Bioactivity guided isolation of mosquito larvicide from *Piper longum*

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**Abstract**

**Objective:** To isolate the larvicidal component from the fruits of *Piper longum* (*P. longum*) against the filariasis vector, *Culex quinquefasciatus* (*C. quinquefasciatus*). **Methods:** Pulverized fruits of *P. longum* were subjected to soxhlet extraction using series of organic solvents of increasing polarity. All the solvent extracts were verified for their larvicidal efficacy against 4th instar larvae of *C. quinquefasciatus* employing standard WHO procedure. Bioassay-guided fractionation through column chromatography lead to the isolation of a bioactive amide, pipyahyine from the petroleum ether extract. **Results:** Petroleum ether extract was found to be the most active fraction among all the extracts tested with LC50 and LC90 being 1.03 and 2.04 ppm respectively. Whereas, pipyahyine, an isolated component of the same fraction was found to be even more effective than the parent extract in terms of LC50 being 0.58 and 1.88 ppm respectively. **Conclusions:** From the results, it is evident that *P. longum* can be considered as a powerful arsenal for the control of mosquito population.

1. Introduction

Mosquitoes not only create a nuisance as biting insects but are etiologic agents for some of the devastating diseases of human history such as malaria, filariasis, chikungunya, dengue etc. Out of 120 million actual cases of lymphatic filariasis in 83 countries, India alone contributes around 39%[1]. *Culex quinquefasciatus* (*C. quinquefasciatus*) is a domestic mosquito vector of lymphatic filariasis. It not only cause a high level of mortality and morbidity but also responsible for great socio-economic loss. Indeed, the present recrudescence of this disease include increasing number of vector breeding places and large number of parasite infected tourists/immigrants. On the other hand, management of filariasis through chemotherapy is still facing a threat, as selective pressure imposed by conventional insecticides is enhancing resistance in various mosquito species resulting in disease outbreak[3]. In certain cases, resistance to microbial agents by several mosquito species have also been elucidated[4]. The recent negative impact of chemical insecticides has shifted the research efforts towards development of new environmentally compatible vector control methods by using naturalistic agents. In search of new vector control strategy, science has intensified the probes to plants in recent decades, so the plant kingdom is receiving renewed attention as mosquitocides. Pavela has verified the efficacy of 56 plants species against the fourth instar larvae of *C. quinquefasciatus*[5]. Nazar et al have tested 100 plants for larvaticidal activity[6]. Recently Scott et al has carried out detailed studies on different species of piper and found that phytochemistry of this genus is rich in terms of insecticidal amides[7]. Park et al have reported the four active isobutylamide alkaloids against *C. pipiens, A. aegypti* and *A. togoi* larvae from *Piper nigrum* (*P. nigrum*)[8].

*P. longum* (Indian long pepper or pippali), belonging to the family *Piperaceae* is a slender aromatic climber with red fruits. These fruits turns greyish black with pungent smell widely accepted approach to control mosquito population, as it will provide rapid solution. On the other hand, the chemical control measures although highly effective, vector control is still facing a threat, as selective pressure imposed by conventional insecticides is enhancing resistance in various mosquito species resulting in disease outbreak[3]. In certain cases, resistance to microbial agents by several mosquito species have also been elucidated[4]. The recent negative impact of chemical insecticides has shifted the research efforts towards development of new environmentally compatible vector control methods by using naturalistic agents. In search of new vector control strategy, science has intensified the probes to plants in recent decades, so the plant kingdom is receiving renewed attention as mosquitocides. Pavela has verified the efficacy of 56 plants species against the fourth instar larvae of *C. quinquefasciatus*[5]. Nazar et al have tested 100 plants for larvaticidal activity[6]. Recently Scott et al has carried out detailed studies on different species of piper and found that phytochemistry of this genus is rich in terms of insecticidal amides[7]. Park et al have reported the four active isobutylamide alkaloids against *C. pipiens, A. aegypti* and *A. togoi* larvae from *Piper nigrum* (*P. nigrum*)[8].

