



Research Article

Phytochemistry and Acute Toxicity Testing of *Balanites aegyptiaca* DEL (*Zygophyllaceae*) Leaf Aqueous Extract in Albino Rats

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ABSTRACT

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Aqueous Extract,  
Albino rats

This study evaluated the phytochemical components and the acute toxicity of *Balanites aegyptiaca* leaf aqueous extract in albino rats. The crude extract was subjected to phytochemical evaluation using standard protocol while the acute toxicity testing was carried out using albino rats and the LD<sub>50</sub> was computed by the arithmetic method of Karbal. Phytochemical screening reveals the presence of high scores for tannins, saponins and flavonoids, and moderate scores for general carbohydrates, terpenes, and alkaloids, while glycosides and reducing sugars showed low scores. The median lethal dose (LD<sub>50</sub>) was calculated as 700 mg/kg and is associated with clinical signs of weakness, depression, transient anorexia, and roughened hair coat which were immediate, while in-coordination, lordosis, ophthalmia, coma and death occurred at about 120 minutes. In conclusion, *Balanites aegyptiaca* leaf aqueous extract contains bioactive components and is moderately safe for therapeutic studies.

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## INTRODUCTION

Phytochemicals are compounds also known as secondary plant metabolites with biological properties of antioxidant activity, antimicrobial effects, modulation and detoxification enzymes, stimulation of the immune system, modulation and hormone metabolism, anti-cancer, free radical quenchers, and inhibitors of lipid peroxidation (Saxena *et al.*, 2013). They are also blueprints for drug synthesis notably alkaloids produce analgesics, antimalarials and tranquilizers; tannins and flavonoids in antimicrobials and glycosides in digoxin and digitoxigenin used in cardiac diseases (Fiot, 2004; Biu *et al.*, 2016). Digitalis is a pharmaceutical obtained from medicinal herbs (Foxglove plant) and used as a heart medication. Aspirin, also known as Salicylic acid, is obtained from willow bark, and taxol, a cancer drug from the Pacific Yew tree. Others include quinine and artemisinin, which are antimalarial and are obtained from the bark of Cinchona and *Artemisia annua* respectively (Nigatu *et al.*, 2017).

Plant-derived compounds/phytochemicals provide natural alternatives to synthetic compounds, and the mechanism of action of bio-products could be sourced from the evaluation of their bio-active components, as this provides opportunities for their structural modification and synthesis to reduce their toxicity, prolong their activity and increase their potency (Sharif *et al.*, 2015).

In the toxicological evaluation of a chemical substance or natural product, acute toxicity testing is the first step and is a method of safety evaluation with a significant function of identifying unexpected toxic effects (Lorke, 1983; Goldberg and Rowan, 1987).

*Balanites aegyptiaca* Del., also known as “desert date”, is a member of the Family *Zygophyllaceae* and is a common plant species in sub-Saharan Africa (Hall and Walker, 1991). It is 4.5-10m tall, woody and

with a small spine scent (Koko *et al.*, 2000), highly resistant to stress resulting from sandy storms and heat waves and low levels of moisture content (Ndoye *et al.*, 2004).

*B. aegyptiaca* has been useful in African natural medicine used in treating cough, typhoid and yellow fever, epilepsy, jaundice, syphilis, diarrhoea and wounds, in addition to its application as anti-inflammatory, anthelmintic, anti-fungal, anti-bacterial, insecticidal, molluscicidal and antidote for snake bites (John *et al.*, 1990; Mohammed *et al.*, 1999; Koko *et al.*, 2000; Inngerdingen *et al.*, 2004; Hammiche and Maize, 2006; Doughari *et al.*, 2007; Kubmarawa *et al.*, 2007; Chapagain *et al.*, 2008; Maregesi *et al.*, 2008; Chothani and Vaghasiya, 2011).

Despite the numerous application and uses of this plant, there is a dearth of data on the toxicity and bioactive components of *Balanites aegyptiaca* species in this northeastern part of Nigeria. Thus, this study attempts to provide such baseline data to establish the safety of its products in research and therapeutics.

