J Vector Borne Dis 49, June 2012, pp. 111–113

Short Research Communications

Larvicidal potential of *Mimusops elengi* against *Aedes aegypti* (L) and *Culex quinquefasciatus* (Say)

Anjali D. Ruikar[1], Pushpa V. Pawar[2], Avalokiteswar Sen[2], Usha D. Phalgune[3], Vedavati G. Puranik[4] & Nirmala R. Deshpande[1]

[1]*Dr T.R. Ingle Research Laboratory, Department of Chemistry, S.P. College, Pune;* [2]*Department of Entomology, Pune;* [3]*Central NMR Facility;* [4]*Centre for Materials Characterization, National Chemical Laboratory, Pune, India*

Key words *Aedes aegypti*; *Culex quinquefasciatus;* larvicidal activity; *Mimusops elengi*

Mosquitoes are known for their public health importance, since they are the vectors of many tropical diseases such as malaria, dengue fever, yellow fever, japanese encephalitis and lymphatic filariasis. One of the methods to control the mosquito borne diseases is to control the vectors for the interruption of disease transmission. In the past, synthetic organic insecticides-based intervention measures for the control of insect pests and disease vectors, have resulted in development of insecticide resistance in some mosquito vectors.

Natural products from the plants are the alternative sources of insect control agents because these contain a range of bioactive chemicals, which are selective and do not harm non-target organisms and the environment[1-3]. Plants have formed the basis of natural pesticides that make excellent leads for new pesticide development[4]. The potential of plants as a source of new drugs is still largely unexplored. Hence, the last decade witnessed an increase in the investigation on plants as a source of new biomolecules for human disease management[5]. Higher plants are much more important in the production of economically important organic compounds, pharmaceuticals and pesticides[6]. During the last decade, various studies on natural plant products against mosquito vectors indicate them as possible alternatives to synthetic chemical insecticides. However, more concerted efforts have to go into these studies to make these environment friendly compounds viable for field use and for large-scale vector control operations.

Therefore, in response to urgent need for the new affordable effective and environment friendly mosquito control agent, the screening of an Indian plant *Mimusops elengi* belonging to family Sapotaceae was carried out for mosquito larvicidal activity.

A scientific and systematic phytochemical investigation of bark of *Mimusops elengi* with regard to various biological activities in general and mosquitocidal activity in particular of this plant is lacking, hence, the present study was undertaken. The present study describes the efficacy of *M. elengi* bark extracts and the isolate against *Aedes aegypti* (L) and *Culex quinquefasciatus* (Say) (Diptera: Culicidae).

Plant material used in this study was collected from the local market of Pune, Maharashtra, India. It was authenticated at Agharkar Research Institute, Pune, India. Its authentication number is AHMA S/B-065.

Air shade dried powdered bark material (50 g) was refluxed with various solvents (150 ml) like acetone, ethyl acetate and ethanol for 18 h. The filtrates were concentrated using rotary evaporator to get respective extracts. Further fractionation of the extracts that showed significant activity (ethyl acetate extract, EA), was carried out using non-polar solvent hexane with increasing percentage of ethyl acetate. Each fraction of 100 ml was collected. The fractions were monitored by thin layer chromatography (TLC). Total four fractions, i.e. 100% hexane (EA1), hexane: ethyl acetate (9:1, EA2), hexane: ethyl acetate (8:2, EA3) and residual methanol soluble part (EA4) were collected.

Air shade dried powdered bark material (300 g) was also extracted with soxhlet extractor using hexane for 18 h. Solvent was removed under reduced pressure to get the crude mass (HEX). The compound 1, Cubebin, was isolated and further purified by preparative TLC from this extract. Repeated crystallization gave colourless fine needles of Cubebin (compound 1, 5.22%).

Aedes aegypti and *Cx. quinquefasciatus* larvae reared in mosquito insectary of the National Chemical Laboratory maintained at 27 ± 2°C temperature and 75 ± 5% relative humidity under 14:10 light and dark cycles were used for the present study. Early IV instar larvae were used for the experiments.

