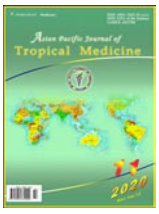

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Phytochemical profiling and biological activity of *Plectranthus amboinicus* (Lour.) mediated by various solvent extracts against *Aedes aegypti* larvae and toxicity evaluation

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ABSTRACT

Objective: To analyze the phytochemical compounds and to investigate the bio-toxic efficacy of various solvent extracts of *Plectranthus amboinicus* against mosquito larvae activity and lethality on non-targeting organisms.

Methods: The methanol, ethyl acetate, hexane, and aqueous extracts of *Plectranthus amboinicus* were subjected to analyze the mosquitocidal activity against the dengue vector, *Aedes aegypti* and toxicity assays on zebra fish and brine shrimp. Three replications were performed, and negative control was also maintained. Amongst, ethyl acetate extract of *Plectranthus amboinicus* was chosen for the determination of bio-active compounds.

Results: The mosquitocidal assays of methanol and ethyl acetate extracts of *Plectranthus amboinicus* showed the maximal activity with minimal concentration against the 4th instar mosquito-larvae of *Aedes aegypti* through the following lethal concentration (LC_{50} and LC_{90}) values: 53.36 & 92.51 μg/mL and 13.64 & 86.09 μg/mL, respectively. In addition, the plant extracts showed no toxicity on zebra fish embryo and brine shrimp assays. The gas-chromatography analysis of the ethyl acetate extract of *Plectranthus amboinicus* revealed the presence of seven different compounds. Among them, PAEA-fraction 60 contained a major active bioactive compound, hexadecanoic acid, methyl ester (270.0).

Conclusions: *Plectranthus amboinicus* possesses mosquitocidal properties and could be used as a potential alternative source for preparing the mosquitocidal agents.

KEYWORDS: *Plectranthus amboinicus*; Cold percolation; Indian borage; Mosquitocidal

1. Introduction

Plectranthus (P.) amboinicus (Lour.) Spreng, as Indian borage is an herbal plant belonging to the family Lamiaceae known as *Coleus aromaticus*, *P. aromaticus* (Benth.) Roxb. Mosquitoes are prime causative agent that spread numerous infectious diseases. In India, the mosquito class of *Aedes (Ae.) aegypti* is a route of Zika infection, yellow fever, dengue and chikungunya which mainly breeds in stagnant water. Globally, 390 million persons are affected by dengue in the year of 2013. In India, 2 million people are reportedly affected by dengue annually[1]. The application of synthetic/chemical-based insecticides, mosquito coils or liquidator for insect repellence reduces the biting from mosquitoes with severe side effects. However, natural products have been used for the treatment of various human diseases and as natural insecticide[2]. Therefore, the search for the discovery of natural plant materials as insecticide is essential towards new insecticides. Previous studies reported that the commonly available terrestrial plant, *P. amboinicus* can be used as an alternate to chemical insecticides because of its low-cost

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resource for controlling the infectious disease spreading mosquito population[3]. Therefore, the present study aimed to investigate the extraction and mosquito-larvicidal assesment of *P. amboinicus* extracted with three different solvents against fourth instar mosquito larvae of *Ae. aegypti*.

2. Material and methods

2.1. Collection of sample and extraction

The fresh healthy plants of *P. amboinicus* were collected from the local area of Salem, India. The morphological and anatomical characteristics of the collected plant were observed and identified using microscopic and macroscopic comparative analyses. And the plant samples were authenticated by Dr. D. Arulbalachandran, Assistant Professor, Department of Botany, School of Life Sciences, Periyar University, Salem, Tamil Nadu. The voucher specimens (No: MBEGL0134) have been kept in the Marine Biotechnology and Ecological Genomics Laboratory, Department of Biotechnology, Periyar University, Salem. The necessary chemicals and organic solvents used in this work were diagnostic grade of extreme purity. The collected plant leaves were washed dried and then finely chopped using kitchen electronic mixer. The powdered plant sample (100 g) was extracted with methanol, ethyl acetate and hexane (PAME, PAEA and PAHE, respectively) and stored in 4 ℃ for further analytical use. One hundred gram of *P. amboinicus* plant dust was mixed with 300 mL of deionized water and then boiled at 60 ℃ for 20 min to prepare the aqueous leaf extract of *P. amboinicus* (PAAE). Subsequently, the aqueous extract, PAAE was filtered by Whatman No. 1 filter paper and kept at room temperature.

2.2. Phytochemical analysis

Preliminary phytochemical analysis of the test samples (PAME, PAEA, PAHE & PAAE) was done by standard protocol[4].

