Journal of Advances in Biology & Biotechnology

15(1): 1-7, 2017; Article no.JABB.28802
ISSN: 2394-1081

Gas Chromotography Mass Spectrometric Analysis and Molluscicidal Effect of Crude Saponin from *Balanites aegyptiaca* (L.) Del.

J. S. Hena[1*], A. Sindama[2] and D. Kulawe[3]

[1]Marine Environment Management Unit, Nigerian Maritime Administration and Safety Agency, Abuja Office, Nigeria.
[2]Department of Biological Sciences, Federal University, Wukari, Nigeria.
[3]Department of Biological Sciences, Gombe State University, Gombe, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author JSH designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AS and DK managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JABB/2017/28802
Editor(s):
(1) Maria Serrano, Department of Applied Biology, EPSO, University Miguel Hernandez, Orihuela, Alicante, Spain.
Reviewers:
(1) Musri Musman, Syiah Kuala University, Indonesia.
(2) Dina Mendonça, Universidade da Beira Interior, Portugal.
(3) Ram Pratap Yadav, DDU, Gorakhpur University, India.
(4) Wagno Alcantara De Santana, University & Country Oswaldo Cruz Foundation, Brazil.
Complete Peer review History: http://www.sciencedomain.org/review-history/20944

Original Research Article

Received 6th August 2016
Accepted 25th August 2017
Published 12th September 2017

ABSTRACT

Crude Saponin extracted from the stem-bark of *Balanites aegyptiaca* was analysed using gas chromatography-mass spectrometry and also evaluated for its molluscicidal properties against *Bulinus globosus* and *Biomphalaria pfeifferri* under laboratory condition. The aim was to develop an environmentally friendly molluscicide from natural products that could be used in the control of snail intermediary host of schistosomiasis. GC-MS reveals fourteen compounds where in 9-octa-decanoic acid ($C_{19}H_{36}O_2$) had the highest mass peak of 107 and a similarity index of 93% while Phenol, 2-methoxy-4-(2-propenyl)-acetate ($C_{12}H_{14}O_3$) recorded the least mass peak of 35 and a similarity index of 79%. 100% mortality was recorded for all the species at 10 mg/L except for adult *Biomphalaria pfeifferi.* Adult form of *Bulinus globosus* recorded the highest LD_{50} of 6.4 mg/L while

**Corresponding author: E-mail: jayhena@yahoo.com;*

the juvenile form of *Biomphalaria pfeifferi* had the least LD_{50} 1.4 mg/L. There was no significant difference ($P > 0.05$) in the LD_{50} Value among the two species of the snails and the level of development. This study therefore suggests the further study of these compounds on the field to ascertain its effect on non-target organisms.

Keywords: Saponin; Balanites aegyptiaca; Bulinus globosus; Biomphalaria pfeifferi; Molluscicide; GC-MS.

1. INTRODUCTION

Snail control continues to be one of the important strategies for the use together with other measures such as treatment of clinical cases [1] the use of natural products from local plants as molluscicide will contribute significantly in reducing the burden of purchasing expensive synthetic mollucicide use in snail control [2], and also regulates the rate at which pollutants are introduced into water bodies. *Bulinus globosus* and *Biomphalaria pfeifferi* are the snail intermediate host of *Schistosoma mansoni* and *Schistosoma haematobium* the causative agents of intestinal and urinary Schistosomiasis respectively. Schistosomiasis also known as bilhaziasis is endemic in 74 tropical developing countries where some 600 million people are at risk of becoming infected and an estimated 200 million people are already infected [3,4,5] The disease is also reported to be endemic in Nigeria [6,7,8,9,10].