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when dried and reported as good remedy for the treatment of gonorrhoea, menstrual pain, tuberculosis, sleeping problems, respiratory tract infections, chronic gut related pain and arthritic condition[9]. Perusal of literature reveals that there have been several attempts to investigate the insecticidal activity of this plant[10,11]. As P. longum has proven to be an eco-friendly, our interest triggered us to explore the phyto-component responsible for the mosquito larvicidal activity of this plant against larvae of C. quinquefasciatus, a bancroftian vector.

2. Material and methods

Fruits of P. longum were purchased from local farmers of Mysore. All the organic solvents used in the experiments were of analytical grade and procured from SD fine chemicals, Bombay. Silica gel was obtained from Merck India limited, Bombay. Early 4th instar C. quinquefasciatus larvae used in the experiments were maintained in the insectarium of the Vector Biology Research Lab of Zoology department, University of Mysore, Mysore. The approval of the Institutional animal ethics committee, University of Mysore was taken for the use of mice to maintain mosquito colony.

2.1. Phytoextracts

A soxhlet extraction was carried out with pulversed fruit material using series of organic solvents of increasing polarity viz, petroleum ether, hexane chloroform, ethylacetate, acetone and ethanol until exhaustion. Solvents were distilled in a vacuum rotary evaporator under reduced pressure of 20–22 mmHg at 35 °C and extract concentrates were further evaporated to complete dryness at room temperature. Known amount of extracts were dissolved in known amount of acetone and stocks solutions were tightly closed and stored in brown bottles at 4 °C. Surface tension of each test solution was measured using stalagmometer to ensure the complete miscibility. Each extracts were assayed for their larvicidal efficacy with variable concentrations and doses were fixed to give larval morality ranging from 10 to 98 percent. As petroleum ether extract was found to be the most bioactive, it was selected for further analysis.

2.2. Isolation and purification of active component

The phytoextract of petroleum ether was dissolved in diethyl ether and subsequently washed with dilute bicarbonate, dilute base, mild acid and repeatedly with distilled water followed by neutralization at each step in a separating funnel. Then, the final diethyl ether fraction was completely dried over anhydrous calcium chloride and chromatographed over thin layer of silica gel (GF254) with standardized mobile phase of hexane and ethyl acetate in the ratio 9:1. Chromatogram was inspected under UV light at wave length of 254 as well as 366 nm and Rf values were calculated for major bands detected. 10 g of petroleum ether extract was ground with 2 g of silica gel (240–400 mesh) and loaded to the silica gel column previously equilibrated with hexane and successfully eluted with a step wise gradient of hexane and ethyl acetate (0:100; 10:90...90:10...100:0).

The 30 sub–fractions so obtained were pooled to 4 major fractions (F1, F2, F3 and F4) based on the bands appeared on TLC (Figure 1). The active F4 was found to be a complex mixture of amides. Further, the most active compound (112 mg) was obtained by repeated column chromatography. Isolated compound was dissolved in hot ethanol and passed through activated charcoal for crystallization and was subjected to RP–HPLC on a Vydac C18 column (250 mm×4.6 mm, 10 μm particle size, 300 Å pour size) in a Shimadzu LC–10 AVP system with dual wavelength detector. Purified compound was carefully evaporated to complete dryness at room temperature and subsequently characterized as pipyahyine on the basis of spectroscopic analytic data. Larval bioassays using pure compound were conducted to find out its efficacy against C. quinquefasciatus 4th instar larvae.

2.3. Spectroscopic analysis

Isolated compound was analysed by IR, UV, 1H, 13C-NMR and mass spectra. Infrared spectra were recorded on a FT, IR, Shimadzu 8300 spectrophotometer. UV spectral data were obtained from Hitachi UV–3900 spectrophotometer. 1H-NMR spectra were recorded on a Bruker 300 MHz spectrophotometer and 13C-NMR spectra were on 300 MHz, JEOL–FX–90Q, FT spectrophotometer in CDCl3, using TMS as internal standard.

