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RESEARCH ARTICLE

Mosquito Larvicidal Activity of Leaf Extracts of Plant *Amomum Subulatum* Roxb. Against *Anopheles Stephensi* (Diptera: Culicidae)

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ABSTRACT

Dengue, malaria, yellow fever, schistosomiasis, filariasis and Japanese encephalitis are the major health diseases which are transmitted by mosquitoes. To prevent the health problem caused by mosquito vectors and to enhance quality of public health and environment, mosquito control is essential. Plants have immense sources of bioactive components that can be develop to form environmentally friendly and safe vectors control agents. The larvicides play a vital role in preventing mosquitoes in their breeding sites, these also have negative effect in areas of beneficial and non-target organisms. The leaves of the plant *Amomum subulatum* were collected and extracted with different solvents such as chloroform, methanol, ethyl acetate and hexane. The larvicidal activity of leaf extracts of the plant was evaluated as per the method recommended by WHO. Batches of 25 third instar larvae were transferred to a small disposable test cups, each containing 200 ml of water. Six replicate were set to for each concentration and an equal number of control were set up simultaneously using tap water. To this 1 ml of appropriate solvent was added. Crude extracts concentration ranging from 60 to 300 ppm was tested. The average larval mortality data were subjected to probit analysis for calculating LC50, LC90. The present investigation has important implications in controlling the mosquito larvae in the polluted aquatic ecosystem. The leaf extracts of *Amomum subulatum* have remarkable larvicidal activity against the mosquito *Anopheles stephensi*.

KEYWORDS

Amomum Subulatum, Anopheles Stephensi, Larvicidal Activity, Leaf Extracts

INTRODUCTION

Dengue, malaria, yellow fever, schistosomiasis, filariasis and Japanese encephalitis are the major health diseases which are transmitted by mosquitoes[1]. The number of diseases transmit by mosquitoes are more than any other group of anthropodes in the world. The diseases caused by mosquitoes hitting more than 100 countries around the world, infecting over 700,000,000 population every year worldwide and 40,000,000 of the Indian population. Mosquitoes have been declared as "public enemy number one" by the WHO[2]. To prevent the health problem caused by mosquito vectors and to enhance quality of public health and environment, mosquito control is essential. By using different methods the diseases transmission by the mosquitoes can be control. But unbalanced and extensive use of synthetic insecticides has raised the problem like increasing resistance towards pesticides[3,4]. The side effects of chemical insecticides used for control of vectors diseases have caused many problems like environmental pollution, toxic

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effects to humans and other non-target organisms[5].

To solve these problems, new approaches has been urgently needed on the use of plant based natural products as larvicides which can be use as an alternate to synthetic insecticides. Plants have immense sources of bioactive components that can be develop to form environmentally friendly and safe vectors control agents[6,8]. Plant derived products have attract attention from scientists and more than 2000 plant species are known to have insecticide properties. The different botanical extracts from the plant leaves, seeds, flowers roots and bark in the concentrate form have been used as conventional insecticides for decades[9]. Natural products are important alternatives to synthetic insecticides. The phytochemicals extracted from the whole plant or particular part of the plant using different solvents also act against the vector mosquitoes as toxicant, repellent, larvicidal, growth regulators and ovipositional deterrent[10,12].

The larvicides play a vital role in preventing mosquitoes in their breeding sites, these also have negative effect in areas of beneficial and non-target organisms. .Hence the objective of the present study was to evaluate the larvicidal activity of leaf extracts of plant *Amomum subulatum.*

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 four different solvents namely hexane, ethyl acetate, methanol and chloroform individually. The solvent from the extract was remove using rotatry vacuum evaporator to collect the crude extract. Standard stock solutions were prepared at 1% by dissolving the residues in acetone. From this stock solution, different concentrations were prepared and these solutions were used for larvicidal bioassays.

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 the10% sugar solution under (28±2) C^0 and (78±2) % relative humidity.

