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Phytochemical Analysis and Evaluation of Larvicidal Property of Leaf Extracts of Pogostemonquadrifolius against Culex quinquefasciatus

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Abstract: *The larvicidal potential of different solvent (Petroleum ether, chloroform, acetone, methanol, aqueous) extracts ofPogostemon quadrifoliusdried leaveswas tested against the 4th instar larvae of Culex quinquifasciatus.Larvicidal bioassaywas carried out using WHO standard method and the mortality was observed after 24 h exposure. The mortality data were subjected to probit analysis to determine the lethal concentration (LC_{50}). The maximum larval mortality was detected in petroleum ether extract (LC_{50} 0.112mg/ml) followed by acetone extract (LC_{50} 0.0234 mg/ml). The phytochemical screening of the potent crude extracts revealed the presence of aikaloids, flavanoids, saponins and terpenoids. The petroleum ether extract was purified by column chromatography and the mobile phase used was designed from several experimental determination of TLC.All the recovered fractions were screened for larvicidal activity and the sixth fraction was found to be active. The active fraction was screened for bioactive phytochemical class by standard qualitative analysis and the presence of terpenoid was identified. This study provides first report on the mosquito larvicidal activity of the P.quadrifolius leaf extract against larvae of Cx. quinquefasciatus.*

Keywords: *Culexquinquefasciatus, Pogostemonquadrifolius, Phytochemicals, Larvicidalactivity.*

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I. Introduction

Over a third of the world population lives in areas at risk for epidemic transmission of mosquito-transmitted diseases like yellow fever, dengue hemorrhagic fever, and the emerging disease chikungunya. Mosquitoes are known to carry many infectious diseases from several different classes of microorganisms, including viruses and parasites(Noure*t al*., 2009).

Repeated use of synthetic insecticides for mosquito control has disrupted naturalbiological control systems and led to resurgences in mosquitopopulations. It also resulted in the development of resistance,undesirable effects on non-target organisms and fosteredenvironmental and human health concern(Thomas*et al*., 2004).The WHO has estimated that there are 25 million cases of acute occupational pesticide poisoning in developing countries and that 20,000 deaths occur worldwide every year.

One of the most effective alternative approaches under the biological control programme is to explore the land floral biodiversity and enter the field of using safer insecticides of botanical origin as a simple and sustainable method of mosquito control. The search for herbal preparations that do not produce any adverse effects in the non-target organisms and are easily biodegradable remains a top research issue for scientists associated with alternative vector control (Chowdhury *et al*., 2008).Many plant species are known to possess biological activity that is frequently assigned to the secondary metabolites. Several studies have identified and reported that plant extracts are effective against mosquitoes at various stages of development. The phytochemicals derived from plant sources possess a complex of chemicals with unique biological activity. The phytochemicals derived from plant resources can act as larvicides, insect growth regulators, repellents, and ovipositional attractants (Rawani*et al*., 2010).Larval control can be an effective tool rather than mosquito repellent devices, which are now widely practiced. Due to the low mobility of larvae especially where the principal breeding habitats are manmade, larval control is very effective.

The *Lamiaceae*is one of the most diverse and widespread plant families in terms of ethnomedicine and its medicinal value is based on the volatile oils concentration (Sarac *et al*,2007).Some of the plants of this family are used as insect repellents because of their aromatic property. Some examples from this family include *Pogostemon, Anisomeles, Colebrookea, Coleus, Hyptis, Leonotis, Leucas, Mentha, Ocimum, Oreganum* and *Salvia*. With an ever increasing public interest and awareness about the environment in both developed and developing countries, positive public perception of natural insecticides is an added incentive for their

development and use.In the present study the larvicidal activity of *Pogostemon quadrifolius*plant extracts was investigated against the fourthinstar larvae of *Cx.quinquefasciatus.*

II. Materials and methods

Plant material

Healthy leaves of *Pogostemonquadrifolius* was collected from in and around the campus of SAFI Institute of Advanced Study, Vazhayoor, Malappuram and the taxonomic identification was made byDr. Rajesh, Associate Professor, Zamorins, Guruvayurappan College, Calicut, Kerala. The leaves were washed, cleaned, air-dried at room temperature for two weeks and coarsely powdered.

