European Journal of Medicinal Plants
4(4): 490-502, 2014

SCIENCEDOMAIN *international*
www.sciencedomain.org

In vivo Antiplasmodial Activity of the Stem Extracts of *Balanites aegyptiaca* (L) Delile

Sulaiman S. Rukayyah[1*] and Ali A. Jigam[2]

[1]*Department of Biochemistry, Ibrahim Badamasi Babangida University, Lapai, Nigeria.*
[2]*Department of Biochemistry, Federal University of Technology, Minna, Nigeria.*

Authors' contributions

Author SSR managed the analyses of the study, literature review and wrote the draft of the manuscript while author AAJ designed and also monitored the study.

Original Research Article

Received 23rd March 2013
Accepted 5th December 2013
Published 18th January 2014

ABSTRACT

Aims: To investigate in vivo antiplasmodial activity of the stem extracts of *Balanites aegyptiaca* (L) Del.
Study Design: Animal model infected with *Plasmodium berghei*.
Place and Duration of Study: Department of Biochemistry, Federal University of Technology, Minna, Niger State, Nigeria, between March 2011 and May 2011.
Methodology: Twenty healthy Swiss albino mice of either sex weighing between 20-30g were selected and divided into five groups. One group served as control, another group as standard and the others as the test groups for hexane, ethylacetate and methanolic extracts respectively. The mice were infected with Chloroquine sensitive strain of *Plasmodium berghei* and left for 72hours for the infection to be established. 600 $mgkg^{-1}$ bw day^{-1} dose was determined as safe and used for the analysis. After 72 hours of infection, the plant extracts were administered subcutaneously once daily for 4 days from D3 to D7. The control group was given 0.9w/v of normal saline. Thick and thin blood smears from the tail blood were examined for parasites. 5mg/kg bw of Chloroquine phosphate, used as control drug, was administered to the positive control.
Results: None of the three fractions (Hexane, Ethylacetate and Methanolic extracts) of *B. aegyptiaca* was completely able to clear the parasites in circulation. In fact, the group of mice given methanolic extract of *B. aegyptiaca* died before the control group while both groups given ethylacetate and Hexane fractions died on the 14th day after infection. The mice administered with hexane and ethyl acetate extracts suppressed parasitaemia on

**Corresponding author: Email: rukkysule82@gmail.com;*

the 6th and 10th day respectively, these suggest that purification and isolation of these crude extracts to know the active compound responsible for the decrease in parasitaemia can be use as drug target in the treatment of malaria,
Conclusion: Stem extracts of *B. aegyptiaca* have suppressive effect on Plasmodium parasites but no curative effect. Further purification and isolation active compounds can help in discovery of a new antimalaria drug. It could thus be assumed that *B. aegyptiaca* is useful in the herbal malarial management by other mechanism other than plasmodicidal effect .Histological studies could establish reason(s) for the death of group treated with methanolic extract before the control group.

Keywords: Balanites aegyptiaca; phytochemicals; curative; Plasmodium beghei.

1. INTRODUCTION

The therapeutic potentials of *Balanites aegyptiaca* (L) *Delile* extracts have been widely reported. In 2007, Otieno et al. reported that *B. aegyptiaca* extract supplemented with a 60-100mg mineral (Kadosero) revealed 100% reduction in bacterial colony in untreated well water [1]. The stem bark extracts also showed high antifungal activity against *C. albicans* and *Aspergillus niger* [2]. Antileishmanial and antihelminthic activities have also been reported [3,4]. *Balanites aegyptiaca* evaluation has revealed its antiproliterative and antidiabetic activity [5,6]. Speroni et al. reported both the anti-inflammatory and antioxidant activities of *Balanites aegyptiaca* [7].

Balanites aegyptiaca is a species of tree that belongs to the plant kingdom, division classified either as a family member of *Zygophyllaceae* or *Balanitacea* [8]. *B. aegyptiacae is* commonly known as desert date or soapberry tree and as *Aduw*a in Hausa language [9]. It is believed that it is indigenous to all dry lands South of the Sahara (Sahel), Middle East and South Asia [8]. It is a multibranched spiny shrub tree that reaches 10m in height, with a stem diameter of about 30cm. The tree coppices well and the trunk is short often branching from near the base and armed with short yellow or green thorns of up to 8cm long. The leaves have two separate leaflets, which are obovate, asymmetric, 2.5-6cm long, bright green, leathery, with fine hair when young. Flowers are in fascicles in the leaf axils and are fragrant, yellowish-green [10]. The fruit is narrow drupe, 2.5-7cm long, 1.5-4cm in diameter. Young fruits are green and tormentose, turning yellow and glabrous when mature. The pulp is bitter-sweet and edible. The seed is pyrene (stone), 1.5-3cm long; light brown, fibrous and extremely hard [8].

