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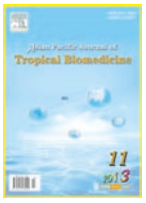

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Evaluation of larvicidal activity of *Pongamia pinnata* extracts against three mosquito vectors

Guna Ranjan Kolli[1], Balakrishnan[2], Vijayan[3], Raja Sundararajan[1]

[1]*GITAM Institute of Pharmacy, GITAM University, Vishakapatnam-530017, Andrapradesh, India*

[2]*National Centre for Disease Control, (Formely Southern India Branch, NICD) Coonoor-643101, Tamil Nadu, India*

[3]*Department of Pharmaceutical Biotechnology, J.S.S College of Pharmacy, Rocklands, P.B No.20 Ootacamund 643001, India*

PEER REVIEW

Peer reviewer

Viroj Wiwanitkit, M.D., Professor, Chulalongkorn University, Bangkok Thailand; visiting professor, Hainan Medical University, China.
Tel: +66892348832
Fax + 6624132436
E-mail: wviroj@yahoo.com

Comments

This is an applied medical plant research. The application on control of mosquito can be seen. The topic can be useful for tropical world, especially for the countries with limited resources. The idea is good and can be applied in tropical biotechnology.
Details on Page 858

ABSTRACT

Objective: To evaluate the mosquito larvicidal activity of *Pongamia pinnata* (*P. pinnata*) extracts against three mosquito vectors.

Methods: The methanol and hydroalcohol extracts of bark part of *P. pinnata* L were tested against fourth instar larvae of *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi*. The mortality was observed 24 h and 48 h after treatment, data was subjected to probit analysis to determine lethal concentration (LC_{50} and LC_{90}) to kill 50 and 90 percent of treated larvae of tested species.

Results: The larval mortality was found in both methanol and hydroalcohol extracts of *P. pinnata* against *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi* with LC_{50} values of 84.8, 118.2 and 151.7 ppm; 97.7, 128.3 and 513 ppm. The highest larval mortality was found in methanol extract of *P. pinnata* when comparable to the hydroalcohol extract.

Conclusions: These results suggest that both methanol and hyrdoalcohol extracts have the potential to be used as an ideal ecofriendly approach for the control of disease vectors. This could lead to isolation of novel natural larvicidal compounds.

KEYWORDS

Pongamia pinnata L, Larvicidal, Mosquito vectors, *Culex quinquefasciatus*, *Aedes aegypti*, *Anopheles stephensi*

1. Introduction

The term malaria comes from 'mal' 'aria', or bad air. A WHO report (called the World Malaria Report 2008)[1] released recently speaks of not only the progress made in controlling malaria but also the challenges posed by it. An estimated 247 million malaria cases out of the 3.3 billion people at risk in 2006 caused nearly a million deaths, mostly of children under 5 years of age. Malaria has been a problem in India for centuries. Details of this disease can be found even in the ancient Indian medical literature like the 'Charaka Samhita'. Malaria has now staged a dramatic comeback in India after its near eradication in the early and mid sixties.

Malaria is a potentially life threatening parasitic disease caused by parasites known as *Plasmodium vivax*, *Plasmodium falciparum*, *Plasmodium malariae* and *Plasmodium ovale*. It is transmitted by the infective bite of *Anopheles* mosquito. Man develops disease after 10 to 14 days of being bitten by an infective mosquito. There are two types of parasites of human malaria (*Plasmodium vivax*, *Plasmodium falciparum*) which are commonly reported from India. Inside the human host, the parasite undergoes a series of changes as part of its complex life cycle. The parasite completes life cycle in liver cells (pre-erythrocytic schizogony) and red blood cells (erythrocytic

*Corresponding author: Guna Ranjan Kolli, Assistant Professor, GITAM Institute of Pharmacy, GITAM University, Visakhapatnam, Andhra Pradesh, India.
Tel: +91-891-2840550
E-mail: sraja61@gmail.com

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schizogony). Infection with *Plasmodium falciparum* is the most deadly form of malaria.