2.4. Larval bioassay

Larval susceptibility tests of different solvent extracts...
and isolated compound were performed employing WHO standard procedure[12]. Various concentrations of the extracts were prepared by serial dilutions of a stock solution in acetone. Batches of 25 early fourth instar larvae were released into glass beakers of 500 mL capacity containing 249 mL of dechlorinated tap water and 1.0 mL of extract. The toxicity of each extract was determined with five various concentrations ranging from 0.2 to 20.0 ppm that provided a range of 10% to 98% mortality. Control beakers contained 25 test organisms and 249 mL of tap water along with 1.0 mL acetone. Treated and control beakers were maintained at same conditions at (25±2)°C, 12 h light/dark regime. No food was provided to the larvae during the test period of 24 h till the morality was monitored. All treatments were replicated 4 times. The larvae were considered as dead or moribund, if they were not responsive to gentle prodding with a fine needle. The results were expressed as percent mortality.

2.5. Statistical analysis

The observed percent mortality was adjusted for the control mortality, using Abbott's formula[3], and then subjected to regression analysis of probit–mortality on log dosage using computerized log–probit analysis (Harvard programming; Hgl.2), providing lethal dosage of 50, 90, 95, 99 ppm as well as their 95% confidence limit. One way variance analysis (ANOVA) was performed followed by post hoc Tukey's Honestly Significant Difference Test (HSD) using SPSS software, version 11.5. *P* value less than 0.05 were considered to indicate statistical significance.

3. Results

The susceptibility levels of fourth instar larvae of filarial vector to different solvent extracts of *P. longum* are shown in Table 1. From the results, it appeared that all the extracts exhibited larvicidal activity at variable concentrations. Out of six solvent extracts, the effect was highly significant (*P*≤0.01) in petroleum ether extracts in terms of LC50 and LC90 being 1.03 and 2.04 ppm respectively. Significant lethality was also exhibited by ethanol and ethyl acetate with respect to acetone with LC50 being 1.37 and 2.98 respectively (*P*≤0.01). However, hexane and chloroform extracts showed significant effect at 5% level with LC50 being 5.66 and 11.24 ppm respectively (*P*≤0.05), whereas, the acetone extract required higher concentrations (21.31 ppm) than all other extracts to produce 90% larval mortality. The slopes of regression lines for different phytoextracts were computed and provided in Figure 3a.

3.1. Bioactive principle

Purified phytoisolate was characterized as pipyahyine and its larval efficacy against mosquitoes are emphasized in Table 2. The compound exhibited larval toxicity with LC50 being 0.58 and 1.88 ppm respectively. *Culex* immatures produced 77% mortality at concentration of 1.2 ppm whereas 11% mortality was observed in 0.2 ppm. However, the dose–mortality responses are depicted in Table 2 and Figure 3b.

Structure of pipyahyine is provided in Figure 2, which was slowly solidified as white needles (112 mg/10 g), IUPAC: 9–Benzo[1,3]dioxol–5–yl–nona–2,4,8–triienoic acid (3–methyl–butyl)–amide IR (Neat): 1400–1600 (C=C aromatic aliphatic), 1650 (C=O), 3330 cm−1 (–NH); ‘H NMR (CDCl3): 1.01 (d, 6H, isopropyl 2CH3), 1.5 (q, 2H, CH2 adjacent to isopropyl group), 1.8 (m, 1H, isopropyl C–H), 2.0 (t, 4H, C6 & C7–H), 2.95 (t, 2H, amide CH2), 5.5 (q, 1H, C6–H), 5.9 (s, 2H, dioxole–H), 6.0 (q, 1H, C7–H), 6.2 (t, 1H, C8–H), 6.4 (d, 1H, C9–H), 6.55 (d, 1H, C9–H), 6.8–7.0 (m, 6H, Ar–H), 7.3 (t, 1H, C10–H), 7.9 (bs, 1H, CONH); C13NMR (CDCl3) 22.0 (q for 2CH3), 25.6 (d for isopropyl C–H), 33.0 (t for C5), 33.4 (t for C4) 40.3 (t for amide CH2), 40.8 (t for CH2 adjacent to isopropyl group), 91.3 (t for dioxole CH3), 112. 8 (d for Ar–CH), 115.0 (d for Ar–CH), 119.5 (d for Ar–CH), 123.8 (d for C6), 126.3 (d for C5), 127.6 (d for C8), 128.2 (s for Ar–C), 128.7 (d for C7), 130.3 (d for C7), 142.7 (d for C7), 146.8 (s for Ar–C), 147.5 (s for Ar–C), 166.5 (s for CO group).

