Bayero Journal of Pure and Applied Sciences, 7(2): 111 – 114
Received: August 2014
Accepted: November 2014
ISSN 2006 – 6996

REPELLENT EFFECT OF THE LEAF EXTRACTS OF *EUPHORBIA BALSAMIFERA* (AIT) AGAINST *ANOPHELES GAMBIAE*

[1]Mudi, S.Y., and [2]Datti, Y.
[1]Department of Pure and Applied Chemistry, Bayero University, Kano.
[2]Department of Chemistry, Northwest University, Kano.
*Correspondence author: symudi@yahoo.com

ABSTRACT
The ethanol extract of the leaves of Euphorbia balsamifera (commonly known as Gwadayi in Hausa) was sequentially extracted with petroleum ether, chloroform, ethyl acetate and methanol. The resulting extracts were designated EB1, EB2, EB3, and EB4 respectively. The ethanol extract designated EB. These extracts were tested for repellence against Anopheles gambiae using the human-bait technique under laboratory conditions, where five volunteers participated in the tests, one each for the extracts. The chloroform extract labelled EB1-02 was found to be the most active with 100% repellence at 25% (w/v) concentration, and 97.2% at 12.5% (w/v) concentration, while the ethyl acetate extract (EB1-03) recorded the least repellence activity of 32.4% at 25% (w/v) concentration, and 21.6% at 12.5% (w/v) concentration. It can thus be concluded that the phytochemicals present in the chloroform extract were responsible for repellent activity of E. balsamifera, and there is a need for further studies in order to ascertain the active compound in the plant.
Keywords: Repellence, Anopheles gambiae, human-bait technique, Euphorbia balsamifera extracts.

INTRODUCTION

Arthropod bites remain a major portal for vector-borne disease transmission. These bites can cause local or systematic effects that may be infectious or inflammatory in nature. Arthropods, notably insects and arachnids, are vectors of potentially serious ailments including malaria, West Nile virus, dengue, and Lyme disease. Measures to curtail the impact of insect bites are important in the worldwide public health efforts to safely protect patients and prevent the spread of disease (Katz *et al.*, 2008). Vector-borne diseases are among the most important public health problems and obstacles to socio-economic development of developing countries, particularly in the tropics, with malaria alone causing an estimated 1.5–2.7 million deaths and 300–500 million cases per year (WHO 1997).

Mosquitoes are one of the most important insect pests that affect the health and well-being of humans and domestic animals worldwide. They can cause a variety of health problems due to their ability to transmit viruses and other disease-causing pathogens even in the arid regions. Female mosquitoes require a blood meal for egg production, and they can produce a painful bite as they feed. While feeding, they can transmit to humans and other animals, viruses that are responsible causes of brain inflammation (encephalitis) (Meisch, 1994), dengue fever (Benette *et al.,* 2012), yellow fever (Reiter *et al.,* 1995), and protozoan parasites such as malaria and filariasis (Schoepke *et al.,* 1998). The most susceptible to the effects of these mosquito-borne pathogens are children and the elderly. However, in some instances life-threatening illness and/or permanent debilitation can occur in infected human hosts of any age (Service, 1993).

Over two billion people, primarily in tropical countries, are at risk from mosquito-borne diseases, such as dengue haemorrhagic 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 (Coleman *et al,.* 1993). However, human toxicity reactions after the applications of synthetic repellents vary from mild to severe reactions (Zadikoff, 1979; Edwards and Johnson, 1987). To avoid these adverse effects, research on repellents that are derived from plant extracts to replace synthetic repellents with such adverse effect has been conducted in many laboratories (Edwards and Johnson, 1987). Recently, extracts of several plants, including neem (*Azadirachta indica*), basil (*Ocimum basilicum*) citronella grass (*Cymbopogon nardus*), galingale (*Alpinia galanga*), clove (*Syzyaium aromaticum*) and thyme (*Thymus vulgaris*), have been studied as possible mosquito repellents (Sharma *et al.,* 1993; Barnard, 1999).

Repellents are substances that are designed to make surfaces unpleasant or unattractive to organisms like insects, animals and plants (Das *et al.,* 2003). They typically contain an active ingredient that repels unwanted organisms as well as secondary ingredients, which aid in delivery and cosmetic appeal. Repellence is known to play an important role in preventing the vector borne diseases by reducing man-vector contact (Das *et al.,* 2003). Synthetic chemicals and insecticides used for control of vectors are causing irreversible damage to the eco-system, as some of them are non-degradable in nature (Das *et al.,* 2003), hence the need to find repellents of natural origin arises.

