International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 02-05-2026, Accepted: 02-06-2026, Published: 17-06-2026
Volume 11, Issue 2, 2026, Page No. 432-438

Allelopathic potential and GC-MS analysis of bioactive compounds from *Pongamia pinnata* (L.) Pierre

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DOI: https://doi.org/10.66856/ijbs.2026.11.2.11150

Abstract
The increasing concerns regarding environmental pollution, herbicide-resistant weeds, and health hazards associated with synthetic agrochemicals have intensified the search for eco-friendly alternatives for sustainable agriculture. Allelopathy, a biological phenomenon involving the release of bioactive compounds by plants, offers considerable potential for the development of natural herbicides and biopesticides. The dried and powdered plant material was subjected to Soxhlet extraction using 70% aqueous ethanol. The obtained extract was analyzed by Gas Chromatography-Mass Spectrometry (GC-MS) and the compounds were identified by comparing their mass spectra with NIST-08 and WILEY-7 library databases. GC–MS analysis revealed the presence of 37 phytochemical constituents comprising both major and minor metabolites. Among the identified compounds, 6-undecyl-5,6-dihydro-2H-pyran-2-one (16.51%) and 1-hexacosanol (14.63%) were the predominant constituents. Other important bioactive compounds detected included neophytadiene, 2,4-di-tert-butylphenol, phytol, tetradecanoic acid, n-hexadecanoic acid and squalene. These metabolites are reported to possess insecticidal, antimicrobial, antifungal, larvicidal, nematicidal, repellent and allelopathic properties. The occurrence of these compounds supports the pesticidal and growth-inhibitory potential of *P. pinnata* and highlights its value as a source of natural bioactive agents. The findings demonstrate that *Pongamia pinnata* is rich in phytochemicals with promising biopesticidal and allelopathic activities. The identified compounds may contribute to the development of environmentally safe botanical pesticides and bioherbicides, providing sustainable alternatives to synthetic agrochemicals for integrated pest and weed management programs.

Keywords: *Pongamia pinnata*, allelopathy, GC–MS, phytochemicals, biopesticide, botanical pesticide

Introduction
Allelopathy is defined as any direct or indirect positive or negative effect of one plant on the other through the release of allelochemicals into the environment. It plays a significant role in natural management of ecosystems, agroecosystems and affects the growths and crop yield. Allelochemicals may be water-soluble substances that are released into the environment through leaching, root exudation, volatilization and decay of the fallen plant parts. Most allelochemicals are secondary metabolites of the plants and their production depends on the plant's genetic and environmental conditions during its growth. Higher plants release diverse allelochemicals (phenolics, alkaloids, organic acids, quinines, terpenoids and flavonoids) into the environment. Allelopathic compounds may regulate the plant growth and development, involving photosynthesis, respiration, transpiration, hormonal activity, antioxidant enzymes, membrane permeability, mitotic activity in roots, ion uptake and even in molecular basis of protein and nucleic acid synthesis. The most frequent reported gross morphological allelopathic effects on plants occur early in the life cycle, influencing the seed germination, coleoptile elongation and shoot and root development (Elqahtani *et al*., 2017) [5].

Some plants such as *Sorghum bicolor* (Cheema and Khaliq, 2000) [2], *Medicago polymorpha*, *Oryza sativa*, *Chenopodium album*, *Amaranthus retroflexus* and *Cynodon dactylon* have the allelopathic potential to suppress the growth of other plants (Rasmussen and Einhellig, 1977) [18]. Allelochemicals that suppress or eliminate the plant species have received special attention due to their agricultural potential as selective natural herbicides. Allelopathic crop species possess strong potential to develop cultivars that are highly weed-suppressive. Furthermore, allelochemicals have the potential to create eco-friendly products for weed management. Because synthetic herbicides pose worldwide risks to health and the environment, the need for alternative methods for weed control has become necessary. The widespread use of synthetic herbicides has resulted in the increasing incidence of weeds' resistance to them and in environmental pollution and associated health problems. Therefore, allelopathy could be regarded as a mean to combat the evolution of herbicide resistance in weeds as well as health and environmental hazards of synthetic herbicides (Heidarzade *et al*., 2010).

