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Research Article

ADULTICIDAL ACTIVITY OF *PITHECELLOBIUM DULCE* (ROXB.) BENTH. AND *DELONIX ELATA* (L.) GAMBLE (FAMILY: FABACEAE) AGAINST THE MALARIA VECTOR *ANOPHELES STEPHENSI* (LISTON) (DIPTERA: CULICIDAE)

Marimuthu Govindarajan[1]*, Mohan Rajeswary[1], S. L. Hoti[2], Giovanni Benelli[3] and A. Amsath[4]

[1] Unit of Vector Control, Phytochemistry and Nanotechnology, Department of Zoology, Annamalai University, Annamalainagar-608 002, Tamil Nadu, India

[2] Regional Medical Research Centre, Nehru Nagar, Belgaum-590010, Karnataka, India

[3]Department of Agriculture, Food and Environment, University of Pisa, Via del Borghetto 80, 56124 Pisa, Italy

[4] Department of Zoology, Khadir Mohideen College, Adirampattinam-614701, Tamil Nadu, India

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ABSTRACT

Bioactive plant materials have attracted growing interest for their potential implications in mosquiro control programs. In this study, we evaluated the adulticidal activity of different solvent crude extracts of *Pithecellobium dulce* and *Delonix elata* against the malaria vector *Anopheles stephensi* (Diptera: Culicidae). Bioassays were carried out following the WHO method for determination of adulticidal activity against mosquitoes. The adult mortality was observed after 24 h of exposure. All extracts showed moderate adulticide effects; however, the highest adult mortality was found in the leaf methanol extract of both *P. dulce* and *D. elata* against the adults of *An. stephensi* with the LC_{50} and LC_{90} values were 197.91, 137.33 mg/L and 372.27, 259.88 mg/L, respectively. This result suggests that the leaf and seed solvent extracts of *P. dulce* and *D. elata* have the potential to be used as an ideal eco-friendly approach for the control of vector mosquito as target species. This is the first report on the mosquito adulticidal activity of *P. dulce* and *D. elata* plants against malarial vector *An. stephensi*.

Keywords: Adulticidal activity, *Pithecellobium dulce*, *Delonix elata*, *Anopheles stephensi.*

INTRODUCTION

Mosquitoes (Diptera: Culicidae) are the oldest human enemy and represent a significant threat to human health because of their ability to vector pathogens that cause diseases that afflict millions of people worldwide (WHO, 2012; Benelli 2015a). Mosquitoes constitute a major public health problem as vectors of serious human diseases. Female mosquitoes are one of the most worldwide important insect pests that affect the health of human being and domestic animals. *Anopheles stephensi* and *Anopheles culicifacies* are the important vectors of malaria (Govindarajan *et al.*, 2012a). Malaria is a protozoan infection of erythrocytes caused in human beings by five species of the genus *Plasmodium* (*P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, and *P. knowlesi*). In most cases, malaria is transmitted via the bite of an infected female anopheline mosquito, but congenital malaria and acquisition through infected blood transfusion are well described (WHO, 2009). More than 40 per cent of the world's population - approximately 3 billion people are exposed to malaria in 108 endemic countries. About one million cases of malaria are reported in India every year. In 2010 an estimated 219 million (range 154-289 million) cases occurred worldwide and 660,000 people died (range 610 000-971 000), mostly in children under five years of age (WHO, 2012).

Presently, organochlorine, organophosphate, carbamate and synthetic pyrethroid insecticides are being used for public health sprays. Successive changes in the insecticides result in multiple insecticide resistant malaria vectors. Malaria vectors (*Anopheles* species) in India are resistant to DDT, HCH, malathion, and deltamethrin (WHO, 2012). Of the principal vector species, *An. stephensi* have shown widespread resistance. In this situation, the change of insecticides has hampered the program with increased costs. The cost of spraying with

