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Larvicidal Efficacy of Plant Extracts against the Malarial Vector *Anopheles stephensi* Liston (Diptera: Culicidae)

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Abstract: Malaria is transmitted by *Anopheles stephensi* and for controlling the malaria parasite *Plasmodium* spp., the vector mosquito has to be controlled. Extensive use of synthetic and chemical insecticides has resulted in environmental hazards and also in development of physiological resistance among vector mosquito species. Plant products are considered to be a potential alternative approach as they are environmentally safe, target specific and biodegradable. The extracts of seven plants *viz*., leaves of *Abutilon indicum*, *Cleistanthus collinus*, *Leucas aspera*, *Murraya koenigii*, aerial parts of *Hyptis suaveolens* and whole plants of *Citrullus colocynthis* and *Sphaeranthus indicus* with four different solvents *viz*., hexane, diethyl ether, dichloromethane and ethyl acetate were tested against the early third instar larvae of *Anopheles stephensi*. The larval mortality was calculated after 24 hours exposure period. The ethyl acetate extract of *Leucas aspera* was found to be effective with a LC_{50} value of 352.84 ppm.

Key words: Larvicidal Activity · *Anopheles stephensi* · Plant Extracts

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 [1, 2]. Mosquitoes constitute a major public health problem as vectors of serious human diseases [3]. WHO has declared the mosquito "public enemy number one" as they are responsible for the transmission of various dreadful diseases [4]. Several species belonging to genera *Aedes, Anopheles* and *Culex* are vectors for the pathogens of various diseases like Dengue fever, Dengue haemorrhagic fever, Malaria, Japanese encepahalitis and Filariasis [5, 6]. *Anopheles stephensi* Liston is the primary vector of malaria in India and other west Asian countries. Malaria remains as one of the most prevalent diseases in the tropical world [7].

Plant products have been used traditionally by human communities [8]. The plant world comprises a rich storehouse of phytochemicals, which are widely used in the place of synthetic insecticides since continuous use of synthetic insecticides cause side effects to non-target organisms and insecticide resistance against mosquitoes [9]. Phytoproducts on account of minimal hazardous effect on the environment and wide range of availability offer promises in future mosquito control programmes. They have revolutionized the fields of vector control as they possess different bioactive components and can be used as general toxicants against various larval stages of the mosquito [10, 11].

Botanical phytochemicals with mosquitocidal potential are now recognized as potent alternative insecticides to replace synthetic insecticides in mosquito control programs due to their excellent mosquitocidal properties and the chemicals derived from plants have been projected as weapons in future mosquito control program as they are shown to function as general toxicant, growth and reproductive inhibitors [12]. Therefore, the present study had been carried out to evaluate the larvicidal activity of seven plants *viz*., *Abutilon indicum* (leaf), *Citrullus colocynthis*

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(whole plant), *Cleistanthus collinus* (leaf), *Hyptis suaveolens* (aerial part), *Leucas aspera* (leaf), *Murraya koenigii* (leaf) and *Sphaeranthus indicus* (whole plant) extracts against the malarial vector *Anopheles stephensi* Liston.

MATERIALS AND METHODS

Plant Extracts: Seven plants *viz*., leaves of *Abutilon indicum*, *Cleistanthus collinus*, *Leucas aspera*, *Murraya koenigii*, aerial parts of *Hyptis suaveolens*, whole plants of *Citrullus colocynthis* and *Sphaeranthus indicus* collected in and around Chennai, Tamilnadu, India were brought to the laboratory, shade dried under room temperature and powdered using an electric blender. Dried and powdered plant parts of each plant (1 kg) was subjected to sequential extraction using 3 L of hexane, diethyl ether, dichloromethane and ethyl acetate for a period of 72 h each to obtain the crude extracts using rotary vacuum evaporator. The hexane, diethyl ether, dichloromethane and ethyl acetate crude extracts thus obtained were lyophilized and a stock solution of 1,00,000 ppm prepared from each crude extract by adding adequate volume of acetone was refrigerated at 4 °C until testing for bioassays.

Test Mosquitoes: Tests were carried out against laboratory reared *Anopheles stephensi* mosquitoes free of exposure to insecticides and pathogens. Cyclic generation of *Anopheles stephensi* were maintained at 25-29 °C and 80-90 per cent R.H. in the insectarium. Larvae were fed on larval food (powdered dog biscuit and yeast in the ratio 3:1) and adult mosquitoes on ten per cent glucose solution. Adult female mosquitoes were periodically blood-fed on restrained albino mice for egg production.

Larvicidal Bioassay: Bioassay for the larvicidal activity was carried out using WHO [13] procedure with slight modifications. From the stock solution, concentrations of 250, 500, 750 and 1000 ppm was prepared. Twenty five early third instar larvae were introduced into a 250 ml beaker containing 200 ml of water with each concentration. A control was prepared by the addition of acetone to water. A total of three trials were carried out with five replicates per trial against vector mosquitoes. Mortality was recorded after 24 h and the control mortality was corrected using Abbott's [14] formula.

