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Research Article

MOSQUITO REPELLENT ACTIVITY OF *DELONIX ELATA* (FABACEAE) LEAF AND SEED EXTRACTS AGAINST THE PRIMARY DENGUE VECTOR *AEDES AEGYPTI* (DIPTERA: CULICIDAE)

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ABSTRACT

Dengue fever causes mortality and morbidity around the world, specifically in tropical and subtropical areas. As a consequence, the search for new anti-dengue agents from medicinal plants has assumed more urgency than in the past. Medicinal plants have been used widely to treat a variety of vector ailments such as malaria. The demand for plant-based medicines is growing as they are generally considered to be safer, non-toxic and less harmful than synthetic drugs. In the present study the repellent activity of hexane, benzene, chloroform, ethyl acetate and methanol extract of *Delonix elata* (*D. elata*) leaf and seed against *Aedes aegypti* (*Ae. aegypti*). One hundred three day old starved female *Ae. aegypti* mosquitoes were kept on a net cage (45 cm×30 cm×45 cm). The repellency was determined against *Ae. aegypti* mosquito species at three concentrations viz., 1.0, 2.5 and 5.0 mg/cm^2 under the laboratory conditions. *Ae. aegypti* were tested during the day time from 07.00 to 17.00 h. In this study, the plant crude extracts gave protection against mosquito bites without any allergic reaction. The repellent activity was dependent on the strength of the plant extracts. Among the tested solvents the maximum efficacy was observed in the leaf and seed methanol extracts. The highest concentrations of 5.0 mg/cm^2 provided over 180 and 150 min protection, respectively. Overall, the crude methanol extract of *D. elata* showed an excellent potential to develop newer and safer control tools the dengue vector mosquito *Ae. aegypti.*

Keywords: Arbovirus; *Delonix elata*, Leaf, Seed, Repellent activity, *Aedes aegypti.*

INTRODUCTION

Mosquito-borne diseases, like malaria, yellow, and dengue fevers, are a major threat to over two billion people in the tropics. Mosquito bites may also cause allergic responses including local skin reactions and systemic reactions such as urticaria and angioedema (Peng *et al.*, 2004). Dengue is a vector-borne disease of tropical and subtropical human populations, which occurs predominantly in urban areas. Dengue is transmitted by *Aedes* mosquitoes that breed in container habitats. The main vector *Aedes aegypti* is a cosmotropical species that proliferates in water containers in and around houses. Secondary vectors include *Aedes albopictus*, an important vector in Southeast Asia and that has spread to the Americas, western Africa, and the Mediterranean rim; *Aedes mediovittatus* in the Caribbean; and *Aedes polynesiensis* and *Aedes scutellaris* in the western Pacific region. *Ae. aegypti* breeds in many types of household containers, such as water storage jars, drums, tanks, and plant or flower containers (Honorio *et al.*, 2003).

Mosquito control relies heavily on synthetic insecticide application. However, over and injudicious application of synthetic insecticides resulted into resistance to these

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insecticides and unwarranted toxic or lethal effects on non-target organisms, as well as environmental health problem (Benelli, 2015a). As an alternate, biological control of mosquitoes could be very promising being eco-friendly as well as cost effective. Hence, there is a constant need for developing biologically active plant materials as insecticides, which are expected to reduce the hazards to humans and other organisms by minimizing the accumulation of harmful residues in the environment. Natural products of plant origin are generally preferred because of their less harmful nature to non-target organisms and their innate biodegradability (Govindarajan and Sivakumar, 2012). Botanicals can be used as alternative synthetic insecticides or along with other insecticides under integrated vector control programs (Benelli, 2015b).