Studies of plants used in traditional medicine for the treatment of malaria in various cultures have yielded important drugs that are critical to modern medicine. Two of the most effective drugs for malaria originate from traditional medicine: quinine from bark of the Peruvian *Cinchona* tree and artemisinin from the Chinese antipyretic *Artemisia annua*. Plants used in traditional medicine may hold keys to the secrets of potent antimalarial drugs. Pharmacological investigations already carried out on crude extracts and pure compounds for antimalarial activity have shown that most plants used traditionally for the treatment of malaria are efficacious, and some of them are even more effective than some currently used antimalarials in clinical use. Many herbal remedies individually or in combination have been recommended in various medical treatises for the cure of different diseases.

Based on the traditional healers claim for the therapeutic usefulness, the plant *Pongamia pinnata* L (*P. pinnata*) was selected for the work. The *P. pinnata* L commonly known as Karanj, has been recognized in different system of traditional medicines for the treatment of different diseases and ailments of human beings[2]. It contains several phytoconstituents belonging to category flavonoids and fixed oils. The oil is used as a liniment for rheumatism. Leaves are active against micrococcus; their juice is used for cold, cough, diarrhoea, dyspepsia, flatulence, gonorrhoea and leprosy. Roots are used for cleaning gums, teeth and ulcers. Bark is used internally for bleeding piles. Juices from the plant as well as oil are antiseptic[3]. In the traditional systems of medicines, such as Ayurveda and Unani, the *P. pinnata* L plant is used for antiinflammatory, anti-plasmodial, antinonciceptive, antihyperglycaemics, anti-lipidoxidative, antidiarrhoeal, anti-ulcer, anti-hyperammonic and antioxidant activities[4]. Based on ethanobotanical information, *P. pinnata* L was screened for their larvicidal potential against three different, public health significant mosquito vectors.

2. Materials and methods

2.1. Collection of plant material

The bark of the plant, *P. pinnata* L. was collected from Puttaparthi of Ananatapur district of Andhra Pradesh during flowering stage in the months of April to July. Then its identification was established with the aid of an expertise botanist, Prof. T.Pullaiah, Sri Krishnadevaraya University, Anantapur, Andhra Pradesh and the samples were compared with herbarium sheets of the authentic sample.

2.2. Preparation of plant extracts

The bark of the plant, *P. pinnata* was dried under shade and then powdered with a mechanical grinder to obtain a coarse powder. Equal quantity of powder was passed through 40 mesh sieve and extracted with methanol in soxhlet apparatus at 60 °C. The solvent was completely removed by rotary vacuum evaporator. The extract (stock solution) was freeze dried and stored in vacuum desiccators. Further, stock solution was prepared by dissolving 2.5 g of extract in water and made up to 250 mL and refrigerated. From this stock solution different concentrations of test solution (250 mL) were prepared in their respective ppm range with tap water. The same procedure was carried out for the preparation of hydroalcohol (alcohol:water, 3:7) extract by used of hydroalcohol solvent instead of methanol.

2.2. Mosquito culture

For the present study various mosquito larvae were obtained from National Institute of Communicable Diseases, Southern India branch field station located at Mettupalayam (Coimbatore District, Tamil Nadu, India), which have been successfully maintaining laboratory colonies of various mosquito vector species, of public health importance. The life cycle of mosquito varies with temperature, climate and region and it was observed that in laboratory colonies, mosquito life cycle periodicity was of 20-30 d, in which egg stage last for 1 d, larva for 8 d, pupa for 2 d and adult stage for 15-20 d. The technique used for maintaining various species in laboratory for rearing of immature eggs, larvae and pupae was followed[5-8].

2.3. Larvicidal bioassay

As per the standard procedure of larval bioassay recommended by the WHO, the experiments were conducted in laboratory. Normally for each experiment, beaker (500 mL) containing 250 mL of test solution was used. Before using, beaker were washed by keeping it in 2% chromic acid bath or potassium dichromate solution for 24 h, washed properly with standard detergent and finally rinsing with acetone, a total of 25 early IV instar larvae were picked in 25 mL of water in 50 mL beakers. They were left to rest for 15-30 min in these beakers for acclimatizing to experimental condition; at this stage unhealthy parasitized or damaged larvae were rejected. The selected test larvae were transferred to test solution, using flat strainer for each concentration of test solution three replicates and controls were also kept. The larval mortality was recorded in the test concentration in each beaker and control solution for 24 h. The numbers of dead and alive larvae were recorded. The larvae that had pupated during the test were discarded. When the control mortality lies between 5% and 20% the corrected mortality percentage was obtained by using Abbot's formula.