*Corresponding author e-mail: drgovind1979@gmail.com, Mobile: +91-9585265999, Fax: 91-4144- 238080

malathion and deltamethrin is 2.5-folds than the costs of spraying of DDT. Thus, the future of vector control mainly relies on the strategies for the management of existing insecticide resistance in malarial vectors and to limit its further spread. The most important aspect of the management of resistance is to either avoid or delay the onset of resistance by effectively manipulating or influencing the factors responsible for the development of resistance. One of the possible ways of avoiding development of insecticide resistance in field is using nonchemical control method, i.e., biopesticides (Rajeswary and Govindarajan, 2014; Benelli 2015a,b). Therefore, it is the hour to launch extensive search to explore eco-friendly biological materials for control of *An. stephensi*. Ravindran *et al.* (2012) to determine the intrinsic toxicity of hexane, ethyl acetate and methanol crude extracts of *Ageratum houstonianum* leaves against adult *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* mosquitoes. The adulticidal efficacy of *Olea vera, Linum usitatissimum* and *Piper nigera* were evaluate against *An. stephensi* and *Ae. aegypti* under laboratory conditions (Nawaz *et al.,* 2011).

Pithecellobium dulce (*P. dulce*) Benth (Fabaceae) is a small to medium sized, evergreen, spiny tree, up to 18 m height, native of tropical America and cultivated throughout the plains of India and in the Andaman islands. It is known as 'Vilayati babul' in Hindi and 'Kodukkapuli' in Tamil. The bark of the plant is reported to be used as astringent in dysentery, febrifuge and it is also used in dermatitis and eye inflammation. The leaves have been reported to possess astringent, emollient, abortifiacient and antidiabetic properties. The presences of steroids, saponins, lipids, phospholipids, glycosides, glycolipids and polysaccharides have been reported in the seeds. The bark contains 37% of tannins of catechol type. quericitin, kaempferol, dulcitol and afezilin have been reported from the leaves (Nigam *et al.,* 1997). *Delonix elata* (Syn. *Poinciana elata*) commonly known as white gold mohur (Fabaceae) is used by folklore for joint pains and in flatulence. In Indochina, the bark is considered as febrifuge and antiperiodic. The leaf and bark in the form of paste are used by local people to reduce inflammation and pain. It has been used in traditional Indian medicine for the treatment of rheumatism and stomach disorders, and its leaves are used in the treatment of bronchitis and pneumonia in infants. Leaf extracts of *D. elata* are reported for strong anti-inflammatory activity (Sethuraman and Sulochana, 1986). As far as our literature survey could ascertain, no information was available on the adulticidal activity of the experimental plants species given here against malarial vector *An. stephensi*. Therefore, the aim of this study was to investigate the mosquito (*An. stephensi*) adulticidal activity of the different solvent extracts of *P. dulce* and *D. elata* plant species from Tamil Nadu, India. This is the first report on the mosquito (*An. stephensi*) adulticidal activity of the different solvent extracts of selected plants.

MATERIALS AND METHODS

Collection and identification of plants

The matured leaves and seeds of *P. dulce* (Roxb.) Benth. and *D. elata* (L.) Gamble. (Family: Fabaceae) were collected (spring summer) from different regions of Thanjavur District (between 9°50′ and 11°25′ of the north latitude and 78°45′ and 70°25′ of the east longitude), Tamilnadu, India. The plants are taxonomically identified by a taxonomist, Department of Botany and voucher specimens have been deposited in the plant Phytochemistry Division, Department of Zoology, Annamalai University.

Preparation of plant extracts

The fully developed fresh leaves and seeds of the plants *P. dulce* and *D. elata* were washed with tap water and shade dried at room temperature (22-24 ^{o}C). The dried leaves and seeds were powdered with the help of electrical blender. The powdered leaf material (1.0 kg) was then subjected to extraction in various solvents viz, hexane, benzene, chloroform, ethyl acetate and methanol (5.0 L) using soxhlet extraction apparatus for 8 hours individually. The extract was filtered through a Buchner funnel with Whatman number 1 filter paper. The filtrate was evaporated to dryness under reduced pressure using rotary vacuum evaporator (Govindarajan *et al.,* 2012b). The residue was then made in to a 1% stock solution with ethanol. From these stock solutions, different concentrations were prepared and these solutions were used for mosquito adulticidal activity.