Pongamia pinnata (L.) Pierre. is a medium-sized evergreen or semi-deciduous tree belonging to the family Fabaceae. It typically attains a height of 15–25m and possesses a straight or slightly twisted trunk measuring 50–80cm or more in diameter. The tree develops a broad crown with spreading or drooping branches. The bark is greyish-brown to dark brown, smooth in young trees and becoming slightly fissured with age. Young branchlets are glabrous and bear distinct stipule scars. The leaves are alternate, imparipinnate and borne on long slender petioles. Young foliage is pinkish-red, gradually turning glossy dark green on the upper surface and pale green beneath with prominent venation. Each leaf consists of 5–9 leaflets, usually arranged in opposite pairs. The leaflets are ovate to elliptic, short-petioled, smooth, entire-margined and slightly thickened along the edges. The apex is generally acute to acuminate, while the base is rounded to cuneate. The inflorescence is an axillary raceme measuring 6–27cm in length and bears clusters of highly fragrant flowers. The calyx is campanulate, about 4–5mm long, truncate and finely pubescent. Flower clusters are slender, drooping, and generally shorter than the leaves. Flowers occur in groups of two to four, are short-stalked, papilionaceous in structure and approximately 15–18mm long. The corolla varies from white to pink with purple markings on the inner surface and brownish veins externally. The standard petal is obovate, 1–2 cm long, provided with basal auricles and often exhibits a green central blotch along with sparse silky hairs on the dorsal side. The wings are elongated and partially adherent to the keel (Nadeem *et al*., 2016) [15].

Pongamia pinnata has attracted considerable attention due to its potent insecticidal and pesticidal properties. Various plant parts, particularly the leaves, seeds, and seed oil, contain bioactive compounds that exhibit significant toxicity against a wide range of insect pests. Ethanolic, methanolic, and petroleum ether extracts of the plant have demonstrated effective insecticidal activity against economically important pests such as *Spodoptera litura* Fabricius, *Tribolium castaneum*, and *Pediculus humanus capitis* (Deshmukhe *et al*., 2009; Kumar *et al*., 2006; Samuel *et al*., 2009) [4, 8, 21]. Reena Singh (2007a, 2007b) investigated the biological effects of different seed extract fractions on *Earias vitella*. Among the bioactive constituents of *Pongamia pinnata*, karanjin has been identified as a major compound responsible for its pesticidal activity. Karanjin demonstrated pronounced termiticidal effects against *Odontotermes obesus* (Rambur), causing 100% mortality within six hours of treatment. The LC_{50} value was reported to be 0.071 $g\ ml^{-1}$ after 24 hours at a 95% confidence limit, indicating its high toxicity and potential utility as an environmentally friendly botanical insecticide. These findings highlight the promise of *Pongamia pinnata* as a valuable source of natural pest management agents for sustainable agriculture.

Material and methods

Plant sample collection and preparation

Plant material of *Pongamia pinnata* was collected from the Mirajgaon village of the Karjat Tehsil. Fresh, disease-free and undamaged stems were selected, separated and thoroughly rinsed with sterile distilled water to remove surface debris. The shade dried samples are powdered using the grinder and exact 25g of powder is used for the Soxhlet extraction.

Soxhlet Extraction (SE)

Soxhlet extraction was carried out using 70% aqueous ethanol solvent, for each extraction, 25 g of powdered material of *Pongamia pinnata* was placed in a Soxhlet apparatus along with 250ml of the 70% ethanol. The extraction process was performed at 68 °C and conducted for 6 to 8hrs. (Kledecka *et al*., 2022) [10]. This procedure provided samples and stored in an airtight container at 4°C for further analysis.

GCMS Analysis

Phyto-components were identified using GC–MS detection system as, however with modification, whereby portion of the extract was analysed directly by headspace sampling. GC–MS analysis was accomplished using an Agilent 7890A GC system set up with 5975C VL MSD. Capillary column used was DB-5MS (30×0.25 mm, film thickness of 0.25 µm). Temperature program was set as follows: initial temperature 50°C held for 1 min, 5°C per min to 100°C, 9°C per min to 200°C held for 7.89 min, and the total run time was 48 min. The flow rate of helium as a carrier gas was 0.811851 mL/ min. MS system was performed in electron ionization (EI) mode with Selected Ion Monitoring (SIM). The ion source temperature and quadruple temperature were set at 230°C and 150°C, respectively.

