Nutraceutical potential of *Balanitesaegyptiaca L. Delile*fruits (Zygophyllaceae): safety and androgenic property

Abstract:*Balanitesaegyptiaca* is a plant of arid lands producing edible fruits called desert date. This study has undertaken to evaluate nutraceutical potential of *Balanitesaegyptiaca*fruits towards its androgenic effect. The bio-guided phytochemical screening of the mesocarp of *B. aegyptiaca*fruits revealed the presence of secondary metabolites including saponins, tannins, coumarins, sterols, polyphenols and reducing sugars.The aqueous extract of *B. aegyptiaca* fruits is devoid of acute oral toxicity, median lethal dose> 5 g/kg/BW. The results have proven that the administration of the *B. aegyptiaca* fruits aqueous extract was able to enhance testicular function, expressed by the increase in muscle mass, physical strength, testicular total cholesterol, testosterone and fructose, as well as mobility and sperm count rats compared to control group ($p<0.05$). The mesocarp of *B. aegyptiaca*fruits has an androgenic activity, and its claimed aphrodisiac virtues depend on it. These results might suggest that mesocarp of the fruits of *B. aegyptiaca* can be used in the treatment of male sexual dysfunction. Desert dates is nutraceutical with androgenic proprieties.

Keywords*:Balanitesaegyptiaca*fruits, safety, testicular function, androgenic activity, nutraceutical

1. Introduction

Balanitesaegyptiaca, is a popular tree belonging to Zygophyllaceae family, genus *Balanites*, found in the dry land areas of Africa, South Asia, and the Middle East, and used in agroforestry (Orwa*et al*., 2009). It is located in the Sahelian zone in Cameroon and called *Tane (in Fulfulde)* by people of this area. All parts of this plant are used either in the pharmacopoeia or as food (Cook *et al*., 1988). For instance, the stem bark is the source of medicine (Mohamed *et al*., 2012), while the leaves and fruits are food (Khamis*et al*., 2020). The fruits of *B. aegyptiaca* are so called desert date. Thanks to their bitter and sweet pulp (mesocarp), they are eaten like candy, after removing the outer layer (epicarp). While the latter is used for the rehabilitation of comatose soils; oil is extracted from the seed (endocarp) (Al Ashaal*et al*., 2010). Previous works

has shown that the pulp of *B. aegyptiaca* fruits contain families of compounds such as: alkaloids, saponins, steroids, flavonoids, and cardiac glycosides (Daya*et al*., 2011; Abdallah*et al*., 2012; Satpute*et al*., 2018; Murthy *et al*., 2021).

The pharmacological studies showed that aqueous extract of *B. aegyptiaca* Del fruit mesocarp protects against CCl_4 – induced liver damage in rats (Salihu*et al*., 2010). Substances extracted from *B. aegyptiaca* Del fruit mesocarp including Balanitoside possess antitumor activity (Al-Ghanam*et al*., 2013). The antioxidant and antimicrobial activities of *B. aegyptiaca* mesocarp have been found by Abdallah*et al*. (2012). In folkloric medicine, many virtues of *B. aegyptiaca* fruits have been reported, including hypoglycemic and antidiabetic, anthelmintic, anticancer, anti-inflammatory, analgesic jaundice, spermicidal, leukoderma and other skin diseases, diuretic, dysentery and constipation (Daya*et al*., 2011; El-Seedi*et al*., 2021).

In the area of health reproductive, recent scientific report mention the positive effect of the seed oil of *B. aegyptiaca* on fertility diseases including impotency and infertility (Abdalbasit and Essa Mohammed, 2022). Men in the northern part of Cameroon consume the mesocarp of the fruits of *B. aegyptiaca*to manage the erectile troubles. However, androgenic properties of mesocarp of *B. aegyptiaca*. The management of male sexual dysfunction requires a dual approach of diet and medication. The use of nutraceutical seems to be a model of such a strategy. The mesocarp of *B. aegyptiaca*can meet this requirement, if it has an androgenic effect. However, the efficacy of the mesocarp of *B. aegyptiaca*fruits in the treatment of erectile dysfunction has not yet been established. The present work was designed to evaluate the androgenic activity of *B. aegyptiaca*mesocarp.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Collection of Plant Material

The plant materials were ripe fruits of *B. aegyptiaca*harvested in December around 4 p.m. during the dry season at Doyang, a locality in the Mayo-Kani Division, Far North Region of Cameroon. These fruits were kindly identified and proved by Herbarium of the Department of Plant Biology and Physiology, Faculty of Science, University of Douala.

