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Bioefficacy of *Biophytum sensitivum* (L.) leaf extracts against Dengue Mosquito Vector *Aedes Aegypti* (L.)

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ABSTRACT

Novel bioactive molecules of plant origin are very important for success of vector control programs. In the present study the larvicidal activity of *Biophytum sensitivum* leaf extracts were analysed on *Aedes aegypti* mosquito. Concentrations of 10, 15, and 25 mg/L of extract were used to determine larvicidal, pupicidal and consequent effect on adult emergence. Results show that acetone extract had a dose-dependent effect and produced higher mortality (LC_{50} = 21.79 and LC_{99} = 139.50 µg ml^{-1}) in larvae. Pupicidal activities of acetone extract showing (LC_{50} = 13.05 and LC_{99} = 137.75 µg ml^{-1}) the highest effect. Acetone extracts also delayed the normal development of adult mosquitoes (LC_{50} = 9.77 and LC_{99} = 11.83 µg ml^{-1}). The study shows that acetone extracts of *B. sensitivum* is effective in controlling the *Aedes aegypti* larvae.

Keywords: *Biophytum sensitivum*, leaf extracts, larvicidal activity, *Aedes aegypti.*

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INTRODUCTION

Mosquitoes pose a major threat to human health by transmitting serious diseases like malaria, filariasis, yellow fever, dengue, chikungunya and Japanese encephalitis [1, 2]. Aedes aegypti role is a vector for the arboviruses responsible for yellow fever and dengue fever, both of which are endemic to Asia and Africa [3, 4]. Vector control methods have relied on the use of synthetic pyrethroids [1]. Development of insecticide resistance, cross-resistance, damaging effect on non target organisms, and possible toxicity hazards associated with synthetic insecticides are some of the reasons for revival of interest in plant based products in recent years [5-8].

India is a sub-tropical country harbouring rich plant diversity. Humans have used plant parts, products and metabolites in pest control since early historical times. Plants are the chemical factories of nature, producing many secondary metabolites, some of which have medicinal and insecticidal properties [9, 10]. Search for cost-effective, safe and highly potent plant-based insecticides for the control of mosquitoes requires the preliminary screening of plants to evaluate their effectiveness in mosquito control. Biophytum sensitivum (L.) is an herbaceous plant belonging to oxalidaceae family. This herb has a tropical distribution and is found in warmer parts of the world in, tropical Africa, Asia. In India is found in the wet lands of southern India. B.sensitivum has several medicinal properties like antiseptic properties, the plant parts are used in the treatment of asthma and phthisis [11], inflammatory diseases, and diabetes [12-14]. The biological activity of the plant shows hypoglycemic [15], immunomodulatory [16], apoptotic effect [17], chemoprotective [18], cell-mediated immune response [19], hypocholesterolemic [20], antiinflammatory [21], antitumor [22], effects on prostaglandin biosynthesis [23, 24], organogenesis and somatic embryogenesis [25] and antibacterial activity [26]. The Phytochemical properties [27] of the plant showed the presence of amentoflavone [28], 3′, 8″-biapigenin [29], proanthocyanidins [30] and phenolic compounds [31]. Looking at the phytochemical constitution of the plant, it is suspected that the plant might hold biomolecules which have insecticidal activity. The present study was undertaken to investigate the larvicidal activity of leaves of B.sensitivum on fourth instar larvae of Aedes aegypti.

MATERIAL AND METHODS

Plant collection

The leaves of B. sensitivum were collected from Pachamalai Hills (latitudes 11°09'00" to 11°27'00" N and longitudes 78°28'00" to 78°49'00" E) Trichy District, Tamil Nadu, India. It was authenticated and herbarium specimen is deposited at the Rapinat herbarium, St. Joseph's College, Tiruchirapalli.

Preparation of the extracts

The leaves were washed with tap water, shade dried, and powdered. The leaf powder was subjected to solvent extraction using soxhlet apparatus using various solvents like methanol, ethanol, chloroform, acetone and dichloromethane. The extract was filtered through a Whatman No. 1 filter paper. The filtrate was evaporated to dryness under room temperature by exposing to open air. The product of extraction was in the form of paste. This paste was stored at 4º C for testing its efficacy on larvae of dengue mosquito vector Aedes aegypti.

Test Organisms

Aedes aegypti larvae were obtained from the stock maintained in the laboratory of Biotechnology, Periyar University Salem. The fourth instar larvae were used in this bioassay.