Balanites aegyptiaca is belongs to the *Kingdom* plantea; Division spermatophyta; subdivision Angiospermea; Class: Dicotyledonea; order: Balanitales; family: Balanitaceae; Genus Balanites; Species: aegyptiaca; local names: soapberry, Adu'a, (Hausa), Tanni (Fulani) and Kingo (Kanuri). It is a large fairly xerophytic savanna tree or shrub indigenous to Egypt, widely distributed throughout Africa, along the tropical belt from Tanzania in the East to Ivory Coast in the West [11]. It is also found in the relatively drier regions of Northern Africa from Mauritania to Nigeria and Ghana, to Egypt, across Palestine, Saudi Arabia and India. The drier regions of Kenya, Uganda and Zaire carry scattered open forests of *Balanites aegyptiaca* [12]. In Nigeria, it is common in the northern part of the country. Extracts from several parts of this tree have been intensively used in Africa and India for various ethno-botanical purposes. Earlier studies have shown that *B. aegyptiaca* contains steroidal saponin. Most of these studies have reported that the presence of saponin is the main cause of these activities. Besides its medicinal uses, *Balanites* trees are widely used as fodder, and for timber purposes [13] in treatment of skin diseases and remedy for stomach ache and jaundice [14], as antidote for snake bite [15], in the treatment of diarrhea (Verbal information) and syphilis [16]. The root was also reported to be used in treatment of inflammation [17]. It was also reported in the treatment of HIV and leukemia [18].

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

The leaves and stem-bark of *Balanites aegyptiaca* was collected in the wild in Zaria (latitude 11.07N and longitude 4.28E). This was taken to the herbarium of the Department of Biological Sciences, Ahmadu Bello University Zaria were it was identified with voucher number 900175. The stem-bark was shade dried and grinded into powder using pestle and mortar. Exposure to sunlight was avoided to prevent the loss of active components [19].

2.2 Test for Saponin

2.2.1 Frothing test

About 0.5 mg of the powdered stem-bark was shaken with 5 mL of distilled water in a test tube. Frothing which persist on warming indicated the presence of saponin [20].

2.2.1.1 Test for steroids and triterpenes

5 mL of acetic anhydride was added to the powdered stem-bark. 1mL of concentrated sulphuric acid was also added down side the tube, the colour change was observed immediately and later. Red, pink or purple colour indicates the presence of triterpenes while blue or blue green indicate steroids [21].

2.3 Extraction of Saponin

Saponin was extracted using the method of [22] as modified by [23]. 20 mL of 20% aqueous ethanol was added to 10 g of the grinded sample and agitated with a magnetic stirrer for 12 h. The

solution was then filtered using Whatman No.1 filter paper and the residue re-extracted with 200 mL 20% aqueous ethanol. The extract was reduced to 40 mL under vacuum and 20 mL diethyl ether added in a separating funnel and shaken vigorously. The aqueous layer was recovered and ether layer discarded. The pH of the aqueous solution was adjusted to 4.5 by adding NaOH, and the solution shaken with 60 mL n-butanol. The combined butanol extracts were washed twice with 10 ml of 5% aqueous NaCl and evaporated to dryness to give a crude saponin which was weighed and recorded.

2.4 Gas-Chromatography – Mass Spectrometry Analysis

Crude Saponins (n-butanol fraction) from the stem of *B. aegyptiaca* was subjected to gas chromatography – mass spectrometry analysis, (GCMS-QP2010 PLUS SHIMADZI, JAPAN) Column of 0.25d mm and 30 mm length was used at column oven temperature of 60.0℃, an injection temperature of 250.00℃ and a pressure of 100.2 kPa, a column flow of content identified was compared identification was performed by matching their retention indices and mass spectra with those obtained from authentic samples and the National Institute of Standards and Technology (NIST)) library. Compounds with close similarity index where identified and recorded.

2.5 Molluscicide Bioassay

2.5.1 Collection of snail species

Two species of mollusc *Biomphalaria glabrata and Bullinus globosu*s were collected in artificial ponds in Dumbin Dutse, Igabi local government area of Kaduna state and transported in a plastic container to the Department of Biological Sciences, Ahmadu Bello University, Zaria where they were separated into adult and juvenile based on their size in milimeter. This was done using a micro meter screw gauge. For *Bulinus* sp., 5-10 mm was considered as adults, 2-4 mm as juvenile. For *Biomphalaria* sp, 3-5 mm was considered juvenile while 6-10 mm was considered adults as described by [24].