$$\text{Corrected mortality} = \frac{\text{Observed mortality} - \text{control mortality}}{100 - \text{Control mortality}} \times 100$$

2.4. Statistical analysis[9]

LD_{50} and LD_{90} values and their 95% confidence limits were estimated by fitting a probit regression model to the observed

relationship between percentage mortality of larvae and logarithmic concentration of the substance. Separate probit models were fitted for each species within each substance. The goodness of fit of the model was tested using *Chi*-square test. A *P*-value of less than 0.05 was considered as a significant departure of the model from observations. In case of significant difference a heterogeneity factor was used to calculate the 95% confidence limit for LD_{50} and LD_{90}. The slopes of the regression models for each species in a substance were compared with a common slope for all species using *Chi*-square test for parallelism.

3. Results

3.1. Larvicidal bioassay

The bioassay experiment was conducted by using the extracts of *P. pinnata* L. against three different mosquito vectors *viz.* *Anopheles stephensi* (*An. stephensi*), *Aedes aegypti* (*Ae. aegypti*) and *Culex quinquefasciatus* (*Cx. quinquefasciatus*). The methanol and hydroalcohol extracts were screened for larvicidal bioassay using water. The procedure followed for determining larvicidal status was the same as described by WHO. The 25 early IV instar larvae obtained were selected for each test solution keeping three replicates and sufficient control. The total number of larvae exposed for each concentration was 150 for all the three mosquito vectors. Different test solutions of various ranges were kept for different mosquito vectors. All the tests were conducted at (28±2) °C and 70%-90% humidity. The observed mortality (crude mortality) was recorded at 24 h of exposure to test solution. From this crude mortality, percentage of crude mortality was obtained. Subsequently control mortality if any was also recorded and percentage mortality was obtained. The crude percentage mortality was corrected by using Abott's formula. These results were tabulated.

3.2. Cx. quinquefasciatus

The observed and expected mortalities of *Cx. quinquefasciatus* larvae based on probit regression analysis for different concentrations of *P. pinnata* with extracts of hydroalcohol and methanol (Figure 1 and Tables 1 and 2). The estimated LC_{50} and LC_{90} values (95% confidence intervals, *CI*) were 97.7 (93.9-101.3) and 175.5 (167.8-184.6) for hydroalcol extract, and 84.8 (78.3- 91.6) and 184.7 (165.3-211.6) for methanol extract respectively. The 95% *CI* for LC_{50} values suggest that the hydroalcol extract was less potent than methanol extract (95% *CI*s do not overlap).

3.3. An. stephensi

Figure 2, Tables 3 and 4 show that the observed and expected mortalities of *An. stephensi* larvae. It was based on probit regression analysis for different concentrations of *P. pinnata* with extracts of hydroalcohol and methanol. The estimated LC_{50} and LC_{90} values with 95% *CI* were 513.0 (465.8-559.1) and 944.7 (868.7-1048.3) for hydroalcol extract and 151.7 (140.7-161.9) and 299.4 (284.8-316.6) for methanol extract respectively. The 95% *CI* for LC_{50} values suggest that *An. stephensi* was highly susceptible to methanol extract than hydroalcohol extract (95% *CI*s do not overlap).