Various parts of *B*. a*egyptiaca* have traditional medicinal properties [11]. The plant stem bark was selected for this study as a result of claims by traditional healers, of its efficacy in the treatment of malaria fever in the northern region of Nigeria [12]. Medicinal plants are considered to be the main sources of biologically active compounds that can be used for the treatment of various ailments including malaria [13]. The major contributory factors to the growing interest in medicinal plant include: rising costs of orthodox medications, low therapeutic index of synthetic compounds and the growing incidence of drug resistance among the pathogens especially in developing countries with very weak economic indices [14]. This plant with many therapeutic potential could also serve as drug target for novel antimalaria pharmacophores against *Plasmodium* parasites which have developed resistance to the commonly available malaria drugs such as chloroquine, quinine and artemisinin derivatives [15]. Five species of the *Plasmodium* parasites can infect humans, the most wide spread and virulent form of the disease is caused by *Plasmodium falciparum*

and is responsible for about 80% of all malaria cases, and about 90% of the deaths [16,17,18]. Malaria mortality rates have been cut by over a quarter worldwide, and by one third in the World Health Organization (WHO) Africa Region. However, malaria transmission still occur in 99 countries and the disease caused an estimated 655,000 death in 2010, mainly among children under five years of age in sub-Saharan Africa [19]. Malaria caused by *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae* have milder symptoms in humans and not generally fatal. A fifth species, *Plasmodium knowlesi*, is a zoonosis that causes malaria in macaques and also in humans [18]. Parasitic *Plasmodium* species also infect birds, reptiles, monkeys, chimpanzees and rodents [20]. Some tribal groups in Nigeria use *B. aegyptiaca* as a component of concoction used in herbal medicine for the treatment of malaria fever. The present analysis has thus been conducted to validate the use of *B. aegyptiaca* traditionally in malaria treatment.

2. MATERIALS AND METHODS

2.1 Plant Materials

The stem bark of *Balanites aegyptiaca* was collected at Batagi in Lemu Local Government Area of Niger State, Nigeria between July and September, after identification and authentication at the Biology Department Federal University Technology, Minna. The plant was air-dried and pulverized using mortar and pestle after the debris are removed. The powder was packaged in an air-tight container, labelled and stored prior to extraction.

2.2 Preparation of Crude Extracts

Eighty grams of air dried sample of *Balanites aegyptiaca* was extracted exhaustively (48hours) in the cold sequentially with two liters each of n-hexane, ethylacetate and methanol (Sigma-Aldrich Europe) in that order. The marc was filtered with muslin cloth and solvent removed under reduced pressure in a rotary evaporator. The pastes were poured into beakers and placed on a water bath at 40ºC for complete evaporation of the organic solvent. Each of the yellow pastes obtained were weighed, and labelled prior to further analysis.

2.3 Animals

Healthy Swiss albino mice of either sex of about 7weeks old weighing between 20-30g were obtained from Nnena Rodents Nigeria Limited NITECO Road, Tunga, Minna, in Niger State, Nigeria and were used for the experiments. The mice were housed under standard laboratory conditions at temperature 27±2ºC, relative humidity 70% and at 12 hr day/night cycle. They had free access to rodent pellets and water. The experiments were conducted in strict compliance with internationally accepted principles for laboratory animals’ use and care as contained in the Canadian Council on Animal Care Guidelines and Protocol Review [21].

2.4 Parasites

Plasmodium berghei Nk65 chloroquine sensitive strain was obtained from Nigerian Institute of Medical Research (NIMR) Lagos, Nigeria and maintained in the laboratory by serial passage in mice.

2.5 Phytochemical Screening

Phytochemical screening was carried out with aqueous extract and pulverized sample using standard procedures. Tannins, phlobatannins, saponins, and flavonoids were screened according to the methods described by Edeoga et al. [22]. Cardiac glycosides (Keller-killani Test) and alkaloids were screened according to the methods described by Akinyemi et al. [23].

Quantitative determination of flavonoids, Ten grams of the pulverized stem bark was extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper No 42 (125mm). The filtrate was transferred into evaporating dish and placed on a water bath at 40^{0C} until a constant weight was maintained [24].

Harbone's Method of alkaloid determination was also used. Five grams of the samples were weighed into 250ml beaker and 200ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 hours. This was filtered and the extract was concentrated on a water bath at 90ºC to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed [22].

The Obadani and Ochuka method was used in saponin determination. Twenty grams of each plant sample was weighed into a conical flask and $100cm^3$ of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 hour with continuous stirring at 55ºC. The mixture was filtered and the residue re-extracted with another 200ml 20% ethanol. The combined extracts were reduced to 40ml over water bath at about 90ºC. The concentrate was transferred into a 250ml seperatory funnel and 20ml of diethyl ether was added and shaken vigorously. The aqueous layer recovered while the ether layer was discarded. The purification process was repeated. 50ml of n-butanol extracts were washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was heated on a water bath at 40^{0C}. After evaporation the samples were dried in the oven to a constant weight, the saponin content was calculated as a percentage of the sample used [22].

Total phenol was determined using Edeoga et al. method. Two grams of the plant sample was defatted with 100ml of diethyl ether using a soxhlet apparatus for 2 hours. The fat free sample was boiled with 50ml of ether for 15 minutes for the extraction of the phenolic component. Five millilitre of the extract was pipetted into a 50ml flask, then 10ml of distilled water was added. Two millilitre of ammonium hydroxide solution and 5ml of concentrated amyl alcohol were also added. The samples were made up to mark and left to react for 30 minutes for colour development. This was measured at 505nm [22].