Guideline for evaluation of molluscicides was adopted for evaluation of the potency of the extracts [25]. Each species was immersed in different concentration of saponin from the stem-bark of *Balanites aegyptiaca* ranging between 2.5 mg to 60 mg. The snail species were placed in the different concentration 10 per concentration in duplicate. After 24 hours, they were rinsed and then transferred into clean containers containing tap water and left to recover for 24 hours. Those that do not show any sign of life after the recovery period are considered dead i.e. showing no sign of movement that could be elicited by mechanical protruding of the head foot [26]. The mortality rate was determined and recorded for each species.

2.5.2 LD_{50} determination

LD_{50} and LD_{90} were determined using probit probability as the percentage mortality value against the logarithm of concentration of the extract. LD_{50} is the concentration of the extract that will kill 50% of the snails.

3. RESULTS AND DISCUSSION

The result reveals the presence of triterpenoidal saponin in the plant this disagrees with the finding of who reported steroidal saponin in the parts of *B. aegyptiaca.* This may be due to the fact that plant secondary metabolites is affected by plant species, genetic origin, the part of the plant examined the environmental and agronomic factor associated with the growth of the plant post harvest treatment such as storage and processing [27]. This is due to the addition of ten-carbon (C10) terpene units to compose a C30 triterpene skeleton [28] often with subsequent alteration to produce a C27 steroidal skeleton [29]. Steroidal saponin, oleanolic acid and glucoside were reported to be biodegradable probably by bacterial action, when suspended in water for a few days they lose their molluscicidal potency [30]. This confers on the plant an advantage of being environmentally friendly molluscicide. Molluscicidal bioassay showed 100% mortality for the two species and the stages of development the fruit, kernel bark, root and branches of *Balanites aegyptiaca* proved lethal to molluscs, miracidia and cercariae of *Schistosoma mansoni* [31,32]. This demonstrates that saponin from *Balanites* as low-cost-technology for snail control was effective in high concentrations at the first exposure for the snails obtained from both areas and the intermittent exposure of the lower concentrations of saponin.

Table 1. LD_{50} and LD_{90} for crude stem-bark saponin on snail species

Snail species	LD_{50} values	LD_{90} values	Slope
Biomphalaria (Adult)	1.6	4.8	6.31
Biomphalaria (Juvenile)	1.4	4.2	6.82
Bulinus (Adult)	6.2	7.2	1.80
Bulinus (Juvenile)	5.7	6.8	2.39