Identification of components

Interpretation on mass spectrum of GC-MS was done using the database of National Institute of standard and Technology NIST-08 LIB. (Mc-Lafferly, 1989) and WILEY-7 LIB. (Stein, 1990) library sources were used for matching the identified components from the plant material having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained (Singariya *et al*., 2012).

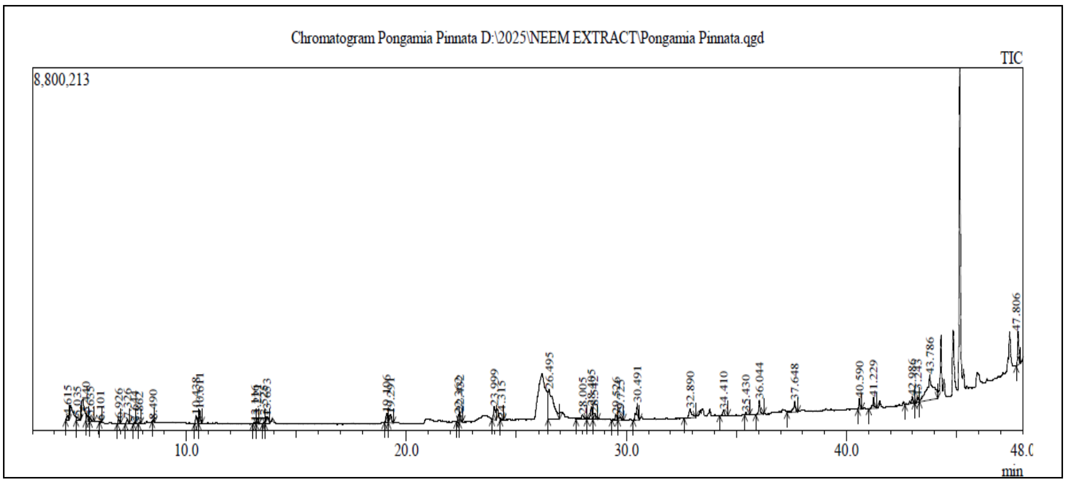
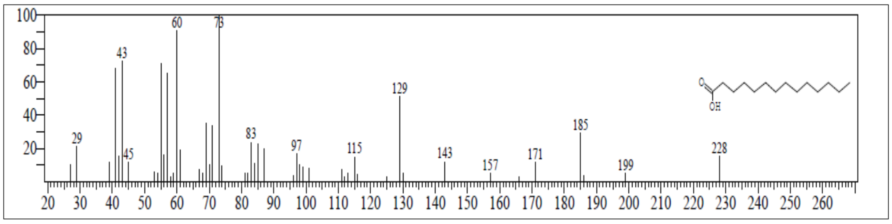


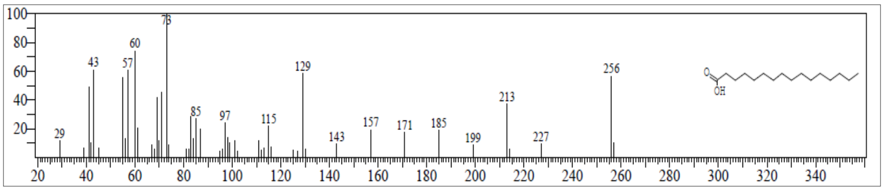
Fig 1: GC-MS chromatogram for whole plant ethanolic extract of *Pongamia pinnata*

