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Phytochemical Screening and Mosquito Repellent Activity of the Stem Bark Extracts of Euphorbia Balsamifera (Ait)

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ABSTRACT
The phytochemical screening of extracts from *Euphorbia balsamifera* was carried out, and the results revealed that the stem bark contains alkaloids, tannins, flavonoids, saponins, glycosides, terpenoids and sterols. The ethanol extract of the stem bark of *Euphorbia balsamifera* (commonly known as *Ayyara* in Hausa) was sequentially partitioned with petroleum ether, chloroform, ethyl acetate and methanol. The extracts were respectively labeled EB1, EB2, EB3, and EB4, with the ethanolic extract labeled EB. The extracts, 12.5% and 25% w/v, were tested for repellency against *Anopheles gambiae*, and the repellent activity was assessed using the human-bait technique. Five volunteers participated in the laboratory tests to ascertain the repellent activity of the extracts, and each volunteer was test with one extract, with each test repeated in triplicate. The chloroform extract labeled EB1-02 was found to be the most active, (97.2% and 100% repellency), while the extract labeled EB1-03 recorded the least activity of 32.4% and 21.6%. It can be concluded that the chloroform extract labeled EB1-02 is responsible for repellent activity of *Euphorbia balsamifera*.

Keywords: *Anopheles gambiae, Euphorbia balsamifera* extracts, Human-bait technique, Phytochemicals, Repellency,

INTRODUCTION

Balsamiferous Spurge or *Euphorbia balsamifera* is one of the plants traditionally used as a mosquito repellent in Nigeria and other West African countries (Adedapo *et al*., 2004). It is native to all Canary Islands in rocky and sunny places, where it forms dense communities in rocky places and on less mobile dune sand (with the exception of the highly mobile sands) in the lower succulent zone (Adedapo *et al*., 2004). It has broad, shortly lanceolate, fresh green to glaucous leaves on broad, light terracotta brown, very succulent stems. It makes a single central flower above light to yellowish green pseudo-petals (cyathies) on each branch followed by a large yellowish to reddish green, sometimes slightly pubescent seed capsule (Antonio *et al*., 1975). *Euphorbia balsamifera* is commonly grown as a hedge and field boundary marker (Burkill, 1994). Its succulent branches carry a copious amount of latex which is generally reported to be toxic (Kerharo and Adam, 1974).

Euphorbia balsamifera has sap (latex) which is rather poisonous if ingested, but widely used in odontology as traditionally antalgic treatment of acute dental pulpitis (Yam *et al*., 1997). The latex is an effective pulpal devitalizer used in dental offices (Yam *et al*., 1997). Most of the fields in Canary Islands are fenced with *Euphorbia balsamifera* that protects the crops from pest attacks and the soil from wind erosion hazards (Thorsell *et al*., 2004). The chemistry of the Euphorbia species is much interesting and diverse among the flowering plants families. The major secondary metabolites present in Euphorbia species can be classified as lipids and its derivatives, terpenes, aromatics, amines, alkaloids and amino acids found to be present in the family (Evans *et al.,* 1986).

Repellents are organic substances that occur naturally, or are designed by synthesis to make surfaces unpleasant or unattractive to organisms like insects, animals and plants (Moore and Debboun, 2007). The repellent formulations typically contain an active primary ingredient that repels unwanted organisms as well as secondary ingredients, which aid in delivery and cosmetic appeal (WHO, 2006). They are available in many forms, from cream to oils, but are most often sold as aerosols (Knowlton and Pearce, 1993). Repellency is known to play an important role in preventing the vector borne diseases by reducing man-vector contact. An ideal repellent should provide protection against a broad spectrum of blood-feeding arthropods for at least 8 hours, be non-toxic, non-irritating, odorless, and non-greasy (Fradin, 1998).