Bioassay

Larvicidal activity

The larvicidal activity of the leaf extract of B. sensitivum was evaluated as per the method recommended by the World Health Organization [32] with some modifications. 100 mg of extracts were suspended in 1 ml DMSO (dimethyl sulfoxide) as a stock concentration prior to use. Extracts were made up to 1 ml using filtered tap water to obtain each of the desired concentrations (10mg/L, 15mg/L, and 25mg/L). The extracts were diluted in 49 ml of filtered tap water in a partition compartment container. The negative control was prepared using 250µl of DMSO to make up to 1ml with filtered tap water then added in 49 ml of water. Commercially available Azadirachtin formulation (10,000 ppm) was serial diluted and which acted as positive control. Ten early fourth instar larvae were then introduced into each solution. For each concentration, 10 replicates were performed, for a total of 100 larvae. Larval mortality was recorded at 24 h after exposure.

Pupicidal activity and Adult Emergence

The larva surviving the bioassay treatment was observed for further development to pupae and further adult emergence.

Statistical Analysis

Mortality was corrected by using Abbott's formula [33]. Probit analysis [34] was conducted on mortality data collected after 24 h exposure to different concentration of extracts using statistical package Minitab15 to determine the lethal concentration for 50% mortality (LC_{50}) and 99% mortality (LC_{99}).

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RESULTS

All the extracts of Biophytum sensitivum were subjected to detailed bioassays in order to determine the respective LC_{50} and LC_{99} values (Table 1, 2 and 3). The acetone extract displayed the highest larvicidal, and pupicidal with LC_{50} values of 21.79 and 13.05 µg ml^{-1}, and LC_{99} values of 139.50 and 137.75 µg ml^{-1} respectively. The emergences of adult mosquitoes were greatly affected by acetone extract with LC_{50} values of 9.77 and LC_{99} values of 11.83 µg ml^{-1} respectively. Ethanol extract expressed moderate larvicidal and pupicidal with LC_{50} values of 633.36 and 23.43 µg ml^{-1}, and LC_{99} values of 2140.9 and 354.18 µg ml^{-1} respectively and also the emergences of adult mosquitoes were significantly affected with LC_{50} values of 21.51 and LC_{99} values of 380.93 µg ml^{-1} respectively. These data show that all the extracts have dose-dependent effect on larval mortality.

Table 1: Larvicidal activity of Biophytum sensitivum leaf extract against Aedes aegypti

LC estimates	Methanol	Ethanol	Acetone	Dichloromethane	Azadirachtin
50 (µg ml^{-1})	62.63244	633.6481	21.7932	30.62903191	3.446652976
99 (µg ml^{-1})	4939.647	2140.90	139.5041	266.6124818	11200.99509

Table 2: Pupicidal activity of Biophytum sensitivum leaf extract against Aedes aegypti

LC estimates	Methanol	Ethanol	Acetone	Dichloromethane	Azadirachtin
50 (µg ml^{-1})	28.42606	23.43694	13.05235	16.7657973	11.5615846
99 (µg ml^{-1})	378.6306	354.1842	137.7537	4832.538483	1443.09314

Table 3: Adult emergence of Biophytum sensitivum leaf extract against Aedes aegypti

LC estimates	Methanol	Ethanol	Acetone	Dichloromethane
50 (µg ml^{-1})	19.45913	21.51833	9.770321	32.35181308
99 (µg ml^{-1})	64.83927	380.9308	11.83745	2788.515268

DISCUSSION

The plant kingdom is by far the most efficient producer of chemical compounds, synthesizing secondary metabolites that are used in defense against herbivores. The essential constituents of secondary metabolites are tannins, alkaloids, polyphenols, terpenoids, and essential oils which have wide range of anti-insect properties [35-37] which include insecticidal activity, repellence, antifeedant effects, insect growth regulation [38-40].

In the present study, acetone extracts were found to be effective larvicidal and pupicidal agent, also interfered with the normal development and emergence of adult mosquitoes (Figure 1, 2 and 3). Similar results were obtained in the studies A. squamosa extracts against mosquito larvae [41, 42]. The leaf extracts of Cassia fistula has been known to have larvicidal and ovicidal activity on Anopheles and Aedes mosquitoes [43, 44]. Walmir et al. [45] have reported that the isolation of bioactive compound ((+)-dicentrine) from Ocotea velloziana has

larvicidal activity. The pesticide activity of crude extracts from a Lauraceous species, Litsea salicifolia, against A. aegypti has been reported by Phukan and Kalita [46]. The results of our study indicate that the plant extracts of B. sensitivum had comparable larvicidal and pupicidal activity as that of azadirachtin which is a proven mosquito larvicide [47]. Further characterization and isolation of bioactive molecules from acetone extracts of B. sensitivum will provide further clarity about the nature of these bioactive compounds. This could become alternative to the conventional insecticides used for the regulation of Aedes aegypti mosquitoes.

