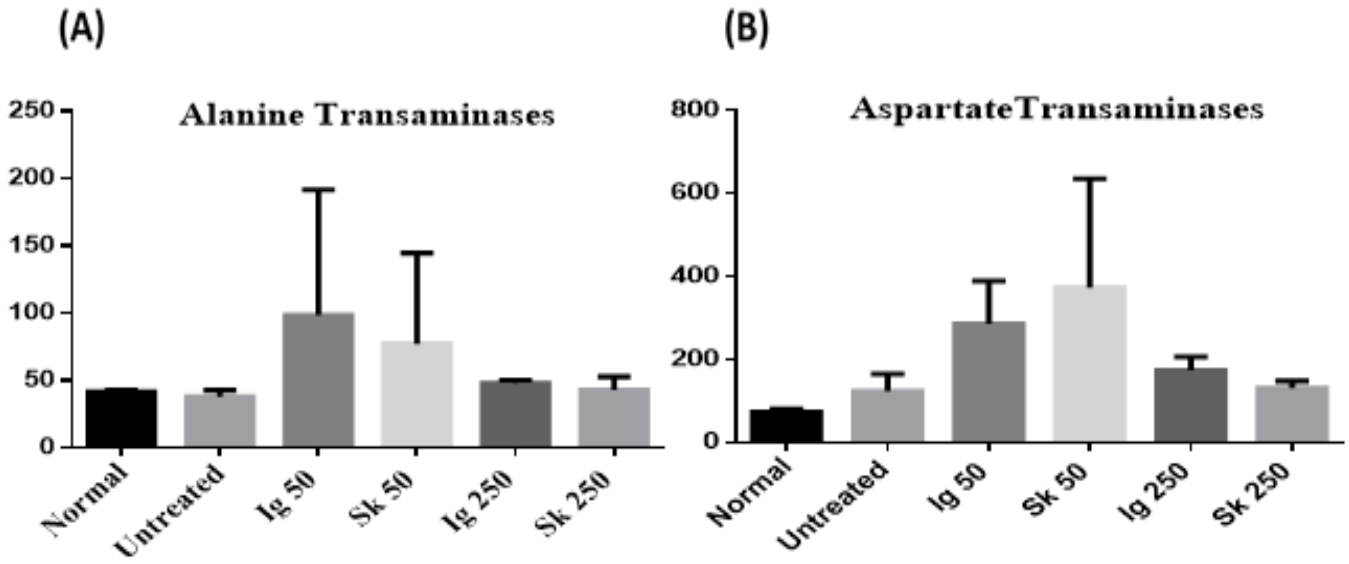

Figure 7:
Modulatory effect of the crude extracts on liver function enzymes: (a) Alanine transaminase (b) Aspartate transaminase. Data expressed as mean ± SD, n=5. The same alphabets show no significant difference (P>0.05).

DISCUSSION

The pathophysiology of erectile dysfunction is multifaceted with several predisposing factors including chronic alcohol. Chronic alcohol causes erectile dysfunction through testicular atrophy, abnormal changes in the histo-architecture of the corpus cavernosum, lowering testosterone and nitric oxide level (Cele *et al*., 2017; Grover *et al*., 2014; Choi *et al*., 2017). In our laboratory, Cele and colleagues, (Cele *et al*., 2017) successfully used n-butanol to mediate testicular dysfunction, hypogonadism, and oxidative stress. It was observed in this study that the crude extracts restored the mounting frequency that had been diminished by butanol in the butanol treated groups. This indicated that butanol induced hypogonadism (testosterone deficient) dependent erectile dysfunction in rats as suggested by Cele *et al* (Cele *et al*., 2017). The increased level of testosterone also supports the enhanced sexual activities and libido boosting efficacy of the crude extracts. Hence, justifies the inclusion of these plants in traditional medicine for improving sexual activities. This finding is asserted by previous studies that some medicinal plants possess aphrodisiac effect (Oboh *et al*., 2019).