EI–MS: m/z 341 (M+, 65). Anal. Calcd. for C21H27 NO3 (341): C, 73.87; H, 7.97; N, 4.10. Found: C, 73.67; H, 7.83; N, 4.19%.

**Figure 3.** a: Log concentration–percent mortality relationship of *C. quinquefasciatus* larvae to *P. longum* fruit extracts. b: Log concentration–percent mortality relationship of *C. quinquefasciatus* larvae to pipyahyine, an active compound obtained from *P. longum* fruit.
Table 1
Larvicidal efficacy of a few solvent extracts of *P. longum* fruits against *C. quinquefasciatus*.

| Solvent extracts        | LC50 (ppm) (LCL-UCL) | LC90 (ppm) (LCL-UCL) | Slope±SE | χ²     | P value |
|-------------------------|-----------------------|-----------------------|----------|--------|---------|
| Petroleum ether         | 1.03(0.98–1.10)       | 2.04(1.82–2.39)       | 4.36±0.40 | 2.76   | 0.0001* |
| Hexane                  | 2.35(2.17–2.58)       | 5.67(4.49–8.61)       | 3.35±0.50 | 0.39   | 0.02    |
| Chloroform              | 7.08(6.79–7.36)       | 11.24(10.44–2.45)     | 6.37±0.57 | 3.48   | 0.04    |
| Acetone                 | 15.64(15.24–16.16)    | 21.31(20.61–26.84)    | 8.77±0.48 | 7.09   | 0.78    |
| Ethyl acetate           | 2.98(1.24–1.39)       | 4.59(2.10–2.74)       | 6.84±0.79 | 1.21   | 0.001** |
| Ethanol                 | 1.37(1.29–1.47)       | 2.55(2.18–3.28)       | 6.98±0.50 | 2.24   | 0.001** |

LCL: Lower confidence limit; UCL: Upper confidence limit. *P<0.05 significant difference at 5%; **P<0.01 Significant difference at 1%.

Table 2
Mosquito larvicidal effects of a purified compound from petroleum ether extract of *P. longum* fruits.

| Test compound   | Concentration (ppm) | Percent mortality | LC50 (ppm) (LCL-UCL) | LC90 (ppm) (LCL-UCL) | Slope±SE | χ²     | P value |
|-----------------|---------------------|------------------|-----------------------|-----------------------|----------|--------|---------|
| Control         | 0.0                 | 0.0              | –                     | –                     | –        | –      | –       |
| Pipyahyine      | 0.2                 | 11.1             | 0.58(0.52–0.65)       | 1.88(1.57–2.58)       | 2.50±0.25 | 0.63   | –       |
| 0.4             | 34.8                | –                | –                     | –                     | –        | –      | –       |
| 0.6             | 54.3                | –                | –                     | –                     | –        | –      | –       |
| 0.8             | 63.0                | –                | –                     | –                     | –        | –      | –       |
| 1.2             | 77.0                | –                | –                     | –                     | –        | –      | –       |

LCL: Lower confidence limit; UCL: Upper confidence limit.

4. Discussion

It is very important to monitor the insecticide susceptibility status of mosquito vectors at different areas in view of the resurgence of various communicable diseases. All the organic components of the tested extracts revealed larvicidal activity against the filarial vector. High significant (P<0.01) results were exhibited by both polar (ethanol) and non polar (petroleum ether) phases of the extracts. Our results are in consonance with the studies conducted by Moawed, against *P. nigrum* and *C. pipiens* using petroleum ether and ethanol extracts of *P. nigrum*.[14] Literature survey has revealed bioassay experiments for exploring the insecticidal activity on mosquito vectors from many piper species. But most of the studies with piper species on mosquitoes are concentrated on *P. nigrum*, the common pepper.[14–19] However, considerable works have also been done with several other plants of the same species. For instance, three plants namely *P. nigrum*, *P. longum* and *P. sarmentosum* have shown adulticidal activity against *Stegomyia aegypti* at Thailand.[10]