Table 1: GC-MS spectral analysis of ethanolic extract of *Pongamia pinnata*

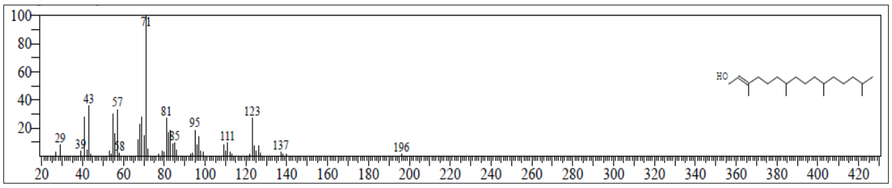
Peak	RT (min)	Name of the compound	Molecular Formula	Molecular Weight	Area%
1	4.615	Benzyloxyamine, TMS derivative	C10H17NOSi	195	0.81
2	5.035	1-Butanol, 3-methyl-, acetate	C7H14O2	130	0.72
3	5.440	p-Xylene	C8H10	106	1.57
4	6.101	Fructofuranose, 2,6-anhydro-1,3,4-tri-O-methyl	C9H16O5	204	0.09
5	7.326	Butane, 1,1-diethoxy-3-methyl-	C9H20O2	160	0.68
6	7.644	Ethane, 1,1,1-triethoxy-	C8H18O3	162	0.15
7	7.862	Decene	C10H20	140	0.09
8	8.490	Hexanoic acid, ethyl ester	C16H30O2	254	0.14
9	10.611	Propane, 1,1,3-triethoxy-	C9H20O3	176	1.59
10	13.221	Neophytadiene	C20H38	278	0.24
11	13.525	Naphthalene	C10H8	128	0.21
12	13.653	1H-Azepine, hexahydro-1-nitroso-	C6H12N2O	128	2.16
13	19.106	1-Pentadecene	C15H30	210	1.69
14	19.291	1-Decanol, 2,2-dimethyl	C12H26O	186	1.97
15	22.432	2,4-Di-tert-butylphenol	C14H22O	206	1.11
16	23.999	n-Pentadecanol	C15H32O	228	2.64
17	24.315	Diethyl Phthalate	C12H14O4	222	0.21
18	26.495	6-Undecyl-5,6-dihydro-2H-pyran-2-one	C16H28O2	252	16.51
19	28.005	Tetradecanoic acid	C14H28O2	228	1.94
20	28.405	1-Nonadecene	C19H38 CAS	266	2.61
21	28.542	Heptadecane	C17H36	240	1.39
22	29.526	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C20H40O	296	0.94
23	29.725	2-Pentadecanone, 6,10,14-trimethyl-	C18H36O	268	0.75
24	30.491	1,2-Benzene dicarboxylic acid, bis(2-methylpropyl) ester	C16H22O4	278	3.24
25	32.890	n-Hexadecanoic acid	C16H32O2	256	2.95
26	34.410	Cyclic octaatomic sulfur	S8	256	1.08
27	35.430	n-Nonadecanol-1	C19H40O	284	0.33
28	36.044	Phytol	C20H40O	296	2.17
29	37.648	Eicosane	C20H42	282	2.59
30	40.590	4,8,12,16-Tetramethylheptadecan-4-olide	C21H40O2	324	1.27
31	41.229	Dotriacontane	C32H66	450	1.76
32	42.986	Hexadecanoic acid, n.-octyl ester	C24H48O2	368	1.21
33	43.243	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	C19H38O4	330	0.79
34	43.786	Bis(2-ethylhexyl) phthalate	C24H38O4	390	16.62
35	47.806	Squalene	C30H50	410	3.96
36	48.844	1-Hexacosanol	C26H54O	382	14.63
37	49.777	δ-Tocopherol	C27H46O2	402	3.65



Tetradecanoic acid



n-Hexadecanoic acid



2-Pentadecanone, 6, 10, 14-trimethyl

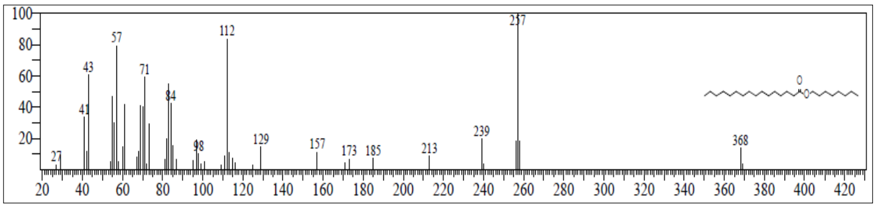
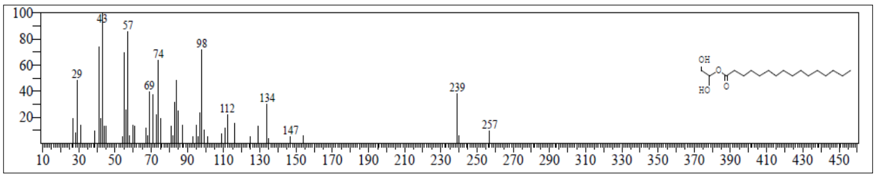
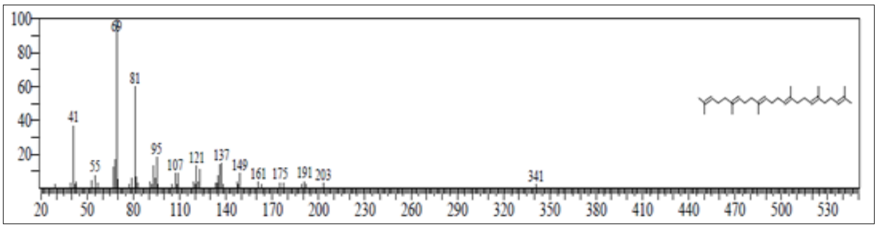