Elevated activities of arginase and acetylcholinesterase result in endothelial dysfunction, a condition characterized by low nitric oxide bioavailability, is a fundamental cause of erectile dysfunction. In this study, the crude extracts attenuated arginase and acetylcholinesterase activities. The augmentation of nitric oxide by the crude extracts also corroborates the fact that arginase activities regulate normal endothelial production of nitric oxide (Olabiyi *et al*., 2017), (Oboh *et al*., 2015). This implies that the plants can correct endothelial dysfunction and therefore stimulate adequate penile erection. This finding agreed with previous studies that medicinal plants' extracts with arginase and acetylcholinesterase activities inhibitory property would exhibit a therapeutic effect on erectile dysfunction (Oboh *et al*., 2019; Ojo *et al*., 2019a; Ojo *et al*., 2019b).

Furthermore, it was evident in this study that the crude extracts lowered ACE activities. Thus, alluding to the fact that the crude extracts could facilitate smooth muscle relaxation, hence, boost erection as suggested by Fraga-Silver and colleagues (Fraga-Silver *et al*., 2013) and Adefegha and team (Adefegha *et al*., 2018) that inhibition of ACE can serve as an alternative therapeutic potential to reversing erectile dysfunction.

High levels of uric acid, AST, and ALT (liver function enzymes) are prognosis for renal and hepatic damage (Nakagawa *et al*., 2006; Yap and AW, 2010). Increased level of uric acid, as well as AST and ALT, observed in the extract-treated rats is a pointer to the fact that indiscriminate use of these plants have the potential to damage the kidney and liver. This was further buttressed by the cytotoxicity of *Inula glomerata*and *Salacia kraussii* against HEK 273 and Hela cell lines.. MTT assays are the most used assays to evaluate the cytotoxicity of the crude extract (Clarkson *et al*., 2004; Magadula, 2014). The MTT toxicity ranges of 10-20, 20-100 and above 100 µg/ml indicating strongly, moderately, and weakly toxic respectively (Magadula, 2014).

In conclusion, *Inula glomerata*and *Salacia kraussii* possess the potential to alleviate erectile dysfunction and the possible mechanism of action could be by the inhibition of arginase and acetylcholinesterase activities as well as boosting the level of testosterone and nitric oxide. However, the strong cytotoxicity and potential of renal and hepatic damage implies that the plants should be taken with caution. This was a cursory study to investigative the alternative therapeutic targets of these plants hence the scope of the work did not include histology, in vivo inhibitory effects of the crude extracts on PDE-5 and the vasoactivity on smooth muscle. Therefore, recommendation for further study will be to evaluate the in vitro and in vivo inhibitory effects of the crude extracts on PDE-5, the vasoactivity on smooth muscle, histology and isolation and characterization of the active compounds.

Ethics and consent to participate: *The project was approved by the University of Zululand Research ethics committee (UZREC) with the number UZREC 171110-030 PGM 2018/ 576.*

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Author contributions: *AR, MC and RA designed and supervised this project; MC performed the experiments (with the exception of cytotoxicity test performed by prof MS) and wrote the manuscript; MC and ND analyzed the data; GE and FO co-supervised and perfected the editing. The authors approved the final draft.*

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REFERENCES

Adefegha S.A., Oboh G., Fakunle B., Oyeleye S.I., Olasehinde T.A. (2018). Quercetin, rutin, and their combinations modulate penile phosphodiesterase-5′, arginase, acetylcholinesterase, and angiotensin-I-converting enzyme activities: a comparative study. *Comp Clin Path.* 27(3), 773-80.

Andersson K.E. (2011). Mechanisms of penile erection and basis for pharmacological treatment of erectile dysfunction. *Pharmcol Rev.* 63(4), 811-59.

Arumugam P., Murugan M., Thangaraj N. (2012). evaluation of anti-inflammatory and analgesic effects of aqueous extract obtained from root powder of Inula racemose Hook.f. *J Med Plant Res*. 6(14), 2801-2806.

Bandeira S.O., Gaspar F., Pagula F.P. (2001). African ethnobotany and healthcare: emphasis on Mozambique. Pharm. Biol. 39, 70-3.

Bella A.J., Lee J.C., Carrier S., Bénard F., Brock G.B. (2015). CUA Practice guidelines for erectile dysfunction. *Can Urol Assoc J.* 9(1-2), 23.

Burrows J.E., Willis C.K. (2005). Plants of the Nyika Plateau Southern African Botanical Diversity Network Report No. 31 SABONET. *Pretoria,* 212pp.