Quantitative determination of Cardiac glycosides: The quantity of glycosides in the pulverized sample was evaluated using Baljet's reagent (95ml 1% picric acid and 5ml 10% NaOH) as described by Oluwaniyi et al. Digitalis cardiac glycosides develop an orange-red colour with Baljet's reagent. The intensity (absorbance) of the colour produced is proportional to the concentration of cardiac glycosides. This colour formation is made use of for the quantitative estimation of cardiac glycosides present in *B. aegyptiaca*. One gram of sample was extracted by soaking overnight with 10ml of 70% alcohol and filtered. The extracts were then purified using lead acetate and Na_2HPO_4 solutions before the addition of freshly prepared Baljet's reagent. The intensity (absorbance) of the colour produced was

measured using a spectrophotometer at 495nm. A blank was carried out at the same time using distilled water and Baljet's reagent. The absorbance of the colour produced is proportional to the concentration of the glycoside. A 0.02% solution of digitoxin solution was prepared in chloroform-methanol (1:1 v/v) mixture (1ml = 0.2mg). Different volumes, viz. 1, 2, 3, 4 and 5ml (equivalent to 0.2, 0.4, 0.6, 0.8 and 1mg of digitoxin respectively) of the solution were transferred, each into a dry Erlynmeyer flask. The solvent in each flask was evaporated on a water bath and dissolved in 0.35ml 90% alcohol. Ten ml of distilled water and 10ml of freshly prepared Baljet's were added. Absorbances were read and standard curve was plotted. The concentration of cardiac glycoside in *B. aegyptiaca* was then extrapolated from the standard curve [25].

Determination of condensed tannins (Proanthocyanidins): The method described by Iqbal et al. was followed for the determination of condensed tannins in the extract. The samples were extracted to quantitatively diffuse the phenolics present in the materials to liquid phase. For the extraction process, aqueous acetone (70%) was used. Each of the dried (finely ground) sample (200 mg) was taken in a glass beaker of approximately 25mL capacity. Ten mL of aqueous acetone (70%) was added and the beaker was suspended in an ultrasonic water bath and subjected to ultrasonic treatment for 20min at room temperature. The contents of the beaker was then transferred to centrifuge tubes and subjected to centrifugation for 10 min at approximately 3000 *g* at 4ºC using a refrigerated centrifuge. The supernatant was collected and kept on ice. The pellet left in the tube was transferred to the beaker using two portions of 5 mL each of 70% aqueous acetone and again subjected the contents to ultrasonic treatment for 20 min. The supernatant was again collected as described above. Butanol–HCl reagent (butanol–HCl 95:5 v/v) was prepared by mixing 95 mL of n–butanol with 5 mL concentrated HCl (37%). Ferric reagent (2% ferric ammonium sulfate in 2N HCl) was prepared by dissolving 2.0 g of ferric ammonium sulfate in 2N HCl (16.6 mL of concentrated HCl was made up to 100 mL with distilled water to make 2N HCl). The reagents were stored in dark bottles. In a 100 mm x 12 mm glass test tube, 0.5 mL of the tannin extract diluted with 70% acetone was pipetted. The quantity of acetone was large enough to prevent the absorbance (550 nm) in the assay from exceeding 0.6. Three mL of the butanol–HCl reagent and 0.1 mL of the ferric reagent was added to the tubes. The tubes capped with a glass marble were shaken using a Vortex and then placed on a heating block adjusted at 97 to 100ºC for 60 min. After cooling the tubes, absorbance was recorded at 550 nm. Absorbance of the unheated mixture (considered as a suitable blank) was subtracted from the absorbance of heated mixture, which was actual reading at 550 nm to be used for calculation of condensed tannins. Condensed tannins (% in dry matter) as leucocyanidin equivalent were calculated by the formula:

$$(A\ 550\ nm \times 78.26 \times \text{Dilution factor})/(\%\ \text{dry matter})$$

This formula assumes that the effective $E^{l\%,\ 1\ cm,\ 550\ nm}$ of leucocyanidin is 460 [26].

2.6 Safe Dose and Acute Toxicity (LD_{50})

Five groups of four mice were used and the animals were given extracts intraperitoneally (i.p) at doses of 200, 400, 800, 1600, and 3000mg/kg body weight (b/w) respectively. Extracts were dissolved with 2ml of dimenthylsulphoxide (DMSO) (Sigma Chemical St Louis, MO, USA) and the volume was made up to 10ml with distilled water.

A control group was given normal saline (0.9% w/v NaCl) at 20ml/Kg bw. Mice were observed over 72 hours for clinical signs and mortality was recorded. LD_{50} was obtained as the intercept of % mortality (y-axis) and dosages (x-axis) [27].

2.7 Antiplasmodal Screening

Mice were pre-screened by microscopy of thin and thick tail tip blood smears. This was necessary to exclude the possibility of test animals harbouring rodent *Plasmodium species*.