The ethanol extracts of *P. beetle* has successfully killed the larvae of 4 mosquito vectors *A. aegypti*, *C. quinquefasciatus*, *A. dirus* and *Monsonia uniformis*.[20] It was noted that most of the compounds responsible for insecticidal activity from these species are amides.[7] Four amide compounds namely pipnoohine, pipyahyine, piptigine and pipwaquarine possessing larvicidal toxicity against *A. aegypti* have been reported from *P. nigrum* fruits in separate studies by Siddiqui et al.[16–18]. Rasheed et al have isolated 14 amide components from seeds of *P. nigrum*, all of which showed insecticidal activity against 4th instar larvae of *A. stephensi* and *A. aegypti*.[19] Findings from the present study too strongly support such observations, as major bioactive fraction (80%) obtained from this plant was a mixture of amides. Many derivatives of piperamides have been synthesized in search of new insecticides and some of them are in the way of successes.[21–23] The literature offers limited publications on the isolation of bioactive components from *P. longum*. Lee and Yang et al have isolated pipernonaline, an alkaloid as mosquito larvicide from methanol extract of this plant.[24,25]. But the authors failed to find such a compound in present investigation. By repeated chromatographic resolution, the active isobutyl amide was characterized as pipyahyine (Figure 2) with molecular formula C₁₉H₂₃NO₃. This has nine unsaturations among which five are accounted by aromatic ring. The IR spectrum showed absorption foe a secondary amide and its NMR spectra supported the presence of isopentyl group and signals for a benzo (1, 3) dioxyl moiety conjugated with double bond. These spectral peaks matched exactly with that of pipyahyine, isolated from the whole fruits of *P. nigrum* by Siddiqui et al.[16]. Further, they have also tested its larvicidal efficacy against *A. aegypti* with LC50 value of 30.0 ppm. In contrast to that, the larvicidal effect was 29.5 times more effective against *C. quinquefasciatus* with LC50 being 0.58 ppm with the same compound from *P. longum* in the present investigation. Moreover, the compound yield (11.5 mg/10 g) from *P. nigrum* was comparatively very less than that of *P. longum* (112 mg/g). The difference in the activity and components may be due to the nature and nurture of the plants and test organisms used in the experiments. It is important to note that the bioactivity of the extracts mainly depend on the chemistry of photoprotein, number of compounds present, nature of the solvent and procedure followed for extraction.[26] Our earlier studies have illustrated a promising synergistic activity of this plant and propoxur with synergistic factor 4.13.[27] Piperonyl butaoxide, the well known synergist derived from Piper species is being used extensively to reduce the dosage of synthetic chemical and to overcome resistance problem in vectors.[28] In this regard, our present work seems to be different and quantitative as it involves simple procedure to isolate more
quantity of larvicide/synergist from this local medicinal plant. The larvicidal activity of this plant is encouraging and might form an eco-friendly and human compatible arsenal in vector management programme. Further, the biochemical mechanisms and mode of action are under investigation and warrants extensive study.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] Molyneux DH, Hotez PJ, Fenwick A, Newman RD, Greenwood B, Sachs J. Neglected tropical diseases and the global funds. Lancet 2009; 373: 296–7.
[2] Addiss DG, Brady MA. Morbidity management in the global programme to eliminate lymphatic filariasis: a review of the scientific literature. Filaria J 2007; 6: 2.
[3] Liu N, Xu Q, Zhu F, Zhang L. Pyrethroid resistance in mosquitoes. Insect Sci 2006; 13: 159–66.