2.1.2.Experimental animals

Animal material consisted of 36 male rats of ages from 6 weeks to 12 weeks with an average weight of 131.62 ± 29.27 g distributed as follows: 6 rats for acute oral toxicity test and 30 rats for the evaluation of androgenic properties of the aqueous fruits extract *B. aegyptiaca*.The rats were purchased from the Animal Biology Department of the Faculty of Science of the University of Douala and allowed to acclimatize to the conditions of the laboratory for one week before the experiment. The animals were treated in accordance with the European Commission's directives on protecting animals used for scientific purposes (EC, 2010).

2.1.3.Laboratorymaterials

The laboratory materials were chemicals, laboratory apparatus and tech tools as presented in table 1

Table 1. Chemicals, lab apparatus and tech tools of the study

Reagents/Solutions	Laboratory apparatus	ICT tools
Testosterone enanthate 250mg/ml inj. solution (androtardyl), 10% Formalin, 0.9% NaCl, NaCl salt, Distilled water, Phosphate buffers, NaOH salt, Ketamine 50mg/ml, H_2SO_4,10% NH_3Draggendorfs reagent, 5% Ferric chloride, Mg chips, ethanol, HCl, Chloroform	Microscope, Hemocytometer, pH meter, Micropipettes, Centrifugation machine, electronic balance, spectrophotometer, Freeze dryer, Desiccator, Lab oven, petri dishes, laboratory glassware, serum vacutainer tubes, Eppendorf tubes, dissecting set, refrigerator	Computer, Statgraphics software, MS word, MS Excel, internet, google scholar, Zotero

2.2. Methods

2. 2.1.Preparation of aqueous extract of the fruits *B. aegyptiaca*

The mesocarp of the *B. aegyptiaca* fruit was peeled off, dried and crushed. Extract was prepared by placing the obtained powder in a stoppered recipient with distilled water (at room temperature) and allowing to stand for 48 hours with frequent agitation. The powder was mixed

with distilled water in the ratio 1:2. After 48 hours, the liquid was then strained off and the marc (solid residue) pressed to recover as much occluded solution as possible. The micelle (mixture of soluble substances and solvent) was then clarified by filtration. The filtrate was concentrated by freeze drying at 30˚C. After crushing the dried fruits, a dry mass was 644g. Maceration of the fruit mesocarp of *B. aegyptiaca* in water produced a brownish-colored extract of mass 360g. The yield of the extraction was 55.9%.

2.2.2. Phytochemical screening of aqueous extract of the fruits *B. aegyptiaca*

Phytochemical screening was carried out using the usual methods for revealing families of secondary metabolites (Mohamed *et al*., 2015) in aqueous extract of *B. aegyptiaca*fruits.

2.2.3. Oral toxicity

The acute oral toxicity test was performed following OECD guidelines, protocol 425 (OECD, 2008). The aqueous extract of *B. aegyptiaca*fruits was administered to healthy and male wistar rats following the limit test procedure at the dose 5000mg/kg. Six female rats aged between 8 and 12 weeks, weighing between 155 and 190 g were used. Prior to dosing,the rats were selected at random, marked to permit individual identification. They were divided randomly into two groups of 3. The rats were starved of food overnight prior to dosing. Following the fasting period, they were weighed, and the dose of the test substance were calculated. Using a feeding probe, the rats were treated with the aqueous extract at dose of 5000mg/kg of body weight. The control group received distilled water. After administration of the aqueous extract of *B. aegyptiaca*fruits, each rat was monitored continuously for the first hour followed by every 2 hours till 12 h and then weighed and observed every day for 14 days. Beside death, signs of toxicity were observed including changes in skin and fur, eyes, mucous membranes, behaviour pattern, tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma. After the 14^{th} day, all animals were deprived of food for 12 hours and then sacrificed after mild ketamine anaesthesia (intra muscular (IM) injection at 1ml/kg). Animals were subjected to partial post-mortem examination to evaluate the toxicity of the *B. aegyptiaca* fruits notably through the assessment of the change in total body mass, relative mass of organs (liver, kidney, heart, lungs and the spleen).