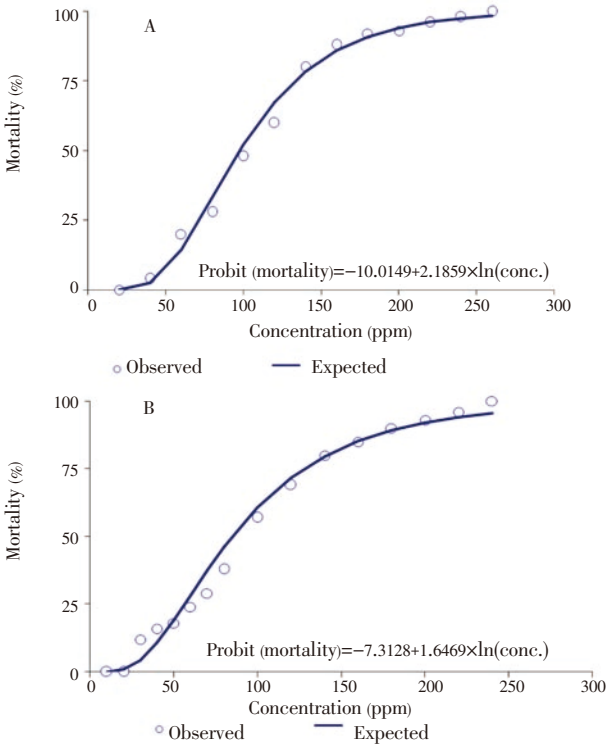

Figure 1. Relation between larval mortality effect of *P. pinnata* and *Culex quinquefasciatus* with extracts of (A) hydroalcohol and (B) methanol. Expected values are based on probit regression analysis.

Table 1
Observed and expected mortality of *Cx. quinquefasciatus* larvae exposed to *P. pinnata* with hydroalcohol extract.

Conc. (μg/mL)	No. of larvae			Expected mortality		
	Exposed	Dead	%	Probit	No. Dead	%
20	150	0	0	-3.47	0.0	0.0
40	150	6	4	-1.95	3.8	2.6
60	150	30	20	-1.06	21.5	14.3
80	150	42	28	-0.44	49.7	33.1
100	150	72	48	0.05	78.1	52.1
120	150	90	60	0.45	101.1	67.4
140	150	120	80	0.79	117.7	78.4
160	150	132	88	1.08	129.0	86.0
180	150	138	92	1.34	136.4	90.9
200	150	140	93	1.57	141.2	94.1
220	150	144	96	1.78	144.3	96.2
240	150	148	98	1.97	146.3	97.5
260	150	150	100	2.14	147.6	98.4
Regression equation: Probit (mortality)				-10.0149+2.1859×ln(conc.)		
Chi-square				16.1		
Degrees of freedom				11		
Probability of significance				0.14		
LC_{50} (95% *CI*)				97.7 (93.9-101.3)		
LC_{90} (95% *CI*)				175.5 (167.8 -184.6)		

Expected mortality is based on probit regression analysis.

Table 2
Observed and expected mortality of *Cx. quinquefasciatus* larvae exposed to methanol extract of *P. pinnata*.

Conc. (μg/mL)	No. of larvae			Expected mortality		
	Exposed	Dead	%	Probit	No. Dead	%
10	150	0	0	−3.52	0.0	0.0
20	150	0	0	−2.38	1.3	0.9
30	150	18	12	−1.71	6.5	4.4
40	150	24	16	−1.24	16.2	10.8
50	150	28	18	−0.87	28.8	19.2
60	150	36	24	−0.57	42.7	28.4
70	150	44	29	−0.32	56.4	37.6
80	150	58	38	−0.10	69.3	46.2
100	150	86	57	0.27	91.0	60.7
120	150	104	69	0.57	107.4	71.6
140	150	120	80	0.83	119.3	79.5
160	150	128	85	1.05	127.8	85.2
180	150	135	90	1.24	133.9	89.2
200	150	140	93	1.41	138.2	92.1
220	150	144	96	1.57	141.3	94.2
240	150	150	100	1.71	143.5	95.7
Regression equation: Probit (mortality)=				−7.3128+1.6469×ln(conc.)		
Chi-square				45.1		
Degrees of freedom				14		
Probability of significance				<0.005		
LC_{50} (95% *CI*)				84.8 (78.3− 91.6)		
LC_{90} (95% *CI*)				184.7 (165.3−211.6)		

Expected mortality is based on probit regression analysis.

