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Identification of Bioactive Antifungal Metabolites from *Melia azadirach* L. via GC–MS Profiling for Sustainable Management of Papaya Fungal Diseases

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ABSTRACT

This study aims to identify antifungal phytochemicals through GC-MS analysis to evaluate their potential applications from *Melia azedarach* L. leaf extract (ethanolic). The phytochemical profile of the extract was analyzed using Gas Chromatography-Mass Spectrometry (GC/MS). The presence of various bioactive constituents, including alkaloids, flavonoids, tannins, saponins, glycosides, and terpenoids, was identified. Six key compounds, including Decane, Squalene, Eicosane, a Benzenepropanoic acid derivative, Phytol, and Hexadecanoic acid ester, were identified. These compounds are documented in the literature for their capacity to disrupt fungal membranes, inhibit spore germination, and function as antioxidants, thereby enhancing the antifungal potential of *M. azadirach*. The broad-spectrum bioefficacy of this plant not only supports its traditional medicinal applications but also underscores its potential in sustainable disease management strategies.

Introduction

Carica papaya L., commonly known as papaya, is a remarkable tropical fruit tree belonging to the Caricaceae family. It boasts an impressive nutritional profile, serving as a rich source of bioactive compounds such as carpaines, benzyl isothiocyanate (BITC), benzyl glucosinolates, and the enzyme papain, which can be found in its seeds, leaves, and succulent fruits (Zhou *et al.,* 2021; Koul *et al.,* 2022). The fruits have essential vitamins A, B, C, E, and K, along with vital nutrients including folate, pantothenic acid, zeaxanthin, lycopene, lutein, magnesium, copper, calcium, and potassium (Pinnamaneni, 2017). Its abundant fiber, antioxidants, and vitamin C are believed to contribute to cardiovascular health by lowering cholesterol levels in the arteries. Furthermore, papaya is noted for its protective effects against arthritis and its potential to reduce visible signs of aging (Kong *et al.,* 2021).

The commercial production of papaya faces significant challenges due to various fungal diseases such as anthracnose, powdery mildew, root rot, and stem end rot. Among these challenges, anthracnose, caused by

Colletotrichum gloeosporioides, is particularly severe, affecting ripened fruits and leading to substantial postharvest losses (Ventura and Tatagiba, 2004; Rawal, 2010; Getnet *et al.,* 2024). Synthetic fungicides, while effective against plant pathogens, pose significant environmental and health risks.

Their repeated use has led to resistance development, environmental pollution, and residual toxicity (Islam *et al.,* 2024). Fungicides can accumulate in soil, water, and air, endangering non-target organisms and ecosystems (Bozdogan, 2014). In response to this pathogen, researchers have investigated a variety of control methods, including botanical extracts and biocontrol agents (Choudhury *et al.,* 2018; Ons *et al.,* 2020). Additionally, antagonistic microorganisms present a potential eco-friendly alternative to conventional chemical treatments for disease management (Sharma *et al.,* 2017). Understanding these diseases and implementing effective management strategies is essential for enhancing papaya cultivation and minimizing economic losses (Tan *et al.,* 2022).

Melia azedarach L., belonging to the Meliaceae family, exhibits substantial potential as a biopesticide. This plant is known to contain compounds with notable antifungal and medicinal properties (Al-Rubae, 2009; Ntalli and Caboni, 2014). Extracts derived from various parts of *M. azedarach* have shown effectiveness against phytopathogenic fungi (Carpinella *et al.,* 2003). Current research is focused on developing more efficient and cost-effective methods for producing botanical pesticides tailored for farmers (Bhandari *et al.,* 2021). This study aims to identify antifungal phytochemicals through GC–MS analysis to evaluate their potential applications from *Melia azedarach* L.

Materials and Methods

Plant Collection, Identification, and Preparation of Plant Material

Fresh leaves of *Melia azadirach* L. were collected from the college campus and subsequently identified and authenticated by Dr. Ashok R. Tuwar, Professor, Department of Botany at the Arts, Commerce, and Science College in Sonai, Tal- Newasa, Dist- Ahmednagar, Maharashtra, India. A total of thirty grams (30 g) of powdered leaf material was extracted using a Soxhlet apparatus. The extraction process employed 250 mL of ethanol, maintained at a temperature range of 60–65 °C for 24 hours. The resulting extract was then concentrated under reduced pressure using a rotary vacuum evaporator, yielding a viscous, semisolid mass. This semi-dry ethanolic crude extract was subsequently utilized for phytochemical profiling.