## MATERIALS AND METHODS

### *Plant Collection and processing*

Fresh leaves of *Balanites aegyptiaca* were collected from surroundings within the University of Maiduguri Campus. They were subsequently taken to the Department of Biological Sciences, University of Maiduguri, Nigeria for identification by a botanist. The leaves were then air-dried under shade (to prevent solar leaching) at laboratory conditions for 14 days. After hand crushing, exactly 500-gram powder was used for extraction with 700 mL distilled water at 60 °C for 8 hours using the Soxhlet extractor. Concentration was done overnight in an oven on an aluminium tray at 60 °C. The drying process removed the water, to obtain 389.5

grams, a 77.9 % w/w yield and stored at room temperature until used.

#### *Phytochemical Screening*

Bioactive components in the crude aqueous leaf extract of *Balanites aegyptiaca* were determined using the standard protocols (Wall *et al.* 1992; Sofowora 1993; Trease and Evans 2002).

#### *Acute Toxicity Testing*

Twenty-five albino rats of both sexes weighing between 112 and 306.3 g were used to determine the median lethal dose (LD<sub>50</sub>) of the aqueous extract of *B. aegyptiaca* leaf. Overall, the rats were sub-divided into five groups (A-E) of 5 rats each and kept in polyacrylic plastic cages within ambient conditions (temperature: 27±1°C; humidity 40± 5%; photoperiod: 12-hour natural light and 12-hour dark). They were fed with pelleted (Vital®) feeds and potable water *ad libitum* and allowed four weeks to acclimatize to the laboratory environment before the commencement of acute toxicity testing.

Rats in each group (A-D) were administered intraperitoneally with graded doses of 200, 400, 800 and 1600 mg/kg of the extract at a concentration of 10g/100ml, while group E rats received only water and served as control. All experimental rats were observed over a

period of 24 hours for clinical signs and possibly death. The LD<sub>50</sub> was calculated using the arithmetic method of Karbal as described by Enegeide *et al.* (2013). All experimental albino rats were handled as recommended by CIOMS, (1985).

## RESULTS

#### *Phytochemical Screening*

Results shows that *B. aegyptiaca* leaf aqueous extract displayed high scores for tannins, saponins and flavonoids (+++), moderate scores for alkaloids, general carbohydrates, and terpenes (++), while reducing sugars and glycosides (cardiac and steroidal), expressed low scores (+) (Table 1).

#### *LD<sub>50</sub> and Clinical Symptoms*

Clinical symptoms observed include weakness, depression, anorexia, starry hair coat, ophthalmia, arched back, coma and death. At the highest dose of 1600 mg/kg, all five of the rats were dead translating to 100% mortality, mortality of three (60%), two (40%) and one (20%) for 800 mg/kg, 400 mg/kg and 200 mg/kg respectively. Using the Karbal method, the calculated LD<sub>50</sub> was 700 mg/kg (Table 2)

**Table 1:** Phytochemical Components of *Balanites aegyptiaca* Aqueous Leaf Extract

Bioactive Component	Tests	Scoring
General Carbohydrates	Molisch's	++
Monosaccharides	Barfoed's	-
Reducing Sugars	Fehling's	+
Ketones	Standard	-
Pentoses	Standard	-
Tannins	Ferric Chloride	+++
Combined Anthraquinones	Borntrager's + H <sub>2</sub> SO <sub>4</sub>	-
Glycosides	Salkowski's	+
Terpenes	Liebermann-Buchard's	+++
Saponins	Frothing	+++
Flavonoids	Pew's	+++
Alkaloids	Dragendorff's	++

**KEYS:** - = Not detected + = Low concentrations ++ = Moderate concentrations +++ = High concentration

**Table 2:** Median Lethal Dose (LD<sub>50</sub>) of *Balanites aegyptiaca* Leaf Aqueous Extract in Albino Rats using Karbal Method