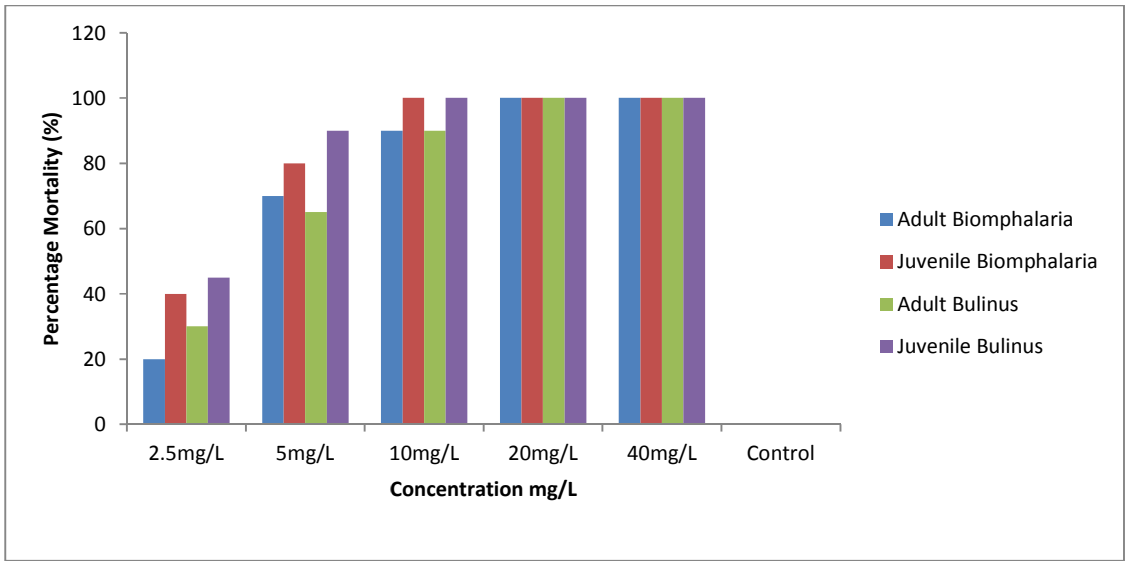

Fig. 1. Percentage mortality of snail species at different concentration of saponin from stem-bark of *Balanites aegyptiaca*

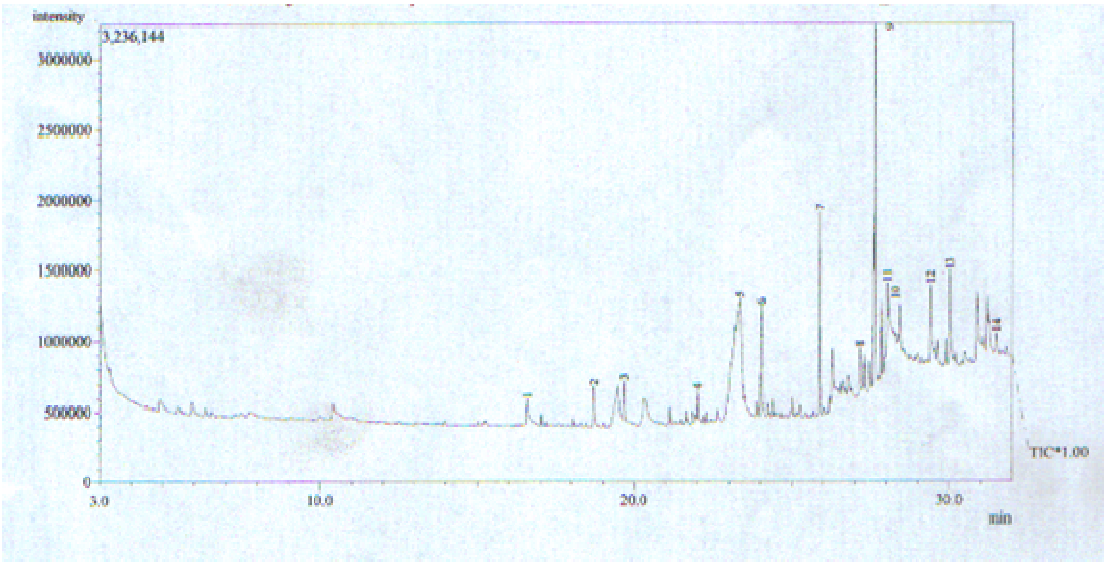

Fig. 2. GC-MS chromatogram of crude saponin from stem-bark of *Balanites aegyptiaca*

This study therefore recommends that similar work be conducted on non-target organisms in the water bodies and also research be conducted on the biodegradability of these compounds in field experiments.