2.2.4. Evaluation of the androgenic properties of the aqueous extract of the fruits of *B. aegyptiaca*

Thirty rats aged between 6 and 8 weeks of body weight between 130.87 ± 19.13 g were used. They were lodged in basins enclosed with cages. A cushion was placed in each cage to ensure the comfort of the rats. The rats had free access to food (pelleted diet) and distilled water. The basins were cleaned, and the cushion replaced every 2 days to ensure perfect hygiene. The rats were randomly distributed in 5 groups of 6 based on the substances they were treated with as follows:

- Group 1: 2ml of Distilled water orally, once daily for 28 days
- Group 2: 1200mg of *B. aegyptiaca* fruit extract orally, once daily for 28 days
- Group 3: 800mg of *B. aegyptiaca* fruit extract orally, once daily for 28 days
- Group 4: 400mg of *B. aegyptiaca* fruit extract orally, once daily for 28 days
- Group 5: A single IM injection of testosterone enanthate at 5mg/kg of body weight (MawdoNgom, 2010).

Rats were made to fast 12 hours before the first administration of the products. The treatment lasted for 28 days. On day 29, the rats were euthanized with ketamine 50mg/ml IM injection at the dose of 1ml/kg. Blood samples were collected through the large blood vessels in serum tubes for biochemical analysis. Thereafter, organs such as the testes, epididymis, prostate, seminal vesicles and the penis were withdrawn, weighed and used for the preparation of homogenates for use in biochemical analysis and sperm analysis (epididymis).

2.2.4.1 Preparation of doses

The test solutions of the aqueous fruit extract of *B. aegyptiaca*were prepared at the following doses: 400mg, 800mg and 1200mg based on previous studies (Gad *et al*.,2006). While testosterone enanthate 5mg/ml was prepared by diluting 250mg/ml of androtardyl in 49ml of olive oil. The volume of testosterone enanthate solution administered to rats was calculated using the formula:

$$V = \frac{DxM}{C}$$

Where: V = Volume; D = Dose (mg/kg); M = Body mass in kg; C = concentration of the solution to administer

Androgenic properties of the aqueous extracts of the fruits of *B. aegyptiaca* were evaluated in the following ways:

- Total gain in body weight;
- The relative mass of sexual organs (testes, epididymis, prostate, penis, seminal vesicles);
- Concentration of spermatozoids in the epididymis;
- Strength test;
- Biochemical analysis of parameters such as serum Calcium, total serum and testicular cholesterol, total testicular and epididymal proteins, seminal fructose.

2.2.4.2 Effects of the extract on variation in mass

The mass of each rat was measured prior to starting treatment and weighed weekly after the first day of treatment for 4 weeks and the average change in mass was weekly calculated.

2.2.4.3 Effects of the extract on the Relative mass of sexual organs

The relative masses of the sexual organs were calculated using the following formula

$$\text{Relative mass of an organ} = \frac{mass\ of\ organ}{body\ mass\ of\ the\ animal} x\ 100$$

2.2.4.4 Effects of the extract on sperm concentration and mobility

This was done using the method described by Uzun*et al* (2009). The principle of this procedure is based on counting with the use of a microscope, sperms contained in the homogenate of the tail of the epididymis. The motility of the sperms will be appreciated by direct microscopic examination of the homogenate. The percentage of mobile forms of the spermatozoa will be calculated as follows:

$$\%\ \text{of mobile spermatozoa} = \frac{numberofmobilespermatozoa}{totalnumberofspermatozoa} \times 100$$

The concentration of spermatozoa was determined using the Malassez counting chamber and a microscope. A drop of the homogenate was pipetted and dropped on a Malassezhemocytometer and then covered with a coverslip and observed under the microscope. Sperms were counted in 5 squared rectangles and their average was determined. The concentration of sperms was then calculated using the following equation:

$$\boldsymbol{N = Xxdfx100}$$

Where: N= Number of sperms per mm^3; df= diluting factor; X= Average number of spermsin 5 squared rectangles. 100 = total number of rectangles

2.2.4.5. Strength test

The strength test consisted of making the rats grip to a suspended bar on 3 occasions and measuring the time that each group will take to fall (figure .1.).