2.3. Quantity-response bioassay

The larvae of the *Ae. aegypti*, obtained from the Centre for Research in Medical Entomology, Madurai, Tamilnadu, India, were kept in plastic trays holding deionized water and kept in laboratory state. Before investigation, the larvae were fed with the mixture of dog food and yeast solution and reared into fourth instar larvae for further experiments[2]. The research works has been performed according to standard guidelines[5]. The larvicidal assays of PAME, PAEA and PAHE were carried out as per the protocol[6] with slight modifications. A total of 25 mosquito-larvae (*Ae. aegypti*) were introduced into a transparent mosquitocidal chamber containing normal tap water (250 mL) with the required concentration of the samples (100, 50, 25, 12.5, 6.25 and 3.125 μg/mL). Three replications were performed, and negative control was also maintained. The larval percentage mortality was calculated after 24 h of exposure using the following method. The morphological deviations of the mosquito larvae treated (with plant extract) and untreated larvae were examined under stereomicroscope (Labomed, LB340).

$$\text{Larval mortality (\%)} = \frac{\text{X-Y}}{\text{X}} \times 100$$

where, X stands for survival in the untreated control and Y stands for survival in the treated sample.

2.4. Toxicity assay with non-targeting organisms

To assess the toxicity of the plant extracts, different non-targeting organisms were used for this study.

2.4.1. Zebra fish embryo toxicity assay

The zebra fish embryos were collected from laboratory adopted zebra fish (*Danio rerio*) (1:2). After 12 h of fertilization, the embryos were taken and rinsed with clear water for the toxicity assessment and unfertilized eggs were discarded. The zebra fish embryos were kept in ELISA plate containing plant extracts with different concentrations ranging from 3.125-100 μg/mL for incubation. The mortality, embryo hatchability and malformations of the treated and control were observed in every 6, 12 and 24 h[7].

2.4.2. Brine shrimp toxicity assay

The toxicity assessment to brine shrimp larvae was performed followed by standard protocol[8]. The eggs brine shrimp could hatch in artificial sea water (37 grams of salt in 1l of filtered distilled water) in the transparent glass chamber with laboratory conditions. After two days, the larvae were collected from the chamber and subjected for lethality assay. The plant extracts lethality was evaluated by adding 10 larvae of brine shrimp with varied concentrations (3.125-100 μg/mL) of three extracts of *P. amboinicus*. The larval death rate was calculated after 24 h of the action. For each sample negative control was maintained. The lethality of the plant extracts was calculated by subjecting the mortality data into probability analysis.

2.5. Isolation of bioactive compound from PAEA

The effective plant extract was subjected for the isolation of bioactive compounds through silica gel column chromatography. The column was packed with silica gel mesh (70-230 mesh). The sample slurry was loaded on the stationary phase to initiate the separation. The column was allowed to run with the eluting solvent initially with 100% chloroform and the polarity was decreased using the methanol at the ratio of (chloroform: methanol) 90:10, 80:20, 70:30, 60:40 and 50:50. The column was eluted at 10 mL/10 min in marked test tubes (15 mL capacity) under gravitational flow. Each test tube fraction was authenticated by TLC and the fractions showing similar bands were pooled together and then concentrated in a fume hood for further use[2].

2.6. Characterization analysis

The Fourier transform infrared-Spectrophotometer (FT-IR, Bruker, D8, Germany model) analysis of plant extracts were noted in the mid-IR region 4 000-400 cm^{-1} at room resolution 4 cm^{-1}. One milligram of plant extract was mixed with 100 mg of potassium bromide and then condensed to prepare salt-disc (3 mm dia), was analysed under FT-IR. The high-performance liquid chromatography (HPLC) analyses of samples/standards were conducted by adopting the standard protocol[9]. The mobile phase, methanol: water in the ratio of 50:50 was used. One milligram of each plant extract was mixed with 1 mL of methanol (HPLC grade) and only 20 μL samples were introduced into HPLC injecting port. The LCGC C18 column was used for isocratic resolution using the portable point at a flow rate of 1.0 mL/min (Shimadzu LC solution 20 AD, Japan).

The gas chromatography analysis was performed to the effective fraction isolated from the PAEA (Perkin Elmer, Massachusetts, USA). The filtered sample of PAEA (1 μL) was introduced into the injecting port and the used mobile phase is Helium. The qualitative and quantitative analysis of PAEA was carried out using a CP 3800 Saturn 2200 Gas Chromatography-Mass Spectrometer. The temperature program was 80 ℃ to 350 ℃ at the rate of 3 ℃/min. Ion temperature was 200 ℃ and scan range was 20-500 AMU (Atomic Mass Unit). The identification of components was based on comparison of their mass spectra with those of Wiley library[2].

2.7. Statistical analysis

All data were analysed through SPSS 16.0 (Software package for Social Studies, Version 16.0, Armonk, NY) to calculate the LC_{50}, LC_{90}, *Chi*-square values and other statistics at confidence limit (*CI*) 95% with upper and lower *CI*.

3. Results

3.1. Phytochemical analysis

The phytochemical analyses of PAME revealed the presence of major phyto-components such as flavonoid, carbohydrate, tannin, glycoside, protein, alkaloid, fixed oil & fat and amino acid. The PAHE has revealed the presence of flavonoid, carbohydrate, protein, alkaloid, fixed oil & fat and amino acid. Whereas PAEA has exerted the presence of flavonoid, carbohydrate, tannin, glycoside, protein, fixed oil & fat and amino acid.