Carlson T.J. (2002). Medical ethnobotanical research as a method to identify bioactive plants to treat infectious diseases. InAdvances in Phytomedicine. *Elsevier*. 1, 45-53.

Castelló-Porcar A.M., Martínez-Jabaloyas J.M. (2016). Testosterone/estradiol ratio, is it useful in the diagnosis of erectile dysfunction and low sexual desire?. *The Aging Male*. 19(4), 254-258.

Cele N.D., Sangweni N.F., Mosa R.A., Penduka D., Lazarus G.G., Singh M., Zharare G.E., Opoku A.R. (2017). Testicular Dysfunction Ameliorative Effect of the Methanolic Roots Extracts of Maytenus procumbens and Ozoroa paniculosa. *Evidence-Based Complementary and Alternative Medicine.*

Choi S.M., Seo D.H., Lee S.W., Lee C., Jeh S.U., Kam S.C., Hwa J.S., Chung K.H., Hyun J.S. (2017). The Effect of Alcohol Administration on the Corpus Cavernosum. *World J Mens Health.* 35(1), 34-42.

Clarkson C., Maharaj V.J., Crouch N.R., Grace O.M., Pillay P., Matsabisa M.G., Bhagwandin N., Smith P.J., Folb P.I. (2004). In vitro antiplasmodial activity of medicinal plants native to or naturalised in South Africa. *J Ethnopharmacol*. 92(2-3), 177-91.

Decaluwé K., Pauwels B., Boydens C., Van de Voorde J. (2014). Treatment of erectile dysfunction: new targets and strategies from recent research. *Pharmacol Biochem Behav*. 121, 146-57.

Dey A., De J.N. (2015). Neuroprotective therapeutics from botanicals and phytochemicals against Huntington's disease and related neurodegenerative disorders. *J Herb Med.* 5(1), 1-9.

Feldman H.A., Johannes C.B., Derby C.A., Kleinman K.P., Mohr B.A., Araujo A.B., McKinlay J.B. (2000). Erectile dysfunction and coronary risk factors: prospective results from the Massachusetts male aging study. *Prev Med.* 30(4), 328-38.

Fraga-Silva R.A., Montecucco F., Mach F., Santos R.A., Stergiopulos N. (2013). Pathophysiological role of the renin–angiotensin system on erectile dysfunction. *Eur J Clin Invest*. 43(9), 978-85.

Fung M.M., Bettencourt R., Barrett-Connor E. (2004). Heart disease risk factors predict erectile dysfunction 25 years later: the Rancho Bernardo Study. *J Am Coll Cardiol.* 43(8), 1405-11.

Gareri P., Castagna A., Francomano D., Cerminara G., De Fazio P. (2014). Erectile dysfunction in the elderly: an old widespread issue with novel treatment perspectives. *Int J Endocrinol*. 878670.

Garza R.G., Lopez M.A., Perez-Vertti R.D., Orasma C.L., Martinez **J.M. (2015).**Testicular damage in Wistar rats caused by methanolic extracts of plants from the North of Mexico. *J Med Plant Res*. 9(25), 724-30.

Grover S., Mattoo S.K., Pendharkar S., Kandappan V. (2014). Sexual dysfunction in patients with alcohol and opioid dependence. *Indian J Psychol Med*. 36(4), 355.

Harbone J.B. (1973). Phenolic compounds. Phytochemical Methods. 271-80.

Helo S., Thomas P., Tadros N.N. (2018). Testosterone Therapy: a Panacea for Sexual Dysfunction in Men?. *Curr Sex Health Rep.* 10(3), 177-85.

Jin L.M. (2010). Angiotensin II signaling and its implication in erectile dysfunction. *J Sex Med.* 6, 302-10.

Lewis R.W., Fugl-Meyer K.S., Corona G., Hayes R.D., Laumann E.O., Moreira Jr E.D., Rellini A.H., Segraves T. (2010). Definitions/epidemiology/risk factors for sexual dysfunction. *J Sex Med.* 7(4pt2), 1598-607.

Lidija B.R., Zdenka K.E., Ita S. (2004). The antidepressant activity of Hypericum perforatum L. measured by two experimental methods on mice, *Acta Pharmaceutical.* 54, 157 – 162.

