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Insecticidal activity of crude extracts from *Pongamia pinnata* (L.) against *Aedes aegypti* (L.) and *Culex quinquefasciatus* (Say)

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Abstract
Insects like mosquitoes, head lice, ticks, and mites, influenced by human activity, transmit several life-threatening diseases to humans and animals, including malaria, dengue, filariasis, and the Zika virus. The use of synthetic chemicals for control can cause illnesses in humans, animals, non-target organisms, and disrupt natural ecosystems. The larvicidal activity of methanolic extract of *Pongamia pinnata* (Fabaceae) against two blood-sucking insects (*Aedes aegypti* and *Culex quinquefasciatus*) was investigated. Phytochemical analysis was performed using UV-Vis spectroscopy to identify and quantify compounds based on their light absorption characteristics, FT-IR spectroscopy to determine functional groups by analyzing infrared light absorption, and GC-MS to separate, identify, and quantify volatile and semi-volatile compounds based on their mass and fragmentation patterns. The crude extract of *Pongamia pinnata* revealed the presence of alkaloids, phenols, tannins, and saponins in the methanol extracts. GC-MS analysis identified isoborneol (14.19%), phloroglucinol (27.44%), and hydantoin (34.54%) as key chemical constituents responsible for insecticidal activity, which aligns with previous research findings. FT-IR analysis revealed vibrations such as stretching, wagging, and bending related to oxygen-containing bonds, as well as the presence of functional groups including alcohols, phenols, carboxylic acids, and alkynes. Our results showed the highest larval mortality in *Cx. quinquefasciatus* (LC_{50} = 75.64 ppm) compared to *Ae. Aegypti* (LC_{50} = 101.36 ppm) after 24 hours. For pupicidal activity, the LC_{50} values were 134.56 mg/L and 185.59 mg/L for *Cx. quinquefasciatus* and *Ae. Aegypti*, respectively. The study proves that the chemicals in *P. pinnata* extracts can kill insects well. This suggests that these plant compounds could be used instead of synthetic insecticides.

Keywords: Methanolic extract, *Pongamia pinnata*, larvicidal activity, *Aedes aegypti*, *Culex quinquefasciatus*

Introduction

Anthropogenic insects like mosquitoes, bugs, lice, and kissing bugs transmit many harmful pathogens, causing 80% of communicable diseases. These blood-feeding insects thrive in urban areas, with female mosquitoes biting to reproduce and human lice feeding to obtain nutrients [1, 2]. Anthropogenic activities increase environmental temperatures, forcing insects to adapt. As poikilothermic animals, insect vectors depend on ambient temperatures for growth and reproduction. Warmer climates favor the proliferation of mosquitoes, lice, and ticks [3]. Climate change has expanded the spatial range of diseases like dengue. In Odisha, mosquito-borne diseases peak from July to November, with dengue prevalent in coastal areas and malaria and Japanese encephalitis in interior regions [4].

Each year, about 400 million people are infected, resulting in 97 million clinical fatalities and 40,000 deaths. Half the global population is vulnerable to vector-borne diseases, with incidence rising 30 to 50 times in tropical and subtropical regions over the last 50 years [5]. In India, 188,401 cases were reported in 2017; 101,192 in 2018; 157,315 in 2019; 44,585 in 2020; and 193,245 in 2021. In 2020, the occurrence of vector-borne diseases dropped by 55-60% due to the impact of the COVID-19 pandemic. The Indian government implemented measures such as public isolation and quarantine, which contributed to the decline in VBD cases [6, 7]. The dengue fever vector is resistant to major insecticide classes like organochlorines, organophosphates, pyrethroids, and carbamates. Resistance mechanisms include target site mutations, metabolic detoxification, reduced insecticide penetration, and behavioral changes in mosquitoes [8].

Plants serve as biocontrol agents for an extended period, offering an alternative source for controlling resistant insect vectors [9]. Nylon fibres treated with lemongrass oil repel Aedes mosquito bites even after 25 washes and prevent microbial infections from bacteria like *Staphylococcus aureus* and *Klebsiella pneumoniae* [10]. Alkaloids present in plant leaves induce aversive feeding behaviour. For instance, quinine and caffeine, along with theophylline, modify insect consumption, metabolism, and gas exchange patterns, leading insects to cease eating [11].

