



RESEARCH ARTICLE

**Repellent Potential of Leaf Extracts of *Amomum Subulatum* Roxb. (*Zingiberaceae*)
Against Malarial Vector, *Anopheles Stephensi* Liston (Diptera: Culicidae)**

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ABSTRACT

Vector borne diseases such as parasitic and viral infection are transmitted by the mosquitoes which are responsible for millions of deaths worldwide. Malaria, dengue, chikengunya, filariasis, encephalitis and yellow fever are major infections transmitted by mosquitoes. The repellent potential of benzene, hexane, ethyl acetate, methanol and chloroform solvent extracts of leaf of the plant *Amomum subulatum* against the mosquito *Anopheles stephensi* were tested at three different concentration of 1.0, 2.5 and 5.0 mg/cm². The arms of volunteer, only 25 cm² dorsal side of the skin on each arms were exposed and the remaining area covered with rubber gloves. Ethanol was served as the control. The control and treated arm were introduced simultaneously into the mosquito cage. In this observation, this plant crude extracts gave protection against mosquito bites without any allergic reaction to the test person, and also, the repellent activity is dependent on the strength of the plant extracts. The results observed suggest that the leaf extracts of the plant *Amomum subulatum* of different solvents have the potential to be used as a non-toxic, non-irritating safe and effective alternative other synthetic mosquito repellents.

KEYWORDS

Leaf Extracts, Repellent, *Anopheles Stephensi*, Skin Test

INTRODUCTION

Nowadays biological resources have become an essential part of our life which also provides us the raw materials for meeting the needs of society. Malaria, dengue, chikengunya, filariasis, encephalitis and yellow fever are the serious health diseases of humans caused by intolerable biting of blood feeding female mosquitoes¹. Malaria is the serious and most common tropical disease in the world. The number of deaths caused by malaria is more than any serious diseases including HIV/AIDS. Urticarial which is a allergic response to skin also caused by mosquito bite.

For personal protection against biting mosquitoes repellents are widely used and reducing the risk of transmission of vector borne diseases^{2,6}. Insecticides treated mosquito nets are the most effective methods for control of malaria. Synthetic chemical are most extensively used and effective in daily life. Organophosphorus carbamates, organochlorine, pyrethroids are some compounds which are toxic to the mosquitoes but the issues related to environment these chemicals have and the increased resistance of mosquitoes against these compounds raised during the last five decades⁷.

Plants now recognized as effective natural insecticides because of non-toxic, biodegradable, selective and have no effect on the environment and non- target organisms^{8,9}. The natural insect

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repellent derived from the extract such as *Syzygium aromaticum*, *Curcuma longa*, *Eucalyptus citriodora*, *Cymbopogon nardus*, *Cymbopogon citrates*, *Azadirachta indica*, *Albizia amara*, *Anethum graveolens*, *Clausena anisata*, *Zanthoxylum piperitum*, *Pogostemon cablin*, *Vetiveria zizanioides*^{10,18}.

The study deals with the investigation of repellent potential of different leaf extracts of plant *Amomum subulatum* against mosquito *Anopheles stephensi*.

MATERIAL AND METHODS

Plant Collection

The plant *Amomum subulatum* was collected in the month of December, 2013 from district Ranikhet, Utter Pradesh, India. The leaf samples were compared, taxonomically identified and authenticated by Dr. (Mrs) Sunita Garg, Chief Scientist, Raw Material Herbarium and Museum, Delhi (RHMD) CSIR-National Institute of Sciences Communication and Information Resources, New Delhi.

Preparation of the Extract

The leaves were washed with tap water, shade dried and finely ground. The finely ground plant material was loaded in soxhlet apparatus and was extracted with five different solvents namely benzene, hexane, ethyl acetate, methanol and chloroform individually. The solvent from the extract was removed using rotary vacuum evaporator to collect the crude extract.

Test Organisms

Anopheles stephensi larvae were collected from the Haryana Agricultural University, under the supervision of Insect collector officer Dr. Ajmer Singh, District Malaria Department, Civil Hospital, Hisar, Haryana. The adults were fed on the 10% sugar solution under (28±2) °C and (78±2) % relative humidity.

Repellent Activity

The repellent study was followed by the method of WHO¹⁹. Three day old blood starved female *Anopheles stephensi* mosquitoes (100) were kept in a net cage (45 cm x 30 cm x 45 cm). The

volunteer had no contact with lotions, perfumes, oils or perfumed soaps on the day of the assay. The arms of volunteer, only 25 cm² dorsal side of the skin on each arm were exposed and the remaining area covered with rubber gloves. The crude extracts were applied at 1.0, 2.5 and 5.0 mg/cm² separately in the exposed area of the forearm. Ethanol was served as the control. The control and treated arm were introduced simultaneously into the mosquito cage, and gently tapping the sides on the experimental cages, the mosquitoes were activated. Each test concentration was repeated six times. The volunteer conducted their test of each concentration by inserting the treated and control arm into the cages at a same time for one full minute for every 5 min. The mosquitoes that land on the hand were recorded and then shaken off before it imbibes any blood. The percentage of repellency was calculated by the following formula.

$$\% \text{ Repellency} = [(T_a - T_b) / T_a] \times 100$$

Where T_a is the number of mosquitoes in the control group and T_b is the number of mosquitoes in the treated group.