Magadula J.J. (2014). Phytochemistry and pharmacology of the genus Macaranga: a review. *J Med Plant Res*. 8(12), 489-503.

Morris Jr S.M. (2009). Recent advances in arginine metabolism: roles and regulation of the arginases. *Br J Pharmacol.* 157(6), 922-30.

Mosmann T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods*. 65(1-2), 55-63.

Nakagawa T., Kang D.H., Feig D., Sanchez-Lozada L.G., Srinivas T.R., Sautin Y., Ejaz A.A., Segal M., Johnson R.J. (2006). Unearthing uric acid: an ancient factor with recently found significance in renal and cardiovascular disease. *Kidney Int*. 69(10), 1722-5.

Oboh G., Adebayo A.A., Ademosun A.O., Abegunde O.A. (2019). Aphrodisiac effect of Hunteria umbellata seed extract: Modulation of nitric oxide level and arginase activity in vivo. *Pathophysiology.* 26(1), 39-47.

Oboh G., Ademiluyi A.O., Ademosun A.O., Olasehinde T.A., Oyeleye S.I., Boligon A.A., Athayde M.L. (2015). Phenolic extract from Moringa oleifera leaves inhibits key enzymes linked to erectile dysfunction and oxidative stress in rats' penile tissues. *Biochem Res Int.*

Odebiyi O.O., Sofowora E.A. (1978). Phytochemical screening of Nigerian medicinal plants II. *Lloydia.* 41(3), 234-46.

Office of Laboratory Animal Welfare. Public Health Service policy on humane care and use of laboratory animals. (2002).

Ojewole J.A. (2007). African traditional medicines for erectile dysfunction: elusive dream or imminent reality?. *Cardiovasc J Afr*. 18(4), 213-215.

Ojo O.A., Afon A.A., Ojo A.B., Ajiboye B.O., Okesola M.A., Aruleba R.T., Adekiya T.A., Oyinloye B.E. (2019). Spondias mombim L. (Anacardiaceae): Chemical fingerprints, inhibitory activities, and molecular docking on key enzymes relevant to erectile dysfunction and Alzheimer's diseases. *J Food Biochem.* 43(3) e12772.

Ojo O.A., Ojo A.B., Oyinloye B.E., Ajiboye B.O., Anifowose O.O., Akawa A., Olaiya O.E., Olasehinde O.R., Kappo A.P. (2019). Ocimum gratissimum Linn. Leaves reduce the key enzymes activities relevant to erectile dysfunction in isolated penile and testicular tissues of rats. *BMC Complement Altern Med*. 19(1), 71.

Olabiyi A.A., Oboh G., Akinyemi A.J., Ademiluyi A.O., Boligon A.A., de Campos M.M. (2017). Tiger nut (Cyperus esculentus L.) supplemented diet modulate key biochemical indices relevant to erectile function in male rats. *J Funct Foods*. 34, 152-8.

Raimondo D., Staden L.V., Foden W., Victor J.E., Helme N.A., Turner R.C., Kamundi D.A., Manyama P.A. (2009). Red list of South African plants 2009. SANBI.

Rew K.T., Heidelbaugh J.J. (2016). Erectile dysfunction. *Am Fam Physician*. 94(10), 820-827.

Saxena A., Prakash P., Porwal M., Sissodia N., Sharma P. (2012). Erectile dysfunction: A review and herbs used for its treatment. *Int j green pharm (IJGP).* 6(2).

Tan R.X., Tang H.Q., Hu J., Shuai B. (1998). Lignans and sesquiterpene lactones from Artemisia sieversiana and Inula racemosa. *Phytochemistry*. 49(1), 157-61.

Whittaker C. (2010). Phosphodiesterase type 5 inhibitors and erectile dysfunction. South African Family Practice. 52(3), 207-11.

Yap C.Y.F. AW T.C. (2010). Liver function tests. Proceedings of Singapore Healthcare. 19(1) (2010).

Yassin A.A., Nettleship J., Almehmadi Y., Salman M., Saad F. (2016). Effects of continuous long-term testosterone therapy (TT h) on anthropometric, endocrine and metabolic parameters for up to 10 years in 115 hypogonadal elderly men: real-life experience from an observational registry study. *Andrologia*. 48(7), 793-9.