Groups (n=5)	Dose (mg/kg/bw)	Dose Difference (DD)	Number Dead	Mean Dead (MD)	DD x MD
A	200		1		
B	400	B-A= 200	2	1.5	300
C	800	C-B = 400	3	2.5	1000
D	1600	D-C = 800	5	4.0	3200
Total					4500

$$LD_{50} = \frac{LD_{100} - (DD \times MD)}{n} \quad 1600 - \frac{4500}{5} = 1600 - 900 = 700 \text{ mg/kg}$$

## DISCUSSION

Phytochemical screening reveals high scores for flavonoids, saponins and tannins, moderate scores for alkaloids, terpenes and carbohydrates and low scores for reducing sugars and glycosides. *Balanites aegyptiaca* plant species have been reported to contain bio-active benzophenones, terpenes, flavonoids, saponins, bioflavonoids, oxalates, xanthenes, tannins, cyanates, and anthraquinones (Asaolu, 2003). These bioactive compounds may be responsible for the medicinal properties of decoctions of *Balanites aegyptiaca*. Flavonoids, tannins and saponins have been reported to have anti-microbial activity and could serve as precursors of steroidal substances with a wide range of physiological activities (Cowan, 1999). In addition, tannins have been reported to stimulate cyclo-oxygenase -2 (Cox-2) which produces prostanoids that mediate pain, fever, and inflammatory processes (Smith *et al.*, 2000). Also, previous reports have it that plant bio-products such as alkaloids, tannins and anthraquinones possess anti-protozoal and anti-trypanosomal activity (Atawodi and Ogunbusola, 2009; Biu *et al.*, 2015).

Acute toxicity testing of the aqueous extract of *Balanites aegyptiaca* leaf in this study has revealed a median lethal dose (LD<sub>50</sub>) of 700 mg/kg and was toxic with clinical symptoms of weakness, depression, anorexia, starry hair coat, ophthalmia, arched back, coma and death, which appeared like those of general phytotoxicity (WHO, 2012; Saganuwan, 2016). The index of acute toxicity has been described as the LD<sub>50</sub> which provides a margin of safety and toxicity of plant products (Lorke, 1983), and acute testing provides preliminary data on the toxic nature of a material and helps in the informed decision when selecting doses for subchronic and chronic toxicity tests (Gad and Chengelis, 1988). However, this extract could be described as moderately toxic as reported by Enegide, *et al.* (2013) and Erhirhie *et al.* (2018), and should be administered with some degree of safety. The administration of plant extracts without any standard dosage coupled with the non-availability of adequate scientific studies on their safety has raised concerns about the safety of the herbs (Saad *et al.*, 2006). Toxicity studies are useful in determining the potential health risk in humans triggered by intrinsic adverse effects of chemical

compounds contained in plant extracts (Oyedemi *et al.*, 2010; Ashafa *et al.*, 2012) and these adverse effects may manifest significant alterations in the levels of biomolecules such as enzymes and metabolic products, normal functioning, and histomorphology of organs (Yakubu *et al.*, 2009). In acute tests, the organism's defence mechanisms are rapidly overwhelmed and have the significance of detecting unexpected

toxic effects, commonly applied for the safety evaluation of crude plant products (Goldberg and Rowan, 1987; Saganuwan, 2016).

## CONCLUSION

*Balanites aegyptiaca* contains pharmacologically active compounds justifying its use in folklore medicine, but it is moderately toxic and should therefore be used with caution.