Statistical Analysis: SPSS [15] was used for determination of LC_{50} and LC_{90} values. Data from mortality and effect of concentrations were subjected to analysis of variance. Difference between the treatments was determined by Tukey's test ($P < 0.05$).

RESULTS AND DISCUSSION

Vector control is facing a serious threat due to the emergence of resistance in vector mosquitoes to conventional synthetic insecticides or development of newer insecticides [16]. Mosquitoes in the larval stage are attractive targets for pesticides because mosquitoes breed in water and thus, it is easy to deal with them in this habitat. The use of conventional pesticides in the water sources, however, introduces many risks to people and/or the environment and due to the continuous increase in resistance of mosquitoes to familiar synthetic insecticides, better alternative means are sought [3]. Natural pesticides, especially those derived from plants, are more promising in this aspect [17]. A considerable number of plant products/derivatives have shown to be effective against mosquitoes with a safe manner. The screening of plants for mosquitocidal property may eventually lead to their use in natural product-based mosquito abatement practices [18]. Larvae from the three medically important mosquito genera *Aedes*, *Anopheles* and *Culex* are all susceptible to a greater or lesser extent to some phytochemicals [19].

The results of the larvicidal bioassay are presented in Table 1. The ethyl acetate leaf extract of *Leucas aspera* was found to be effective with a LC_{50} value of 352.84 ppm against the larvae of *Anopheles stephensi* followed by ethyl acetate leaf extract of *Cleistanthus collinus* with 399.72 ppm. Various plant species have been screened for their larvicidal activity against different species of mosquitoes [12, 19-21]. Plants that showed larvicidal activity are methanolic stem extracts of *Satureja hortensis* (LC_{50} 28ppm), *Ocimum basilicum* (LC_{50} 32ppm), *Thymus vulgaris* (LC_{50} 48ppm), flower extracts of *Lavandula officinalis* (LC_{50} 59ppm) and stem extracts of *Stachys byzantica* (LC_{50} 65ppm). Plants that showed moderate larvicidal activity (LC_{50} values ranging from 100-250ppm) are methanlolic extracts of *Salvia virdis* flower (LC_{50} 110ppm), *Hysopus officinalis* stem (LC_{50} 150ppm), *Salvia officinalis* stem (LC_{50} 159ppm), *Salvia farinaceae* (LC_{50} 195ppm) [22, 23], ethyl acetate

Table 1: Probit analysis of larvicidal efficacy of plant extracts against *Anopheles stephensi*

Extracts	LC_{50} (ppm)	LC_{90} (ppm)	Chi-square value	Regression value
Abutilon indicum				
Hexane	1411.16	7276.48	0.59*	0.58
Diethyl ether	725.09	2198.88	0.24*	0.81
Dichloromethane	1376.03	6285.00	0.17*	1.94
Ethyl acetate	790.27	3236.78	3.40*	2.09
Cleistanthus collinus				
Hexane	1398.29	5833.61	0.82*	2.07
Diethyl ether	602.04	5451.98	0.43*	1.12
Dichloromethane	776.13	3066.50	18.76	2.15
Ethyl acetate	399.72	1251.76	39.92	2.58
Leucas aspera				
Hexane	1592.05	8501.06	0.54*	1.76
Diethyl ether	2262.20	5547.41	0.65*	1.07
Dichloromethane	1077.84	6384.63	0.04*	1.25
Ethyl acetate	352.84	1033.60	23.26	2.75
Murraya koenigii				
Hexane	418.74	891.99	54.67	3.90
Diethyl ether	856.70	3771.08	4.37*	1.99
Dichloromethane	944.39	2624.53	7.19	2.89
Ethyl acetate	647.55	2750.57	0.12*	1.66
Hyptis suaveolens				
Hexane	1523.19	6964.42	0.39*	1.22
Diethyl ether	1490.78	6908.41	2.93*	1.92
Dichloromethane	1396.41	4854.16	7.63	2.37
Ethyl acetate	944.08	2129.29	0.25*	3.63
Citrullus colocynthis				
Hexane	1451.29	7895.79	0.77*	1.74
Diethyl ether	503.39	3678.13	0.23*	1.01
Dichloromethane	1209.59	8213.79	5.60*	1.09
Ethyl acetate	3467.26	7258.59	0.21*	1.84
Sphaeranthus indicus				
Hexane	590.06	2600.38	0.17*	0.87
Diethyl ether	838.35	3606.22	3.11*	2.02
Dichloromethane	1311.98	5547.49	0.06*	2.05
Ethyl acetate	526.50	1220.46	82.47	2.05

* Significant at $P < 0.05$ level

leaf extract of *Strychnos nuxvomica* (LC_{50} 222ppm) [24] and methanlolic extracts of *Sideritis euxina* (LC_{50} 250ppm). Plants possessing LC_{50}values less than 500ppm include methanolic stem extracts of *Origanum vulgare* (LC_{50} 256.0ppm), *Stachys cretica* (LC_{50} 292ppm), *Salvia verbenaca* (LC_{50} 311ppm), *Teucrium hircanicum* (LC_{50} 316ppm) and *Salvia verticillata* (LC_{50} 410ppm) against the larvae of *Culex quinquefasciatus* [22, 23]. The results of the above mentioned reports were comparable with the LC_{50} value of the present study thus exhibiting moderate larvicidal activity. Further investigations on evaluation, identification and isolation of the bioactive component(s) of *Leucas aspera* extracts and its systemic effects on target mosquitoes are needed.