Larvicidal bioassay

The extracts, fractions and the isolates were dissolved in DMSO solvent. These were tested to determine the larvicidal activity by making serial dilutions from 500 to 10 ppm in bioassays against larvae of the two mosquito species. Larvicidal activity was assessed by the procedure of WHO with slight modification. The bioassays were performed at a room temperature of 27 ± 1°C by exposing 10 larvae in each concentration of the extract in the final volume of 50 ml in 100 ml beaker. Five replicates of each concentration were tested for larval bioefficacy. Each experiment was repeated three times. Thus, the mean percentage mortality is an average of 15 replicates. The larval mortality in each concentration and control was recorded after 24 h of continuous exposure. Where there was no 100% kill the larvae were allowed to stay in water and cumulative mortality was recorded after 48 and 72 h. The corrected mortality was determined using Abbott's formula whenever required[7]. The dose mortality data were analyzed by log probit method of Finney[8] and lethal concentrations for 50 and 90% mortality were calculated (i.e. LC_{50} and LC_{90}).

RESULTS & DISCUSSION

The Compound 1, Cubebin, 3,4 bis (1,3-benzodioxol-5-yl methyl) tetrahydrofuran-2-ol (Fig. 1) was isolated as white crystalline solid, obtained by repeated crystallization using mixed solvent system of chloroform and hexane from hexane extract. The structure of the compound was assigned by modern spectral analysis and further confirmed by single X-ray diffraction study. The data obtained were found to be similar as reported earlier[9].

Larvicidal activity of various extracts of bark was studied. Hexane (HEX) and ethyl acetate (EA) extracts showed promising larvicidal activity. Toxicity of these extracts, fractions EA1, EA2, EA3 of EA along with a pure compound 1 against two species of mosquitoes was recorded. In this study, IV instar larvae of both mosquitoes were subjected to dose dependent efficacy of *M. elengi* under normal conditions. In case of EA, 90 and 80% mortality against *Ae. aegypti* and *Cx. quinquefasciatus* respectively was noticed after 24 h exposure period, however, after 48 h exposure the mortality was 100% in both the species. LC_{50} values were 390.46 and 453.23 ppm

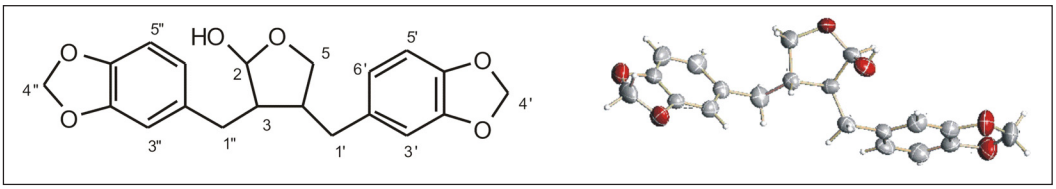

Fig. 1: Schematic representation and ORTEP diagram of Compound 1.

Table 1. Lethal concentrations of test samples against IV instar larvae of *Ae. aegypti*

Test sample	LC_{50} (ppm) (24 h)	LC_{90} (ppm) (24 h)	Regression equation	χ^2-value
HEX	50.86	82.18	Y = –5.495 + 6.150 × S.E. ± 1.45	18.36
Compound 1	42.33	52.48	Y = –17.330 + 13.727 × S.E. ± 4.19	12.90
EA	390.46	845.27	Y = 4.903 + 3.82 × S.E. ± 1.25	22.35
EA1	59.47	95.46	Y = –6.064 + 6.235 × S.E. ± 2.35	14.24
EA2	82.64	170.01	Y = –2.844 + 4.091 × S.E. ± 1.096	12.85
EA3	115.53	307.82	Y = –1.21 + 3.01 × S.E. ± 0.99	15.51

Table 2. Lethal concentrations of test samples against IV instar larvae of *Cx. quinquefasciatus*

Test sample	LC_{50} (ppm) (24 h)	LC_{90} (ppm) (24 h)	Regression equation	χ^2-value
HEX	53.68	89.90	Y = –4.903 + 5.75 × S.E. ± 2.29	17.40
Compound 1	46.30	64.89	Y = –9.60 + 8.76 × S.E. ± 1.73	15.56
EA	453.23	1003.42	Y = –4.804 + 3.713 × S.E. ± 1.27	17.23
EA1	66.48	103.23	Y = –7.225 + 6.707 × S.E. ± 2.58	19.25
EA2	95.62	211.00	Y = –2.386 + 3.729 × S.E. ± 0.834	21.07
EA3	130.63	960.66	Y = 4.440 + 2.341 × S.E. ± 0.722	17.54

respectively against *Ae. aegypti* and *Cx. quinquefasciatus* (Tables 1 & 2).