Figure 1: Percentage mortality of 4th instar *Aedes aegypti* larvae exposed to different doses of *Biophytum sensitivum.*

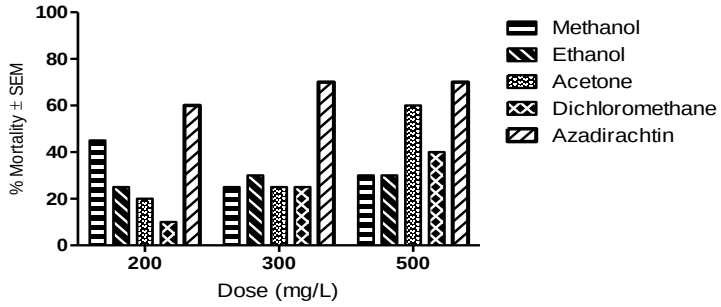


Figure 2: Percentage mortality of *Aedes aegypti* pupae exposed to different doses of *Biophytum sensitivum.*

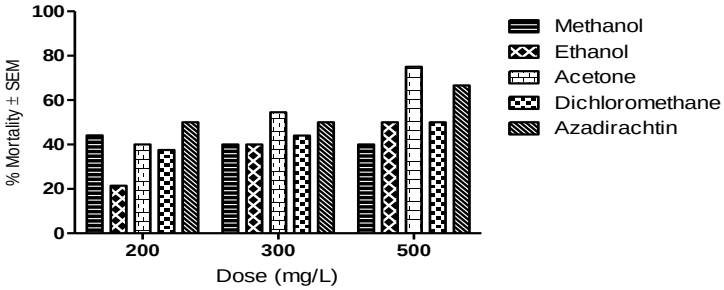
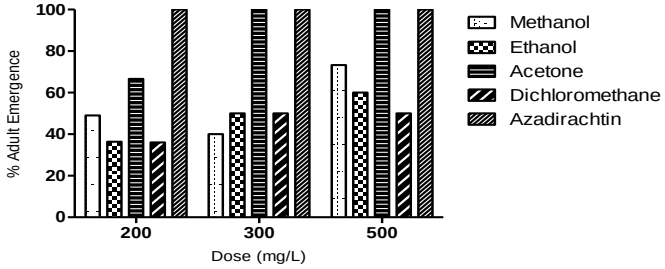


Figure 3: Percentage Adult emergences of *Aedes aegypti* exposed to different doses of *Biophytum sensitivum.*



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CONCLUSIONS

There has been no information about the larvicidal activity of B. sensitivum this is first hand information shows B. sensitivum having an excellent potential as larvicidal agent against A. aegypti. The bioassay guided fractionation, purification and isolation of pure compounds from the cured chloroform and ethyl acetate extracts of leaves of B. sensitivum are in future.

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REFERENCES

[1] World Malaria Report. 2009; available fromhttp://www.who.int/malaria/world_malaria_report_2009/en/index.html.
[2] Jang YS, Kim MK, Ahn YJ, Lee HS. Agric Chem Biotechnol 2002; 45(3):131–41.
[3] Consoli RAGB, Oliveira RL. Principais Mosquitos de Importância Sanitária no Brasil. Fiocruz, Rio de Janeiro Editor Fiocruz 1994; 225.
[4] Neves DP. Parasitologia Humana. São Paulo Editora Atheneu 2000; 257-261.
[5] Suwannee P, Amara N, Maleeya K, Ushavadee T. Insect Sci 2006; 13:179–84.
[6] Isman MB. Annu Rev Entomol 2006; 51: 45-66.
[7] Severini C, Rom R, Marinucci M, Rajmond M. J Am Mosq Control Assoc 1993; 9:164–8.
[8] Macedo ME, Consoli RA, Grandi TS, dos anjos AM, de Oliveira AB, Mendes NM, Queiroz RO, Zani CL. Mem Inst Oswaldo Cruz 1997; 92(4):565–570.
[9] Ansari MA, Razdan RK, Tandon M, Vasudevan P. Biores Technol 2000; 73:207-213.
[10] Ahmed S, Graivge M, Hylin JW, Mitchell WC, Listinger JA. Some promising plant species for use as pest control agents under traditional farming system. Second International Neem conference 1984; 565–80.
[11] Pullaiah T. Medicinal plants in India Vol I Regency publications New Delhi 2002; 96-97.
[12] Puri D, Baral N, Upadhyaya BP, Indigenous plant remedies in Nepal used in heart diseases. J Nepal Med Association 1997; 36:334–337.
[13] Mitra AP, Ambasta SP. The Wealth of India Raw Materials Vol II-B CSIR New Delhi 1988; 151-152.
[14] Kirtikar KR, Basu BD. Indian Medicinal Plants, Vol. I, B.S.M.P. Singh, Deharadun 1984; 440-441.
[15] Puri D, Baral N. Ind J Physi Pharm 1998; 42:401–406.
[16] Guruvayoorappan C, kuttan G. Asian Pac J Cancer Prev 2007; 8:27-32.
[17] Guruvayoorappan C, Kuttan G. Integrative cancer therapies 2007; 6:373–380.
[18] Guruvayoorappan C, kuttan G. Drug Metabol Durg Interact 2007; 22:131-150.
[19] Guruvayoorappan C, Kuttan G. Immunopharmacology and immunotoxicology 2007; 29:37–350.