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REFERENCES

1. WHO, 1992. Vector resistance to pesticides. Fifteenth report of the WHO expert committee on vector biology and control. Technical Report Series, 818: 1-62.
2. WHO, 1998. Malaria prevention and control. WHO Report, Geneva.

3. Hag, E., E.A.A.H. Nadi and A.A. Zaitoon, 1999. Toxic and growth retarding effects of three plant extracts on *Culex pipiens* larvae (Diptera: Culicidae). Phytotherapy Research, 13: 388-392.
4. WHO, 1996a. The World Health Report, Geneva.
5. Service, M.W., 1983. Management of vectors. In: Youdeowei, A. and M.W. Service (Eds.), Pest and Vector Management in Tropics, pp: 265-280.
6. Gubler, D.J., 1998. Resurgent vector-borne diseases as a global health problem. Emerging Infectious Diseases, 4(3): 442-450.
7. WHO, 2010. Malaria fact sheets No. 94. WHO Report
8. Jacobson, M., 1958. Insecticides from plants: a review of the literature, 1941-1953. U.S. Dept. Agricultural Hand Book, pp: 154.
9. Kelm, M.A., M.G. Nair and R.A. Schutzki, 1997. Mosquitocidal compounds from *Magnolia salicifolia*. International Journal of Pharmacognosy, 35: 84-90.
10. Sharma, P., L. Mohan and C.N. Srivastava, 2004. Larval susceptibility of *Ajuga remota* against anopheline and culicine mosquitoes. Southeast Asian Journal of Tropical Medicine and Public Health, 35(3): 608-610.
11. Mohan, L., P. Sharma and C.N. Srivastava, 2005. Evaluation of *Solanum xanthocarpum* extracts as mosquito larvicides. Journal of Environmental Biology, 26(2): 399-401.
12. Sukumar, K., M.J. Perich and L.R. Boobar, 1991. Botanical derivatives in mosquito control: A review. Journal of the American Mosquito Control Association, 7(2): 210-237.
13. WHO, 1996b. Report of the WHO informal consultation on the evaluation and testing of insecticides. CTD/WHOPES/IC/96.1.
14. Abbott, W.S., 1925. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology, 18: 265-267.
15. SPSS, 2007. SPSS for windows, Version 11.5. SPSS, Chicago, IL
16. Chandre, F., F. Darriet, M. Darder, A. Cuany, J.M.C. Doannio, N. Pasteur and P. Guillet, 1998. Pyrethroid resistance in *Culex quinquefasciatus* from West Africa. Medical and Veterinary Entomology, 12: 359-366.
17. Amer, A. and H. Mehlhorn, 2006. Larvicidal effects of various essential oils against *Aedes*, *Anopheles* and *Culex* larvae (Diptera, Culicidae). Parasitology Research, 99: 466-472.
18. Bowers, W.S., B. Sener, P.H. Evans, F. Bingol and I. Erdogan, 1995. Activity of Turkish medicinal plants against mosquitoes *Aedes aegypti* and *Anopheles gambiae*. Insect Science and its Application, 16(3/4): 339-342.
19. Shaalan, E.A.S., D. Canyonb, M.W.F. Younesc, H.A. Wahaba and A.H. Mansoura, 2005. A review of botanical phytochemicals with mosquitocidal potential. Environment International, 31: 1149-1166.
20. Sakthivadivel, M. and T. Daniel, 2008. Evaluation of certain insecticidal plants for the control of vector mosquitoes viz., *Culex quinquefascaitus*, *Anopheles stephensi* and *Aedes aegypti*. Applied Entomology and Zoology, 43(1): 57-63.
21. Samuel, T., K. John Ravindran and S. Arivoli, 2012. Screening of twenty five plant extracts for larvicidal activity against *Culex quinquefasciatus* Say (Diptera: Culicidae). Asian Pacific Journal of Tropical Biomedicine, (Article in press).
22. Pavela, R., 2008. Larvicidal effects of various Euro-Asiatic plants against *Culex quinquefasciatus* Say larvae (Diptera: Culicidae). Parasitology Research, 105: 887-892.
23. Pavela, R., 2009. Larvicidal effects of some Euro-Asiatic plants against *Culex quinquefasciatus* Say larvae (Diptera: Culicidae). Parasitology Research, 102: 555-559.
24. Arivoli, S. and T. Samuel, 2012. Larvicidal efficacy of *Strychnos nuxvomica* Linn. (Loganiaceae) leaf extracts against the filarial vector *Culex quinquefascaitus* Say (Diptera: Culicidae). World Journal of Zoology, 7(1): 6-11.