3.2. Quantity-response bioassay

The larval mortality percentages of *P. amboinicus*-extracts against 4th instar larvae of *Ae. aegypti* are shown in Figure 1. The PAEA have shown effective larvicidal efficacy with the LC_{50} and LC_{90} values (13.64 and 86.09 μg/mL) against *Ae. aegypti*, followed by PAME (53.36 and 92.51 μg/mL), PAHE (68.85 and 156.65 μg/mL) and PAAE (135.36 and 293.29μg/mL), respectively as shown in Table 1. The control larvae (untreated) were found to be normal without any morphological and mortality change (Figure 1). Whereas, the larvae treated with PAEA, PAME and PAHE have exposed the progressive irregularities in the lateral hairs of the mosquito-larvae (Figure 1).

Table 1. Mosquito-larvicidal activity of *Plectranthus amboinicus* extracts (μg/mL).

Extract[x]	LC_{50} (95% *CI*)	LC_{90} (95% *CI*)
PAME	53.36 (41.89-72.62)	92.51 (57.21-160.37)
PAEA	13.64 (10.80-16.98)	86.09 (85.32-219.58)
PAHE	68.85 (52.86-98.14)	156.65 (33.30-178.74)
PAAE	135.36 (93.23-315.62)	293.29 (128.32-359.38)

[x]*Plectranthus amboinicus* extracts with methanol, ethyl acetate, hexane and aqueous (PAME, PAEA, PAHE and PAAE, respectively).

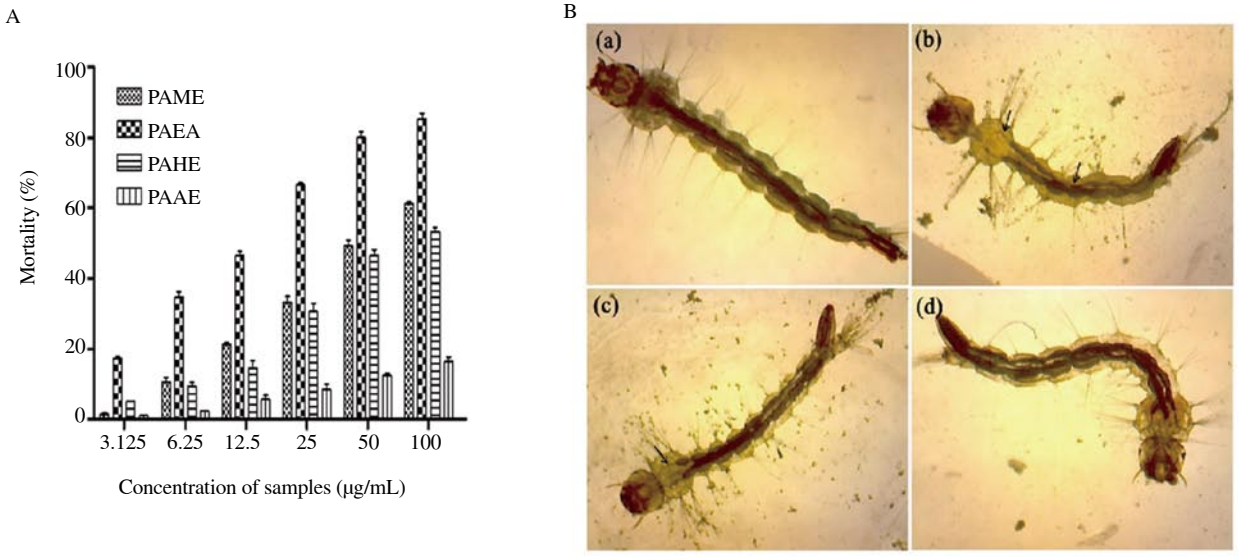

Figure 1. (A) Mosquito-larvicidal activity of *Plectranthus amboinicus* of PAME, PAEA, PAHE and PAAE against *Aedes aegypti*. (B) *Aedes aegypti* treated with (a) PAAE, (b) PAEA, (c) PAME and (d) PAHE. *Plectranthus amboinicus* extracts with methanol, ethyl acetate, hexane and aqueous (PAME, PAEA, PAHE and PAAE, respectively). Arrows (in b and c) indicate the seaweed induced deformation on the mosquito larvae.

3.3. Toxicity assay with non-targeting organisms

The zebra fish embryo toxicity assay was conducted up to 24 h post fertilization (hpf). The PAEA have shown no deformities even after 24 hpf. But the PAHE and PAME showed that the progressive deformities during each developmental stage (Figure 2). The percentage of brine shrimp toxicity was determined after 24 h of treatment (Figure 3A). The maximum mortality (76.66±1.83) percentage of brine shrimp [*Artemia (A.) salina*] was recorded with PAME (100 ppm) followed by PAHE (50.00±1.02), PAAE (40.66±0.15) and PAEA (33.33±1.52). Further, the microscopic images of brine shrimp (*A. salina*) using PAEA were shown in Figure 3B.