Test organism

Mosquito larvae and eggs were collected regularly in the month of January- March. The collected larvae were transferred to clean plastic bags filled with water from the same breeding place and brought to the laboratory. The larvae hatched were identified, and recorded (Oguoma *et al*., 2010).

Maintenance of mosquito larvae

After hatching of the egg, the first instar larvae of *Culex quinquefaciatus* were transferred to an enamel tray of $30\times25\times5$ cm^3 containing well water. The larvae were fed on a diet of finely powdered biscuits and yeast in the ratio 3:1.The water in the tray was changed every day and dead larvae were removed (Gerber *et al*., 1994).

Preparation of Plant extracts

Leaves of *Pogostemon quadrifolius* were collected and washed thoroughly with tap water, dried in air and powdered. Extracts of the collected plant materials were taken by a modified method of Minjas and Sarda, 1986; Tandon and Sirohi, 2010. Powdered plant material (40g) was extracted sequentially using 400ml of different solvents of increasing polarity (petroleum ether, acetone, chloroform, methanol and distilled water) in shaking incubator for 12 h and the extracts were filtered through Whatman filter paper No. 4 and then concentrated at 40º C and stored at 4º C until testing for subsequent bioassays.

Phytochemical screening

Phytochemical screening was carried out using standard procedure (Harborne,1984).

Larvicidal Bioassay

The larvicidal activity of crude extracts of the selected plants was assessed by the protocol of WHOwith some modifications (Rahumann*et al.*,2000). For the bioassay 50-250ppm concentrations of the extracts were prepared using 1% of Dimethyl sulfoxide (DMSO) as an emulsifier. Controls were prepared by adding 1% DMSO in water. Twenty, 4^{th} instar larvae of *Culex quinquefaciatus* were released into 250ml glass beaker containing 100ml water with each concentration independently. All experiments were conducted in six replicates along with control. Observation on mortality of the larvae was recorded after 24h of continuous exposure. After 24 hours exposures the dead larvae were counted and total was expressed as percentage of larval mortality for each concentration. When the Control mortality ranged from 5-20 per cent, the observed percentage mortality was corrected by Abbott's formula(Abbott WS.,1925).

Purification of larvicidal phytochemicals

Thin Layer Chromatography of Petroleum Ether extract

Mobile phases in Column chromatographic purification of the Petroleum ether extract was designed from several experimental determination of TLC. Chromatographic plate was prepared using silica gel under standard specifications. The mobile phase used for separation of the petroleum ether extract, was toluene: ethyl acetate solvents in 9:1. The plates were dried at room temperature and they were placed in iodine chamber for the development of chromatogram.

Column chromatography of Petroleum Ether extract

The potent crude extract was subjected to column chromatographic separation [Column length: 25cm, diameter: 2.5cm] using silica gel as stationary phase. 50mg extract mixed with 1.5ml petroleum ether, was applied on the column. Toluene: ethyl acetate was added as mobile phase and eluate was collected at a flow rate of 1ml/min. All fractions were monitored by thin layer chromatography until a single spot was obtained. The pure fractions were evaporated to dryness and were screened for their larvicidal activity.

Statistical analysis

Data were analyzed using one-way ANOVA. Significant differences between treatments were determined using Tukey's test ($P \leq 0.01$). LC_{50} values were calculated using probit analysis.

III. Results

The preliminary phytochemical screening is a means of evaluating the potential phyto compounds in the leaf extracts of *Pogostemon quadrifolius* and the results are summarised inTable 1.