2.8 Curative Test

This is a procedure whereby mice are infected and left for 72 hours before treatment with test and standard drug as in the Rane test or established infection [28]. Twenty albino mice were selected and divided into five groups. One group served as control, another group as standard and the others as the test groups for hexane, ethylacetate and methanolic extracts respectively. The mice were inoculated with 0.2ml of diluted donor blood (*Plasmodium berghei* approximately 1 x 10^7 infected red cells) by the intraperitoneal route. The animals were left for 72hours for the infection to be established. The control group was given 0.2ml of 0.9w/v of normal saline. 600 $mgkg^{-1}$ bw day^{-1} dose was selected for the plant extract, because it was the safe dose. On D3 i.e, after 72 hours of infection, the plant extracts were administered subcutaneously once daily for 4 days from D3 to D7. Thick and thin blood smears from the tail blood were examined for parasite every morning. Chloroquine (5mg/kg bw) was used as standard drug because the parasites are sensitive to this compound (Chloroquine sensitive strain of *Plasmodium berghei*) and was hence ran for comparison.

3. RESULTS

3.1 Extract Yields

The extract yields of *Balanites aegyptiaca* obtained with different solvents are in the order: methanol (5.73g) > hexane (4.00g) > ethyl acetate (1.42g).

3.2 Phytochemical Contents

The results of qualitative and quantitative phytochemical screening of *Balanites aegyptiaca* is given in Table 1. Total phenols were most abundant in the plant.

3.3 Safe Dose Determination (pre LD_{50})

The result of safe dose determination of *B. aegyptiacae* is in Table 2. The extract was generally devoid of adverse symptoms up to 600mg/kg bw hence the dose chosen as safe. The safe dose chosen was used for subsequent administration of the extracts to the experimental animals.

Table 1. Phytochemical contents of *B. aegyptiaca* extracts

Phytochemicals	Test	*B. aegyptiaca*	%
Condensed Tannins	0.1% FeCl	+++	9.25
	Bromine H_2O	-	
Pholobatannins	Lead acetate	-	
	1% HCl	-	
Saponins	Frothing	+++	16.10
	Emulsion	+++	
Flavonoids	Dilute NH_3/Conc H_2SO_4	+	12.50
	Lead Acetate	-	
	Dilute NaOH	-	
Cardiac glycosides	Keller-killeni	-	0.00
Alkaloids	Mayer's test	+	4.00
Total phenol	Spectrophotometric test		

+++= highly present ++= moderately present, += slightly present, - = absent

Table 2. Results of dose determination (Pre LD_{50}) for *Balanites aegyptiaca*

Dose (mg/kg bw i.p)	Observation	Mortality
200	No observable change	0/4
400	Apparently Normal	0/4
600	Appear Stable and Normal	0/4
800	Somnolence but Normal	0/4
1600	Somnolence Normal	0/4
3000	Laboured Breathing	0/4

Selected dose = 600 mg/kg bw,

3.4 Results of Parasite Suppression

The suppression of parasitaemia in mice treated with different fractions of *B. aegyptiaca* is in Fig. 1. None of the three fractions (Hexane, Ethylacetate and Methanolic extracts) of *B. aegyptiaca* was able to clear the parasite completely from circulation but hexane and ethyl acetate extracts showed decrease in parasitemia. Mice treated with hexane extract showed suppression on the 6th and 10th day while mice treated with ethylacetate extract showed suppression on the 4th and 10th day. In fact, the group of mice given methanolic extract of *B. aegyptiaca* died before the control group while both groups given ethylacetate and Hexane fractions died on the 14th day after infection.