3.4. FT-IR analysis of plant extract

The FT-IR analysis of PAME revealed the presence of major peaks at the range of 3 322.75, 2 946.81, 2 850.27, 2 309.34, 1 633.41, 1 417.42, 1 376.93, 1 031.73 and 992.196 cm^{-1}. The band at 3 322.75 cm^{-1} could be allotted to the dimer OH stretching vibration of carboxylic acid. The bands at 2 946.81, 2 850.27 cm^{-1} could be attributed to the CH stretch and -CH2- stretching vibrations of alkanes. The band at 2 309.34 cm^{-1} corresponds to the P-H-phosphine sharp stretching vibration of miscellaneous group. Similarly, the bands at 1 633.41 cm^{-1}, represents the C=O stretch (free) vibration of Amides. The band at 1 417.42 cm^{-1} could be assigned to the Ar C-C stretching vibrations of aromatics. The band

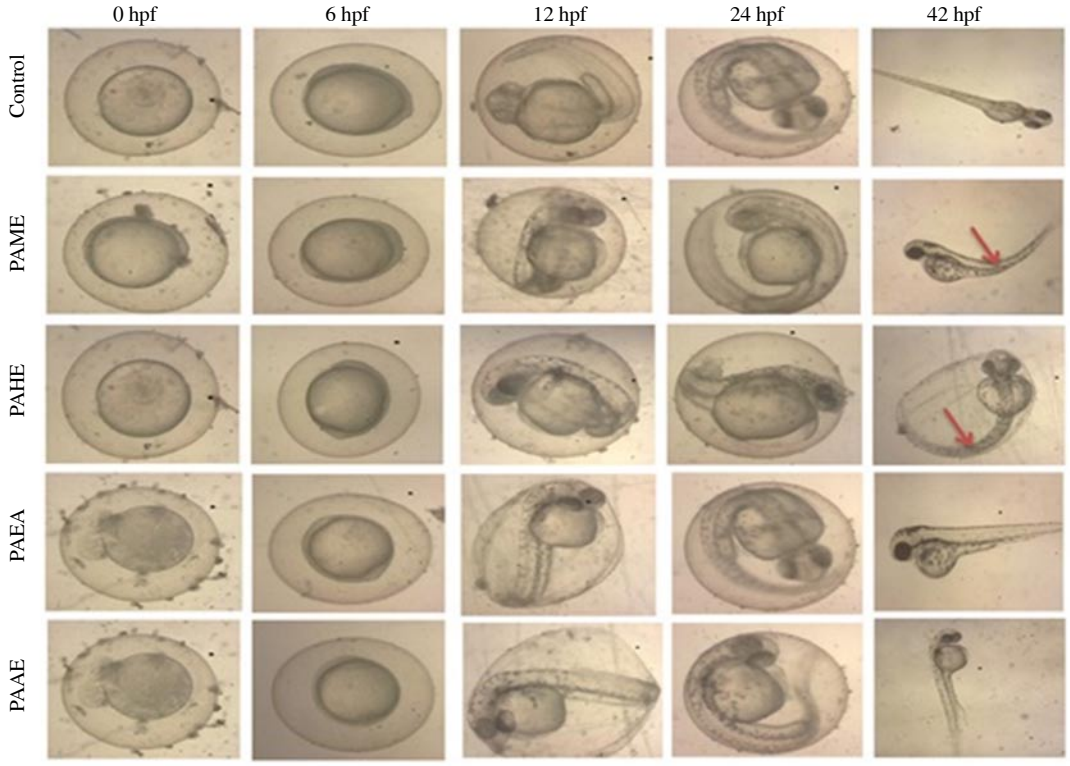

Figure 2. Toxicity of *Plectranthus amboinicus* against zebra fish (*Danio rerio*) embryo. The red arrows indicate the progressive deformities in the developing zebra fish embryo.