Table 2. GC-MS result of n-butanol fraction of stem-bark of *B. aegyptiaca*

Line	Compound name	Formular	Mol. weight	Retention (Min.)	Similarity index (%)
1.	2-methoxy-4-vinylphenol phenol	$C_9H_{10}O_2$	150	16.600	83
2.	Benzaldehyde	$C_8H_8O_3$	152	18.708	77
3.#	Phenol, 2-methoxy-4-(2-propenyl)-acetate	$C_{12}H_{14}O_3$	206	19.683	80
4.	Ethanone, 1-(4hydroxphenyl)-	$C_9H_{10}O_3$	166	22.008	74
5.	Alpha-d-6,3-furanose				
6.	4-((3-hydroxy-1-propenyl)-2-methoxyphenol	$C_{10}H_{12}O_3$	180	24.025	81
7.	Hexadecanoic acid	$C_{17}H_{34}O_2$	270	25.892	94
8.	2-Butanone	$C_{13}H_{22}O$	194	27.158	81
9.*	9- octa decanoic acid (Z)	$C_{19}H_{36}O_2$	296	27.642	93
10.	Octadecanoic acid	$C_{19}H_{38}O_2$	298	27.850	93
11.	13-Octadecenal	$C_{18}H_{34}O$	266	28.042	81
12.	Tetradecanoic	$C_{30}H_{60}O_2$	452	29.400	52
13.	9-Octadecenamide	$C_{18}H_{35}N0$	281	30.025	89
14.	Di-n-octylphalate	$C_{24}H_{38}O_4$	390	31.517	79

**Compound with the highest mass peak of 107 and a base peak of 55.00. #compound with lowest mass peak of 35 and base peak of 103*

4. CONCLUSION

This research highlights the potency of *Balanites aegyptiaca* in the control of intermediary hosts of the schistosomiasis. It provides an environmentally friendly substitute to the harzadous chemicals that have been used for such control. We therefore recommend futher investigation of the extract on non-target organism and the isolation of the bioactive saponin for further elucidation.

ETHICAL APPROVAL

As per international standard or university standard ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Schall VT, De Vasconcellos MC, De Souza CP, Baptista DF. The molluscicidal activity of crown of christ (*Euphorbia splendens* var. *hislopii*) latex on snails acting as intermediate hosts of *Schistosoma mansoni* and *Schistosoma haematobium.* Am. J. Trop. Med. Hyg. 1998;58(1):7–10.
2. Osman EA, Mohamed EM, Elreesh BI, Elegami AA. Molluscicidal activity of *Combretum glutinosum*. International Journal of Molecular Medicine and Advanced Science. 2007;3(4):151–154.
3. WHO. The control of schistosomiasis: Second report of the WHO Expert Committee WHO Technical Report Series No. 830; 1993.
4. Chitsulo L, Engels D, Montresor A. and Savioli L. The global status of schistosomiasis and its control. Acta. Tropica. 2000;77:41-51.
5. Gibodat M. Post-transmission schistosomiasis: A new agenda. Acta Tropica. 2000;77:3-7.
6. Okafor FC. *Schistosoma haematobium* cercariae transmission pattern in freshwater systems of Anambra state, Nigeria. Angwe Parasitol. 1990;31:159-166.
7. Okpala HO, Agwu E, Agba MI, Chimiezie OR, Nwobu GO, Ohihoin AA. A survey of the prevalence of schistosomiasis among pupils of Apata and Laranto areas in Jos, Plateau state. Online J Health Allied Scs. 2004;3:1.