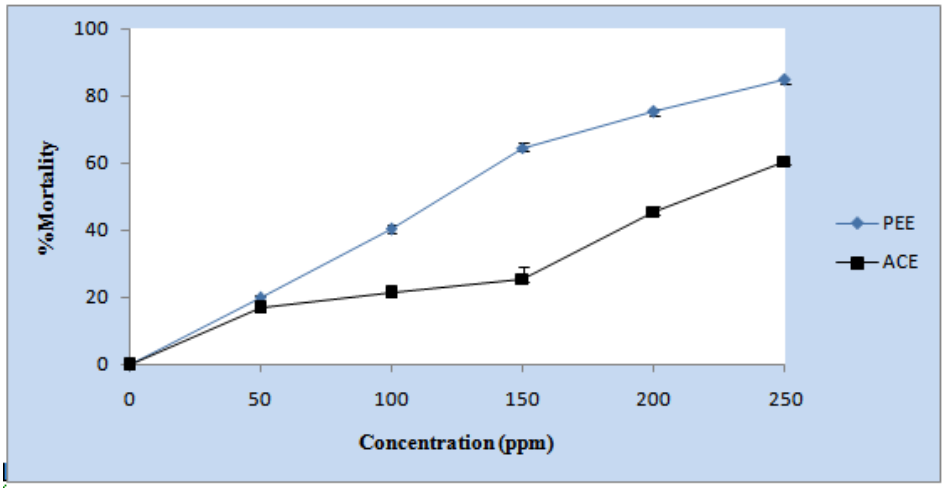
Table 1: Phytochemical screening of leaf extracts of *Pogostemon quadrifolius*

Phytochemical	Leaf extract											
	Petroleum ether		Chloroform		Acetone		Methanol		Aqueous			
Alkaloid	+	+	+	+	–		+		+	+		
Flavanoids	+		+	+	+		+	+	+	+		
Steroids	–		+		–		+	+	+			
Saponins	–		+		+		+	+	+	+		
Terpenoids	+	+	–		+	+	+		+			
Proteins	–		–		–		–		+			
Carbohydrates	–		–		+		+		+	+		
Phenols	–		–		+		+		+	+		

++ Positive + Trace - Not detected

Among the five different solvent extracts tested for larvicidal activity against *Cx. Quinquefasciatus*the activity was shown by petroleum ether (PE) and acetone (AC) extractsas presented in figure 1. It was observed that larvae became slowly inactive after 10^{th} h and began to sink down, except in control. Larvicidal bioassay revealed the potential of these extracts for the control of mosquito larvae with an LC_{50} of112ppm and 234ppm for petroleum ether and acetone extracts respectively.

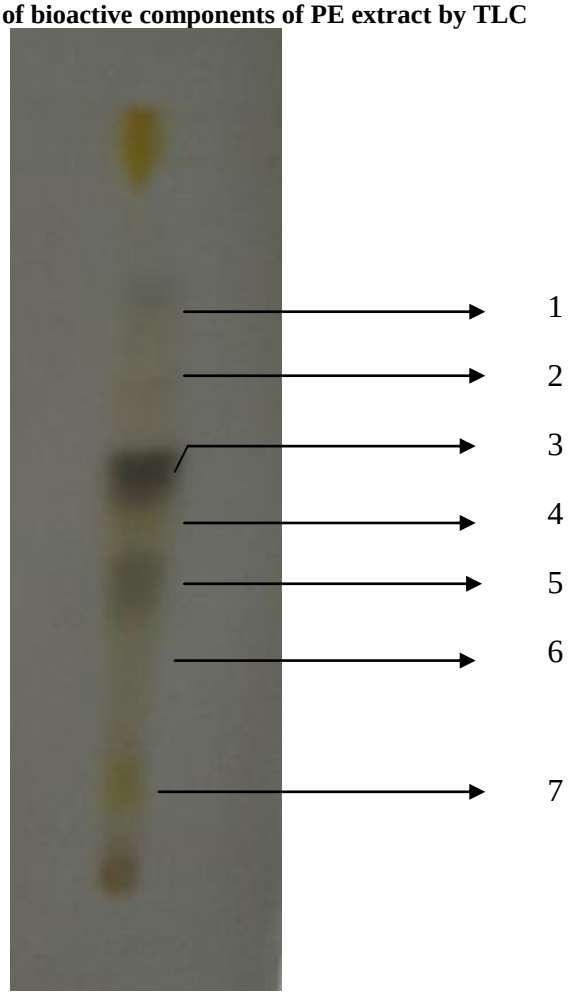
Figure 1: Larvicidal activity of PE and AC extracts of *Pogostemon quadrifolius*against 4^{th} instar larvae of *Cx.quinquefasciatus.*