Pongamia pinnata (L.) commonly known as Pongamia, an evergreen tree native to Southeast Asia, is extensively used in traditional medicine. Various parts of the *P. pinnata* plant are utilized for different purposes such as biofuel (with seeds containing 40% oil), the production of antimicrobial soap, bioinsecticides (utilizing Karanjin found in seeds), insect repellents, and edible oil [12]. *P. pinnata* leaves were tested for wound-healing properties in Wistar rats, showing upregulation of Hydroxyproline, hexosamine, TNF-α, and IL-6 levels, aiding faster wound healing. They also prevent microbial infections by organisms like *Staphylococcus aureus*, *Escherichia coli*, *Staphylococcus pyogenes*, and *Candida albicans* [13-14].

To overcome the issues, the methanol extract of *P. pinnata* leaves was tested against mosquitoes to determine its chemical composition and LC_{50} values.

Materials and methods

Plant materials

The leaf of *P. pinnata* was harvested from St. Xavier's college campus, Palayamkottai (N 8° 43' 3.2484", E 77° 44' 20.2056", altitude 47 m), Tamil Nadu. Dr. V. Chelladurai, ex-Research Officer at the Central Council for Research in Ayurveda and Siddha, authenticated the plant. The leaves were washed with tap water and air-dried in the shade at room temperature for 15 days.

Preparation of plant extracts

The dried leaves (350 g) were ground into a fine powder using a commercial electric stainless-steel blender. This powdered material was then extracted with methanol in a Soxhlet apparatus (1,700 ml) until the extraction was complete. The methanol extract was concentrated under reduced pressure at 45°C, and the resulting residue was stored at 4°C.

UV-Vis spectroscopic analysis

UV-Visible spectroscopy analysis was conducted by dissolving one gram of the extracted powder in 10 ml of the same solvent. The resulting extracts were scanned across a wavelength range of 200 to 800 nm using an Agilent Cary 8454 model (US), and the individual peaks were recorded [15-16].

Fourier Transform-Infrared spectrophotometry (FT-IR)

FTIR, known for its accuracy, is employed to identify functional groups and structural components in compounds, offering a quick and non-destructive fingerprint analysis of plant extracts. The methanolic leaf extract was applied to a potassium bromide (KBr) disc, and the spectrum was recorded in the wave number range of 4000 cm^{-1} to 450 cm^{-1} using an FTIR spectrometer (Perkin-Elmer – Spectrum, US) in absorption mode [17].

GC-MS analysis

The analysis was conducted using the Clarus 680 GC with an Elite-5MS column (5% biphenyl, 95% dimethylpolysiloxane). Components were separated with helium as the carrier gas at 1 ml/min. The injector was set at 260°C, and a 1µL sample was injected. The oven temperature was programmed from 60°C (2 minutes) to 300°C (10°C/min) and held at 300°C for 6 minutes. Mass detector settings included a transfer line temperature of 240°C, ion source at 240°C, electron impact ionization at 70 eV, with a scan time of 0.2 seconds and a range of 40 to 600 Da. Component spectra were matched with the GC-MS NIST (2008) library.

Mosquito culture

Ae. aegypti and *Cx. quinquefasciatus* colonies were kept in an insectary (45×40×40 cm) at 27 ± 2°C and 80 ± 2% relative humidity with a 14:10-hour light-dark cycle. Eggs were sourced from the ICMR-Vector Control Research Centre in Madurai, Tamil Nadu, India, and placed in dechlorinated tap water to hatch. Larvae were fed a diet of yeast and dog biscuits in a 3:1 ratio, and plastic bowls with 50 ml of tap water were provided for oviposition.

Larvicidal Activity Procedure

For larvicidal activity, a 2% solution of the tested material was prepared in methanol as the stock solution [18]. To assess mortality, concentrations of 25.0, 50.0, 100.0, 200.0, and 400.0 mg/L were prepared. In 200 mL plastic cups with 100 mL of tap water, the sample was added and 10 third-instar larvae were introduced. Each concentration was tested in quintuplicate, and larval mortality was recorded after 24 hours. The same method was used for pupicidal activity. All cups were kept in a mosquito colony, and mortality was noted. Larval death was indicated by lack of response to mechanical stimulation, and the Abbott formula was used to calculate mortality percentages.

Statistical analysis

The LC_{50} and LC_{90} values were estimated using Probit analysis with SPSS software version 22. The results for larvicidal and pupicidal activities were analyzed using ANOVA in SPSS software.