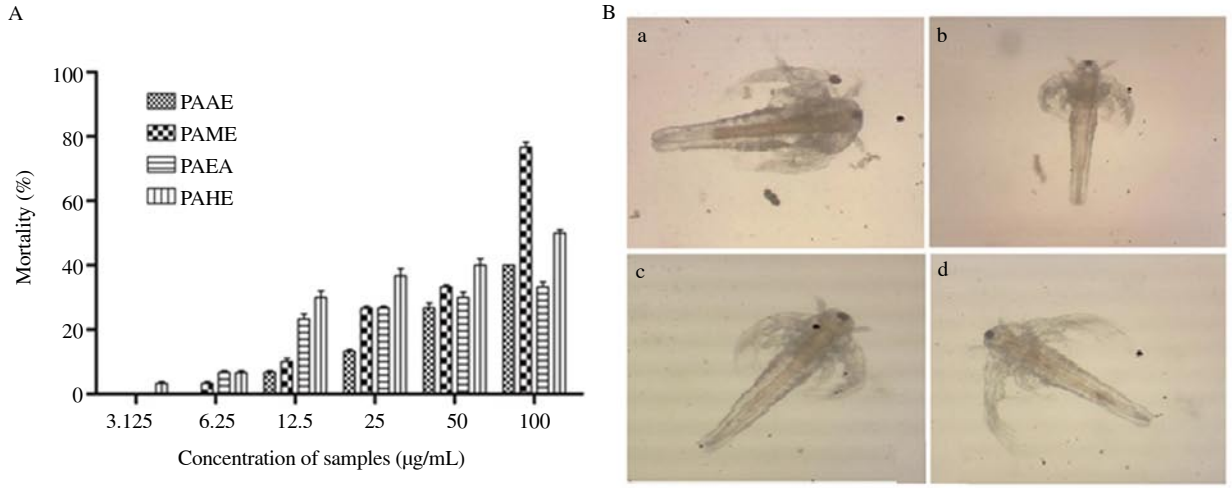

Figure 3. (A) Percentage mortality of brine shrimp (*Artemia salina*) using plant extracts. (B) Microscopic images of brine shrimp *Artemia salina* using PAEA after (a) 0 h, (b) 6 h, (c) 12 h and (d) 24 h.

at 1 031.73 cm^{-1} could be assigned to the C-N stretching vibration of amines. The band at 992.196 cm^{-1} could be attributed to the =CH out of place stretching vibration of alkanes (Figure 4). Similar peaks were observed in FT-IR spectrum of PAEA at 3 287.07, 2 957.30, 2 850.27, 2 359.48, 1 713.44, 1 161.90, 1 035.59, 832.13 and 718.35 cm^{-1}. The band at 3 287.07 cm^{-1} corresponds to the dimer OH stretching vibrations of carboxylic acids. The band at 2 957.30 cm^{-1} could be assigned to the CH-stretching vibration of alkanes. The band at 2 850.27cm^{-1} represents the P-H phosphine sharp of miscellaneous group. The band at 1 713.44 cm^{-1} could be assigned to C=O stretching vibration of ketones. Whereas, PAHE has shown peaks at 3 280.32, 2 957.30, 2 840.35, 2 359.48, 1 715.37, 1 615.09, 1 023.05 and 719.31 cm^{-1}. The bands at 3 280.32 and 2 840.35 cm^{-1} signals the dimer OH vibration of carboxylic acids. The band at 2 957.30 cm^{-1} could be assigned to the CH-stretching vibration of alkanes. Similarly, the bands at 1 715.37 and 1 615.09 cm^{-1} corresponds to the C=O stretching vibrations of ketones. The PAAE has shown the following peaks at 3 280.32, 2 918.73, 2 360.44, 1 716.34, 1 591.95, 1 394.28, 1 235.18, 1 029.80 cm^{-1}. The bands at 3 280.32 and 2 918.73 cm^{-1} could be attributed to the dimer OH stretching vibrations of carboxylic acids. The bands at 1 235.18 and 1 029.80 cm^{-1} could be allotted to the C-F stretching vibrations of alkyl halides. The corresponding stretching and functional groups of the samples are presented in supplementary tables (Table S1-S4).

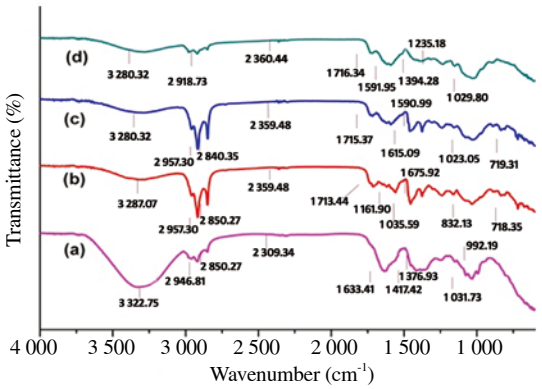

Figure 4. FT-IR spectrum analysis (a) PAME, (b) PAEA, (c) PAHE and (d) PAAE.

3.5. HPLC analysis of plant extracts

The HPLC analysis of standards ascorbic acid and gallic acid has showed the sharp intense peak at a retention time of 5.207 and 5.274. Similarly, the extracts of PAME, PAEA, PAHE and PAAE have shown a sharp peak at a retention time of 4.159, 4.820, 4.089 and 3.818 along with some slight peaks (Figure 5).