Phytochemicals could be valuable weapons in the fight against mosquito-borne diseases (Govindarajan, 2010a). For instance, the mosquito repellent activity of phytochemical extracts from peels of five citrus fruit species, *Citrus sinensis, C. limonum, C. aurantifolia, C. reticulata* and *C. vitis* (Effiom *et al.,* 2012). The ovicidal and repellent activities of methanol leaf extract of *Ervatamia coronaria* and *Caesalpinia pulcherrima* against *Cx. quinquefasciatus, Ae. aegypti*, and *An. stephensi* (Govindarajan *et al.,* 2011). Furthermore, the repellent efficacy of the flower extracts of *Calotropis gigantea* against *Cx. quinquefasciatus* mosquito and to screen the bioactive compounds present in the flower extract (Dhivya and Manimegalai, 2013). The ethanolic extracts of leaves of *Datura stramonium* were evaluated for larvicidal and mosquito repellent activities against *Ae. aegypti, An. stephensi* and *Cx. quinquefasciatus* (Swathi *et al.,* 2012). The larvicidal and repellent properties of essential oils from various parts of four plant species *Cymbopogan citrates, Cinnamomum zeylanicum, Rosmarinus officinalis*, and *Zingiber officinale* against *Cx. tritaeniorhynchus* and *An. subpictus* (Govindarajan, 2011).

The larvicidal, pupicidal, repellent, and adulticidal activities of methanol crude extract of *Artemisia nilagirica* were assayed for their toxicity *against Anopheles stephensi* and *Aedes aegypti* (Panneerselvam *et al.,* 2012). The repellent activity of crude extracts of ten indigenous plant species, *Azadirachta indica, Cymbopogon citratus, Ocimum gratissimum, Ageratum conyzoides, Annona squamosa, Hyptis suaveolens, Tridax procumbens, Citrus sinensis, Lantana camara* and *Solanum nigrum* against the malaria vector *An. stephensi* (Egunyomi *et al.,* 2010).

The repellent activity of the essential oils of *Thymus* and *Mentha* species against *Ochlerotatus caspius* were evaluated by (Koc *et al.,* 2012). Gu *et al*. (2009) to investigate the repellent properties of essential oils from *Cryptomeria japonica* against adults of mosquitoes *Ae. aegypti* and *Ae. albopictus*. The essential oil (EO) extracted from fresh leaves of *Hyptis suaveolens*, and its main constituents were evaluated for larvicidal and repellent activity against the Asian tiger mosquito, *Ae. albopictus* (Conti *et al.,* 2013). The undiluted oils of *Zingiber cassamunar* exhibited maximum repellency against the larvae of *Leptotrombidium* chiggers (Eamsobhana *et al.,* 2009). In view of the increased interest in developing plant origin insecticides as an alternative to chemical insecticides, this study was undertaken to assess the repellent potential of the extracts from the medicinal plant *D.elata* against the primary dengue vector mosuito *Ae. aegypti.*

MATERIALS AND METHODS

Collection of plants

Fully developed leaves and seeds of the *D. elata* were collected from Thanjavur District (Between 9°50’ and 11°25’ of the north latitude and 78°45’ and 70°25’ of the east longitude), Tamilnadu, India. It was authenticated by a plant taxonomist from the Department of Botany, Annamalai University. A voucher specimen was deposited at the Herbarium of Plant Phytochemistry Division, Department of Zoology, Annamalai University.

Extraction

The leaves and seeds were washed with tap water, shade dried, and finely ground. The finely ground leaf and seed powder (1.0 kg/solvent) was loaded in soxhlet extraction apparatus. Five different solvents, namely, hexane, benzene, chloroform, ethyl acetate and methanol were used for extraction. The solvents were removed from the extracts using a rotary vacuum evaporator to collect the crude extract. Standard stock solutions were prepared at 1% by dissolving the residues in ethanol. From this stock solution, different concentrations were prepared and these solutions were used for repellent bioassay.

Test organisms

Ae. aegypti was reared in the Vector Control Laboratory, Department of Zoology, Annamalai University. The larvae were fed on dog biscuits and yeast powder in 3:1 ratio. Adults were provided with 10% sucrose solution and 1-week old chicks for blood meal. Mosquitoes were held at (28±2) °C, 70%-85% relative humidity, with a photo period of 12-h light and 12-h dark.

Repellent activity

The repellency was evaluated by using the percentage of protection in relation to dose method (World Health

Organization, 2009). One hundred three day old starved female *Ae. aegypti* mosquitoes were kept on a net cage (45 cm×30 cm×45 cm). Two cages with hungry mosquitoes for test and control were kept aside. The volunteer had no contact with lotions, perfumes, oils or perfumed soaps on the day of the assay. The arms of the volunteer skin washed and cleaned with ethanol and ethanol served as control, respectively. After air drying, the each arm was exposed and the remaining area covered by rubber gloves. The different concentrations of crude extracts were applied. *Ae. aegypti* were tested during the day time from 07.00 to 17.00 h. 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. The volunteer conducted their test of each concentration by inserting the treated and control arm into 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 formula.