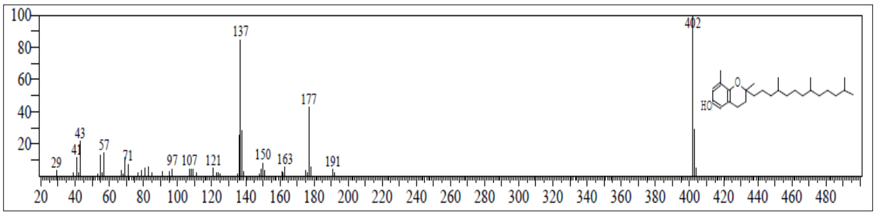
Fig 2: Mass spectra of reported biopesticidal activity compound from *Pongamia pinnata*



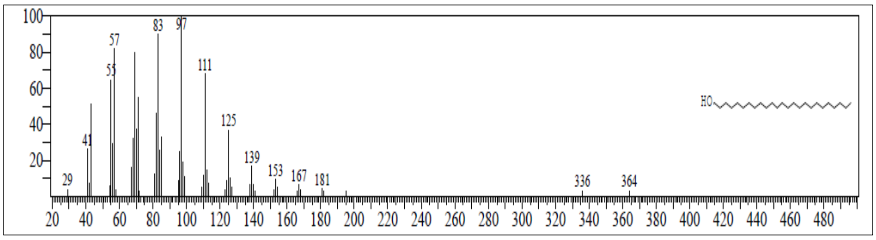
Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester



Squalene



δ-Tocopherol



1-Hexacosanol

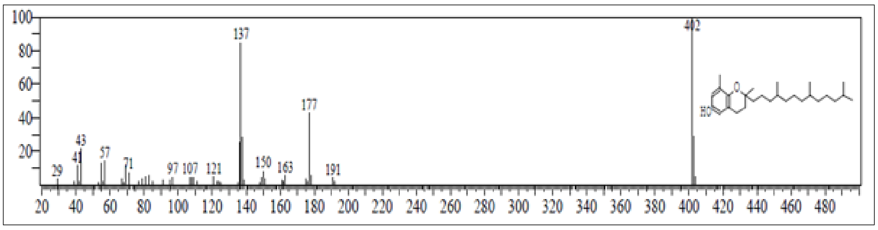


Fig 3: Mass spectra of reported biopesticidal activity compound from ethanolic extract of *Pongamia pinnata*

Results and Discussion

Pongamia pinnata has potent insecticidal and pesticidal properties. The analysis and extraction of plant material play an important role in the development, standardization and quality control of biopesticidal formulations. Hence the present study was undertaken to find out the bioactive compounds present in the ethanolic extract of *Pongamia pinnata* by using Gas chromatography and Mass spectroscopy. The active principles with their retention time (RT), molecular formula, molecular weight (MW), concentration (peak area %) are presented in Table 1 and Fig. 1 which shows the presence of 37 bioactive phytochemical compounds in the ethanolic extract of *Pongamia pinnata*. The mass spectra of identified compounds from *Pongamia pinnata* were presented in Fig. 2 and Fig. 3.

Totally 37 bio-active constituents were identified in the present study from the ethanolic extracts of the whole plant of *Pongamia pinnata* which including both major and minor constituents. The GC–MS analysis revealed the presence of several bioactive metabolites with potential biopesticidal properties, including neophytadiene, 2, 4-di-tert-butylphenol, phytol, tetradecanoic acid, n-hexadecanoic acid, squalene and 6-undecyl-5,6-dihydro-2H-pyran-2-one. Among these, 6-undecyl-5, 6-dihydro-2H-pyran-2-one (16.51%) and 1-hexacosanol (14.63%) were the predominant constituents. These compounds have been reported to possess insecticidal, antimicrobial, antifungal, larvicidal, repellent and allelopathic activities, suggesting that they may contribute significantly to the observed biopesticidal and bioherbicidal potential of the plant extract.