Larvicidal Bioassay

The larvicidal activity of leaf extracts of the plant was evaluated as per the method recommended by WHO[13]. Batches of 25 third instar larvae were transferred to a small disposable test cups, each containing 200 ml of water. The appropriate volume of dilution was added 200 ml water in cups to obtain the desired target dosage, starting with the lowest concentration. Six replicate were set to for each concentration and an equal number of control were set up simultaneously using tap water. To this 1 ml of appropriate solvent was added. The LC_{50} value was calculated after 24 h by probit analysis[14].

Statistical Analysis

The average larval mortality data were subjected to probit analysis for calculating LC50, LC90, 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 $p<0.005$ were considered to be statistically significant.

RESULTS

The toxicity of different solvents crude extract of leaf of *Amomum subulatum* was tested against larve *Anopheles stephensi* (table no.). The data were observed and statistical data regarding LC_{50}, LC_{90}, LCL, UCL and *chi*-square values

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

Name of the extract	**Concentration (ppm) Control**	**% of mortality ± SD**	**LC_{50} ppm (LCL-UCL)**	**LC_{90} ppm (LCL-UCL)**	**X^2**
Hexane	Control 60 120 180 240 300	0.0±0.0 24.6±1.2 40.00±1.1 55.4±1.0 73.0±1.5 95.7±1.2	151.44 (109.60-190.20)	272.65 (223.40-379.94)	20.205*
Ethyl acetate	Control 60 120 180 240 300	0.0±0.0 23.0±1.3 38.4±1.5 60.0±1.0 75.6±0.4 87.2±1.1	152.38 (121.36.185.30)	285.57 (243.01-367.62)	12.320*
Methanol	Control 60 120 180 240 300	0.0±0.0 25.2±1.5 44.0±1.1 57.5±1.0 75.3±0.5 87.1±1.2	125.32 (93.27.158.24)	243.69 (201.19-331.83)	15.800*
Chloroform	Control 60 120 180 240 300	0.0±0.0 28.2±1.5 40.0±1.2 60.4±1.6 80.3±1.3 92.4±1.0	142.24 (110.10-179.52)	273.38 (229.22-357.05)	14.683*

Each value (X S:D:) represents mean of six values LCL lower confidence limit, UCL upper confidence limit a Significant at $p<0.05$ level

were calculated. The LC_{50} and LC_{90} values of hexane ethyl acetate, methanol and chloroform extract of *Amomum subulatum* against early third instar larvae of *Anopheles stephensi* were 151.44, 152.38, 125.32 and 142.24 ppm and 272.65, 285.57, 243.69 and 273.38 ppm resp. Methanol extract was observed having maximum larvicidal activity followed by chloroform, ethyl acetate and hexane leaf extract. The chi-square values are significant at $p< 0.05$ level. The *chi*-square values are significant at $p< 0.05$ level.