Results

UV and FTIR Studies

The optical properties of *P. pinnata* extract were analysed using an Agilent Cary 8454 (US) within the wavelength range of 200–100 nm. The absorption bands observed were 270 nm (polyphenols), 204 nm (carboxyl groups), 271 nm (nitrate groups), and 284 nm (carbonyl groups). FTIR analysis, within 400–4000 cm^{-1}, revealed peaks at 3305.74 cm^{-1} (O–H stretch), 2943.53 cm^{-1} (CH stretch), 2115.10 cm^{-1} (N=C=S stretch), and 1604.38 cm^{-1} (C=C stretch). Peaks at 1515.39 cm^{-1} (N-O bending), 1392.42 cm^{-1} and 1288.34 cm^{-1} (S=O stretch), and 666.33 cm^{-1} and 518.35 cm^{-1} (C–Br stretching) were also identified.

Determination of bioactive compounds using GC MS analysis

The GC-MS analysis of *P. pinnata* leaf extract identified several bioactive compounds along with their molecular weights and structures. As shown in Table 2, the extract contained a range of components, including ethers, amides, fatty acids, alcohols, and heterocyclic compounds. The most abundant compounds were Hydantoin (34.54%), Phloroglucinol (27.44%), and Isoborneol (14.19%).

Larvicidal and pupicidal activity

The larvicidal and pupicidal effects of *P. pinnata* methanol extract were tested on 3rd instar larvae of *Ae. aegypti* and *Cx. quinquefasciatus* over 24 hours. The extract showed strong larvicidal activity, with LC_{50} values of 75.64 mg/L for *Ae. aegypti* and 101.36 mg/L for *Cx. quinquefasciatus*. For pupicidal activity, the LC50 values were 134.56 mg/L for *Cx. quinquefasciatus* and 185.59 mg/L for *Ae. aegypti*, indicating the extract's effectiveness against both species.

Discussion

This study identified the methanolic extract of P. pinnata as having as a promising bioinsecticide agent against mosquitoes. *P. pinnata* constituent, Karanjin, was quantified at 0.2 to 0.35% using an HPLC method [21], demonstrating high efficiency against *Culex pillens* (LC_{50} = 14.61mg/L), *Ae. aegypti* (LC_{50} = 16.13 mg/L), and *Ae. albopictus* (LC_{50} = 35.26 mg/L) [22]. Isoborneol (14.19%) was identified as a major constituent of *P. pinnata*, which repels female mosquitoes by selectively activating OR49 and OR9-expressing neurons and MD3 neuron antennal lobe [23]. (-)-Bornyl chloroacetate, a derivate of (-)-borneol, exhibited potent larvicidal potency (LC_{50} = 20.3 ppm) against *Ae. aegypti* while being non-toxic to Artemia sp. (LC_{50}=170.7 ppm) [24]. Phloroglucinol (27.44%), a monoterpenoid present in *P. pinnata*, has demonstrated good antibacterial activity against *S. aureus* d demonstrated strong inhibitory activity

against *Ae. aegypti* and *Ae. albopictus*. Hydantoin (34.54%) has also been reported to exhibit larvicidal properties [25-27].

The methanol and hydroalcoholic extracts of *P. pinnata* showed significant larvicidal activity against *Cx. quinquefasciatus*, *Ae. aegypti*, and *An. stephensi*. The LC_{50} values for the methanol extract were 84.8 ppm, 118.2 ppm, and 151.7 ppm, respectively, while for the hydroalcoholic extract, the values were 97.7 ppm, 128.3 ppm, and 513 ppm, respectively [28]. Moreover, the extract of *P. pinnata* leaves demonstrated insecticidal properties against the cassava pink mealy bug, *Phenacoccus manihoti*, with higher effectiveness observed in first instars compared to second instars at a concentration of 2% [29]. Neem and pongam soap pastes, developed by IIHR Bangalore, are recommended as plant biopesticides. Tested against *Cx. quinquefasciatus* larvae, their LC_{50} values were 112.67 ppm for pongam soap, 48.57 ppm for neem soap, and 60.35 ppm for neem + pongam soap [30].