Aklilu *et al.,* (2002) were able to quantify the repellent activity of live-potted plants against *Anopheles gambiae*. The repellent effect of some six aboriginal plant species was confirmed against *Trogoderma granarium* by Dwivedi and Shekhwat (2004). While Shah *et al.,* (2008) were able to ascertain the repellent effect of some plants' extract against saw-toothed grain beetle (*Oryzaephilus surinamensis* (L). The potential of volatile oils derived from some plant species, turmeric, citronella and hairy basil, for use as topical repellents against both diurnal and nocturnal mosquitoes, was demonstrated by Tawatsin *et al*., (2001) The oviposition deterrent and skin repellent activities of *Solanum trilobatum* leaf extract was confirmed against the malarial vector *Anopheles stephensis*, by Rajkumar and Jebanesan (2005). Similarly, Tawatsin *et al,*. (2006) were able to evaluate the repellence 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*. Also, Pugazhvendan *et al.,* (2009) tested and ascertained the repellent activity of the leaves of three plant species; *Argemone mexicama*, *Tephrosia purpurea* and *Prosopus juliflora*, against *Tribolium castaneum*, the red flour beetle, which is a major pest of grain based products (e.g. flour mills, ware houses and retail stores). Maharaj and Gayaram (2008), showed the repellent activities of some plant species against mosquito, using the time lag trials. Lastly, the effectiveness of an essential oil derived from *Zanthoxylum piperatum* was confirmed to be an alternative to some standard synthetic repellents in a research conducted by Kamsuk et *al.,* (2006).

Balsamiferous Spurge or *Euphorbia balsamifera* 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. It has broad, shortly lanceolate, fresh green to glaucous leaves on broad, light terracotta brown, very succulent stems (Antonio et al., 1975). *Euphorbia balsamifera* has sap (latex) which is rather poisonous if ingested, but widely used in odontology as traditionally antalgic treatment of acute dental pulpitis. The latex is an effective pulpal devitalizer used in dental offices (Yam et al., 1997). Native Americans used the plant for many medicinal purposes including treatment of skin infections (applied on the skin) and gonorrhea (internally) (Adedapo et al., 2004).

The present study was aimed at studying the repellent effects of *Euphorbia balsamifera* extracted using different solvents against *Anopheles gambiae* mosquito.

MATERIALS AND METHODS

Three hundred gram of the dried and ground form of the leaves of *Euphorbia balsamifera,* was put in 2.5L brown capacity brown bottle, and 1L of 90% ethanol was added. The set up was left for two weeks with constant shaking after which the mixture was filtered and then concentrated using rotavapor, to get the crude residues labelled EB. Twenty gram of the crude residue was dissolved in aqueous methanol and then extracted with 100cm^3 of petroleum ether (3 times). The petroleum ether extract was concentrated, dried, weighed and labelled EB1-01. The aqueous methanol portion was again extracted with 100cm^3 chloroform (3 times), and the extract was concentrated, dried, weighed and labelled as EB1-02. The aqueous methanol portion was again extracted with 100cm^3 ethyl acetate (3 times), and the extract was concentrated, dried, weighed and labelled as EB1-03. The aqueous methanol portion was finally concentrated, dried, weighed and labelled as EB1-04 (Colin and Cooke, 2000; Mohan and Ramaswamy, 2007; Dreyer and Kragl, 2008).

Test mosquitoes and the Repellence test

The mosquitoes used in this study were laboratory-reared *Anopheles gambiae* (aged 3-5 days). These were reared at the Department of Biological Sciences of Bayero University Kano, who willingly supplied the researchers with the larvae used in this research.

The repellence of the five extracts from *Euphorbia balsamifera,* were all assessed in the laboratory using a human-bait technique (WHO, 1996). Five volunteers (aged 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 *A. gambiae* are nocturnal biters. Evaluations were carried out in a large room, at room temperature. An area of 3x10 cm on each forearm of each of the human volunteers was marked out with a permanent marker. The test extract 12.5% and 25% W/V (extract/ethanol) was 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. 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 (80x40x40 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 hand, the repellence 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).