DISCUSSION

The most important feature of insecticides is environmental safety. An insecticide does not allow causing high mortality on target organisms[15]. Phytochemicals may serve as these are relatively safe, inexpensive and readily available in many parts of the world. Several plants are used in traditional medicines for the mosquito larvicidal activities in many parts of the world. The studies on ethanolic extract of *Annona squamosa* leaves has shown LD_{50} (20.70 ppm), LD_{90} (76.73 ppm) against *Aedes albopictus* and LD_{50} (6.96 ppm), LD_{90} (31.80 ppm) against *Culex quinquefasciatus*[16]. The ethanolic extract of *Capsicum annum* fruits has shown LD_{50} (0.011ppm), LD_{90} (0.027 ppm) against *Anopheles stephensi*[17]. The result of the present investigation showed that the methanol, hexane, chloroform and ethyl acetate extracts of leaf of *Amomum subulatum* have significant larvicidal activity against important vector mosquitoes *Anopheles stephensi*. This result is also similar to earlier reports of Singh et al. Who observed the larvicidal activity of *Ocimum canum* oil against vector mosquitoes, namely, *Aedes aegypti* and *Culex quinquefasciatus* (LC_{50}, 301 ppm) and *Anopheles stephensi* (234 ppm)[18]. Leaf methanol extracts of *Pavonia zeylanica* and *Acacia ferruginea* were tested for their larvicidal activity against the late third instar larvae of *Culex quinquefasciatus* showed LC_{50} values of 2,214.7 and 5,362.6 ppm, respectively[19]. The methanol extract peel of *Citrus sinensis* and the ethyl acetate extracts of leaf and flower of *Occimum canum* were tested against the larvae of *Anopheles stephensi* (LC_{50}=95.74, 101.53, and 28.96 ppm; LC_{90}=303.20, 492.43 and 168.05 ppm), respectively[20]. The leaves extract of petroleum ether of the plant *Vitex negundo* were evaluated for larvicidal activity against larval stages of *Culex tritaeniorhynchus* with LC_{50} and LC_{90} values of 2.4883 and 5.1883 mg/l, respectively[21]. The methanol leaf extracts of *Vitex trifolia*, *Vitex negundo, Vitex peduncularis* and *Vitex altissima* showed varying levels of larvicidal activity against *Culex quinquefasciatus* and *Anopheles stephensi* and were found with LC_{50} value of 212.57, 41.41, 76.28, and 128.04 ppm, respectively[22]. Mullai and Jebanesan have reported that petroleum ether, methanol and ethyl acetate leaf extracts of *Cucurbit* and *Citrullus colocynthis* showed LC_{50} values of 117.73, 171.64 and 75.91 ppm and 66.92, 118.74 and 47.58 ppm, respectively, against larvae of *Culex quinquefasciatus*. The petroleum ether and methanol extracts of *R. nasutus* and *Derris elliptica* showed larvicidal effects against *Aedes aegypti*, *Culex quinquefasciatus*, *Anopheles dirus*, and *Mansonia uniformis* with LC_{50} values between 3.9 and 11.5 mg/L, while the Methanol extract gave LC_{50} values of between 8.1 and 14.7 mg/L.

CONCLUSION

The present investigation has important implications in controlling the mosquito larvae in the polluted aquatic ecosystem. The leaf extracts of *Amomum subulatum* have remarkable larvicidal activity against the mosquito *Anopheles stephensi*. Further studies on identification of active compounds for larval control and commercial preparation of repellent products and field trials are needed to recommend for the development of eco-friendly insecticides.

REFERENCES

1. Murugan, K., Murugan, P., & Noortheen, A. (2007). Larvicidal and repellent potential of Albizzia amara Boivin and Ocimum basilicum Linn against dengue vector, Aedes aegypti (Insecta: Diptera: Culicidae). *Bioresource Technology*, *98*(1), 198-201.

2. Cutris, C. F. (1992). Personal protection methods against vector disease. *Review of*

Medical and Veterinary Entomology, *80*(10), 543-553.