Tran *et al.* [31] reported that Pongam leaf extracts were found to be highly effective in managing the first and second instars of the cabbage webworm, *Hellula undalis*. At a concentration of 1.0%, mortality rates of 93.2% and 68.4% were observed in first and second instar larvae, respectively. Yogeshwar *et al.* [32] found that methanolic leaf extracts of *P. pinnata* demonstrated potent antifungal effects against Pythium debaryanum, inhibiting 97.3% of mycelial growth at 1 mL concentration after 3 days. These findings suggest its potential use as a fungicide.

Various plant-derived secondary metabolites and essential oils can effectively kill or repel head lice, body lice, mites, and ticks [33]. Samuel *et al*. [34] found that extracts of *P. pinnata*, including chloroform, petroleum ether, methanol, and water, were effective against head lice. Petroleum ether extract had the highest mortality rate in nymphs (100%), followed by methanol extract (82.9%) at 20% (w/v). This is likely due to sterol derivatives in the petroleum ether extract, which penetrate the insect cuticle. Additionally, *P. pinnata* leaf extract has been shown to alleviate skin issues like psoriasis and infections. A hydroalcoholic extract significantly reduced skin thickness and scaling in a psoriasis mouse model [35].

The chloroform, ethyl acetate, 70% methanol, and hexane extracts of *P. pinnata* leaves were tested against three gram-negative bacteria (*P. aeruginosa* ATCC 6432, *E. coli* ATCC 43888, *E. coli* ATCC 8739, *S. typhimurium* ATCC 2515) and three gram-positive bacteria (*B. subtilis* ATCC 6633, *S. aureus* ATCC 6538, *L. monocytogenes* ATCC 19118, *L. monocytogenes* ATCC 19166). The 70% methanol extract showed notable antibacterial activity against the gram-positive bacteria, likely due to the lack of a hydrophilic lipopolysaccharide cell wall in gram-negative bacteria. The hexane extract, however, exhibited no antibacterial activity against either gram-positive or gram-negative bacteria [36].

In toxicology studies, administering the leaf extract of *P. pinnata* at a dose of 5000 mg/kg orally to adult albino Wistar rats (150-200 g) proved to be safe, with no instances of mortality observed during a 14-day observation period. Overall, *P. pinnata* has been shown to be effective in controlling mosquitoes and is very safe for both humans and animals [37].

Table 1: List of the chemical compounds detected from the methanol extract of *P. pinnata* through GC–MS.

S. No	Name of the Compound	Retention Time	Molecular Weight	Formula	Area %
1	Isoborneol	8.836	154	$C_{10}H_{18}O$	14.196
2	Pyridine, 1,2,3,6-Tetrahydro-1-Methyl-	9.956	97	$C_6H_{11}N$	1.920
3	Hydantoin	16.064	114	$C_4H_6N_2O_2$	34.549
4	Phloroglucinol	16.934	132	$C_6H_{12}O_3$	27.443
5	Asparagine, Dl-	17.079	132	$C_4H_8N_2O_3$	9.258
6	4-Hydroxy-N-Methylpiperidine	17.509	173	$C_6H_{13}NO$	1.837
7	Nonanoic Acid	18.400	158	$C_9H_{18}O_2$	4.304
8	2-T-Butyl-4-Methyl-5-Oxo-[1,3]Dioxolane-4-Carboxylic Acid	18.620	202	$C_9H_{14}O_5$	1.850
9	Oxalic Acid, Allyl Octyl Ester	19.715	242	$C_{13}H_2$	0.901
10	2,6,6-Trimethyl-Bicyclo [3.1.1]Hept-3-Ylamine	20.060	163	$C_{10}H_{19}N$	1.521
11	3-Decyn-2-Ol	20.165	154	$C_{10}H_{18}O$	0.860
12	Bicyclo [3.1.0]Hexan-3-Ol,4-Methyl-1-(1-Methylethyl)-	20.220	224	$C_{15}H_{28}O$	1.360

Table 2: Larvicidal activity of methanolic extract of *P. pinnata* leaf extract against third instars of *Culex quinquefasciatus* and *Aedes aegypti* after 24 hours

Animal Species	Conc (mg/L)	Mortality (%) ± SE	LC_{50} (mg/L)	LC_{90} (mg/L)	95% fiducial limit for LC_{50} (mg/L)		χ^2
					LCL	UCL	
Cx. quinquefasciatus	0.0	0.0 ± 0.0	75.640	363.214	66.451	85.734	13.20
	25.0	15 ± 2.24					
	50.0	34 ± 4.30					
	100.0	56 ± 4.00					
	200.0	81 ± 2.92					
	400.0	100 ± 0.0					
Ae. aegypti	0.0	0.0 ± 0.0	101.364	434.670	87.848	117.036	9.28
	25.0	11 ± 2.92					
	50.0	28 ± 4.64					
	100.0	48 ± 5.39					
	200.0	71 ± 5.57					
	400.0	90 ± 2.74					

LC_{50}, lethal concentration that kills 50% of the exposed larvae; LC_{90}, lethal concentration that kills 90% of the exposed larvae; $\chi 2$, chi-square test; LCL lower confidence limit, UCL upper confidence limit.