Preliminary Phytochemical Screening

Qualitative phytochemical analysis was conducted using standard methodologies as outlined by Pandey and Tripathi (2014); Egbuna *et al.,* (2018); Balamurugan *et al.,* (2019); Nagori *et al.,* (2025). This analysis aimed to identify the presence of various bioactive constituents, including alkaloids, flavonoids, tannins, saponins, glycosides, and terpenoids.

Gas Chromatography-Mass Spectrometry (GC–MS) Analysis

Ethanolic crude extract of *Melia azedarach* L. was analyzed using a Shimadzu GC-MS-TQ8050 system with an AOC-20i+s autosampler at Central Instrumentation Facility, Shivaji University, Kolhapur, India.. Separation was achieved on an HP-5 MS capillary column (30 m × 0.25 mm, 0.25 µm film). Injection was performed in split mode (1:50), 1 µL volume, at 250 °C. The oven temperature was programmed from 50 °C (2 min), raised to 180 °C at 5 °C/min (2 min hold), then to 250 °C and 260 °C with 2 min holds at each step. Helium was used as the carrier gas in linear velocity mode (1.01 mL/min; 36.5 cm/s). Total flow was 54.6 mL/min with a purge flow of 3.0 mL/min and pressure at 54.4 kPa. Autosampler settings included high plunger speed, 5 rinses pre/post-injection, 8 µL wash volume, and 0.3 s dwell time. MS conditions: ion source at 200 °C, interface at 270 °C, Q3 scan mode (45–500 m/z), scan speed 1666 amu/s, solvent cut-off at 4 min, detector gain at 1.29 kV.

Identification of Compounds

Compounds were identified based on their retention times and mass spectra using the National Institute of Standards and Technology (NIST) Mass Spectral Library (NIST II), which contains over 62,000 known compounds. The relative abundance of each compound was calculated by normalizing the peak area.

Results and Discussion

Preliminary Phytochemical Screening of Melia azedarach L.

The qualitative phytochemical analysis of *Melia azedarach* L. leaf extract (ethanolic) identified several bioactive compounds (Table 1). The presence of alkaloids and flavonoids suggests a strong antimicrobial and antifungal potential. Meanwhile, the detection of tannins, saponins, and steroids indicates moderate activity, contributing to membrane disruption and astringency. Notably, the abundance of terpenoids aligns with their well-documented potent antifungal properties. The presence of phenols further supports the extract's antifungal and antioxidant efficacy.

However, the absence of glycosides suggests a limited role in bioactivity. These results affirm that the extract contains key antifungal agents that are valuable for sustainable disease management in papaya cultivation. Similar observations were noted by Jafari *et al.,* (2013); Deb *et al.,* (2018) and Sultana *et al.,* (2013).

GC-MS Analysis of *Melia azedarach* L.

The GC–MS analysis of the ethanolic leaf extract of *Melia azedarach* revealed 15 major peaks, indicating a diverse array of phytochemical constituents (Fig.1). Compound identification was performed using the NIST library, leading to the detection of several bioactive molecules with established biological properties (Table.2). Notably, six key compounds exhibited antifungal activity: Decane, Squalene, Eicosane, a Benzenepropanoic acid derivative, Phytol, and Hexadecanoic acid ester. The chemical structures of these six significant antifungal compounds identified through GC–MS profiling of the ethanolic extract of *Melia azedarach* are illustrated in Fig. 2. These compounds are documented in the literature for their capacity to disrupt fungal membranes, inhibit spore germination, and function as antioxidants, thereby enhancing the antifungal potential of the extract. The presence of additional antimicrobial, antioxidant, and insecticidal compounds further underscores the broad-spectrum bioefficacy of *M. azedarach,* affirming its application in sustainable fungal disease management.

Numerous studies have identified a range of compounds in *M. azedarach*, including fatty acid derivatives, steroids, terpenes, and phenolic compounds. Among the notable bioactive compounds are hexadecanoic acid, phytol, and squalene, all of which exhibit antifungal, antimicrobial, and antioxidant properties (Habib *et al.,* 2017; Khan and Javaid, 2021; Bhat *et al.,* 2024; Uka *et al.,* 2022). Extracts from this plant have proven effective against pathogenic fungi such as *Alternaria alternata* and *Neoscytalidium dimidiatum* (Jawad and Garaawi, 2022). In addition to its antifungal properties, *M. azedarach* has demonstrated potential in the treatment of various ailments, showcasing antiviral, antidiabetic, and cytotoxic effects. The extensive phytochemical profile and broad-spectrum bioactivities of *M. azedarach* not only support its traditional medicinal applications but also underscore its potential in sustainable disease management strategies (Jawad and Garaawi, 2022; Hieu *et al.,* 2023; Habib *et al.,* 2017; Duc *et al.,* 2023).