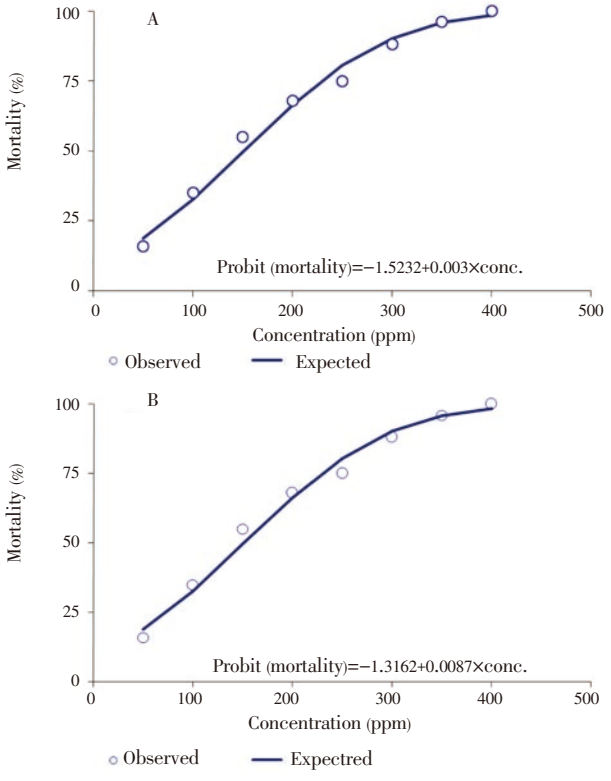

Figure 2. Relation between *An. stephensi* larval mortality against *P. pinnata* with extracts of (A) hydroalcohol and (B) methanol.
Expected values are based on probit regression analysis.

3.4. Ae. aegypti

The observed and expected mortalities of *Ae. aegypti* larvae based on probit regression analysis for different concentrations of *P. pinnata* with extracts of hydroalcohol and methanol was shown in Figure 3 and Table 5. The estimated LC_{50} and LC_{90} values (95% confidence intervals, *CI*) were 128.3 (121.6−134.9) and 278.9 (261.7−299.9) for hydroalcol extract and 118.2 (106.9−129.3) and 227.0 (201.3−267.7) for methanol extract respectively. The 95% *CI* for LC_{50} values suggest that *Ae. aegypti* was equally susceptible to both hydoalhocol and methanol extracts (95% *CI*s overlap).

Table 3
Observed and expected mortality of *An. stephensi* larvae exposed to *P. pinnata* with hydroalcoholic extract.

Conc. (μg/mL)	No. of larvae			Expected mortality		
	Exposed	Dead	%	Probit	No. Dead	%
100	150	18	0	−1.22	16.6	11.1
200	150	24	4	−0.92	26.7	17.8
300	150	48	20	−0.62	40.0	26.7
400	150	54	28	−0.32	56.0	37.3
500	150	72	48	−0.02	73.6	49.1
600	150	84	60	0.28	91.4	60.9
700	150	108	80	0.58	107.7	71.8
800	150	112	88	0.88	121.5	81.0
900	150	126	92	1.18	132.1	88.0
1000	150	150	93	1.48	139.5	93.0
Regression equation: Probit (mortality)				−1.5232+0.003×conc.		
Chisqure				20.8		
Degrees of freedom				8		
Probability of significance				0.008		
LC_{50} (95% *CI*)				513.0 (465.8−559.1)		
LC_{90} (95% *CI*)				944.7 (868.7−1048.3)		

Expected mortality is based on probit regression analysis.

Table 4
Observed and expected mortality of *An. stephensi* larvae exposed to *P. pinnata* with methanol extract.

Conc. (μg/mL)	No. of larvae			Expected mortality		
	Exposed	Dead	%	Probit	No. Dead	%
50	150	24	16	−0.88	28.4	18.9
100	150	52	35	−0.45	49.2	32.8
150	150	82	55	−0.01	74.3	49.6
200	150	102	68	0.42	99.6	66.4
250	150	112	75	0.86	120.7	80.5
300	150	132	88	1.29	135.3	90.2
350	150	144	96	1.73	143.7	95.8
400	150	150	100	2.16	147.7	98.5
Regression equation: Probit (mortality)				−1.3162+0.0087×conc		
Chisqure				9.1		
Degrees of freedom				6		
Probability of significance				0.17		
LC_{50} (95% *CI*)				151.7 (140.7−161.9)		
LC_{90} (95% *CI*)				299.4 (284.8−316.6)		

Expected mortality is based on probit regression analysis.

Table 5
Observed and expected mortality of *Ae. aegypti* larvae exposed to *P. pinnata* with hydroalcoholic extract and methanol extract.