The major compounds identified through GC–MS analysis, including neophytadiene, 2,4-di-tert-butylphenol, phytol, n-hexadecanoic acid, squalene, 1-hexacosanol and pyranone derivatives, have previously been reported to possess insecticidal, antimicrobial, larvicidal, nematicidal, antifungal and allelopathic activities. The occurrence of these bioactive metabolites supports the potential use of the studied plant extract as a botanical biopesticide and eco-friendly pest management agent. Among the identified constituents, 6-undecyl-5,6-dihydro-2H-pyran-2-one (16.51%) was the most abundant compound. Pyranone derivatives have been reported to possess antifungal, antimicrobial, insecticidal, and phytotoxic activities, making them promising candidates for the development of bioherbicidal formulations (Mishra *et al*., 2019). The high concentration of this compound may contribute significantly to the allelopathic and pesticidal properties of *P. pinnata*.

The second major constituent, 1-hexacosanol (14.63%), is a long-chain fatty alcohol known to possess antimicrobial and insect-deterrent properties. Long-chain alcohols have been reported to influence insect feeding behavior and may contribute to plant defense mechanisms against herbivorous pests (Jiang *et al*., 2013). Its abundance in the extract suggests a possible role in the biopesticidal activity of the plant.

Neophytadiene, another important compound detected in the extract, has been reported to exhibit insecticidal, antimicrobial, anti-inflammatory, and antioxidant activities (Swamy *et al*., 2017). Similarly, phytol, a diterpene alcohol, possesses strong antimicrobial, larvicidal, and allelopathic properties and has been identified in several medicinal plants with pesticidal potential (de Moraes *et al*., 2014) [3]. The presence of phytol further strengthens the possibility of utilizing *P. pinnata* extracts for eco-friendly pest control.

The phenolic compound 2,4-di-tert-butylphenol identified in the extract has attracted considerable attention due to its broad-spectrum antimicrobial, antioxidant, antifungal, and phytotoxic activities. Previous studies have reported that this compound can inhibit seed germination and seedling growth in several plant species, indicating a significant allelopathic role (Kanchiswamy *et al*., 2015) [9]. Therefore, its occurrence in *P. pinnata* may be associated with the plant's ability to suppress competing vegetation.

Fatty acids such as tetradecanoic acid and n-hexadecanoic acid (palmitic acid) were also identified in the present study. These compounds have been reported to exhibit insecticidal, nematicidal, antimicrobial, and growth-regulating activities (Rahuman *et al*., 2000). Several researchers have suggested that fatty acids contribute to the pesticidal effectiveness of plant extracts by disrupting cell membranes and interfering with insect metabolism.

Squalene, another bioactive compound detected in the extract, is recognized for its antioxidant and antimicrobial properties. Although primarily known as a precursor in sterol biosynthesis, squalene has been reported to enhance the biological activity of plant extracts and may contribute synergistically to their pesticidal effects (Rao *et al*., 2017) [17].

The presence of these diverse bioactive metabolites supports previous reports on the pesticidal efficacy of *Pongamia pinnata*. Karanjin, a well-known furanoflavonoid from *P. pinnata*, has been reported to exhibit strong insecticidal and termiticidal activities against several agricultural pests (Reena *et al*., 2007a; Reenaet a., 2007b). Although karanjin was not detected in the present stem extract, the occurrence of several other biologically active compounds suggests that different plant parts may possess distinct phytochemical profiles contributing to overall pesticidal activity. Overall, the GC–MS profile obtained in the present study demonstrates that *Pongamia pinnata* contains a complex mixture of phytochemicals with potential allelopathic, insecticidal, antimicrobial, and bioherbicidal activities.

These findings provide scientific evidence supporting the use of *P. pinnata* as a source of environmentally safe biopesticides and natural weed management agents. Further laboratory and field studies are necessary to evaluate the phytotoxic effects of individual compounds and to determine their efficacy against specific weeds and insect pests under agricultural conditions.