3.6. Bioactive compound isolation

Based on the preliminary mosquitocidal activity, PAEA was

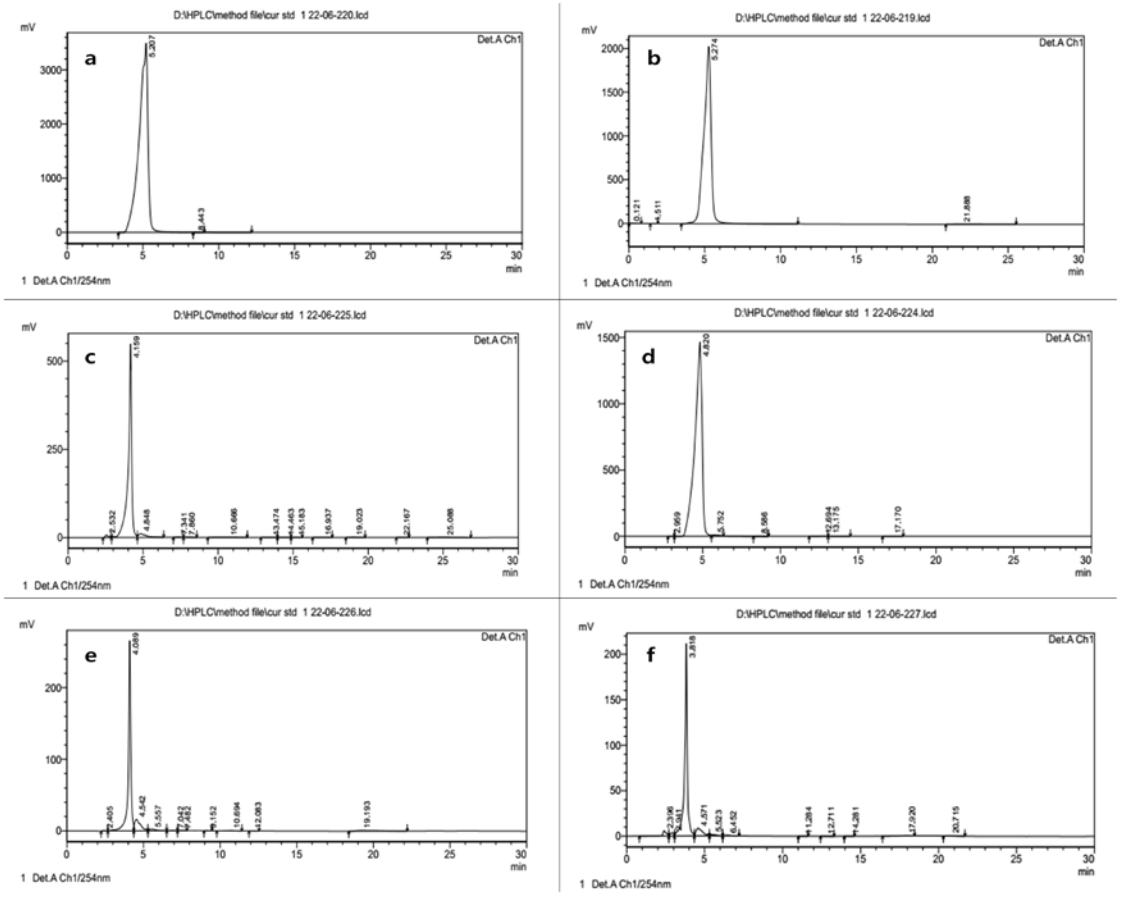

Figure 5. HPLC profile of (a) Ascorbic acid, (b) Gallic acid, (c) PAME, (d) PAEA, (e) PAHE and (f) PAAE.

selected for the bioactive compound isolation. The column chromatography analysis of the PAEA has yielded the partition of the crude extract. The TLC authentication was performed to categorize the fractions having the same R_f values. The TLC authentication studies of the PAEA-fraction 60 have revealed the single band formation. The R_f value of the effective fraction was found to be 0.213. Hence, fraction 60 is subjected for the gas chromatography analysis.

3.7. GC-MS analysis of PAEA-Fraction 60

The gas chromatography analysis of PAEA-fraction 60 has revealed the presence of a major active compound, hexadecanoic acid, methyl ester (270.0) (Figure 6). The chemical name and molecular weight of the other bioactive compounds were identified as ether (210.0), 1-docosene (308.0), pentanoic acid 10-undecenyl ester (254.0), 9-octadecenoic acid (Z)-methyl ester (296.0), oxirane dodecyl- (212.0), oleic acid (282.0). The retention time, molecular formula, chemical name and peak percentage of the bioactive compounds of the PAEA-fraction 60 were reported in the Table 2.

4. Discussion

Many useful drugs have been formulated through the exploration of whole or parts of medicinal plants. Previous research works have showed that *P. amboinicus* is a unique source of many potential phytochemicals that can be used for human welfare[10]. The phytochemical test results of this study revealed the presence of different phytochemicals in the 4 different solvent extracts of *P. amboinicus*, *viz*. carbohydrate, flavonoid, tannin, saponin, protein, glycoside, fat, alkaloid, amino acid and fixed oil. There are slight variations in the concentrations of these phytochemicals among the solvent extracts of the plant. Such phytochemicals of the plant extracts have been already reported in by some researchers that showed insecticidal activity[11]. These bioactive metabolites of the medicinal/herbal plants were found to possess activity against pathogenic microbes such as, bacteria and yeast but differ in their quality and quantity based on the presence of bioactive compounds/ metabolites and origin of the plant and the *Plectranthus* species have been reported for mosquito-insecticidal and repellent property[9].