Conc. (μg/mL)	*Ae. aegypti* larvae exposed to hydroalcohol extract						*Ae. aegypti* larvae exposed to methanol extract					
	No. of larvae			Expected mortality			No. of larvae			Expected mortality		
	No. Exposed	Dead	%	Probit	No. Dead	%	No. Exposed	Dead	%	Probit	No. Dead	%
20	150	0	0	−3.06	0.2	0.1	150	0	0	−3.49	0.0	0.0
60	150	18	12	−1.24	16.2	10.8	150	18	12	−1.33	13.7	9.1
100	150	52	35	−0.39	52.3	34.9	150	52	35	−0.33	55.6	37.1
120	150	67	45	−0.09	69.8	46.5	150	67	45	0.03	76.7	51.1
160	150	97	65	0.39	97.7	65.1	150	97	65	0.59	108.5	72.4
200	150	114	76	0.76	116.4	77.6	150	114	76	1.03	127.3	84.9
220	150	120	80	0.92	123.1	82.0	150	120	80	1.22	133.3	88.9
260	150	128	85	1.19	132.5	88.4	150	128	85	1.55	140.9	93.9
300	150	136	90	1.43	138.6	92.4	150	136	90	1.83	144.9	96.6
360	150	144	96	1.73	143.8	95.8	150	144	96	2.19	147.8	98.6
400	150	150	100	1.91	145.8	97.2	150	150	100	2.39	148.7	99.2
Regression equation: Probit (mortality)				−8.0178+1.6565×ln(conc.)						−9.376+1.9644×ln(conc.)		
Chisqure				6.8						13.8		
Degrees of freedom				9						6		
Probability of significance				0.66						0.03		
LC_{50} (95% *CI*)				128.3 (121.6−134.9)						118.2 (106.9−129.3)		
LC_{90} (95% *CI*)				278.9 (261.7−299.9)						227.0 (201.3−267.7)		

Expected mortality is based on probit regression analysis.

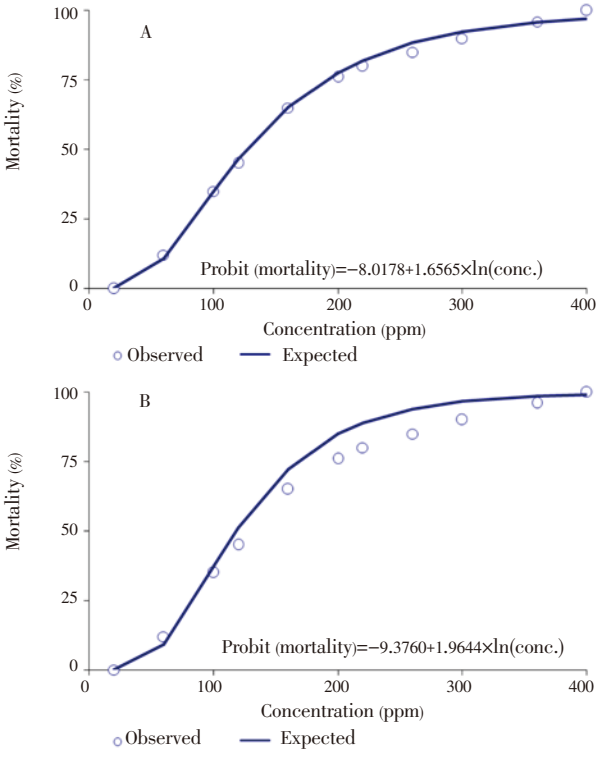

Figure 3. Relation between *Ae. aegypti*i larval mortality against *P. pinnata* with extracts of (A) hydroalcohol and (B) methanol.
Expected values are based on probit regression analysis

4. Discussion

Mosquitoes are the most important single group of insects in terms of public health importance, which transmit a number of diseases, such as malaria, filariasis, dengue, Japanese encephalitis, *etc*. causing millions of deaths every year. Repeated use of synthetic insecticides for mosquito control has disrupted natural biological control systems and led to resurgences in mosquito populations. It has also resulted in the development of resistance[10], undesirable effects on non-target organisms and fostered environmental and human health concern[11], which initiated a search for alternative control measures. Plants are considered as a rich source of bioactive chemicals and they may be an alternative source of mosquito control agents[12].