RESULTS

The results of the hexane, ethyl acetate, benzene, chloroform and methanol extract of *Amomum subulatum* show significant repellency against *Anopheles stephensi*. In this observation, plant crude extracts gave protection against mosquito bites without any allergic reaction to the test person, and also, the repellent activity is dependent on the strength of the plant extracts.

DISCUSSION

Different parts of plants having a complex of chemicals with specific biological activity. Toxins and secondary metabolites are responsible for that which act as attractants or deterrents. This study showed that leaf extracts of different solvents of *Amomum subulatum* have significant repellent activity against *Anopheles stephensi* mosquito. The results are given in table no 1, which are similar with an earlier research done by Elango *et al*²⁰.

Table 1: Repellency of different leaf extracts of *Amomum subulatum* against *Anopheles stephensi*

Solvent	Conc ⁿ (mg/ cm ²)	% of Repellency ± SD							
		Time Post Application of Repellent (min.)							
		15	30	60	90	120	150	180	210
Hexane	1.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± ± 0.0	63.4 ± 1.2	54.4 ± 1.3	40.3 ± 1.8
	2.5	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± ± 0.0	69.3 ± 1.5	61.2 ± 1.8	46.7 ± 2.0
	5.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± ± 0.0	100.0 ± ± 0.0	72.6 ± 1.4	64.4 ± 1.5
Ethyl acetate	1.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	72.3 ± 1.4	73.6 ± 1.6	66.0 ± 1.5	50.7 ± 1.7
	2.5	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	79.6 ± 2.3	79.9 ± 2.0	71.8 ± 1.2	52.6 ± 1.3
	5.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± ± 0.0	97.8 ± 1.4	81.4 ± 2.0	70.9 ± 2.1
Benzene	1.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± ± 0.0	58.3 ± 1.2	49.2 ± 1.6	33.9 ± 1.4
	2.5	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± ± 0.0	62.7 ± 1.1	52.7 ± ±1.4	41.3 ± 1.5
	5.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± ± 0.0	74.6 ± 1.2	64.7 ± 1.7	52.6 ± 1.9
Chloroform	1.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± ± 0.0	73.5 ± 1.8	67.9 ± 1.9	54.7 ± 1.2
	2.5	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± ± 0.0	80.6 ± 1.5	73.3 ± 2.1	61.9 ± 2.1
	5.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± ± 0.0	100.0 ± ± 0.0	80.9 ± 1.5	72.6 ± 1.8
Methanol	1.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± ± 0.0	79.7 ± 1.6	63.4 ± 2.3	57.3 ± 1.4
	2.5	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± ± 0.0	85.6 ± 1.1	71.0 ± 1.5	66.7 ± 2.2
	5.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± ± 0.0	100.0 ± ± 0.0	85.3 ± 1.7	72.5 ± 1.6

Each value ($\chi \pm SD$) represents average of six values.

Who found that the maximum repellent activity observed at 500 ppm of methanol extracts of *Aegle marmelos* and *Acacia lineate* and ethyl acetate extract of *Cytisus hirsutus*, the average protection time ranged from 90 to 120 min. by the different extracts. The repellent efficacy of wood vinegar was studied against mosquitoes at 1, 5, 10, 20, 40, 60 and 80% concentrations. The results showed that wood vinegar has mosquito repellence of varying degree depending on the concentration used. Repellence against *Cx. pipiens pallens* was high being 90.3% at 20% concentration, 92.2% at 40% concentration, 93.9% at 60% concentration and 100% at 80% concentration. The duration of protection time tests showed that the 40% and 60% concentrations of the wood vinegar give protection from landing of *Ae. togoi* for a period of up to 7 h, though the lower concentration gave lower protection after the first five hours²¹. The repellent activity of *Lavandula officinalis* and *R. officinalis* essential oils against *Cx. pipiens* showing an effective repellent effect mainly to adult mosquitoes due to α -terpinene, carvacrol, and thymol(20). The leaves of *Echinops sp.* (92.47%), *Ostostegia integrifolia* (90.10%), and *Olea europaea* (79.78%) were also effective and efficient to drive away mosquitoes and the roots of *Silene macroserene* (93.61%), leaves of *Echinops sp.* (92.47%), *O. integrifolia* (90.10%), and *O. europaea* (79.78%) were exhibited the significant repellency by direct burning²².

CONCLUSION

In conclusion, an attempt has been made to evaluate the role of medicinal plant extracts for their repellent bioassay against *Anopheles stephensi*. The results reported in this study open the possibility for further investigations of the efficacy of repellent properties of natural products.

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