3. Service, M. W. (1983). Management of vectors. In: Youdeowei, A., Service, M.W. (Eds.), *Pest and Vectors Management in the tropics*, 265-280.
4. Senthilkumar, A., & Venkatesalu, V. (2012). Larvicidal potential of Acorus calamus L. essential oil against filarial vector mosquito Culex quinquefasciatus (Diptera: Culicidae). *Asian Pacific Journal of Tropical Disease*, *2*(4), 324-326.
5. Sakulku, U., Nuchuchua, O., Uawongyart, N., Puttipipatkhachorn, S., Soottitantawat, A., & Ruktanonchai, U. (2009). Characterization and mosquito repellent activity of citronella oil nanoemulsion. *International Journal of Pharmaceutics*, *372*(1), 105-111.
6. Ponlawat, A., Scott, J. G., & Harrington, L. C. (2005). Insecticide susceptibility of Aedes aegypti and Aedes albopictus across Thailand. *Journal of Medical Entomology*, *42*(5), 821-825.
7. Choochote, W., Tuetun, B., Kanjanapothi, D., Rattanachanpichai, E., Chaithong, U., Chaiwong, P., & Pitasawat, B. (2004). Potential of crude seed extract of celery, Apium graveolens L., against the mosquito Aedes aegypti (L.)(Diptera: Culicidae). *Journal of Vector Ecology*, *29*(2), 340-346.
8. Webb, C., Russell, R. C. (2006). Evaluation of two 80% DEET formulations of bushman insect repellent against *Aedes aegypti* mosquitoes in laboratory trials. *Institute of Clinical Pathology and Medical Research*.
9. Revay, E. E., Junnila, A., Xue, R. D., Kline, D. L., Bernier, U. R., Kravchenko, V. D., & Müller, G. C. (2013). Evaluation of commercial products for personal protection against mosquitoes. *Acta Tropica*, *125*(2), 226-230.
10. Govindarajan, M. (2011). Larvicidal and repellent properties of some essential oils against Culex tritaeniorhynchus Giles and Anopheles subpictus Grassi (Diptera: Culicidae). *Asian Pacific Journal of Tropical Medicine*, *4*(2), 106-111.
11. Mandal, S. (2011). Repellent activity of Eucalyptus and Azadirachta indica seed oil against the filarial mosquito Culex quinquefasciatus Say (Diptera: Culicidae) in India. *Asian Pacific Journal of Tropical Biomedicine*, *1*(1), S109-S112.
12. Tennyson, S., Ravindran, J., Eapen, A., & William, J. (2012). Repellent activity of Ageratum houstonianum Mill.(Asteraceae) leaf extracts against Anopheles stephensi, Aedes aegypti and Culex quinquefasciatus (Diptera: Culicidae). *Asian Pacific Journal of Tropical Disease*, *2*(6), 478-480.
13. Gokulakrishnan, J., Kuppusamy, E., Shanmugam, D., Appavu, A., & Kaliyamoorthi, K. (2013). Pupicidal and repellent activities of Pogostemon cablin essential oil chemical compounds against medically important human vector mosquitoes. *Asian Pacific Journal of Tropical Disease*, *3*(1), 26-31.
14. Nerio, L. S., Olivero-Verbel, J., & Stashenko, E. (2010). Repellent activity of essential oils: a review. *Bioresource Technology*, *101*(1), 372-378.
15. Choochote, W., Chaithong, U., Kamsuk, K., Jitpakdi, A., Tippawangkosol, P., Tuetun, B., & Pitasawat, B. (2007). Repellent activity of selected essential oils against Aedes aegypti. *Fitoterapia*, *78*(5), 359-364.
16. Phasomkusolsil, S., & Soonwera, M. (2011). Efficacy of herbal essential oils as insecticide against Aedes aegypti (Linn.), Culex quinquefasciatus (Say) and Anopheles dirus (Peyton and Harrison). *Southeast Asian Journal of Tropical Medicine and Public Health*, *42*(5), 1083.
17. Krishnappa, K., & Elumalai, K. (2012). Toxicity of Aristolochia bracteata methanol leaf extract against selected medically important vector mosquitoes (Diptera:

Culicidae). *Asian Pacific Journal of Tropical Disease*, *2*, S553-S557.

18. World Health Organization. (2009). Guidelines for efficacy testing of mosquito repellents for human skin, p. 4-18
19. Elango, G., Bagavan, A., Kamaraj, C., Zahir, A. A., & Rahuman, A. A. (2009). Oviposition-deterrent, ovicidal, and repellent activities of indigenous plant extracts against Anopheles subpictus Grassi (Diptera: Culicidae). *Parasitology Research*, *105*(6), 1567-1576.
20. Kiarie-Makara, M. W., Yoon, H. S., & Lee, D. K. (2010). Repellent efficacy of wood vinegar against Culex pipiens pallens and Aedes togoi (Diptera: Culicidae) under laboratory and semi-field conditions. *Entomological Research*, *40*(2), 97-103.
21. Choi, W. S., Park, B. S., Ku, S. K., & Lee, S. E. (2002). Repellent activities of essential oils and monoterpenes against Culex pipiens pallens. *Journal of the American Mosquito Control Association*, *18*(4), 348-351.
22. Karunamoorthi, K., Ramanujam, S., & Rathinasamy, R. (2008). Evaluation of leaf extracts of Vitex negundo L. (Family: Verbenaceae) against larvae of Culex tritaeniorhynchus and repellent activity on adult vector mosquitoes. *Parasitology Research*, *103*(3), 545-550.