8. Ibidapo CA, Mafe MA, Awobimpe OL. Comparison of three diagnostic methods for the determination of prevalence of urinary Schistosomiasis among residents and pupils of Badagry area of Lagos state, Nigeriai. African Journal of Biotechnology. 2005;4(11): 1325-1328.
9. Okoli EL, Odaibo AB. Urinary Schistosomiasis among school children in ibadan, an urban community in South-Western Nigeria. Trop Med Int. Health. 1999;4:308-315.
10. Kanwai S, Ndams IS, Gyam ZG. Prevalence of urinary Schistosomiasis in Dumbin Duste, Igabi Local Government Area, Kaduna State. In: Book of proceedings, conference of Medical Doctors in University Health Services. 2009;12-15.
11. Mohamed AH, Eltahir KH, Ali M, Galal M, Ayeed IA, Adam SI, Hamid OA. Some Pharmacological and Toxicological Studies on *Balanites aegyptiaca* Bark. Phytother. Res. 1999;13:439–441.
12. Suliman AG, Jackson JK. The Heglig tree. Sudan Silva. 1959;9:1-8.
13. Arbonier M. Trees, shrub and lianas of West African dry zones. The Netherlands: Girad Margraf Publishers. 2002;189-426.
14. Hammiche H, Maiza K. Traditional medicine of leaves in Sahara. Pharmacopocia of Tassili. Journal of Ethnopharmacology. 2006;105.
15. Innggerdingen KS, Diallo ND, Mounkoro BS. An ethnopharmacological Survey of plants use for wound healing in Dogonland Mali West Africa. Journal of Ethnopharmacology. 2004;92:233-244.
16. Boulos L. Medicinal plants of North Africa. Reference Publication Inc. 1992;286.
17. Kubmarawa D, Ajoko GA, Enwerem NM, Okorie DA. Preliminary phytochemical and antimicrobial screening of 50 medicinal plants of Nigeria. African Journal of Biotechnology. 2007;6:(14):1690-1696.
18. World Intellectual property Organisation (WIPO). *Balanites aegyptiaca* extract for the treatment of HIV/aids and Leukemia. (WO/2001/049306). 2001;3-4.
19. Thakare M. Pharmacological screening of some medicinal plants as antimicrobial and feed additive. Masters' Thesis. Department of Animal and Poultry Science, Virginia Polytechnic Institute and State University, Blackburg, Virginia, USA; 2004.
20. Sofoworo A. Medicinal plants and traditional medicine in Africa. Spectrum Books, Ltd Lagos. 2006;231-234.
21. Trease GE, Evans WC. A textbook of pharmacology, 13th Ed. Ballieria T. Nal Ltd. London; 1989.
22. Birk Y, Bondi A, Gestetner B, Ishaya IA. Thermo stable hemolytic factor in soybeans. Nature. 1963;197:1089-1090.
23. Edward JB, Sogbesan OA. Toxicity effect of Temephos on *Bulinus globossus* and *Lymnaea natalensis*. Advances in Biological Research. 2007;1(3-4):130-133.
24. WHO. Guidelines for evaluation of plant molluscicides. In: Lemma, (A), Heyneman (D), Silangwa (S), ed. *Phytolacca dodemonda* (Endod). Dublin, Tycooly International Publishing Limited. 1983; 121–124.
25. WHO Guidelines for evaluation of plant molluscicides. In: Lemma, (A), Heyneman (D), Silangwa (S), ed. Phytolacca dodemonda (Endod). Dublin, Tycooly International Publishing Limited. 1983; 121–124.
26. Evans NA, Whitefield PJ, Squire BJ, Fellows LE, Evans SV, Mollott SM. Molluscicidal activity in the seeds of *Millettia thrningii* (Legummosae: Papilionideae). Med. and Hyg. 1986;80: 451-453.
27. Fenwick A. Experience in molluscicide to control schistosomiasis. Parasitology Today. 1987;3:70–73.
28. Hartmut F. MetaCyc pathway: Saponin Biosynthesis I; 2006.

    Available:http://BioCyc.org/META/NEW-IMAGE?type=PATHWAY&object=PWY-5203&detail-level=3

    (Retrieved on February 23, 2009)
29. Hostettmann K. and Marston A. Saponins. Cambridge: Cambridge University Press. (1995). p. 3. ISBN 0-521-32970-1. OCLC 29670810.
30. McCollough FS, Gayral PH, Duncan J, Chrstie, JD. Molluscicide in Schistosomiasis control. Bulletin of World Health Organization (WHO). 1980;58(5): 681- 689.

31. Archibald RG. The use of the fruit of tree *Balanites aegyptiaca* in the control of schistosomiasis in the Sudan. Transaction of Royal Society of Tropical Medicine and Hygiene. 1933;27:207.

32. Bashir AK, ElKheir YM, Ahmed EM, Suliman SM. Investigation of moluscicidal activity of certain Sudanese plants used in folk medicine. The first Arab Conference on Medicinal Plants. Cairo, Eygpt; 1984.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/20944