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

$$\%\,Repellency = \frac{C - T}{C} \, X\, 100$$

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

RESULTS AND DISCUSSION

The physical properties of *Euphorbia balsamifera* (Table 1) reveal higher amount of the extracts in EB1-02, EB1-03 and EB1-04 (6.34g, 4.66g and 7.64g respectively) indicating the presence in large concentration, of moderately and highly polar compounds in the leaves of the plant. On the other hand, it can be seen (from Table 2) that different extracts show certain level of repellence, with the chlorofor, extract (EB1-02) showing the highest degree of repellence of 97.2% and 100% (at 12.5% and 25% w/v extract concentration respectively), while the ethyl acetate extract (EB1-03) showed the least degree of repellence of 32.4% and 21.6% (at 12.5% and 25% w/v extract concentration respectively). Other extracts have also shown some degree of repellence ranging from 21.6% 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 repellence 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 repellence, we can say that the most active of these extract is the ethly acetate extract EB1-02. Hence, this result agrees with the traditional use of *E. balsamifera* as insect repellent as reported by Duke (1985).

Table 1: Physical features of the five extracts of *E. balsamifera*

S/N	EXTRACT	WEIGHT (g)	COLOUR
1	EB1	33.61	Dark green
2	EB1-01	3.56	Dark Green
3	EB1-02	6.34	Green
4	EB1-03	4.66	Greenish-Brown
5	EB1-04	7.65	Brown

Table 2: Results of Repellence of the Extracts at Three Different Concentrations

S/No	Extract	Conc. (w/v %)	Average No. of Mosquitoes Repelled	Average No. of Mosquitoes not Repelled	% Repellence
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	

CONCLUSION

From the results obtained from this research, it can be concluded that extract EB1-02 is the most active, and is more effective in repelling mosquitoes, while EB1-03 is the least effective. The repellent activity of EB1-02 is a valuable scientific proof of the repellence effect of *Euphorbia balsamifera* against 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 extract, EB1-02.

REFERENCES

Adedapo, AA., Abatan, MO. and Olorunsogo, OO. (2004). Toxic Effects of Some Plants in the Genus Euphorbia on Haematological and Biochemical Parameters of Rats. *Veterinary archives* 74:53-62.

Antonio, G., Braulio, MF., Fedro, G. and Angel, GR. (1975). Triterpenes From Latex of *Euphorbia balsamifera*. *Phytochemistry* vol 15, p 427.

Aklilu, S., Kabiru, EW., Wande, WL., Killeen, F. and Ali, AH. (2002). Repellence of Live-Potted Plants Against *Anopheles gambiae* From Human-Baits in Semi-Field Experimental Huts. *American Journal of Tropical Medicine and Hygiene* 67(2):191-195.

Barnard, DR. (1999) Repellence of Essential Oils to Mosquitoes (Diptera: Culicidae). *Journal of Medical Entomology* 36:625-629.

Bennett, KE., Olson, KE., Munoz, ML., Fernandez-Salas, L., Farfan-Ale, JA. and Higgs, S. (2002). Variation in Vector Competence for Dengue Virus among Twenty Four Collections of *Aedes aegypti* From Mexico and the United States. *American journal of Tropical Medicine and Hygiene* 67(1):85-92.

Coleman, RE., Robert, LL., Roberts, LW., Glass, JA., Seeley, DC., Laughinghouse, A., Perkins, PV. and Wirtz, RA. (1993). Laboratory Evaluation of Repellents Against Four Anopheline Mosquitoes (Diptera: Culicidae) and Two Phlebotomine Sand Flies (Diptera: Psychodiae). *Journal of Medical Entomology* 30:499-502.

Colin, P. and Cooke, M. (2000). Extraction. *Encyclopedia of Separation Science* 10:465-467.

Das, NG., Baruah, I., Talukdar, PK. and Das, SC. (2003) Evaluation of Botanicals as Repellents Against Mosquitoes. *Journal of Vector Borne Diseases* 40:49-53.

Dreyer, S. and Kragl, U. (2008). Ionic Liquids for Aqueous Two-Phase Extraction and Stabilization of Enzymes. *Biotechnology and Bioengineering* 99(6):1416-1424.

Dwivedi, SC. and Shekhwat, NB. (2004) Repellent Effect of Some Indigenous Plant Extracts Against *Trogoderma granarium*. *Asian Journal of Experimental Biological Sciences* 18:1&2 47-51.

Edwards, DL. and Johnson, CE. (1987) Insect-Repellent-Induced Toxic Encephalopathy in a Child. *Journal of Clinical Pharmacology* 6:496-498.