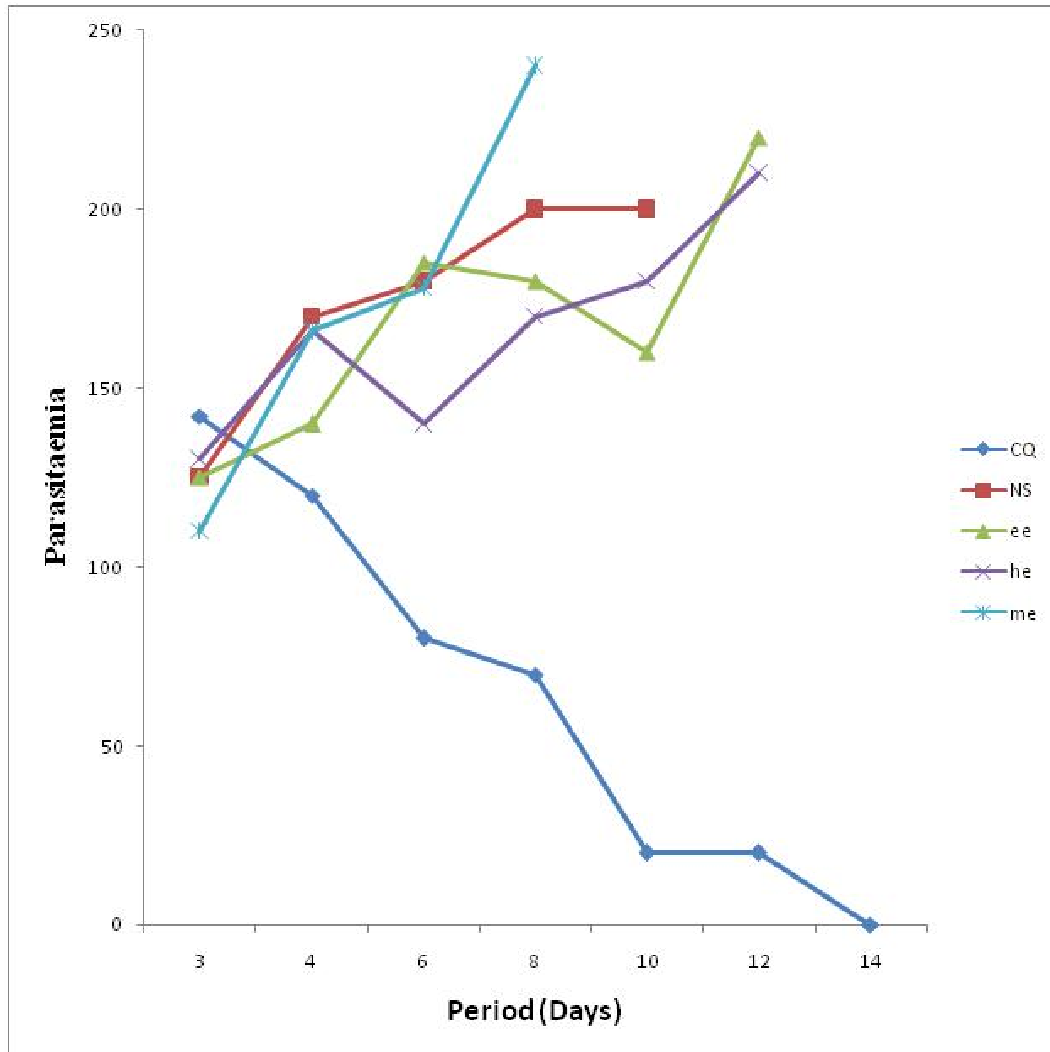

Fig. 1. Suppressive activity of *B. aegyptiaca* in *P. berghei* infected mice
Key: CQ= Parasite suppression for chloroquine (standard), NS= Parasite suppression for normal saline, ee = Parasite suppression for ethylacetate extract, he =Parasite suppression for hexane extract, me = Parasite suppression for methanolic extract.

4. DISCUSSION

Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Properties of a good solvent in plant extraction include, low toxicity, ease of evaporation at low heat, promotion of rapid physiologic absorption of the extract, preservative action, inability to cause the extract to complex or dissociate. The factors affecting the choice of solvent are quantity of phytochemicals to be extracted, rate of extraction, diversity of different compounds extracted, diversity of inhibitory compounds extracted, ease of subsequent handling of the extracts, toxicity of the solvent in the bioassay process, potential health hazard of the

extractants [29]. Polar solvents will extract polar chemical and the same is true for non-polar solvents. Polar solvent include methanol, ethanol and water, medium polar solvents examples are ethylacetate, acetone and dichloromethane and non-polar solvent include toluene, chloroform and hexane [30].

The extract yield is in the order of methanol > hexane > ethylacetate. Sequential extraction is an efficient process to recover various products based on differential solubility, molecular mass and charge distribution of polysaccharides from plant [31]. Solvent extraction is a diffusion process. To extract phytochemicals from an insoluble matrix, solvent must first penetrate the matrix, mobilize the substance, and only then can the substance diffuse from the matrix into the surrounding extraction solvent. This is dependent on the duration for the extraction, which invariably have an effect on extraction yield. McCloud (2010) reported that extraction of small, organic solvent soluble compounds from *Asclepias syriaca* has reached 50(wt/wt) in the first half hour [32]. But double the yield was obtained if steeping in organic solvent is allowed to continue for 20hours. In addition, the mass of materials extracted from a specimen will be dependent on the number of times the substrate is extracted and also the nature of the solvent in relation to the substrate. In a series of studies, it has been demonstrated that following 15 hours of contact, majority (38gm) of organic solvent soluble material is contained in the first pass of 1:1 dichloromethane/methanol. Three additional passes gave extract yield of 2.9, 1.7 and 1.6 grams respectively. So as the number of passes increase, the yield decreases progressively. Two additional passes with 100% Methanol gave 1.7 and 1.0 gram respectively. Reextraction of the residue with water yielded 4.6 and 2.3 grams in the first and second passes [32]. This indicates that sequential extraction decreases extract yield. If the second and third extraction solvents were used to be use alone the yield would have been higher than the yield of the same solvent from sequential extraction. Therefore sequential extraction depends on the number of passes, nature of solvent in relation to the substrate, duration of extraction process and the order of extraction (starting either with non-polar to polar medium or from polar to non-polar medium). The increase in methanolic extract yield clearly indicates that polar substances which are not appreciably ethylacetate soluble are being mobilized. This also shows that most of the phytochemicals present in *B. aegyptiaca* are polar compounds and would be more soluble in polar solvents. The fact that stem bark were use could have affect the yield due to intraspecific variation of secondary metabolites [33].

The phytochemical compounds detected were tannins, saponins, flavonoids, alkaloids and total phenol; consequently this plant could serve as a good source for these compounds.