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

Where *T*a is the number of mosquitoes in the control group, and *T*b is the number of mosquitoes in the treated group. Repellency data was analyzed by two-way ANOVA, with two factor (dosage and compound). Tukey's HSD test was used to separate means ($P<0.05$).

RESULTS

In the present observation, the results from the skin repellent activity of hexane, ethyl acetate, benzene, chloroform and methanol extract of *D. elata* leaf and seed against blood starved adult female of *Ae. aegypti* were given in Table 1 and 2. The present result shows that the percentage protection in relation to dose and time (minutes). Among the tested solvents the maximum efficacy was observed in the leaf and seed methanol extract. The higher concentration of 5.0 mg/cm^2 leaf and seed methanol extract provided over 180 and 150 min protection and the lower concentration of 1.0 mg/cm^2 provided 120 and 90 min protection against *Ae. aegypti*, respectively. In this observation, the 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 tested plant crude extracts have exerted promising repellent against dengue vector mosquito.

Table 1. Repellency of different solvent leaf extracts of *Delonix elata* against *Aedes aegypti.* Within each row, different letters indicate significant differences (Tukey's HSD test, $P<0.05$).

Solvent	Concentration (mg/cm^2)	Repellency % ±SD							
		Time of post application (minutes)							
		30	60	90	120	150	180	210	240
Methanol	1.0	100±0.0^a	100±0.0^a	100±0.0^a	100±0.0^a	93.5±1.0^b	75.8±1.5^c	61.2±2.0^d	48.3±1.9^e
	2.5	100±0.0^a	100±0.0^a	100±0.0^a	100±0.0^a	100±0.0^a	94.2±1.8^b	78.6±1.6^c	63.7±1.2^d
	5.0	100±0.0^a	100±0.0^a	100±0.0^a	100±0.0^a	100±0.0^a	100±0.0^a	95.3±1.0^b	81.0±1.3^c
Ethyl acetate	1.0	100±0.0^a	100±0.0^a	100±0.0^a	95.1±1.4^b	79.6±1.7^c	64.8±1.5^d	49.2±1.8^e	33.5±1.6^f
	2.5	100±0.0^a	100±0.0^a	100±0.0^a	100±0.0^a	93.6±1.9^b	80.7±1.9^c	65.2±1.4^d	51.4±2.0^e
	5.0	100±0.0^a	100±0.0^a	100±0.0^a	100±0.0^a	100±0.0^a	94.2±1.8^b	81.2±2.5^c	66.3±1.7^d
Chloroform	1.0	100±0.0^a	100±0.0^a	100±0.0^a	90.3±1.6^b	76.8±1.9^c	58.7±1.5^d	40.9±2.1^e	26.3±1.1^f
	2.5	100±0.0^a	100±0.0^a	100±0.0^a	100±0.0^a	91.1±1.4^b	77.6±2.0^c	62.7±1.4^d	46.5±1.6^e
	5.0	100±0.0^a	100±0.0^a	100±0.0^a	100±0.0^a	100±0.0^a	92.7±1.6^b	79.3±1.6^c	64.8±2.0^d
Benzene	1.0	100±0.0^a	100±0.0^a	100±0.0^a	89.1±1.0^b	74.0±1.2^c	55.3±1.4^d	38.1±2.0^e	22.4±1.6^f
	2.5	100±0.0^a	100±0.0^a	100±0.0^a	94.8±1.2^b	80.7±2.3^c	66.8±1.3^d	51.7±1.6^e	35.7±1.4^f
	5.0	100±0.0^a	100±0.0^a	100±0.0^a	100±0.0^a	96.3±2.1^b	81.7±1.2^c	65.3±1.2^d	52.3±1.6^e
Hexane	1.0	100±0.0^a	100±0.0^a	95.2±1.6^b	80.8±1.9^c	67.3±1.8^d	52.6±1.9^e	38.1±1.8^f	23.2±1.5^g
	2.5	100±0.0^a	100±0.0^a	100±0.0^a	91.5±1.5^b	76.1±1.6^c	60.2±2.1^d	48.0±1.9^e	35.1±1.0^f
	5.0	100±0.0	100±0.0^a	100±0.0^a	100±0.0^a	92.3±1.5^b	77.4±2.6^c	62.8±2.2^d	46.8±2.4^e