Mosquitoes

The mosquitoes *An. stephensi* were reared in the vector control laboratory, Department of Zoology, Annamalai University. The larvae were fed on dog biscuits and yeast powder in the 3:1 ratio. Adults were provided with 10% sucrose solution and 1-week-old chick for blood meal. Mosquitoes were held at 28±2°C, 70–85% relative humidity, with a photoperiod of 14:10-h light/dark cycle.

Adulticidal activity

Sugar-fed adult female mosquitoes (5 to 6 days old) were used. Based on the wide range and narrow range tests, the *P. dulce* and *D. elata* leaf and seed crude extracts were tested at 100, 200, 300, 400, 500 and 120, 240, 360, 480, 600 mg/L and 70, 140, 210, 280, 350 and 90, 180, 270, 360, 450 mg/L concentrations and were impregnated on filter papers (14×12 cm^2). A blank paper consisting of only ethanol was used as control. The papers were left to dry at room temperature to evaporate off the ethanol overnight. Impregnated papers were prepared fresh prior to testing. The bioassay was conducted in an experimental kit consisting of two cylindrical plastic tubes both measuring 125×44 cm following the method in WHO (1981). One tube served to expose the mosquitoes to the plants extracts

and another tube was used to hold the mosquitoes before and after the exposure periods. The impregnated papers were rolled and placed in the exposure tube. Each tube was closed at one end with a 16 mesh size wire screen. Twenty female mosquitoes (2-5 days old glucose fed, blood starved) were transferred into a plastic holding tube. The mosquitoes were allowed to acclimatize in the holding tube for 1 h and then exposed to test paper for 1 h. at the end of exposure period; the mosquitoes were transferred back to the holding tube and kept 24 h for recovery period. A pad of cotton soaked with 10 per cent glucose solution was placed on the mesh screen. Mortality of mosquitoes was determined at the end of 24 h recovery period. The above procedure was carried out in triplicate.

Statistical analysis

The average larval mortality data were subjected to probit analysis (Finney, 1971) for calculating LC_{50}, LC_{90}, and other statistics at 95% confidence limits of upper confidence limit (UCL) and lower confidence limit (LCL), and chi-square values were calculated using the SPSS12.0 (Statistical Package of Social Sciences) software. Results with $p<0.05$ were considered to be statistically significant.

RESULTS

The adulticidal activity of different solvent leaf and seed extracts of *P. dulce* and *D. elata* against *A. stephensi* is presented in Table 1 and 2. The results revealed that the *P. dulce* and *D. elata* leaf methanol extract had the significant adulticidal activity with LC_{50} values of 197.91 and 137.33 mg/L, respectively. The lowest adult mortality was observed with the hexane extract with the LC_{50} values of 273.45 and 203.74 mg/L, respectively. Seed extracts have moderate activity, the higher adulticidal activity was observed in methanol extract with the LC_{50} values of 237.57 and 178.14 mg/L and the lowest adulticidal activity was observed in the hexane extract with the LC_{50} values of 363.68 and 259.23 mg/L, respectively. No mortality was observed in control.

Table 1. Adulticidal activity of different solvent leaf and seed extracts of *P. dulce* against *An. stephensi* in the laboratory.

Parts used	Solvents	LC_{50} (mg/L)	LCL-UCL (mg/L)	LC_{90} (mg/L)	LCL-UCL (mg/L)	χ^2
Leaf	Methanol	197.91	135.19-253.94	372.27	306.45-503.77	18.779
	Ethyl acetate	216.33	164.25-265.28	393.77	333.34-502.96	14.300
	Chloroform	233.05	187.93-276.46	419.66	363.25-513.54	10.752
	Benzene	254.23	187.93-276.46	455.19	395.56-554.39	9.868
	Hexane	273.45	227.13-321.03	493.62	427.33-606.75	9.676
Seed	Methanol	237.57	138.75-321.13	470.34	374.90-691.23	24.556
	Ethyl acetate	275.69	209.14-338.54	516.47	436.30-663.49	13.924
	Chloroform	315.85	226.60-444.90	603.50	489.38-868.96	19.397
	Benzene	336.63	258.77-420.94	631.21	520.82-868.13	15.521
	Hexane	363.68	300.88-434.76	648.71	550.89-834.36	11.359

P> 0.05= represent heterogeneity in the population of tested larvae, LCL= Lower Confidence Limits, UCL= Upper Confidence Limits, χ^2 – Chi square.