Figure .1. Measurement of physical strength

2.2.5. Biochemical analysis

Biochemical analyses were carried out on both blood and male sex organ homogenates (testes, epididymis and seminal vesicles). The following parameters were determined, namely: total cholesterol, testosterone, fructose, calcium and protein; using commercial kit (ELISA testosterone of CALBIOTECH, LABKIT…).

2.2.6. Statistical analysis

The analysis of results was done with the help of STATGRAPHICS Plus version 5.0. Results were expressed as the mean ± Standard deviation. To analyze the results of the various elements, the ANOVA (Analysis of variances) was used, then the test of Student-Newman-Keuls permitted to compare the means of the test and control groups. The $P < 0.05$ were considered significant.

3. Results and discussion

3.1. Secondary metabolites in aqueous extract of *B. aegyptiaca*fruits

The extractof *B. aegyptiaca*fruits revealed the presence of secondary metabolites such as: saponins, tannins, coumarins, sterols, polyphenols and reducing sugars. This findings is similar to that of Absalom *et al*. (2013). However, the phytochemical screening of *Balanitesaegyptiaca*seeds revealed the presence of alkaloids in addition to the families of compounds identified in the mesocarp (Vijaykumar*et al*., 2024)

3.2. Safety of the aqueous extract of the fruits of *B. aegyptiaca*

To evaluate the oral acute toxicity, the rats were divided into two groups of three. One group was treated with the extract at the dose of 5000mg/kg and the other received distilled water (2ml) and observed for 14 days. During this period, no death or any visible sign of toxicity was observed in both groups. From the first hours of treatment to day 14, skin and fur, respiration, eyes, tremors, convulsion, salivation, diarrhoea, lethargy, sleep were normal. Furthermore, the autopsy performed did not show any abnormalities. Following the weighing of the organs, a balance between organ mass and body mass was noted. The median lethal dose (LD50) was greater than 5 g/kg BW. This result contrasts with that of Absalom*et al*. (2013), who, found lethal toxic dose of 12.9 g/L in catfish.According to Globally Harmonized System of Classification and Labelling of Chemicals (GHS),*B. aegyptiaca* extract is not classified as acutely toxic (OECD, 2008). Moreover, Koubé*et al*. (2017) consider any substance or mixture of substances with a median lethal dose (LD50) above 5000 mg/kg as lacking oral toxicity. Thus, the aqueous extract of *B. aegyptiaca* fruits is devoid of acute oral toxicity.

3.3. Evaluation of the androgenic properties of the aqueous extract of the fruits of *B. aegyptiaca*

Evaluation of physical parameters (including body weight, relative mass of sexual organs, strength), sperm count and mobility, and biochemical parameters such as concentrations of testicular and serum constituents like protein, cholesterol, testosterone can give useful information on the androgenic and or anti-androgenic potential of plant extracts (Schroeder *et al*., 2003; Gupta *et al*., 2004; Watcho*et al*., 2004; Yakubu*et al*., 2007; Yakubu*et al*., 2008).

3.3.1. Effectsof *B. aegyptiaca*on the weekly variation in mass

Variation in the masses of rats that underwent treatment is presented in table 2. There was a weekly increase in mass in all the groups from week 1 to week 4. After week 1, there was a non-significantly higher increase in the TE, BA 1200 mg, BA 800 mg, and BA 400 mg groups (in increasing order from the smallest to the biggest) when compared to the DW group. At the end of week 2, we observed a non-significantly greater increase in the TE, BA 400 mg and BA 800 mg groups (in increasing order) compared to the DW group. Meanwhile, there was a non-significantly lower increase in the BA 1200 mg group. After week 3, there was a non-significantly higher increase in mass in the TE group while the other groups were non-significantly lower when compared to the DW group. It was noted that the masses of the rats that received the extract (BA) and the reference androgen (TE) increased in a greater but non-significant proportion than those of the normal control group (DW) by week 4. The proportion of the TE group was greatest. The growth of the animals can be explained by the fact that consumption of the mesocarp of *Balanitesaegyptiaca* improves the increase in muscle mass.