In recent years, many researchers have focused their attention towards developing effective insecticides/pesticides from natural background. It is a recent trend due to the disadvantage of synthetic chemical pesticides, such as the impact on atmosphere, development of pesticide resistance mechanism and the induction of toxicity to non-targeting organisms as well as humans and the development of resistance in targeted insect populations. The insecticidal property of the *P. amboinicus* could be a promising control strategy against mosquito vectors, which may lead to the development of side effect free insecticide, especially in developing countries. Several plant extracts have been reported to be biologically active against insect pests. The presence of different functional groups in the plant extracts have confirmed the better biological activity, including alkenes, alkynes, phenolics, alkaloids and terpenoids[12]. The toxicity percentage of plant extract against insect pest and non-targeted organisms may differ because of the solvent-polarity used which might be influenced by the chemical combinations of

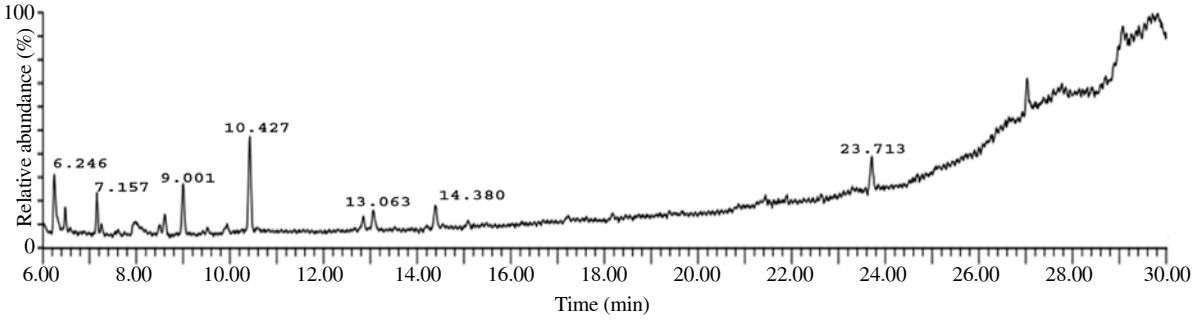

Figure 6. Gas chromatography analysis of isolated fraction of PAEA.

Table 2. Chemical constituents of PAEA analyzed by gas chromatography-mass spectrometry.

R_t	Name of the compound	Molecular formula	Molecular weight	Peak area (%)	Biological properties
6.246	Ether	210.0	$C_{14}H_{26}O$	3.160	No activity found
7.157	1-Docosene	308.0	$C_{22}H_{44}$	1.820	No activity found
9.001	Hexadecanoic acid, methyl ester	270.0	$C1_7H_{34}O_2$	6.087	Antioxidant, hypocholesterolemic, nematicide, insecticidal and hemolytic[31]
10.427	Pentanoic acid, 10-undecenyl ester	254.0	$C_{16}H_{30}O_2$	5.418	No activity found
13.063	9-Octadecenoic acid (Z)-, methyl ester	296.0	$C_{19}H_{36}O_2$	1. 328	Anti-inflammatory[32]
14.380	Oxirane, dodecyl-	212.0	$C_{14}H_{28}O$	1.622	No activity found
23.713	Oleic acid	282.0	$C_{18}H_{34}O_2$	2.430	Antibacterial, antiviral, antioxidant, antifungal[33]

R_t: retention time.

the plant extracts. Presently, the percentage mortality of the plant extracts (PAME, PAEA, PAHE, and PAAE) against 4th instar larvae of *Ae. aegypti* were found to be: 61.3%, 85.3%, 53.3% and 16.4%, respectively. Some earlier researchers have found the toxicological properties of bioactive metabolites of *Plectranthus* species that were effective against insect pest such as mosquitoes and *Haemonchus contortus*. The toxicological studies of aqueous and hydro-alcoholic extracts of *P. punctatus* against *Haemonchus contortus* have shown the effective dose (ED_{50}) values (μg/mL): 18.8 and 18.45, respectively[13]. Recently, the mosquito-larvicidal and pupicidal properties of the terrestrial plant *P. glandulosus* based essential oils against fourth instar larvae and pupae of *Ae. aegypti*, *A. gambiae* and *C. quinquefasciatus* were reported. Among them, the essential oil was found to show an effective larvicidal activity on *Ae. aegypti* with the LC_{50} values (μg/mL) of 2.66, like that of the present findings[14]. Similarly, the mosquito-larvicidal activity of essential oil extracted from *P. amboinicus* against African malaria vector, *Anopheles (An.) gambiae* was found to be more effective with LC_{50} and LC_{90} values (μg/mL) of 98.56 and 147.40 (at 12 h) 55.20 and 99.09 (at 24 h) and 32.41 and 98.84 (at 48 h)[15]. The mosquito-repellency efficacy of the essential oil (prepared from *P. incanus*) has revealed the strong repellant property against *An. stephensi* and *Culex (C.) fatigans* with the following LC_{50} value (μg/mL) of 23.8 and 19.6, respectively[16]. According to Danga *et al*[17], stated that the mosquito-larvicidal activity of different organic solvents (chloroform, methanol, ethyl acetate and hexane) and water extract of *P. glandulosus* were against the late third instar larvae of three mosquito species: *An. gambiae, C. quinquefasciatus* and *Ae. aegypti*. Among the crude extracts, hexane extract of *P. glandulosus* showed the effective larvicidal activity with minimal concentration of LC_{50} value (μg/mL): 610.40, 17.11 and 89.08 against *An. gambiae*, *Ae. aegypti* and *C. quinquefasciatus*, respectively. The mosquito larvae treated with plant extracts were shown the effect like darkening and shrunken cuticle of anal papillae. Likewise, the morphological changes when methanolic and acethonilic extracts of *Ipomoea cairica* were treated in contradiction of the early fourth instar larvae of *C. quinquefasciatus*[18].