Insect-transmitted disease remains a major source of illness and death worldwide. Mosquitoes alone transmit disease to more than 700 million people annually (Taubes, 1997). Malaria alone kills

3 million each year (Shell, 1997). Over two billion people primarily in tropical countries, are at risk of mosquito-borne diseases, such as dengue hemorrhagic fever, malaria and filariasis (Service, 1993). The search for effective vaccines against these diseases is still in progress. Mosquito control and personal protection from mosquito bites are currently the most important measures to control these diseases. The use of repellents is an obvious practical and economical means of preventing the transmission of these diseases to humans (Yap, 1998; Coleman *et al*., 1993; Walker *et al*., 1996).

The repellency of plant material has been exploited for thousands of years by man, most simply by hanging bruised plants in houses, a practice that is still in wide use throughout the developing countries (Moore *et al.,* 2006). Tawatsin *et al*. (2006) evaluated the repellency effect of essential oils extracted from some plants in Thailand against mosquito vectors, Diptera and Culicidae, as well as the extracts oviposition deterrent effects against *Aedes aegypti*. On the other hand, Maharaj and Gayaram (2008) showed the repellent activities of some plant species against mosquito, using the time lag trials, while Sophia and Pandian (2009) successfully evaluated the efficacy of the repellent property of certain phytochemicals (Eucalyptus oil and Lemon grass oil) and Rhizomes (*Curcuma longa* and *Acorus calamus*) against the filarial vector mosquito, *Culex quinquefasciatus*. Datti and Idris (2013) tested the leaf extract of *Lawsonia inermis* for repellent activity against adult female mosquito *Anopheles gambiea*. Plants have also been used for centuries in the form of crude fumigants where plants were burnt to drive away nuisance mosquitoes and later as oil formulations applied to the skin or clothes which was first recorded in writings by ancient Greek, Roman and Indian scholars (Carey *et al.*, 2010). Plant-based repellents are still extensively used in this traditional way throughout rural communities in the tropics because for many of the poorest communities they are the only means of protection from mosquito bites that are available (Moore *et al.*, 2006), and indeed for some of these communities (Johnson, 1998) as in Europe and North America (Trumble, 2002) 'natural' smelling repellents are preferred because plants are perceived as a safe and trusted means of mosquito bite prevention.

The aim of this study is to determine the phytochemical constituents and repellent activity of the stem bark extracts of *Euphorbia balsamifera*.

MATERIALS AND METHODS

The plant, *Euphorbia balsamifera* (Ait), was collected in September 2011 from Dawakin Tofa Local Government Area of Kano Nigeria. Taxonomic identification was conducted at the Department of Biological Sciences, Bayero University, Kano, and a voucher specimen was deposited at the herbarium. The bark of the plant was air-dried in the laboratory and grounded into powder with pestle and mortar.

Extraction of the Plant Materials

Three hundred grams (300 g) of the powdered form of the stem bark of *Eurphobia balsamifera* was put in an Amber winchester bottle and percolated with 1.5 L of ethanol for two weeks with shaking at regular interval. It was filtered and then concentrated to dryness on rotavapor (R110) at $40^{O}C$, to give the crude ethanol extract labelled as EB1. Twenty grams (20 g) of the crude extract (EB1) was dissolved in 200 ml aqueous methanol (80% v/v) and then partitioneed with 100 ml each of petroleum ether, chloroform and ethyl acetate (3 times each), and the combined extracts were evaporated and the successive extracts were weighed and respectively labelled as EB1-01, EB1-02 and EB1-03. The aqueous methanol portion was finally evaporated and the extract weighed and labelled as EB1-04 (Colin and Cooke, 2000; Mohan and Ramaswamy, 2007; Dreyer and Kragl, 2008).