Table 2. Effects of the aqueous extractof*Balanitesaegyptiaca* on weekly change in mass (g)

Week	DW	BA 1200 mg	BA 800 mg	BA 400 mg	TE
1	1.33 ± 0.33	6 ± 2.52	7 ± 3	14.67 ± 5.7	4.67 ± 1.76
2	22.33 ± 0.67	17.33 ± 3.76	27.67 ± 8.09	24.33 ± 10.4	23.33 ± 1.67
3	49.33 ± 1.86	29 ± 10.79	48.33 ± 15.07	38.33 ± 8.69	55.33 ± 2.4
4	57.67 ± 3.18	63.33 ± 4.37	65 ± 10.5	62 ± 8.08	76.67 ± 0.88

**Significantly different (P < 0.05) compared to the control group (DW)*

DW= distilled water, BA= aqueous extract of Balanitesaegyptiaca, TE= Testosterone enanthate, SEM= Standard error of the mean. Each value represents the mean ± SEM; n = 6

3.3.2. Effects of the aqueous extract of*Balanitesaegyptiaca* on the relative mass of sexual organs

The effects of the aqueous extract on the relative mass of sexual organs are presented in Table 3. For the seminal vesicles, we observed higher values of the relative masses of the TE group and

BA group at dosesof 1200 mg and 800 mg. The difference was statistically significant compared to the DW group (by 29.73%, 24.32%, and 24.23% respectively) at $P < 0.05$. Meanwhile,a lower relative mass was noted in the BA 400 mg group, which was non-significant compared to the DW. For the testes, the BA 400mg and TE groups had non-significantly lower relative masses compared to the DW group. Meanwhile, the BA 1200 mg and BA 800 mg had higher relative masses (by 23.19% and 10% respectively) compared to the DW group but only the relative mass of the BA 1200 mg group was significant ($P < 0.05$). There was a non-significantly higher relative mass of the epididymis of the BA 800mg and BA 1200 mg group (by 25% and 21% respectively) compared to the DW group. Whereas the TE group had a non-significantly lower relative mass. An increase in the relative masses of the seminal vesicles (at doses 1200 mg and 800 mg) and testes (1200 mg) was observed. This is probably as a result of the enhancement of the structural and functional integrity of these organs which are androgen dependent (Mooradian*et al*., 1987). This result is backed by the elevated levels of testicular testosterone and cholesterol shown in our present study. This is an indication of the anabolic and androgenic effects of the aqueous extract of the fruits of *B. aegyptiaca* which is in accord with the findings of Watcho*et al*., 2004 and Yakubu*et al*., 2007 following the administration of *Mondiawhitei*Hook. F Skeels (Periplocaceae) roots and *Massulariaacuminata*stem, respectively, to male rats.

Table 3. Effect of the aqueous extract of*Balanitesaegyptiaca* on Relative mass of sexual organs (in %)

Organ	DW	BA 1200mg	BA 800mg	BA 400mg	TE
S. vesicles	0.37 ± 0.01^{a}	0.46 ± 0.02^{b}	0.46 ± 0.02^{b}	0.35 ± 0.02^{a}	0.48 ± 0.03^{b}
Testes	0.69 ± 0.03^{a}	0.85 ± 0.07^{b}	0.76 ± 0.04^{a}	0.66 ± 0.02^{a}	0.61 ± 0.01^{a}
Epididymis	0.28 ± 0.03^{a}	0.34 ± 0.05^{a}	0.35 ± 0.01^{a}	0.28 ± 0.01^{a}	0.21 ± 0.05^{a}
Penis	0.14 ± 0.03^{a}	0.13 ± 0.03^{a}	0.13 ± 0.01^{a}	0.12 ± 0.02^{a}	0.14 ± 0.01^{a}
Prostate	0.14 ± 0.03^{a}	0.17 ± 0.02^{a}	0.17 ± 0.01^{a}	0.18 ± 0.02^{a}	0.15 ± 0.01^{a}

Values with the same superscript letters in the same column are not significantly different at $P < 0.05$. S= seminal, DW= Distilled water, BA= aqueous extract of B. aegyptiaca, TE=

Testosterone enanthate, SEM= Standard error of the mean. Each value represents the mean ± SEM; n = 6