[20] Puri D. Pharma Bio 2003; 41(4):253-258.
[21] Jachak SM, Bucar F, Kartnig Th. Phytotherapy Research 1999;13:73–74
[22] Bhaskar VH, Rajalakshmi V. Ann Biol research 2010; 3:76-80
[23] Bucar FS, Jachak M, Kartnig Th, Noreen Y, Bohlin L, Schubert-Zsilavecz M. Phenolic compounds of Biophytum sensitivum and their activities on COX catalyzed prostaglandin biosynthesis, International Symposium of Bioassay methods in Natural Product Research and Drug Development Uppsala University Swedish Academy of Pharmaceutical Sciences Uppsala Sweden 1997; 49.
[24] Bucar FS, Jachak M, Noreen Y, Kartnig Th, Bohlin L, Schubert-Zsilavecz M. Planta medica 1998; 64:373-374.
[25] Shivanna MB, Vasanthakumari MM, Mangala MC. Ind J Biotechnol 2009; 8:127-131.
[26] Natarajan D, Shiva kumar MS, Srinivasan R. J Pharm Sci & Res 2010; 2(11):717-720.
[27] Lin YL, Wang WY. Chinese Pharmaceutical Journal 2003; 55:71–75.
[28] Ravishankara MN, Pillai AD, Padh H, Rajani M. Journal of Planar Chromatography–Modern TLC 2003; 16:201–205.
[29] Jachak SM, Bucar F, Kartnig Th, Schubert-Zsilavecz M. C-glycosylflavones from Biophytum sensitivum leaves, 44th Annu Cong of the Soc for Med Plant Res Prague Czech Republic 1996; 188.
[30] Bucar FS, Jachak M, Kartnig Th, Noreen Y, Perera P, Bohlin L and Schubert- Zsilavecz M. Amentoflavone, a cyclooxygenase-1 inhibitor from Biophytum sensitivum DC. 13th Sci Conf of the Austrian Pharm Society Vienna Austria K-4 Sci Pharm 65 Supplement 1997; 1:22.
[31] Bucar FS, Jachak M, Kartnig Th, Schubert-Zsilavecz M. Pharmazie 1998; 53:651-653.
[32] World Health Organization. WHO Expert Committee on Insecticide, Instructions for determining the susceptibility or resistance of mosquito larvae to insecticides, 1981; WHO/VBC/8.807.
[33] Abbott WS. J Econ Entomol 1925;18:265–267.
[34] Finney DJ. 1971; Probit Analysis. Cambridge University Press London p 38.
[35] Jantan I, Yalvema MF, Ahmad NW, Jamal JA. 2005; Pharm Biol 43:526–532.
[36] Kiran SR, Bhavani K, Devi PS, Rao BRR, Reddy KJ. Bioresour Technol 2006; 97:2481–2484.
[37] Knio KM, Usta J, Dagher S, Zournajian H, Kreydiyyeh S. Bioresour Technol 2008; 99:763–768.
[38] Prakash A, Rao J. Botanical Pesticides in Agriculture Boca Raton FL CRC Press 1997; p 461.
[39] Arnason JT, MacKinnon S, Isman MB, Durst T, Philogène BJR, Hasburn C, Sanchez P, Poveda L, Roman LS, Isman MB, Satasook C, Towers GHN, Wiriyachitra P, MacLaughlin JL. 1993; Phytochemistry 27:107–131.
[40] Secoy DM, Smith AE. Econ Bot 1983; 37:28-57.
[41] Mehra BK, Hiradhar PK. J Entomol Res 2000; 24(2):141–146.
[42] George S, Vincent S. J Vect Borne Dis 2005; 159–163.
[43] Govindarajan M, Jebanesan A, Pushpanathan T. Parasitol Res 2008; 102:289–292
[44] Govindarajan M. Euro Rev Med Pharm Sci 2009; 13:99-103.

[45] Walmir S, Garcez, Fernanda R, Garcez, Lilliam MGE, da Silva, Lidilhone Hamerski. Biores Technol 2009; 100:6647–6650.
[46] Phukan S, Kalita MC. Ind J Exp Biol 2005; 43:472–474.
[47] Modue L, Nisbet AJ. Ann Entomol Soc Brail 2000; 29:615-632.