Repellency Test for the Extracts

The mosquitoes used in this study were laboratory-reared *Anopheles gambiae*. The standard rearing protocol as described by Mullai and Jebanesan, 2007; Alexander *et al.,* 2010; Dhanasekaran *et al.,* 2013, were adopted. The repellency activity of the five extract fractions obtaine from the back of *Eurphobia balsamifera* were all assessed in the laboratory using a human-bait technique (WHO, 1997). Five volunteers (age 21-34 years) participated in the laboratory tests, with each volunteer exposed to only one of the extracts at a time. The testing period lasted up to eight hours spread over 14 days, depending on the efficacy of repellent. The timing of the tests depended on the fact that *Anopheles gambiae* are night-biters. Evaluations were carried out in a large room, at room temperature. An area of 3x10 cm on each forearm of the human volunteers was marked out with a permanent marker. Each extract was tested for repellency at two different concentrations. The test extracts 12.5% and 25% w/v (extract/ethanol) for each plant extract were applied to the marked area of one forearm of each volunteer, while the other forearm was treated with only ethanol free from the extracts, to serve as a control. This procedure was repeated three times for each concentration and the average was taken. During the test, the forearm was covered by a paper sleeve with a hole corresponding to the marked area. Each volunteer put the test forearm in a mosquito cage (80 x 40 x 40 cm^3), containing 50 female mosquitoes (3-5 days old), for the first three minutes of every half-hour exposure. However, before the start of each exposure, the bare hand, used as control area of each volunteer, was exposed for up to 30 seconds. If at least two mosquitoes landed on the bare hand, the repellency test was then continued. This was done to ensure that the mosquitoes were host seeking. The number of mosquitoes probing the treated area of each volunteer was noted for half-hour (Tawatsin *et al.*, 2001; Karunamoorthi *et al.*, 2008; Sarita *et al.*, 2011).

Percentage repellency (% repellency) in the field evaluation was analysed according to the formula described by Yap *et al*., (1998).

$$\% \, Repellency = \frac{C-T}{C} \, X \, 100\% \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

where C is the number of mosquitoes that landed on the control and T is the number of mosquitoes that landed on the treated volunteers.

Phytochemical Screening of the Extracts

All the extracts from the plant were screened for the presence of alkaloids, tannins flavanoids saponins, sugar, glycosides, terpenoids and steroids were screened according to the methods employed by Harbone (1984) and Imran *et al.*, (2010).

Test for Alkaloids

The extracts, 3 ml each, were introduced into 3 different test tubes, then acidified with 1 ml of 1% hydrochloric acid. About 0.5 g of extract was diluted in 10 ml of 1% aqueous hydrochloric acid, to each of these solutions, 4 drops of Mayer, Wagner and Dragendroff reagents were separately added. A creamy white (Mayer), reddish brown (Wagner) and orange brown (Draggendorff) precipitates indicated the presence of alkaloids.

Test for Tannins

Two drops of 5% ferric chloride was added to 1 ml of the test extract. A dirty green precipitate indicated the presence of tannins in the extract.

Test for Flavonoids (The Shinoda Test)

Magnesium powder (10 mg) was added to 3 ml of the test extracts followed by 5 drops of concentrated hydrochloric acid. A red colouration indicated the presence of flavonoids.

Test for Saponins (Frothing Test)

The test extracts, 2 ml each, were vigorously shaken in a test tube for 2 minutes, and observed for a stable persistent froth. Frothing in the test extract indicated the presence of saponins.

Test for Sugar (Fehling's Test)

A mixture of Fehling's solutions A and B (5 ml) was added to 2 ml of the test extract in a test tube. The resulting mixture was boiled for 2 minutes. A brick-red precipitate of copper (I) oxide indicated the presence of free reducing sugars.

Test for Glycosides (Keller-Killiani test)

A mixture of 10 ml of 50% sulfuric acid and 1 ml of the test extracts in a test tube was heated in boiling water for 15 minutes, then 10 ml of Fehling's solution was added to this mixture and boiled for another 10 minutes. A brick-red precipitate indicated the presence of glycosides in the extract.

Test for Terpenoids (Salkowski test)

Concentrated sulphuric acid (3 ml) was carefully added to 2 ml of each extract to form a layer. A reddish brown colouration of the interface indicated the presence of terpenoids.

Test for Sterols

Concentrated sulfuric acid (1 ml) was added to 1 ml of the text extract. A red colour indicated the presence of steroidal ring or sterol.

RESULTS AND DISCUSSION

The powdered plant material was first percolated with ethanol and the crude extract obtained was subsequently partitioned with petroleum ether, chloroform, ethyl acetate and methanol to obtain the fractions EB1-01, EB1-02, EB1-03 and EB1-04 respectively. The physical properties of the fractions obtained is as tabulated below in Table 1. The results for the phytochemical screening of the fractions are presented in Table 2. While the results for the repellent activity test of the fractions against the Anopheles mosquito are given in Table 3.