Table 2. Adulticidal activity of different solvent leaf and seed extracts of *D. elata* against *An. stephensi* in the laboratory.

Parts used	Solvents	LC_{50} (mg/L)	LCL-UCL (mg/L)	LC_{90} (mg/L)	LCL-UCL (mg/L)	χ^2
Leaf	Methanol	137.33	94.99-175.10	259.88	215.07-346.60	17.530
	Ethyl acetate	152.57	116.65-186.45	276.82	234.84-352.14	13.988
	Chloroform	168.26	131.24-203.98	313.07	266.36-396.90	12.623
	Benzene	184.71	146.18-223.80	343.98	291.26-442.38	12.481
	Hexane	203.74	169.74-240.34	368.95	317.00-461.29	9.813
Seed	Methanol	178.14	97.02-245.69	352.13	277.09-538.67	27.894
	Ethyl acetate	198.91	137.01-255.42	383.12	314.35-539.98	18.889
	Chloroform	214.69	143.82-281.38	421.23	339.78-610.48	20.982
	Benzene	236.24	169.59-304.47	455.00	369.17-653.22	18.856
	Hexane	259.23	205.02-318.94	480.98	400.78-645.44	13.716

P> 0.05= represent heterogeneity in the population of tested larvae, LCL= Lower Confidence Limits, UCL=Upper Confidence Limits, χ^2 – Chi square.

DISCUSSION

A large number of synthetic chemicals have been tested for their repellent activity against mosquitoes. However, the prohibitive retails cost of proprietary formulations of chemicals like DEET (N, N-diethyl-m-toluamide) restricts their usage by the poor in countries such as India. Hence, the search for a safer, better, and cheaper repellent is an ongoing effort. Since cost is an important factor, investigation on the use of local plants as repellents is strongly recommended. Repellents of plant origin should be nontoxic, nonirritating and long lasting. Plants of terrestrial origin have been reported to be a source of mosquito repellents (Govindarajan and Sivakumar, 2012a). Our results showed that crude extract of *P. dulce* and *D. elata* have significant adulticidal activity against *An. stephensi* mosquito. This result is also comparable to earlier reports of, the hexane fraction from methanol extract of *Acorus calamus* rhizome , the methanol fraction of *L. elliptica* and hexane fraction of the *P. aduncum* crude extract were displayed good adulticidal property with the LC_{50} and LC_{90} values of 0.04, 0.11, 0.20 $mgcm^{-2}$ and 0.09, 6.08, 5.32 $mgcm^{-2}$ respectively (Hidayatulfathi *et al.,* 2004). The KDT_{50} and KDT_{90} values of the *L. camara* were 20, 18, 15, 12 and 14 min and 35, 28, 25, 18 and 23 min against *Aedes aegypti*, *Culex quinquefasciatus*, *Anopheles culicifacies*, *An. fluvialitis* and *An. stephensi* with of 93.3, 95.2,100, 100 and 100 per cent respectively (Dua *et al.,* 2010). The five different solvent extracts of *E. alba* and *A. paniculata* were assayed adulticidal activity against *Cx. quinquefasciatus* and *Ae. aegypti*. The highest adult mortality was found in methanol extract of *A. paniculata* against the adults of *Cx. quinquefasciatus* and *Ae. aegypti* with the LC_{50} and LC_{90} values were 149.81, 172.37 mg/L and 288.12, 321.01 mg/L, respectively (Govindarajan and Sivakumar, 2012b). The adulticidal activity of hexane, ethyl acetate, benzene, chloroform and methanol leaf and seed extract of *P. dulce* against *Cx. quinquefasciatus*. The LC_{50} and LC_{90} values of leaf and seed methanol extracts of *P. dulce* against *Cx. quiquefasciatus* were 234.97, 309.24 mg/L and 464.86, 570.80 mg/L, respectively (Govindarajan *et al.,* 2012b).