[4] Saleh MS, Meniawi EFA, Kelda NL, Zahram HM. Resistance development in mosquito larvae Culex pipiens to the bacterial agent Bacillus thuringiensis var. israelensis. J Appl Entomol 2003; 123: 29–32.
[5] Pavela R. Larvicidal effects of various Euro-asiatic plants against Culex quinquefasciatus say larvae (Diptera: Culcidae). Parasitol Res 2009; 36: 821–3.
[6] Nazar S, Ravikumar S, Parakash Williams G, Syed Ali M, Saganthi P. Screening of Indian coastal plant extracts for larvicidal activity of Culex quinquefasciatus. Indian J Sci Technol 2009; 2: 2427.
[7] Scott IM, Jensen HR, Philogene BJR, Amason JT. A review of Piper spp (Piperaceae) phytochemistry, insecticidal activity and mode of action. Phytochemistry Rev 2008; 7: 65–75.
[8] Park IK, Lee SG, Shin SC, Park JD. Larvicidal activity of isobutyal amides identified in Piper nigrum fruits against three mosquito species. J Agr Food Chem 2002; 50: 1866–70.
[9] Nadkarni KM. Indian materia medica (2 vol set). Indian: Popular Prakashan Pvt Ltd 2007, p. 2288.
[10] Choochote W, Chaithong U, Kamsuk K, Rattanachampichai E, Jitpakdi A, Tippawangkosol P, et al. Adulticidal activity against Stegomyia aegypti (Diptera: Culicidae) of three Piper spp. Rev Inst Med Trop de São Paulo 2006; 48: 33–7.
[11] Park BS, Lee SE, Choi WS, Jeong CY, Song C, Cho KY. Insecticidal and acaricidal activity of pipernonaline and piper octadecalidine derived from dried fruits of Piper longum L. Crop Prot 2002; 21: 249–51.
[12] World Health Organization: Guidelines for laboratory and field testing of mosquito larvicides. 2005, WHO/CDS/WHOPES/GC/2005. Geneva: WHO; 2005.
[13] Abbot WS. A method of computing the effectiveness of an insecticide. J Econ Entomol 1925; 8: 265–7.
[14] Moawed HAM. Joint action of some plant extracts against the mosquito larvae of Culex pipiens and their physiological impact. M.Sc, Thesis. Mansoura University: Faculty of Science–Dmietta; 1998.
[15] Vasudevan K, Malarmagar H, Charulatha H, Sarawatura VL, Palakaran K. Larvicidal effects of crude extract of dried ripened fruits of Piper nigrum against Culex quinquefasciatus larval instars. J Vect Borne Dis 2009; 46: 153–6.
[16] Siddique BS, Gulzar T, Mahmood A, Begum S, Khan B, Afshan F. New insecticidal amides from petroleum ether extract of dried Piper nigrum L. whole fruits. Chem Pharm Bull 2004; 52: 1349–52.
[17] Siddique BS, Gulzar T, Mahmood A, Begum S, Khan B, Afshan F, et al. Insecticidal amides from fruits of Piper nigrum Linn. Nat Prod Res 2005; 19: 143–50.
[18] Siddique BS, Gulzar T, Begum S, Afshan F, Sultana R. A new natural product and insecticidal amides from seeds of Piper nigrum Linn. Nat Prod Res 2008; 22: 1107–11.
[19] Rasheed M, Afshan F, Tariq RM, Siddiqui BS, Gulzar T, Mahmmod A, et al. Phytochemical studies on the seed extract of Piper nigrum Linn. Nat Prod Res 2005; 19: 703–12.
[20] Komalasmira N, Trongtokit Y, Rongsriyam Y, Apiwathnasorn C. Screening for larvicidal activity in some Thai plants against four mosquito vector species. Southeast Asian J Trop Med Public Health 2005; 36: 1413–22.
[21] de Paula VF, Barbosa LC, Demuner AJ, Piló-Veloso D, Piccano MC. Synthesis and insecticidal activity of new amide derivatives of piperine. Pest Manag Sci 2000; 56: 168–74.
[22] Dyer LA, Richards J, Dodson CD. Isolation, synthesis and evolutionary ecology of Piper amides. In: Dyer LA, Palmer A. Piper: A model genus for studies of phytochemistry, ecology and evolution. New York: Kluwer/Plenum; 2004, p. 139.
[23] Pridgeon JW, Meelpagala KM, Becnel JJ, Clark CC, Pereira RM, Mahmood A, et al. Phytochemical studies on the seed extract of Piper nigrum Linn. Nat Prod Res 2005; 19: 703–12.
[24] Madhu SK, Vijayan VA. Evaluation of the larvicidal efficacy of piperonyl butoxide in formulations for the testing of mosquito larvicides. 2005, WHO/CDS/WHOPES/GC/2005. Geneva: WHO; 2005.
[25] Yang YC, Lee SG, Kim MK, Lee SH. A piperidine amide from papaya fruit shows activity against Aedes aegypti females. J Agric Food Chem 2002; 50: 2457.