Figure.1 GC-MS chromatogram of ethanolic extract of *Melia azadirach* L.

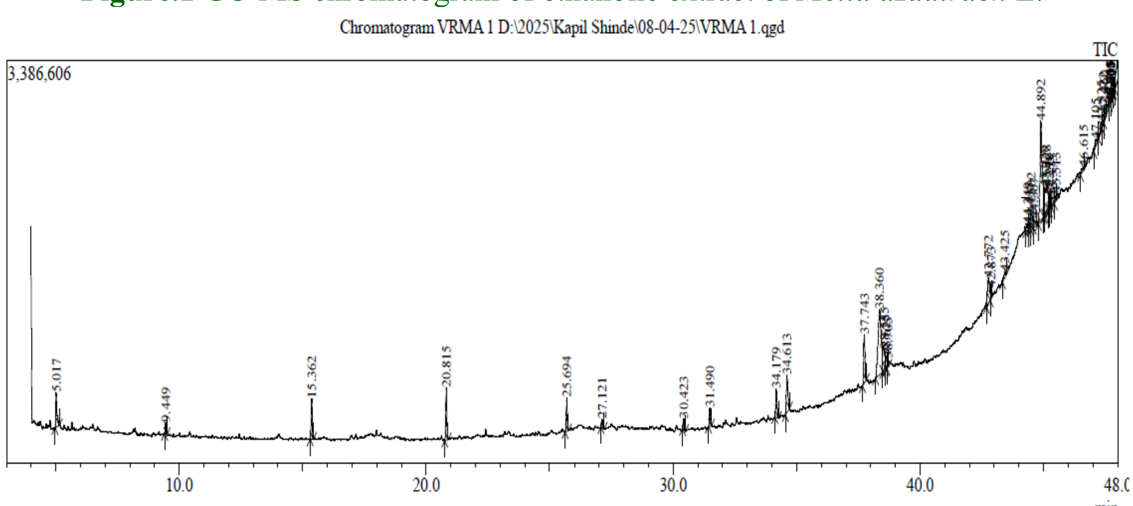


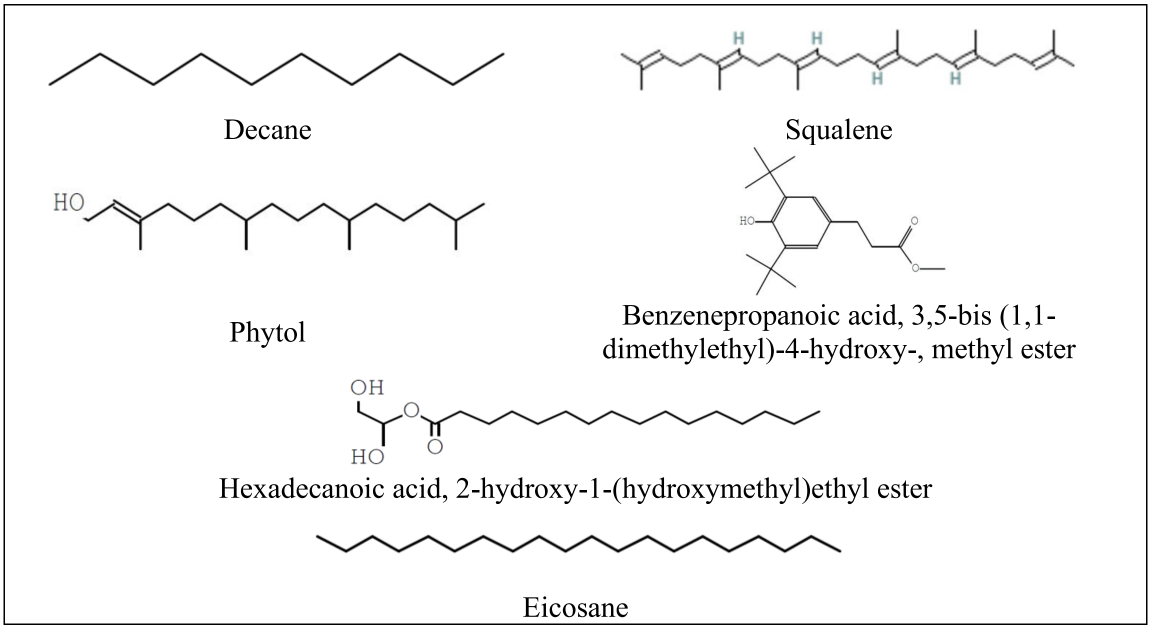
Table.1 Preliminary Phytochemical Screening of *Melia azedarach* L.