A high level of tannins was detected in *B. aegyptiaca* extracts. Tannins are potential metal ion chelators, protein precipitating agents and biological antioxidants [34]. The administration of *B. aegyptiaca* extracts could predispose to any of the mentioned processes in vivo. Tannins have been shown to bind and precipitate proteins and other organic compounds which in some case inhibit the absorption of nutrients [35]. If ingested in excess quantities, tannins inhibit the absorption of minerals such as iron, which may, if prolonged, lead to anemia [36]. This is because tannins are metal iron chelators and tannin-chelated metal irons are not bioavailable. Tannins only reduce bioavailability of plant sources of iron, also known as non-heme. Animal sources or heme iron absorption are not affected by tannins [37]. Vitamin C or lemon juice help neutralize the tannin effect on iron absorption [37]. Despite the entire negative effects of tannins Banzouzi et al. isolated ellagic acid (tannin) from *Alchornea cordifolia* as the active ingredient against *Plasmodium berghei* infected mice [38]. This is to show tannins also have beneficial activity. Alkaloids exhibit important pharmacological properties; example *Remijia species* contain quinine which is an

antimalarial agent. Most alkaloids show a good ability to scavenge singlet oxygen [39]. The seed extracts of *Tetracerpidium canophorum* were found to contain alkaloids and also have antibacterial activity [40]. Alkaloids also have toxic effects especially the cyclic esters like retrorsine. This or any alkaloid administered in large doses have cytotoxic effects on vital organs and affect the central nervous system, pyrolizidine alkaloid during hepatic metabolism generate on active intermediates (pyrrole alkylation agent) which is toxic [41]. It is possible that the alkaloids present in the plant are responsible for its toxic property. Bioassay guided fractionation have shown that numerous alkaloids have antiplasmodial activities. Examples of such alkaloids are quinine, chloroquine, araliopdimerine A, indole alkaloids and alkaloid akuamine [42]. Quinine being the first alkaloid to be isolated as antimalarial, acts as a blood schizonticide and weak gametocide against *Plasmodium vivax* and *Plasmodium malariae*. As an alkaloid, it is accumulated in the food vacuoles of *Plasmodium species*, especially *Plasmodium falciparum*. It acts by inhibiting hemozoin biocrystallization, thus facilitating an aggregation of cytotoxic heme [43]. Quinine can cause hypoglycaemia through its action of stimulating insulin secretion [44]. So the, hypoglycaemic effect of *B. aegyptiaca* could be as a result of presence of alkaloid.

Saponins were the largest of phytochemical contents detected after quantitative analysis of *B. aegyptiaca* stem. Steroidal saponins have been shown to have antimicrobial properties [45]. Azebaze et al. reported the antimalaria efficacy of *Allanblackia monticola*, of which stigmasterol-3-o- beta-D-glucopyranosides saponin was detected as one of its phytochemicals [46].

Flavonoids are the other form of the two plant phenolic structures detected in this study. Flavonoids are known to lower vein permeability and are hence used in the treatment of circulatory disorders. Different flavonoids and phenolic compounds react with free radicals to reduce the degeneration of membranes [46]. They also possess antibacterial properties [47]. Flavonoids can also be used as antioxidants and in vitro as enzyme inhibitors [48]. Flavonoids have been reported with significant antiplasmodial activity against different strains of malaria parasites [49].

The death of mice in the group given methanolic extracts before the control group might be due to toxic principal(s) or due to extraction solvent used. The high level of tannins could precipitate some proteins needed for growth and development. Tannins are metal iron chelators and tannin-chelated metal irons are not bioavailable, this can predispose to anemia which could have cause death of the mice before the control group. Metabolism of some alkaloids yields toxic metabolites that affect vital organs like the liver, kidney and central nervous system. Methanol is polar, but due to its cytotoxic nature, it is unsuitable for extraction in certain kind of studies as it may lead to incorrect results [34]. Parasite response to ethylacetate and hexane extract in relation to time (days) indicated some initial suppression but similar to methanol extract, parasitaemia was not resolved. It is thus clear that stem extracts of *B. aegyptiaca* even though being used by herbalist in malaria fever does not demonstrate curative effects but possesses suppressive effect. There is thus the likelihood of identifying with therapeutic effects [50]. Rather the high phenolic and flavonoid contents may be suggestive of its antioxidative properties as earlier reported. Again, the need to test the extracts against *P. falciparum* in vitro is imperative.

5. CONCLUSION

Stem extracts of *B. aegyptiaca* does not have curative effect against *Plasmodium berghei* in mice. It may still be relevant in the management of malaria fever due to its suppressive

effect and also its reported antioxidant and anti-inflammatory effects. Further histological studies could establish reason(s) for the death of group treated with methanolic extract before the control group.