3.3.3. Effects of the aqueous extract of*Balanitesaegyptiaca* on the mobility and concentration of sperm cells

The effects of the treatment on the mobility and concentration of rats' sperm cells are presented in table 4. After analysis of data on mobility, it was observed that group BA 1200 mg, BA 800 mg, BA 400 mg and TE had more mobile sperm cells than DW by 6.2%,15.5%,18% and 18% respectively and only the values of group BA 400 mg and TE were significant statistically ($P < 0.05$). Groups BA 400mg, BA 800 mg, TE and BA 1200 mg all had significantly higher concentrations of sperms by 57.6%, 56.5%, 54.1% and 52.9% respectively when compared to the DW group. Apart from targeting spermatogenesis, normal levels of androgens are required for the development of male secondary sexual characteristics such as enhanced growth of muscle mass and sexual organs, strength, and libido amongst others (Monk *et al*., 2018).Phytochemical screening of our extract revealed the presence of saponins. Based on the reports made by the previous studies stated above, we can deduce that saponins could be responsible for the elevated levels of testosterone, sperm count, and motility observed after administration of our aqueous extract.

Table 4. Effect of the aqueous extract of*Balanitesaegyptiaca* on Mobility and Concentration of sperm cells

Group	Mobility (%)	Concentration ($x10^6$ cells/ml)
DW	84.5 ± 6.17^a	5.84 ± 1.39^a
BA 1200mg	89.8 ± 9.01^b	12.4 ± 1.51^b
BA 800mg	97.6 ± 1.56^b	13.44 ± 0.26^b
BA 400mg	100 ± 0^b	13.77 ± 1.21^b
TE	100 ± 0^b	12.71 ± 1.81^b

Values with the same superscript letters in the same column are not significantly different at $P < 0.05$. DW= Distilled water, BA= aqueous extract of Balanitesaegyptiaca, TE= Testosterone enanthate. Each value represents the mean ± SEM; n = 6

3.3.4. Effects of the aqueous extract of *B. aegyptiaca* on physical strength

The results obtained from this test showed that group BA1, BA2, BA3 and TE had greater strength than group DW by 10.69%, 19.43%, 60.21% and 38.86% respectively (fig. 2). Only BA3 had a statistically significant difference ($P < 0.05$). The increase in the concentration of sperms, physical strength mass observed in our study could be a manifestation of elevated testosterone. This is therefore another indication of the anabolic and androgenic potential of our extract which agrees with the findings of Matyja et al. (2023)

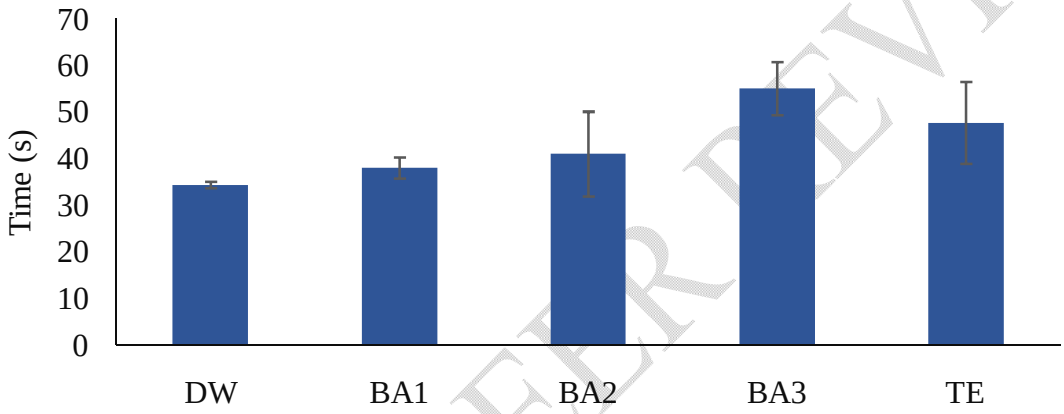

Figure 2. Effects of the of the aqueous extract of*Balanitesaegyptiaca* extract of Balanitesaegyptiaca on physical strength

*DW= distilled water; BA1= Balanitesaegyptiaca 1200mg, BA2= Balanitesaegyptiaca800mg, BA3= Balanitesaegyptiaca400mg; TE= Testosterone enanthate. Each value represents the mean ± SEM; n = 6; *Significantly different (P < 0.05) compared to the control group (DW).*