Elango *et al.* (2011) evaluate the adulticidal activity and adult emergence inhibition (EI) of leaf hexane, chloroform, ethyl acetate, acetone, and methanol extracts of *Aegle marmelos*, *Andrographis lineate*, *A. paniculata*, *Cocculus hirsutus*, *Eclipta prostrata* and *Tagetes erecta* tested against malarial vector, *An. subpictus*. All plant extracts showed moderate adulticidal activity and EI effects after 24 h of exposure at 1,000 mg/L; however, the highest adulticidal activity was observed in ethyl acetate extract of *A. lineata*, chloroform extract of *A. paniculata*, acetone extract of *C. hirsutus*, and methanol extract of *T. erecta* (LD_{50}=126.92, 95.82, 109.40, and 89.83 mg/L; LD_{90}= 542.95, 720.82, 459.03, and 607.85 mg/L); and effective EI was found in leaf acetone extract of the *A. marmelos*, ethyl acetate extract of *A. lineata*, methanol extracts of *C. hirsutus*, and *T. erecta*, (EI_{50}=128.14, 79.39, 143.97, and 92.82 mg/L; EI_{90}= 713.53, 293.70, 682.72, and 582.59 mg/L), respectively, against *An. subpictus*. The larvicidal and adulticidal activities of ethanolic and water mixture (50:50) of plant extracts *Eucalyptus globulus*, *Cymbopogan citratus*, *Artemisia annua*, *Justicia gendarussa*, *Myristica fragrans*, *Annona squamosa*, and *Centella asiatica* were tested against *An. stephensi*, and the most effective between 80% and 100% was observed in all extracts (Senthilkumar *et al.,* 2009). Hafeez *et al.* (2010) evaluated the adulticidal action of ten citrus oils against *Aedes albopictus* through exposure tube method. The results showed that toxic effects of citrus oils varied with time and concentration. *Citrus sinensis* oil was the most lethal (LC_{50}= 53.61, 11.07 and 3.41%) at all recorded times (6, 12 and 24 respectively) and concentrations (5, 10, 15 and 20%) with LT_{50} (18.70, 14.08, 10.42 and 6.59% respectively. Compared with earlier reports, our results revealed that the experimental plant extracts were effective to control *An. stephensi*.

CONCLUSION

From these results, it was concluded that the plant leaf and seed extracts of *P. dulce* and *D. elata* exhibits adulticidal activity against important vector mosquito *An. stephensi*. The results reported in this study open the possibility for further investigations of the efficacy of adulticidal properties of natural product extracts. Plants can provide safer alternatives for modern deadly poisonous synthetic chemicals. The isolation and purification of crude extract of leaf methanol extracts of *P. dulce* and *D. elata* are in progress.

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REFERENCES

Benelli, G., 2015a. Research in mosquito control: current challenges for a brighter future. *Parasitol. Res*. 114: 2801-2805.

Benelli, G., 2015b. Plant-borne ovicides in the fight against mosquito vectors of medical and veterinary importance: a systematic review. *Parasitol. Res*., 114: 3201-3212.

Dua, V.K., Pandey, A.C. and Dash, A.P., 2010. Adulticidal activity of essential oil of *Lantana camara* leaves against mosquitoes. *Indian J. Med. Res.,* 131: 434-439.

Elango, G., Rahuman, A.A., Kamaraj, C., Bagavan, A. and Zahir, A.A., 2011. Efficacy of medicinal plant extracts against malarial vector, *Anopheles subpictus* Grassi. *Parasitol. Res.,* 108: 1437-1445.

Finney, D.J. 1971. Probit analysis. Cambridge University Press, London, p. 68-72.