Table 2. Repellency of different solvent seed extracts of *Delonix elata* against *Aedes aegypti.* Within each row, different letters indicate significant differences (Tukey's HSD test, P<0.05).

Solvent	Concentration (mg/cm^2)	Repellency% ±SD							
		Time of post application (minutes)							
		30	60	90	120	150	180	210	240
Methanol	1.0	100±0.0^{a}	100±0.0^{a}	100±0.0^{a}	90.7±2.1^{b}	77.3±2.3^{c}	63.5±2.4^{d}	50.2±1.7^{e}	38.7±1.3^{f}
	2.5	100±0.0^{a}	100±0.0^{a}	100±0.0^{a}	100±0.0^{a}	92.1±2.4^{b}	79.1±1.2^{c}	65.3±1.8^{d}	52.9±1.9^{e}
	5.0	100±0.0^{a}	100±0.0^{a}	100±0.0^{a}	100±0.0^{a}	100±0.0^{a}	93.8±1.5^{b}	80.4±1.5^{c}	66.5±1.8^{d}
Ethyl acetate	1.0	100±0.0^{a}	100±0.0^{a}	94.1±1.6$_{b}$	81.2±1.6$_{c}$	66.8±1.1$_{d}$	52.6±1.8$_{e}$	39.7±1.7$_{f}$	24.6±2.1$_{g}$
	2.5	100±0.0^{a}	100±0.0^{a}	100±0.0^{a}	92.3±2.0^{b}	78.2±1.2^{c}	64.3±1.1^{d}	51.2±2.6^{e}	36.4±2.3^{f}
	5.0	100±0.0^{a}	100±0.0^{a}	100±0.0^{a}	100±0.0^{a}	95.4±1.7^{b}	82.0±1.7^{c}	67.9±2.1^{d}	55.1±1.4^{e}
Chloroform	1.0	100±0.0^{a}	100±0.0^{a}	92.0±2.4^{b}	76.8±1.6^{c}	64.3±1.6^{d}	49.3±1.6^{e}	36.1±1.7^{f}	24.1±2.1^{g}
	2.5	100±0.0^{a}	100±0.0^{a}	94.7±2.0^{b}	79.9±1.3^{c}	67.2±2.4^{d}	54.8±1.0^{e}	38.9±1.3^{f}	24.0±1.6^{g}
	5.0	100±0.0^{a}	100±0.0^{a}	100±0.0^{a}	93.8±1.8^{b}	80.2±1.9^{c}	65.7±1.3^{d}	48.9±2.0^{e}	32.9±1.8^{f}
Benzene	1.0	100±0.0^{a}	100±0.0^{a}	91.2±1.6^{b}	74.4±1.4^{c}	62.1±1.4^{d}	46.6±2.1^{e}	34.0±1.9^{f}	21.5±1.0^{g}
	2.5	100±0.0^{a}	100±0.0^{a}	92.4±1.2^{b}	75.3±1.9^{c}	64.7±2.0^{d}	51.6±2.0^{e}	35.1±1.5^{f}	21.8±1.9^{g}
	5.0	100±0.0^{a}	100±0.0^{a}	100±0.0^{a}	92.1±2.0^{b}	78.5±1.3^{c}	64.3±1.3^{d}	45.7±1.6^{e}	31.1±2.0^{f}
Hexane	1.0	100±0.0^{a}	100±0.0^{a}	89.5±1.9^{b}	72.7±1.6^{c}	58.8±1.2^{d}	43.8±1.4^{e}	31.3±2.1^{f}	18.3±1.7^{g}
	2.5	100±0.0^{a}	100±0.0^{a}	90.4±1.6^{b}	74.1±1.5^{c}	62.5±1.8^{d}	49.0±1.0^{e}	33.4±1.6^{f}	20.8±1.3^{g}
	5.0	100±0.0^{a}	100±0.0^{a}	100±0.0^{a}	91.4±1.7^{b}	76.4±1.6^{c}	63.1±2.1^{d}	43.2±1.6^{e}	30.2±1.0^{f}