Sr. No.	Phytochemical Test	Result (+ / -)	Inference	References
1	Alkaloids (Mayer's Test)	++	Presence indicates possible antimicrobial activity	Karou *et al.*, (2006).
2	Flavonoids (Lead acetate)	++	Known for its antioxidant and antifungal properties	Rodríguez, *et al.*, (2023).
3	Tannins (Ferric chloride)	+	Confers astringency and antifungal effects	Scalbert, (1991).
4	Saponins (Foam test)	+	May enhance the bioavailability of active compounds	Singh and Chaudhuri, (2018).
5	Steroids (Salkowski Test)	+	Implicated in anti-inflammatory and antifungal roles	Dembitsky, (2023).
6	Terpenoids (Chloroform test)	+++	Reported to possess antifungal activity	Haque, *et al.*, (2016).
7	Glycosides (Borntrager's)	-	Not detected in the extract	--
8	Phenols (Ferric chloride)	++	Contribute to antioxidant, antifungal activity, and antimicrobial actions	Ansari, *et al.*, (2013).

Table.2 Compound identified in GC-MS Analysis of *Melia azedarach* L.

Sr. No.	Name of Compound	Retention Time (min)	Molecular Weight	Molecular Formula	Biological Activity*	References
1	Propane, 1-methoxy-2-methyl-	5.02	88	$C_5H_{12}O$	Antimicrobial	Al-Omar and Amr, (2010
2	Decane	9.45	142	$C_{10}H_{22}$	Antifungal, antibacterial	Khan, and Javaid, (2021).
3	Dodecane	15.36	170	$C_{12}H_{26}$	Antimicrobial, insecticidal	Padma *et al.*, (2019).
4	Tetradecane	20.82	198	$C_{14}H_{30}$	Antibacterial	Nasr *et al.* (2022)
5	Hexadecane	25.69	226	$C_{16}H_{34}$	--	---
6	Squalene	27.121	410	$C_{30}H_{50}$	Antimicrobial Antifungal	Bhat *et al.*, (2023).
7	Eicosane	30.42	282	$C_{20}H_{42}$	Antifungall	Bhat, *et al.*, (2024).
8	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	31.49	296	$C_{20}H_{40}O$	Antioxidant, antimicrobial	Ryu *et al.*, (2017).
9	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	34.18	396.52	$C_{18}H_{28}O_3$	Antioxidant, Antifungal	Bashir *et al.*, (2012).
10	l-(+)-Ascorbic acid 2,6-dihexadecanoate	34.61	652	$C_{38}H_{68}O_8$	Antioxidant, Antibacterial	Begum *et al.*, (2017).
11	Phytol	37.74	296	$C_{20}H_{40}O$	Antimicrobial, Antifungal	Lima *et al*, (2020).
12	13-Tetradecenal	38.36	210	$C_{14}H_{26}O$	--	--
13	Tetracosane	42.77	338	$C_{24}H_{50}$	Antimicrobial	Nasr *et al.* (2022)
14	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	44.89	330	$C_{19}H_{38}O_4$	Antioxidant, antifungal	Uka *et al.*, (2022).
15	Octadecanoic acid, 2,3-dihydroxypropyl ester	48.79	358	$C_{21}H_{42}O_4$	Antimicrobial	Canli *et al.*, (2023)

Figure.2 Chemical Structures of Antifungal Compounds Detected in the GC–MS Analysis of *Melia azedarach* L.



The GC–MS profiling of ethanolic leaf extract from *Melia azedarach* L. identified several potent bioactive compounds known for their antifungal properties. Among these metabolites are Phytol, Squalene, and Hexadecanoic acid ester, highlighting the plant's potential as a natural resource for managing fungal infections in papaya. These findings support the creation of eco-friendly and sustainable disease control strategies, thereby decreasing dependence on synthetic fungicides.

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Author Contributions

Varsha N. Rawade: Investigation, formal analysis, writing—original draft. Ashok R. Tuwar: Validation, methodology, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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