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

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PEER REVIEW

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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.
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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

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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

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