Table 2: Compounds with reported biopesticidal potential

Sr. No.	Compound	Reported biopesticidal activity	Reference
1.	Neophytadiene	Insecticidal, antimicrobial, larvicidal, and repellent activities against agricultural pests.	Mohan *et al*. (2016) [14]
2.	2,4-Di-tert-butylphenol	Strong antimicrobial, antifungal, nematicidal, insecticidal and allelopathic activities; reported as a biocontrol metabolite.	Vasu *et al*. (2025) [25]
3.	Tetradecanoic acid (Myristic acid)	Insecticidal and antimicrobial effects; contributes to pest suppression.	Tyagi & Agarwal (2017) [24]
4.	n-Hexadecanoic acid (Palmitic acid)	Insecticidal, larvicidal, nematicidal and herbicidal activities reported in several plant extracts.	Tyagi & Agarwal (2017) [24]
5.	Phytol	Well-documented insecticidal, larvicidal, antifeedant, mosquito-control and antimicrobial activity.	Almeida *et al*. (2025)
6.	4,8,12,16-Tetra-methylheptadecan-4-olide	Insect-repellent and antimicrobial properties; precursor of phytol-derived bioactive compounds.	Shahin *et al*. (2022) [22]
7.	2-Pentadecanone, 6,10,14-trimethyl-	Reported as an insect attractant/repellent and defense-related metabolite in plants.	Kordali *et al*. (2008) [11]
8.	Hexadecanoic acid, n.-octyl	Exhibits antimicrobial and insecticidal potential.	Tyagi & Agarwal

	ester		(2017) [24]
9.	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	Antimicrobial and pesticidal activities reported in botanical extracts.	Luo *et al*. (2024) [12]
10.	Squalene	Repellent, antioxidant and antimicrobial activities; may contribute indirectly to pest resistance.	Lozano-Grande *et al*. (2018) [13]
11.	δ-Tocopherol	Antioxidant and defensive metabolite involved in plant resistance against insect and pathogen attack.	Falk & Munné-Bosch (2010) [6]
12.	1-Hexacosanol	Long-chain alcohol associated with insect deterrence, wax-based plant defense and antimicrobial activity.	Singariya *et al*. (2015)
13.	6-Undecyl-5,6-dihydro-2H-pyran-2-one	Pyranone derivatives are known for antimicrobial, antifungal, insecticidal and allelopathic properties; likely one of the major bioactive constituents.	Vinale *et al*. (2008) [26]

Conclusion

The present study demonstrated that the ethanolic extract of *Pongamia pinnata* contains a rich diversity of bioactive phytochemical constituents with potential allelopathic and biopesticidal activities. GC–MS analysis identified a total of 42 compounds, including major constituents such as 6-undecyl-5,6-dihydro-2H-pyran-2-one and 1-hexacosanol, along with other biologically important metabolites such as neophytadiene, phytol, 2,4-di-tert-butylphenol, n-hexadecanoic acid, tetradecanoic acid, and squalene. These compounds have been reported to possess insecticidal, antimicrobial, antifungal, larvicidal, nematicidal, repellent, and allelopathic properties. The occurrence of these phytochemicals suggests that *Pongamia pinnata* may serve as an effective natural source of bioactive compounds for sustainable pest and weed management. The presence of allelopathically active metabolites further indicates its potential application in the development of eco-friendly bioherbicides capable of suppressing undesirable plant species while minimizing environmental contamination.
Moreover, the identification of several pesticidal compounds supports the use of *P. pinnata* as a promising botanical pesticide for integrated pest management programs. Overall, the findings provide scientific evidence supporting the agricultural value of *Pongamia pinnata* and highlight its potential as a renewable source of natural agrochemicals. Further studies involving bioassays, isolation of active constituents, and field-level evaluations are required to validate the allelopathic and biopesticidal efficacy of the identified compounds and to facilitate their commercial application in sustainable agriculture.

Acknowledgement

The first author is thankful to the Chhatrapati Shahu Maharaj Research, Training and Human Development Institute (SARTHI), Pune, for providing financial assistance (Ph.D. Fellowship). I am very grateful to Principal Tuljaram Chaturchand College of Arts, Science and Commerce, Baramati-413102, affiliated to Savitribai Phule Pune University, Pune, (MS), India, for providing research facilities, guidance and constant inspiration.

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