Value represents mean±SD of five replications. ANOVA followed by TUKEY test performed.Values are significantly different at P ≤ 0.01level.

Since petroleum ether extract exhibited high larvicidal activity it was selected for purification by column chromatography.The mobile phase for the purification of components of most PE extract was screened by several experimental determination of TLC. Themaximum separation of the components was found in toluene: ethyl acetate solvents in 9:1 proportion and yielded seven components represented in figure 2.

Figure 2: Separation of bioactive components of PE extract by TLC



Rf values of the separated components were calculated and thecompounds detected in band 1 to 7 showed Rf values of 0. 0.8, 0.57, 0.44, 0.38, 0.31, 0.22 and 0.11.The PE extract is separated by column chromatography and yielded eight different compounds. The fractions were monitored by TLC to obtain single spots. All the recovered compounds were screened for their larvicidal activity and the results are listed in Table 2. The activity was exhibited by the sixth fraction and was designated as PEAF-6. Preliminary studies on the larvicidal activity of PEAF-6 against *Cx quinquifasciatus* showed 100% mortality at a concentration of 100ppm. On exposure, larvae were found to be inactive within 15min and mortality was observed in 1h.

Table 2.Larvicidal activity of compounds separated from PE extract by column chromatography.

Fraction number	Colour	Concentration(ppm)	Larvicidal activity
1	Colourless	100	–
2	Yellow		–
3	Dark green		–
4	Light green		–
5	Yellowish green		

			–
6	Light brown		+
7	Light brown		–
8	Colourless		–

The phytoconstituent of the active fraction PEAF-6 was found to be a Terpenoid by standard qualitative analysis.

IV. Discussion

Vector control is facing a serious threat due to the emergence of resistance in vector mosquitoes to conventional synthetic insecticides. 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 conventionalpesticides 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. Natural pesticides, especially those derived from plants, are more promising in this aspect.

The findings of the present investigation revealed that the leaf extracts of *Pogostemon quadrifolius* possess larvicidal activity against *Cx.quinquefasciatus* and PE extract showed maximum inhibitory activity with LC_{50} value of 112ppm. The purified PE fraction named PEAF-6 was found to be a potent inhibitor by causing 100% mortality of the larvae at 100ppm. The standard qualitative analysis revealed the presence of terpenoid as an active constituent of PEAF-6. Triterpenoids are generally credited with mosquito larvicidalactivities (Gbolade, 2000). Thus, high mortality rate recorded in the presentstudy could be due to the presence of terpenoids andtriterpenoids which are hydrocarbons present in the extract thatinhibits the developmental stages of insects (Peerzada, 1997).

The efficacy of phytochemicals against mosquito larvae can vary significantly depending on plant species, plant parts used, age of plant parts (young, mature or senescent), solvent used during extraction as well as upon the available vector species. Many reports are available regarding the changes in the larvicidal efficacy of the plant extracts based on changes in geographical origin of the plant.

V. Conclusion

The bioactive terpenoid from the leaf extracts of *P. quadrifolius* showed higher larvicidal efficiency against fourth instar larvae of *Cx. quinquefaciatus.* Further studies on the purification and characterisation of bioactive phytochemical constituent followed by in-depth laboratory and field bioassays are needed as the present study shows that there is scope to use *Pogestemon quadrifolius* leaf extracts to control the immaturestages of vector mosquitoes.

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