ACKNOWLEDGEMENTS

There was no funding by any organization or agency.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Otieno JN, Hosea KM, Lyaruu HV. The effect of local minerals Kadsaro towards the antimicrobial activity of medicinal plants extract:Case of Lake Victoria Basen, Tarim Tanzania, Afri J. Tradit., Complement and Altern Med. 2007;4:6.
2. Maregesi SM, Pieters L, Ngassapa OD, Apers S, Vinegerhoets R, Cos, P. Screening of some Tanzanian medicinal plants from Bunda district for antibacterial, antifungal and antiviral activities, J. Ethnopharmacol. 2008;119:58-66.
3. Khalid FA, Abdaslla NM, Mohomed HO,Toum AM, Magzxouab MA, Ali MS. *In vitro* assessment of anti-cutaneouas leishmaniasis activity of some Sudanese plants, Turkiye Parazitoloji Dergisi. 2005;29:3-6.
4. Koko WS, Abdalla HS, Galal M, Khalid HS. Evaluation of oral therapy on Mansonial Schistosomiasis using single dose of *Balanites aegyptiaca* fruit and praziquantel, Fitoterapia. 2005;76:30-34.
5. Gnoula C, Megalizzi V. Neve ND, Sauvage S, Ribaucour F, Guisso P. Balanitin-6 and 7: Diosgenyl saponins isolated from *Balanites aegyptiaca* Del. Display significant anti tumor activity in vitro and in vivo, Inter J. Oncol. 2008;32:5-15.
6. Mohammed ZG, Maha MS, Manal FI, Nibal DT. Biochemical study of the antidiabetic action of the Egyptian plants Fenugreek and Balanites, Mol Cell Biochem. 2006;281:173-183.
7. Speroni E, Cervellati R., Innocenti G, Costa S, Guerra MC, Dall S. Anti-S, Journal of Ethnopharmacology. 2005;98:117-125.
8. Yadav JP, Panghal M. *Balanites aegyptiaca* (L) Del.(Hingot): A review of its traditional uses, phytochemistry and pharmacological properties, International Journal Green Pharmacology. 2010;4:140-146.
9. Yves G, Dechassa L. Wildfood plant in southern Ethiopia: Reflection on the role of ‘famine-foods’ at a time of drought”UN-OCHA Report; 2009.
10. Schmidt L, Joker D. *Balanites aegyptiaca* (L.) Del., Seed leaflet. 2000;21:1-2.
11. Hamidou TH, Kabore H, Ouattara O, Ouedraogo S, Guissou IP, Sawadogo L. Efficacy of *Balanites aegyptiaca* (L) DEL. Balanitaceae Anthelminthic and Mulluscicid used by Traditional Healer in Burkino Faso, International Conference on Emerging Infectious Diseases. 2002;37.
12. Mann A, Gbate M, Ndaumar A. Medicinal and Economic plants of Nupeland. Jube Evans Books and Publication, Bida. 2003;160.
13. George VC, Kumar N, Rajkumar V, Suresh PK, Kumar AR. Quantitative Assessment of the relative antineoplastic potential of the n-butanolic leaf extract of Annona muricata Linn. In normal and immortalized human cell lines, Asian pacific J Cancer Prev. 2012;13:699-704.

14. Bulus T, Atawodi SE, Mamman M. Acute toxicity effect of the aqueous extract of Terminalia avicennioides on white albino rats, Sci. World Journal. 2011;6(2):1-4.
15. Omoregie ES, Sisodia BS. In vitro antiplasmodial activity and cytotoxicity of leaf extracts of *Jatropha tanjorensis* J.L. Ellis and Soroja, Bayero Journal of Pure and Appl Sci. 2012;5(1):90-97.
16. Mendis K, Sina B, Marchansini P, Carter R. The neglected burden of *Plasmodium vivax* malaria, Ameri J. Tropl Med Hyg. 2001;62:97-106.
17. Jigam AA, Akanya HO, Emmanuel OO, Dauda BEN, Evans CE. *In vivo* antiplasmodial, analgesic and anti-inflammatory activities of the leaf extract of *Lippa multiflora* mold, J. Med Plant Res. 2009;3:48-154.
18. Singh B, Kim SL, Matusop A. A large focus of naturally acquired *Plasmodium knowlesi* infections in human beings, Lancet. 2004;363:1017-1024.
19. World Health Organization. Scaling up diagnostic testing, treatment and surveillance for malarial; 2012.
20. Escalante A, Ayala F. Phylogeny of malarial genus Plasmodium, derived from rRNA gene sequences, Proc National Academic Sci USA. 1994;19:11373-11377.
21. Ernest D, Olfert DV, Brenda M, Cross VM, Mcwilliam AA. A Guide to Care and Use of Experimental Animals. 2nd edition, Canadian Council on Animal Care; 1993.
22. Edeoga, HO, Okwu DE, Mbaebie BO. Phytochemical Constituents of some medicinal plants, Afri J. Biotec. 2005;49:685-687.
23. Akinyemi, OK, Oladapo O, Okwara CE, Ibe CC, Fasure KA. Screening of crude extract of six medicinal plants used in south west Nigerian unorthodox medicine for anti-methicillin resistance *Staphyolococcus aureus* activity, Biomedicial Central Complement Alternate Medicine. 2005;5:6.
24. Kwada AD, Tella OI. Determination of infochemicals and the phytochemical screening of the foliage and stem bark of *Senna siamea* (lam.) in Yola, Adamawa State, J. Med Plants Res. 2009;3(9):630.
25. Oluwaniyi OO, Ibiyemi AS, Usman AL. Effect of Detoxification on the Nutrient content of Thevetia peruviana seed cake, Res. J. Applied Sci. 2007;2(2):188-191.
26. Iqbal Z, Sajid MS, Abbas RZ, Shindu ZUD. Determination of condensed tannin contents from different plants of kharimurat rangeland (Attock, Pakistan), J. Agric. Soc. Sci. 2001;7:114-116.
27. Ogbadoyi EO, Garba MH, Kabiru AY, Mann A, Okogun JI. Therapeutic evalution of Acacia nilotica (Linn) stem bark extract in experimental African typanosomiasis, Intl. J. App. Res. Natrl. Pro. 2011;4:11-18.
28. Jigam AA, Akanya HO, Emmanuel OO, Dauda BEN. In vivo antiplasmodial, analgesic and anti-inflammatory effects of the root extracts of *Acacia nilotica* Del (Leguminosae), Asian J. Exp Biol Sci 2010;1:315-320.
29. Prashant T, Bimlesh K, Mendeep K, Gurpreet K, Harleen K. Phytochemical screening and extraction: A Review, Int Pharm Sci. 2011;1:98-106.
30. Lekgari L, Extraction of phytochemicals, Biotechnology. 2010;14:29.
31. Suresh V, Kumar NS, Murugan P, Palani P, Rengasamy R, Anbazhagan C. Antioxidant properties of Sequential extracts from brown seaweed *Sargassum plagiophyllum* C. Agardh, Asian Pacific J of Tropical Disease. 2012,5:938.
32. McCloud TG. High through put extraction of plant, marine and fungal specimen for preservation of biologically active molecules, Molecules. 2010;15:4526-4563.
33. Meyer KD, Paul VJ. Intraplant variation in secondary metabolites concentration in three species of Caulerpa (Chlorophyta: Caulerpales) and its effects on herbivorous fishes, Mar. Ecol. Prog. Ser. 1992;82:249-257.