Kamsuk, K., Choochote, W., Chaithong, U., Jitpakdi, A., Tippawangkosol, P., Riyong, D. and Pitasawat, B. (2006). Effectiveness of *Zanthoxylum piperitum*-Derived Essential Oil as an Alternative Repellent Under Laboratory and Field Applications. *Journal of Parasitology Research* 100(2):339-45.

Katz, MD., Tracy, M., Jason, H. Miller, MD., Adelaide, A. and Hebert, MD. (2008). Insect Repellents: Historical Perspectives and New Developments. *Journal of the American Academy of Dermatology* 58:865-871.

Maharaj, R. and Gayaram, R. (2008). Testing Plant Samples for Mosquito Repellency, Time Lag Trials. *Malaria Research Lead Programme* 1:96.

Meisch, MV. (1994). The Dark Ricefield Mosquito, *Psorophora columbiae*. *Wing Beats* 5(1):8.

Mohan, DR., and Ramaswamy, M. (2007) Evaluation of Larvicidal Activity of the Leaf Extract of a Weed Plant, *Ageratina adenophora*, Against Two Important Species of Mosquitoes, *Aedes aegypti* and *Culex quinquefasciatus. African Journal of Biotechnology* 6(5):631-638.

Pugazhvendan, SR., Elumalai, K., Ronald-Ross, P. and Soundararajan, M. (2009). Repellent Activity of Chosen Plant Species Against *Tribolium castaneum. World Journal of Zoology* 4(3):188-190.

Rajkumar, S. and Jebanesan, A. (2005). Bioactivity of Flavonoids Compounds From *Poncirus trifoliate* Against the Dengue Vector, *Aedes aegypti*. *Journal of Parasitology Research* 104:1 19-25.

Reiter, P., Amador, MA., Anderson, RA. and Clark, GG. (1995). Short Report: Dispersal of *Aedes* aegypti in a Urban Area After Blood Feeding as Demonstrated by Rubidium-Marked Eggs*. American journal of Tropical Medicine and Hygiene* 52:177-179.

Service, MW. (1993). Mosquitoes (*Culicidae*). In: R.P. Lane and R.W. Crosskey (Eds.), *Medical Insects and Arachnids* Chapman & Hall, London, Pp 723

Shah, MMR., Prodhan, MDH., Siddique, MNA., Mamun, MAA. and Shahjahan, M. (2008). Repellent Effect of Some Indigenous Plant Extracts Against Saw-Toothed Grain Beetle *Oryzaephilus surinamensis. International Journal of Sustainable Crop Production* 3(5):51-52 *.*

Sharma, VP., Ansari, MA. and Razdan, RK. (1993) Mosquito Repellent Action of Neem (*A. indica*) Oil. *Journal of the American Mosquito Control Association* 9:359-360.

Schoepke, A., Steffen, F. and Norman, G. (1998). Effectiveness of Personal Protection Measures Against Mosquito Bites for Malaria Prophylaxis in Travelers. *Journal of Travel Medicine* 5:188-92.

Tawatsin, A., Wratten, SD., Thavara U. and Scott, RR. (2001) Repellency of Volatile Oils from Plants Against Three Mosquito Vectors. *Journal of Vector Ecology* 26(1):76-82.

Tawatsin, A., Thavara, U., Bansidhi, J. and Mulla, MS. (2006). Repellency of Essential Oils Extracted From Plants in Thailand Against Four Mosquito Vectors, and Oviposition Deterrent Effect Against *Aedes aegypti*. *South-East Asian Journal of Tropical Medicine* 37:5.

WHO (1996) Report of the WHO Informal Consultation on the Evaluation and Testing of Insecticides. CTD/WHOPES/IC/96.1, Control of Tropical Diseases Division. World Health Organization, Geneva, Pp 69.

Yam, AA., Gaye, F., Dieme, FA., Bassene, E. and Ba, I. (1997). Application of Phytotherapy in Odontology: The Case of *Euphorbia balsamifera*. *Dakar Medical Journal* 42(2):169-71.

Yap, HH., Jahangir, K., Chong, ASC., Adanan, CR., Chong, NL., Malik, YA. and Rohaizat, B. (1998). Field Efficacy of a New Repellent, KBR 3023, Against *Aedes albopictus* (Skuse) and *Culex quinquefasciatus* (Say) in a Tropical Environment. *Journal of Vector Ecology* 23:62–68.

Zadikoff, CM. (1979) Toxic Encephalopathy Associated With Use of Insect Repellent. *Journal of Pediatrics* 95:140-142.