3.3.5. Effects of the aqueous extract of*Balanitesaegyptiaca* on biochemical parameters

Table 5 shows effects of the extract of *B. aegyptiaca* on biochemical parameters, including serum calcium, serum total cholesterol, serum testosterone, testicular total cholesterol, testicular total protein, testicular testosterone, epididymal protein and vesicular fructose**.**

Table 5. Effect of the aqueous extract of*Balanitesaegyptiaca*biochemical parameters

Parameters	DW	BA 1200mg	BA 800mg	BA 400mg	TE

Serum calcium (mg/dl)	22.17 ± 1.21[a]	24.94 ± 3.02[b]	25.22 ± 2.15[b]	22.31 ± 1.41[a]	24.33 ± 3.53[b]
Serum total cholesterol (mg/dl)	153.45 ± 5.07[b]	163.84 ± 5.91[c]	146.54 ± 7.02[ab]	138.05 ± 5.18[a]	187.42 ± 8.04[c]
Serum testosterone (ng/dl)	5.10 ± 1.03[a]	8.64 ± 1.15[b]	10.73 ± 1.81[b]	3.11 ± 1.21[a]	15.14 ± 2.05[c]
Testicular cholesterol (mg/dl)	10.39 ± 2.10[a]	10.48 ± 1.93[a]	14.95 ± 2.11[b]	8.91 ± 0.02[a]	17.95 ± 3.11[b]
Testicular protein (mg/dl)	0.12 ± 0.02[a]	0.08 ± 0.02[a]	0.11 ± 0.01[a]	0.12 ± 0.02[a]	0.11 ± 0.01[a]
Testicular testosterone (ng/dl)	3.82 ± 1.81[a]	5.48 ± 1.62[b]	6.45 ± 0.92[b]	1.61 ± 0.19[a]	7.43 ± 1.95[b]
Epididymal protein (mg/dl)	0.30 ± 0.02[b]	0.22 ± 0.03[b]	0.15 ± 0.01[a]	0.38 ± 0.02[c]	0.22 ± 0.01[b]
Vesicular fructose	15.88 ± 2.13[b]	11.74 ± 2.43[b]	16.72 ± 3.26[a]	11.14 ± 2.05[c]	16.52 ± 2.57[b]

Values with the same superscript letters in the same column are not significantly different at P < 0.05.. Each value represents the mean ± SEM; n = 6

*DW= distilled water; BA1= Balanitesaegyptiaca 1200mg, BA2= Balanitesaegyptiaca800mg, BA3= Balanitesaegyptiaca400mg; TE= Testosterone enanthate. Each value represents the mean ± SEM; n = 6; *denotes a significant difference (P < 0.05) compared to the normal control (DW)*

Serum calcium measurement showed greater values in BA1, BA2, BA3 and TE (12.49%, 13.73%, 0.62% and 9.72% respectively) when compared to the control group. Only BA1 and BA2 showed a significant difference (P < 0.05). Serum total cholesterol measurement showed superior values in BA1 and TE (by 6.76% and 22.13% respectively) which were significant when compared to DW. Meanwhile BA2 and BA3 had lower values (4.51% and 10.04% respectively) and only BA3 was significant when compared to DW. Serum testosterone measurement showed significantly superior values in BA1, BA2, and TE (by 69.46%, 110.42% and 197% respectively) when compared to DW while BA3 serum testosterone was lower (by 38.88%) but not significant. Testicular total cholesterol measurement showed greater values in

BA1, BA2 and TE (by 0.9%, 43.82% and 72.21% respectively) but only BA2 and TE were significant when compared to DW. While in BA3 total testicular cholesterol was non-significantly lower (by 14%) when compared with DW. We observed a higher value of total proteins in BA3 (by 6.43%) which was non-significant when compared to DW. While BA1, BA2 and TE values were lower (22.82%, 9.56% and 3.63% respectively) and non-significant. Measurement of testicular testosterone revealed higher values in BA1, BA2 and TE (by 43.29%, 68.63% and 94.18% respectively). Values of BA2 and TE were significantly different when compared to DW. Whereas BA3 had a lower testicular testosterone and the difference (58.06%) was non-significant when compared to DW. Measurement of total protein in the epididymis revealed lower values in BA1, BA2, and TE (by 26.97%, 51.4% and 27.15% respectively). Only the value in BA2 was significantly different when compared to DW. Whereas a superior value was observed in BA3 (27.36%) which was non-significant when compared to DW. Lower significant fructose values were observed in BA1 and BA3 (by 26.08% and 29.9% respectively) when compared to (DW) while BA2 and TE fructose values were greater (by 5.31% and 4% respectively) when compared with DW but not significant.