## REFERENCES

- Asaolu, M.F. (2003). Chemical Composition and Phytochemical Screening of the Seeds of *Garcinia kola*. *Pakistan Journal of Science and Industrial Research*, 46:145-147.
- Ashafa, A. O. T., Orekoya, L. O. and Yakubu, M. T. (2012). Toxicity profile of ethanolic extract of *Azadirachta indica* stem bark in male Wistar rats. *Asian Pacific Journal of Tropical Biomedicine*, 2(10): 811-817.
- Atawodi, S.E. and Ogunbusola, F. (2009). Evaluation of antitrypanosomal properties of four extracts of leaves, stem, and root barks of *Prosopis africana* in laboratory animals. *Biokemistri* 21:101-108.
- Biu, A.A., Buratai, L.B., Hambali, I.U., Auwal, M.S., Lawan, F.A., Mustapha, F.B., Yakaka, Wakil, Ruth, P.A., Ibrahim, M.M. and Mbaya, A.W. (2015). *In vitro* trypanocidal efficacy of *Balanites aegyptiaca* DEL (*Zygophyllaceae*) leaf aqueous extract. *Annals of Borno*, 25:171-177.
- Biu, A.A., Buratai, L.B., Hambali, I.U., Auwal, M.S., Lawan, F.A., Mustapha, F.B., Yakaka, Wakil, Ruth, P.A., Ibrahim, M.M. and Mbaya, A.W. (2016). Studies on the Phytochemistry, Acute Toxicity and *In vitro* Trypanocidal Efficacy of *Guiera senegalensis* Leaf Aqueous Extract. *Annals of Borno*, 26: 201-208.
- C.I.O.M.S. (1985). International Guiding Principles for Biomedical Research Involving Animals. Developed by Council for International Organization of Medical Sciences in Collaboration with World Health Organization (WHO) 1211 Geneva, 27 Switzerland.
- Chapagain, B. P., Saharan, V. and Wiesman, Z. (2008). Larvicidal activity of saponins from *Balanites aegyptiaca callus* against *Aedes aegyptiaca*. *Bioresour Technology* 99: 1165-1168.
- Chothani, D. L. and Vaghasiya, H. U. (2011). A review on *Balanites aegyptiaca* Del. (Desert Date): phytochemical constituents, traditional uses, and pharmacological activity. *Pharmacognosy Research*, 5(9): 55-62.
- Cowan, M.M. (1999). Plant products as antimicrobial agents. *Clinical Microbiology Review*, 12(4): 564-582.
- Doughari, S, H., Pukuma, M.S. and De, N. (2007). Antibacterial effects of *Balanites aegyptiaca* L. Del. and *Moringa oleifera* Lam. on *Salmonella typhi*. *African Journal of Biotechnology*, 6(19): 2212-2215.
- Enevide, C., David, A. and Fidelis, S.A. (2013). A new method for determining acute toxicity in animal models. *Toxicology International*, 20(3):224-226.
- Erhirhie, E.O., Ihekwereme, C.P. and Iiodigwe, E.E. (2018). Advances in acute toxicity testing: strengths, weaknesses and regulatory acceptance. *Interdisciplinary Toxicology*, 11(1):5-12.