34. Hamgerman AE, Riedl KM, Alenander GJ, Sovik NK, Ritchard TN, Hartz Feld PW, Riechd LT. High Molecular weight plant ployphenolics(Tannins) Biolgical Antioxidants, J. Agric and Food chem. 1998;46:1887-1892.
35. Katie EF, Thorington RW. Squirrels: the animal answer guide, Baltimore; Johns Hopkins University press. 2006;91.
36. Brune M, Rossander L, Hallberg L. Iron absorption and phenolic compounds: Importance different phenolic structures, Euro J Clinc Nutr. 1989;43:547–557.
37. Harrell RF, Reddy M, Cook, J.D. Inhibition of non–haem iron absorption in man by polyphenolic – containing beverages, British Journal of Nutrition. 1999;81:289-295.
38. Banzouzi JT, Pblache Y, Rado R, Menan H, Valentin A, Roumestan E, et al. *In vitro* antiplasmodial activity of extracts of *Alchornea cordiola* and identification of active constituent: ellagic acid, J. Ethnopharmacol. 2002;81:399–401.
39. Trease GE, Evans WC. Pharmacognosy English language Book Society. 13th edition, Bailliere Tindall, London. 1999;683-684.
40. Isaac OO, Chinwe JA. The phytochemical analysis and antibacterialscreening of extracts of *Tetracarpidium conophorum*, J. Chem Soc Nig. 2001;26:53-55.
41. Aiello C, Susan E. The Merck Veternarys Manual, 8th edition, Merck and Company. 1998;2028-2029.
42. Tane P, Wabo H, Tchimere M, Okunhi C, Iwu M, Schuster B, Connolly J. Lead antiplasmodial substance from some Cameroonian medicinal plant, Acta Tropica. 2005;95:195.
43. White NJ. Antimalarial drug resistance. Journal of Clinical Investigation. 2004;113:1084–1092.
44. Seltzer HS. Drug – induced hypoglycemia. A review of 1418 cases, Endocrino and Meta. Clin North Amer. 1989;18:163-183.
45. Azebaze AG, Mayer M, Valentin A, Nguemfor, EL, Fomum ZT, Nkengfack AE. Prenylated Xanthone derivatives with antiplasmodial activity from *Allanblackia monticola.* Chem Pharmacol Bul. 2006;54:111-113.
46. Hertzogi DI and Tica O. Molecular mechanism underlying the anticancerous action of Flavonoids, Cur Health Sci Journ. 2012;38:145-149.
47. Hostettmann K, Martson AJ, Wolfender I, Maillard M. Screening for flavonoids and related compounds in medicinal plants by LC-UV-MS and subsequent isolation of bioactive compounds, Akademiai, Kiaho, Buhapest. 1995;35-52.
48. Evans CR. Flavonoid antioxidants, Curr Med Chem. 2001;8(7):797-807.
49. Ngemenya M, Tilanji V, Akam T, Yong J, Tane P, Fanso-free S, Berzins K. Antiplasmodial activity and toxicity of extracts and products from selected medicinal plant used in Cameroon, Acta Tropica. 2005;96:1–506.
50. Mathee O, Wright AD, Konig OM. HIV reverse transcriptase inhibitor of natural origin. Planta Med. 1999;65:493.

© 2014 Rukayyah and Jijam; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=400&id=13&aid=3389