Recently, it is reported that the embryo toxic effect of effluents from sewage in zebrafish embryo has resulted in malformation in notochord, eye retardation and non-paired muscle development[19]. It has been reported that the exposure of zebra fish embryos into titanium dioxide nanoparticles (TiO_2NP) did not affect the rate of hatchability and no mortality/deformity appreaed in the embryos[20]. The earlier research recorded that exposure of zebra fish embryo into silver and gold nanoparticles synthesized from aqueous plant extract of *Spinacia oleracea* revealed 100% mortality with the higher concentration (300 mg/mL) but the aqueous extract did not show any mortality which is in agreement with our current study[21].

Similarly, the introduction of zebra fish (*Danio rerio*) embryos into TiO_2NP at the maximum concentrations of up to 500 mg/L for 96 h did not affect the hatching rate and no deformity was observed in embryonic zebra fish[22]. Toxicity of crude clove essential oil against *A. salina* has shown the very less (LC_{50}=0.599 3 μg/mL) lethal concentration[23]. According to Bergami *et al*[24], the toxicity analysis of polystyrene nanoplastics against *A. franciscana* has revealed the low mortality in brine shrimps (LC_{50}=0.83 μg/mL). Recent study has revealed that the toxicity of TiO_2NP against *A. salina* has shown no mortality up to 5 mg/mL concentration in which similar findings were also reported in our study.

The GC-MS analyses of *P. amboinicus*-essential oil revealed the presence of major phyto-components, carvacrol (28.65%) is the major cause of the larvicidal activity in contrast to 4th instar larvae of *An. stephensi* that were reported to have the following LC_{50} and LC_{90} values (μg/mL): 33.54 and 70.27 after 12 h of treatment[3]. The observed morphological variations of the mosquito larvae treated with plant extracts might be due to the bioactive compounds of the *P. amboinicus*, which might be the reason for the spoiled anal papillae, distorted body, darken body and pale body and such malformations were previously reported in *Leucas aspera*, *Adenanthera pavonina* and *Sargassum binderi*[25-27]. The biological activity of any plant extract is primarily depending on the presence of the bio-active molecule. Hence, the FT-IR analysis of the plant extract may expose the biological property of the plant extract[28]. The earlier report of pupicidal property of *Cymbopogan citrtus* against 4th instar larvae of *C. quinquefasciatus* was found to have the following major peak vibrations at 2 918 and 2 360 cm^{-1} in the FT-IR analysis. Interestingly, in the present study, the FT-IR analysis of PAEA has revealed the similar peaks which might have influenced the mosquito-larvicidal effect. Similar peaks were also earlier found in the aqueous leaf extract of *Feronia elephantum* that exhibited larvicidal activity against the mosquito species of *C. quinquefasciatus*, *An. stephensi*, and *Ae. aegypti*[29].

The comparable peaks were observed in the HPLC profile of the PAEA to the standards. The major peaks observed in the PAME, PAEA and PAHE have revealed the presence of active metabolites as reported[30]. The comparative analyses of HPLC profile of samples and standards revealed the presence of a reduction of closely related metabolites. The earlier research has reported that the gas chromatography analysis of petroleum ether extract of *Lepidagathis scariosa* has revealed the presence of bioactive compound hexadecanoic acid which showed the nematicide, insecticidal activity[31]. Similarly, in the present study, the GC-MS analysis *P. amboinicus* was found to have such active compound which might be responsible for the maximum larvicidal effect. The presence of octadecenoic acid has been found to have antifungal effect[32]. The previous research of antiviral and antifungal activity of plant leaves

of *Pseudoglochidion anamalayanum* has resulted the presence of a bioactive compound, oleic acid like that of the present study[33]. Thus, the present analysis revealed the active principle for the mosquito-toxicological efficacy of *P. amboinicus* for human health care.

In conclusion, the herbal based formulation of a medicine, insecticide and mosquitocidal agent are low-cost, safe for consuming and gives side effect free treatment. Hence, the present study could form a base-line for making awareness of an alternate and side effect-free preparation of mosquitocidal formulation against dengue vector, *Ae. aegypti*. The GC-MS analysis of the isolated fraction has been identified as hexadecanoic acid. Moreover, it is also suggested that further research should be performed to evaluate the effect of *P. amboinicus* and consider other aspects to get their more usage.

Conflicts of interest statement

The authors of this article declare that there is no conflict of interest.

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Authors' contributions

D.P. and P.P. designed the research, conceived the project, wrote the original manuscript draft, performed the experiments. B.B interpreted the design and results, helped to revise, edit and re-write the manuscript. T. K, P.A, W.C.L and S.P helped in revising the manuscript.

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