- Fiot, J., Ollivier, E., Timon-David, P. and Balanzard, G. (2004). *Guiera senegalensis* J.F. Gmel. (Combretaceae). *Recent Research Development in Plant Science*, 2:267-277.
- Gad, S.C. and Chengelis, C.P. (1988). In: *Acute toxicity testing perspectives and horizons* (S.C. Gad and C.P. Chengelis eds.) The Tetford Press. Caldwell, N.J. Pp. 2-167.
- Goldberg, A.M., and Rowan, A.N. (1987). Conceptual approaches to alternate methods in toxicological testing. *Defence Science Journal*, 37(2):99-112
- Hall, J. B. and Walker, D. H. (1991). *Balanites aegyptiaca* Del. A Monograph, School of Agriculture and Forest Sciences. Banger: University of Wales. Pp. 1-12.
- Hammiche, V. and Maiza, K. (2006). Traditional medicine in Central Sahara: pharmacopoeia of Tassili N'ajjer. *Journal of Ethnopharmacology*, 105(3):358-367.
- Inngerdingen, K. C. S., Diallo, N. D. and Mounkoro, B. S. (2004). An ethnopharmacological survey of plants used for wound healing in Dogonland, Mali, West Africa. *Journal of Ethnopharmacology*, 92:233-244.
- John, T., Kokwaro, J. O. and Kimanani, E. K. (1990). Remedies of Luo of Siaya District, Kenya. Establishing Quantitative Criteria for Census. *Economic Botany*, 44 (3): 369-381.
- Koko, W. S., Galal, M. and Khalid, H. S. (2000). Fasciolicidal efficacy of *Albizia anthelmintica* and *Balanites aegyptiaca* compared with albendazole. *Journal of Ethnopharmacology*, 71: 247-252.
- Kubmarawa, D., Ajoko, G. A., Enwerem, N. M. and Okorie, D. A. (2007). Preliminary phytochemical and antimicrobial screening of 50 medicinal plants of Nigeria. *African Journal of Biotechnology*, 6 (14): 1690-1696.
- Lorke, D. (1983). "A new approach to practical acute toxicity testing". *Archives of Toxicology*, 54(4): 275-287.
- Maregesi, S. M., Pieters, L., Ngassapa, O. D., Apers, S., Vingerhoets, R., Cos, P., Berghe, D. A. and Vlietinck, A. (2008). Screening of some Tanzanian medicinal plants from Bunda district for antibacterial, antifungal, and antiviral activities. *Journal of Ethnopharmacology*, 119:58-66.
- Mohammed, A. H., El- Tahir, K. E. H., Ali, M. B., Galal, M., Ayeed, I. A., Adam, S. I. and Hamid, O. A. (1999). Some pharmacological and toxicological studies on *Balanites aegyptiaca* bark. *Phytotherapy Research*, 13: 439-441.
- Ndoye, M. (2004). Reproductive biology in *Balanites aegyptiaca*(L) Del. a semi-arid forest tree. *African Journal of Biotechnology*, 3(1): 40-46.
- Nigatu, T. A., Afework, M., Urga, K., Ergete, W. and Makonnen, E. (2017). Toxicological investigation of acute and chronic treatment with *Gnidia stenophylla* Gilg root extract on some blood parameters and histopathology of spleen, liver and kidney in mice. *BMC Research Notes*, 10:625
- Oyedemi, S. O., Yakubu, M.T. and Afolayan, A. J. (2010). Effect of aqueous extract of *Leonotis leonorus*(L) R. Br. leaves in male Wistar rats. *Hum. Experimental Toxicology*, 29: 377-384.
- Saad, B., Azaizeh, H., Abu-Hijleh, G., and Said, O. (2006). Safety of traditional Arab herbal medicine. *Evidence-Based Complementary and Alternative Medicine*, 3(4), 433-439.
- Saganuwan, S.A. (2016). Toxicity study of drugs and chemicals in animals. An overview. *Bangladesh Journal of Veterinary Medicine*, 1311-1477.
- Saxena, M., Saxena, J., Nema, R., Singh, D. and Gupta, A. (2013). Phytochemistry of Medicinal Plants. *Journal of Pharmacy and Phytochemistry*, 1(6): 168-182.
- Sharif, H.B., Mukhtar, M.D., Mustapha, Y. Gabi Baba, and Lawal, A.O. (2015). Acute and Subchronic Toxicity Profile of *Euphorbia pulcherrima* Methanol Extract on Wistar Albino Rats. *Advances in Pharmaceutics*, 539646: 9

- Smith, W.L., Dewitt, D.L. and Garavito, R.M. (2000). Cyclo-oxygenases: Structural, Cellular and Molecular Biology. *Annual Review of Biochemistry*, 69: 145-182.
- Sofowora, A. (1993). *Medicinal plants and traditional medicine in Africa*. Spectrum Books Ltd., Ibadan, Nigeria. Pp. 289.
- Trease, G.E. and Evans, W.C. (2002). *A Textbook of Pharmacognosy*, 14<sup>th</sup> ed. W. B. Saunders Co. Ltd. 24-28 Oval Road, London NW1 and DX, UK. Pp. 13-511.
- Wall, M.E., Eddy, C.R., McClanna, M.L. and Klump, M.E. (1992). Detection and estimation of steroid saponinogens in plant tissues. *Analytical Chemistry*. 24:32-37.
- WHO media centre (2012). Fact sheet NO. 259: African trypanosomiasis or sleeping sickness.
- Yakubu, M. T., Bukoye, B. B., Oladiji, A. T. and Akanji, M. A. (2009). Toxicological implications of aqueous extract of *Bambusa vulgaris* leaves in pregnant Dutch rabbits. *Hum. Experimental Toxicology*, 28: 591-598.