The plant world comprises a rich untapped pool of phytochemicals that may be widely used in place of synthetic Insecticides in mosquito control programme. Kishore *et al.*[13] reviewed the efficacy of phytochemicals against mosquito larvae according to their chemical nature and described the mosquito larvicidal potentiality of several plant derived secondary materials, such as, alkanes, alkenes, alkynes and simple aromatics, lactones, essential oils and fatty acids, terpenes, alkaloids, steroids, isoflavonoids, pterocarpans and lignans.

Phytochemicals are botanicals which are naturally occurring insecticides obtained from floral resources. Applications of phytochemicals in mosquito control have been in use since the 1920s[14], but the discovery of synthetic insecticides such as DDT in 1939 side tracked the application of phytochemicalsin mosquito control programme. After facing several problems due to injudicious and over application of synthetic insecticides in nature, re-focus on phytochemicals that are easily biodegradable and have no ill-effects on non-target organisms was appreciated. Safe and efficacious insecticides of plant origin have gained importance in recent years and are considered as less hazardous to human and cattle health. Studies on the larvicidal action of terrestrial plant extracts against the mosquito larvae were carried out tremendously.

Many authors have studied the larvicidal action of terrestrial plant extracts against different mosquito larvae at very high concentrations of the plant extracts for achieving significant mortality of mosquito larvae[15]. Phytochemicals derived from plant sources can act as larvicide, insect growth regulators, repellent and ovipositor attractant and have different activities observed by many researchers[16]. However, insecticides of plant origin have been extensively used on agricultural pests and to a very limited extent, against insect vectors of public health importance.

The present study was to compare the LC_{50} values across species against *P. pinnata* L. with methanol extract suggesting that *Cx. quinquefasciatus* was 1.4 and 1.8 times more susceptible than *Ae. aegypti* and *An. stephensi* respectively whereas *Ae.*

aegypti was 1.3 times more susceptible than *An. stephensi* and hydroalcohol extract suggesting that *Cx. quinquefasciatus* is 1.3 and 5.3 times highly susceptible than *Ae. aegypti* and *An. stephensi* respectively, *Ae. aegypti* was 4 times highly susceptible than *An. stephensi*. The result reported open possibilities of further investigation of efficacy on their larvicidal properties of natural product extracts.

Use of these botanical derivatives in mosquito control instead of synthetic insecticides could reduce the cost and environmental pollution. Plant could be an alternative source for mosquito larvicides because they constitute a potential source of bioactive chemicals and generally free from harmful effects. These results could encourage the search for new active isolated compounds offering an alternative to synthetic insecticides from other medicinal plants.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

In the traditional systems of medicines, such as Ayurveda and Unani, the *P. pinnata* L plant is used for antiinflammatory, anti-plasmodial, antinonciceptive, antihyperglycaemics, anti-lipidoxidative, antidiarrhoeal, anti-ulcer, anti-hyperammonic and antioxidant activities.

Research frontiers

The work seems to be a good idea. An application to local tropical developing countries is possible. But this might not be high original. There are many local reports on plants for mosquito controls. The literature review to cover those publications is needed and such mentioned papers should be added and listed into the references.

Related reports

There are relating reports for sure. As noted, the literature review has to be extended to cover the local databases (many databases for Ayurveda in India, Chinese Medicine, Thai Medicine, etc). The thesis on this aspect can also be seen in many tropical medicine faculties with medical entomology unit. These related reports have to be concerned, summarized and included.

Innovations and breakthroughs

The innovation can be seen. However, there must be good literatures to support. At least, additional data on botanical aspect of the used plant must be included. The preparation procedure should also be further described to support the innovation of the authors's idea.

Applications

The application can be possible. To verify the application is needed, the author(s) has(have) to investigate the application in many settings, real life not the laboratory settings. Acceptability of local people is important for implication of the technique. Also, the work must be tested on the economical aspects.

Peer review

This is an applied medical plant research. The application on control of mosquito can be seen. The topic can be useful for tropical world, especially for the countries with limited resources. The idea is good and can be applied in tropical biotechnology.

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