The extract EA was less effective but its three fractions EA1, EA2 and EA3 show remarkable toxicity to the larvae. EA1 was the most active. It exhibited 100% mortality after 24 h exposure at 200 ppm dose in case of both the species, whereas EA2 and EA3 were active at 300 ppm showing 100% kill after 48 h of exposure. The LC_{50} values of these three fractions were 59.47, 82.64 and 115.53 ppm respectively in case of *Ae. aegypti* while it was 66.48, 95.62 and 130.63 ppm respectively in case of *Cx. quinquefasciatus*. Hexane extract was found to be highly active since it exhibited 100% kill at 100 ppm within 24 h in both the mosquito species. Cubebin, which was isolated from the hexane extract, exhibited 100% mortality at the lowest dose of 60 ppm in case of *Ae. aegypti* and 100 ppm in case of *Cx. quinquefasciatus* after 24 h. LC_{50} of the hexane extract was 50.86 and 53.68 against *Ae. aegypti* and *Cx. quinquefasciatus* larvae respectively. Cubebin showed LC_{50} of 42.33 and 46.30 ppm against *Ae. aegypti* and *Cx. quinquefasciatus* larvae respectively. Potency of these extracts can be graded as cubebin>hexane> EA1> EA2> EA3>EA. *Aedes aegypti* larvae were found to be more susceptible than *Cx. quinquefasciatus* since LC_{50} values of all the samples tested were lower.

These findings suggested that the activity of the samples may be because of the lignan compound, cubebin.

CONCLUSION

The present study demonstrates the potential of *Mimusops elengi* against IV instar larvae of *Ae. aegypti* and *Cx. quinquefasciatus* and its benefits in developing cost-effective and environment friendly new type of larvicide for mosquito control. This suggests that the plant is accountable for larvicidal activity. The isolate possess potent activity. Hence, further studies may be directed with appropriate formulation in order to ascertain whether this compound can be used as an effective larvicide.

ACKNOWLEDGEMENTS

Authors are thankful to the Principal, S.P. College Pune, the Head, Department of Chemistry, S.P. College, Pune and the Director, National Chemical Laboratory, Pune, India.

REFERENCES

1. Arnason JT, Philogene BJR, Morand P. Insecticides of plant origin. ACS Symp. Ser No. 387. Washington DC: American Chemical Society 1989: p. 213.
2. Hedlin PA, Holingworth RM, Masler EP, Miyamoto J, Thopson DG, editors. *Phytochemicals for pest control.* ACS Symp Ser No. 658. Wahington DC: American Chemical Society 1997; p. 372.
3. Sukumar K, Perich MJ, Boober LR. Botanical derivatives in mosquito control: a review. *J Am Mosq Control Assoc* 1991; *7:* 210–37.
4. Newman, DJ, Cragg GM, Snader KM. The influence of natural products upon drug discovery. *Natural Product Res* 2000; *17:* 215–34.
5. Grierson DS, Afolayan AJ. Antibacterial activity of some indigenous plants used for the treatment of wounds in the Eastern Cape, South Africa. *J Ethnopharmacol* 1999; *66:* 103–6.
6. Hostettmann K, Wolfender JL. The search for biologically active secondary metabolites. *Pesticide Sci* 1997; *51:* 471–82.
7. Abbott WS. A method of computing the effectiveness of an insecticide. *J Econ Entomol* 1925; *18:* 265–7.
8. Finney DJ. Probit analysis, III edn. London : Cambridge University Press 1971; p. 1–333.
9. Bruno-Colmenárez J, Usubillaga A, Khouri N, Díaz de Delgado G. Cubebin: a lignan isolated from *Aristolochia odoratissima* L. *Acta Cryst E* 2007; *63:* o2046–o2047.

Correspondence to: Dr Anjali D. Ruikar, Fergusson College (Jr. Section), Shivajinagar, Pune–411 004, India.
E-mail: anjaliruikar07@yahoo.com

Received: 9 January 2012 *Accepted in revised form:* 1 May 2012