A high level of testicular cholesterol was noted upon administration of our extract at 800mg. Cholesterol is the starting material for androgen synthesis in the Leydig cells of the testes (Kothandapani et al., 2021). Since the Leydig cells cannot store androgens, de novo synthesis takes place continuously. Hence the high level of cholesterol in the testes is an indication that the aqueous extract of the fruits of *Balanitesaegyptiaca* enhanced steroidogenesis resulting in an increased concentration of androgens (Moutard*et al*., 2023). This could be responsible for the high level of serum and testicular testosterone seen in our study. The elevated level of cholesterol observed in this study showcases the anabolic effects of the extract (Mbongue*et al*., 2005; Egwurugwu*et al*., 2005; Muthu and Krishnamoorthy, 2011).

LH, testosterone and FSH are the main endocrine factors controlling testicular functions. Testosterone is the principal circulating androgen. It is secreted by Leydig cells under LH stimulation, and plays an important role in the maintenance of spermatogenesis (Weinbauer et al., 2010). The elevated level in testosterone concentration observed in our study is probably due to the induction of its synthesis by the Leydig cells (Chung *et al*., 2019). Our extract may therefore play a role in stimulating androgen synthesis. This is supported by the elevated level of

cholesterol which is the starting material for androgen synthesis. The increase in the concentration of sperms, physical strength, the relative mass of the testes and seminal glands, and general body mass observed in our study could be a manifestation of elevated testosterone (Yakama *et al*., 2025). This is therefore another indication of the anabolic and androgenic potential of our extract which agrees with the findings of Yakubu*et al*. (2008) and Yakubu*et al*. (2010), following the administration of *Bulbinenatalensis*Baker (Asphodelaceae) stem, *Mucunapruriens*Linn. (Fabaceae) seeds, *Fadogiaagrestis*(Schweinf. Ex Hiern) stem and *Massulariaacuminata*(G. Don) Bullock ex Hoyl. (Rubiaceae) stem in male Wistar rats.

Several studies have shown that steroidal saponins contribute to the improvement of male sexual function. Pashapour*et al*. (2023)reported that saponins ameliorated the testicular tissueand sperm parameters over a treatment period of 56 days in streptozotocin-induced diabetic mice. Gauthaman and Ganesan (2008) have also reported that protodioscin has an androgen increasing property. Another study reported that the saponin fraction of the extract *Tribulusterrestris* was shown to possess a stimulatory action on libido and spermatogenesis (Tomova*et al*., 1981; Balanathan*et al*., 2001). The administration of 800 mg of the aqueous extract of the fruits of *Balanitesaegyptiaca* is able to enhance testicular function, expressed by the increase in testicular testosterone and sperm count in male rats. This is an indication that the plant has an androgenic potential, and therefore suggests that the aphrodisiac claims is a function of its androgenic property.

4. CONCLUSION

Male sexual disorders often arise as a result of a deficiency in androgens. Traditional medicinal plants are progressively becoming a recourse in the management of these ailments. The present study was aimed principally at evaluating the nutraceutical potential of the fruits of *B. aegyptiaca* towards the androgenic properties. The results of this research have proven that the administration of the aqueous extract of the fruits of *Balanitesaegyptiaca* is able to enhance testicular function, expressed by the increase in testicular testosterone and sperm count in male rats. This is an indication that the plant has an androgenic activity, and therefore suggests that the claimed aphrodisiac virtues depend on it. The mesocarp of the fruits of *Balanitesaegyptiaca* is a nutraceutical recommended in the treatment of male sexual dysfunction

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Ethical considerations

Conflict of interest: The authors declare that they have no conflict of interest

Ethical approval: This study did not involve human participants. It was approved by the ethical committee of the Faculty of Medicine and Pharmaceutical Sciences of the University of Douala. The work was conducted in accordance with the Organization for Economic Co-operation and Development (OECD) requirements on animal experimentation.

Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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