Govindarajan, M., Sivakumar, R. 2012. Adulticidal and repellent properties of indigenous plant extracts against *Culex quinquefasciatus* and *Aedes aegypti* (Diptera: Culicidae). *Parasitol. Res.* 110: 1607-1620.

Govindarajan, M., Sivakumar, R. 2012. Repellent properties of *Cardiospermum halicacabum* Linn. (Family: Sapindaceae) plant leaf extracts against three important vector mosquitoes. *Asian Pac. J. Trop. Biomed*, 2(8): 602-607.

Govindarajan, M., Sivakumar, R., Rajeswari, M. and Yogalakshmi, K., 2012a. Larvicidal and Ovicidal properties of leaf and seed extracts of *Delonix elata* (L.) Gamble (Family: Fabaceae) against malaria (*Anopheles stephensi* Liston) and dengue (*Aedes aegypti* Linn.) (Diptera: Culicidae) vector mosquitoes. *Parasitol. Res.*, 111: 65-77.

Govindarajan, M., Sivakumar, R., Rajeswary, M., Yogalakshmi, K., 2012b. Adulticidal activity of *Pithecellobium dulce* (Roxb.) Benth. against *Culex quinquefasciatus* (Say). *Asian Pac. J. Trop. Dis*, 2(2): 124-128.

Hafeez, F., Akram, W., Suhail, A. and Aslam Khan, M., 2010. Adulticidal action of ten citrus oils against *Aedes albopictus* (diptera: culicidae). *Pak. J. Agri. Sci.*, 47(3): 241-244.

Hidayatulfathi, O., Sallehuddin, S., Ibrahim, J., 2004. Adulticidal activity of some Malaysian plant extracts against *Aedes aegypti* Linnaeus. *Trop. Biomed.* 21(2): 61-67.

Nawaz, R., Rathor, H.R., Bilal, H., Hassan, S.A., Khan, I.A., 2011. Adulticidal Activity of *Olea vera*, *Linum usitatissimum* and *Piper nigera* against *Anopheles stephensi* and *Aedes aegypti* under laboratory. conditions. *Iran. J. Arthropod. Born. Dis*, 5(2): 2-9.

Nigam, SK., Misra, G., Uddin, R., Yoshikawa, K., Kawamoto, M., Arihara, S., 1997. Pithedulosides A-G, oleanane glycosides from *Pithecellobium dulce*. *Phytochemistry*, 4: 1329-1334.

Rajeswary, M. and Govindarajan, M., 2014. Mosquito adulticidal properties of *Delonix elata* (Family: Fabaceae) against dengue vector, *Aedes aegypti* (Diptera: Culicidae) *J. Coast Life Med.* , 2(5): 389-393.

Ravindran, J., Samuel, T., Alex, E. and William, J., 2012. Adulticidal activity of *Ageratum houstonianum* Mill. (Asteraceae) leaf extracts against three vector mosquito species (Diptera: Culicidae). *Asian Pac. J. Trop. Dis.*, 2(3): 177-179.

Senthilkumar, N., Varma, P. and Gurusubramanian, G., 2009. Larvicidal and adulticidal activities of some medicinal plants against the malarial vector, *Anopheles stephensi* (Liston). *Parasitol. Res.*, 104: 237-244.

Sethuraman, M.G. and Sulochana, N., 1986. The anti-inflammatory activity of *Delonix elata*. *Curr Sci*, 55: 343.

WHO (World Health Organization), 1981. Instructions for determining the susceptibility or resistance of adult mosquitoes to organochlorine, organophosphate and carbamate insecticides: diagnostic test. WHO/VBC/81–807, Geneva.

WHO (World Health Organization), 2009. World malaria report. Available from: *http://www.who.int/ malaria/world_malaria_report_2009/en/index.html*, ac- cessed on February 16, 2010.

WHO (World Health Organization), 2012. World malaria report. Switzerland Geneva, Available from: *http:// www.who.int/malaria/publications/world_malaria_rep ort _report_2012/en/*, accessed on July 18.