Table 1: Some Physical Properties of Bark Extracts of *Euphorbia balsamifera* EB

S/No	Fractions	Weight (g)	% Yield	State	Colour
1	EB1	27.9	9.3	Liquid	Dirty green
2	EB1-01	3.7	18.5	Liquid	Green
3	EB1-02	6.8	34.0	Liquid	Green
4	EB1-03	6.7	33.5	Liquid	Green
5	EB1-04	2.6	13.0	Liquid	Green

Table 2: Results of the Phytochemical Screening of the Extracts from the Bark of *Euphorbia balsamifera* EB

S/No	Tests	EB1	EB1-01	EB1-02	EB1-03	EB1-04
1.	Alkaloids	+	-	-	+	+
2.	Tannins	+	+	+	+	-
3.	Flavanoids	+	+	+	+	-
4.	Saponins	+	-	-		+
5.	Sugar	-	-	-	-	-
6.	Glycosides	+	+	+	-	-
7.	Terpenoids	+	+	+	-	+
8.	Sterols	+	-	+	-	+

Key: (-) = Negative test (+) = Positive test

Table 3: Results of Bioactivity Test of *Euphorbia balsamifera* EB

S/No	Fractions	Conc. (%)	Average No. of Mosquitoes Repelled	Average No. of Mosquitoes not Repelled	% Repellency
1	EB1	12.5	47	3	90.9
		25.0	49	1	96.9
	CONTROL	0	17	33	
2	EB1-01	12.5	23	23	41.0
		25.0	27	27	30.7
	CONTROL	0	11	39	
3	EB1-02	12.5	49	1	97.2
		25.0	50	0	100
	CONTROL	0	14	36	
4	EB1-03	12.5	25	25	32.4
		25.0	21	29	21.6
	CONTROL	0	13	37	
5	EB1-04	12.5	31	19	53.6
		25.0	28	22	46.3
	CONTROL	0	9	41	

The results obtained show high amount of the extract in EB1-02 and EB1-03 (6.8 and 6.7g respectively), indicating that moderately polar compounds are present in large quantity. The results of the phytochemical screening of the extracts showed that the stem bark is rich in most of the secondary metabolites analyzed using different solvents as shown in Table 2. It should be noted that compounds demonstrating steroidal activity are of importance and interest in pharmacy due to their relationship with sex hormones (Okwu, 2001), and it has been reported that several phenolic compounds like tannins present in the cells of plants are potent inhibitors of many hydrolytic enzymes such as proteolytic macerating enzymes used by plant pathogens. Other compounds like saponins also have antifungal properties (Aboaba and Efuwape, 2001), while some non-toxic glycosides can be hydrolyzed to phenolic compounds that can repel insects (Evans *et al.,* 1986). It can therefore be assumed that the repellent activity in *Euphorbia balsamifera* may be due to the presence of these metabolites (glycocides). On the other hand, it can be seen that different extracts show certain level of repellency, with EB1-02, which is the chloroform fraction showing the highest degree of repellency of 97.2% (at 12.5% concentration) and 100%, (at 25% concentration), while EB1-03 showed the least degree of repellency of 32.4% (at 12.5% concentration) and 21.6% (at 25% concentration), while other extracts have also shown some degree of repellency ranging from 30.7% to 53.6%. Going back to the technique employed by Tawatsin *et al*, 2001, where the number of mosquitoes probing the treated hand is counted, and the repellency evaluation technique adopted by Yap *et al.,* (1998), the most active of the four extracts will be the one with the least number of mosquitoes probing the hand, which will consequently be the one with the highest degree of repellency, we can say that the most active of these extract is the chloroform extract EB1-02.

CONCLUSION

From the results obtained, it can be concluded that the repellent activity of the chloroform fraction from the bark of *Euphorbia balsamifera* is an important discovery in our struggle to find a lasting solution to the menace of mosquitoes in particular, and insects in general. Based on this result, further work should be geared towards isolating and characterizing the active compounds in the chloroform fraction.

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