Table 3: Pupicidal activity of methanolic extract of *P. pinnata* leaf extract against third instars of *Culex quinquefasciatus* and *Aedes aegypti* after 24 hours

Animal species	Conc (mg/L)	Mortality (%) ± SD	LC_{50} (mg/L)	LC_{90} (mg/L)	95% fiducial limit for LC_{50} (mg/L)		χ^2
					LCL	UCL	
Cx. quinquefasciatus	0.0	0.0 ± 0.00	134.56	431.31	67.58	175.82	12.41
	25.0	0.0 ± 0.00					
	50.0	14.00±10.12					
	100.0	32.00±14.31					
	200.0	61.07±13.81					
	400.0	79.00±13.38					
Ae. aegypti	0.0	0.0±0.0	185.59	705.00	161.16	223.82	7.20
	25.0	0.00±0.00					
	50.0	11.00±7.50					
	100.0	25.00±7.50					
	200.0	35.00±7.50					
	400.0	68.00±14.31					

LC_{50}, lethal concentration that kills 50% of the exposed larvae; LC_{90}, lethal concentration that kills 90% of the exposed larvae; $\chi 2$, chi-square test; LCL lower confidence limit, UCL upper confidence limit.

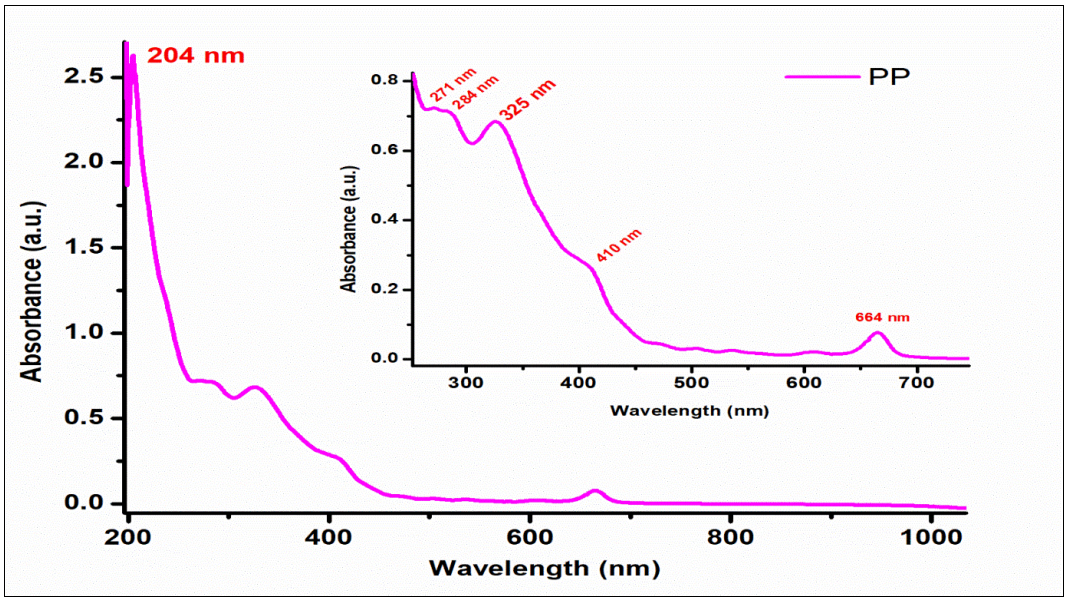

Fig 1: UV–Visible spectra *of Pongamia pinnata* leaf extract

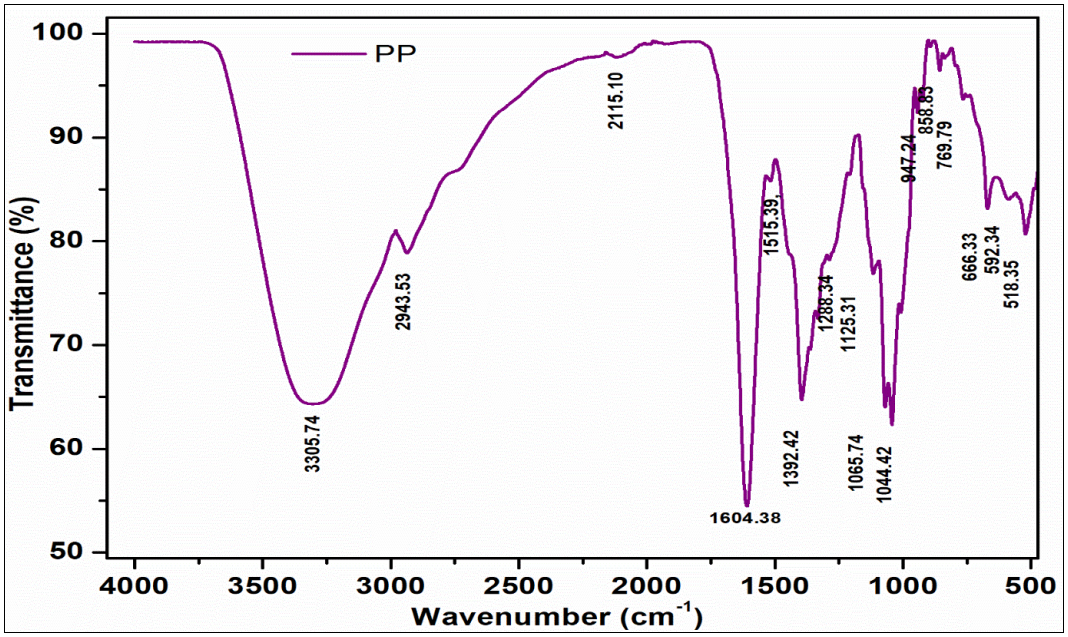

Fig 2: FTIR spectra of *Pongamia pinnata* leaf extract

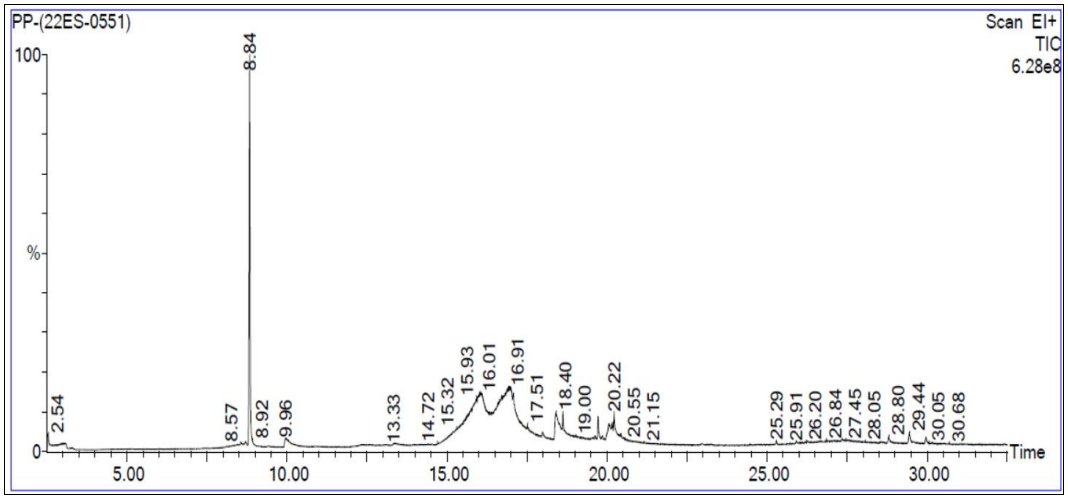

Fig 3: GC-MS analysis of *Pongamia pinnata* leaf extract

Conclusion

In summary, GC-MS analysis of methanol extracts from *P. pinnata* identified 13 major phytochemicals. This study indicates that the leaf extract of *P. pinnata* is rich in bioactive compounds, which may be promising as natural insecticides for controlling blood-feeding pests such as mosquitoes. Furthermore, pongam leaves may have potential therapeutic effects in conditions such as psoriasis, arthritis, and bacterial infections. In conclusion, this study recommends *P. pinnata* leaf extract as a biopesticide for controlling mosquitoes.

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