DISCUSSION

Current research highlights the information available for various parts and extracts types of medicinal plants for the treatment of dengue. However, some of the plants that have not yet been fully explored may have a broad range of potential therapeutic applications. The development of new anti-dengue products from bioactive compounds is necessary in order to find more effective and less toxic anti-dengue drugs. Therefore, any extensive study on the potential of plants with isolated active compounds that have shown anti-dengue activity should go through additional in vitro and in vivo animal testing followed by toxicity and clinical tests. This route may reveal a promising compound to be optimized and thus be suitable for application in the production of new anti-dengue compounds. In our results showed that, crude extract of *D. elata* have significant repellent activity against *Ae. aegypti* mosquito. This result is also comparable to earlier reports of Elango *et al*. (2009) stated that the maximum repellent activity was observed at 500 ppm in methanol extracts of *A. marmelos*, *A. lineata*, and ethyl acetate extract of *C. hirsutus*, and the mean complete protection time ranged from 90 to 120 min against *An. subpictus*. The repellent efficacy of wood vinegar was assessed against mosquitoes under laboratory conditions at 1, 5, 10, 20, 40, 60 and 80% concentrations. The results showed that wood vinegar provided mosquito repellence of varying degree depending on the concentration used. The observed repellence averaged from as low as 39.6% at 5.0% concentration to as high as 100% at 80% concentration against *Ae. togoi*. 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 hours (Kiarie-Makara *et al.,* 2010).

The essential oils of *Ocimun basilicum* used as promising new natural repellents at 0.1 % concentration against *Anopheles* and *Aedes* mosquitoes (Nour *et al.,* 2009). The extracts of *F. vulgare* leaves were toxic against *Cx. pipiens* larvae, and terpineol and 1,8-cineole were the most effective components in repellency tests (Traboulsi *et al.,* 2005).The repellent activity compare to previous report at the dose of 25.0 mg/mat, thymol provided complete repellency, whereas *Trachyspermum ammi* seed oil could achieve a repellency of 45.0% and the repellent doses (RD_{50}) observed were 25.02 and 11.63 mg/mat for *T. ammi* seed oil and thymol, respectively, against *An. stephensi* (Pandey *et al.,* 2009). The repellent activity of *Lavandula officinalis* and *R. officinalis* EOs against *Cx. pipiens* pallens, showing an effective repellent effect mainly to adult mosquitoes due to α-terpinene, carvacrol, and thymol (Choi *et al.,* 2002). 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 burning (Karunamoorthi *et al.,* 2008).

The mosquito-repellent and mosquitocidal activities of the volatile oil of *Ocimum gratissimum* used at three different locations. Topical application of each of the four different lotions significantly (p <0.05) reduced the biting rate of mosquitoes in all the three locations tested (Oparaocha *et al.*, 2010). The 30% (v/v) concentration in olive oil base exhibiting highest average percentage repellencies of 97.2, 95.7 and 96.3% at three centers respectively, while the 20% (v/v) concentration in palm kernel oil base had the least repellency of 36.3, 41.6 an d 36.3%, respectively. Skin repellent test at 1.0, 2.5 and 5.0 mg/cm^2 concentration of *P. acidula* gave 100% protection up to 2.30, 4.00 and 6.45 hrs and 2.45, 4.30 and 7.0 hrs respectively (Samidurai *et al.*, 2009). The larvicidal activity of crude extract of *Sida acuta* against three important mosquitoes with LC_{50} values ranging between 38 to 48 mg/l; the crude extract had strong repellent action against three species of mosquitoes as it provided 100% protection against *An. stephensi* for 180 min. followed by *Ae. aegypti* (150) min. and *Cx. quinquefasiatus* (120) min (Govindarajan, 2010b).

CONCLUSION

In conclusion, an attempt has been made to evaluate the role of medicinal plant extracts for their repellent bioassay against *Ae. aegypti*. 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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