Phytochemical Screening and in vitro Pharmacological Evaluation of Passiflora foetida L. Leaf Extract Found in the Vicinity of Lonar Lake: A Distinctive Saline Crater Lake in India

Sharmishtha Doifode

shamadoifode@gmail.com

Balbhim College

Sunita Bhosle

Balbhim College

Research Article

Keywords: GC-MS, Phytochemicals, Lonar Lake, Passiflora foetida L, Antibacterial activity, Antifungal activity

Posted Date: November 21st, 2025

DOI: https://doi.org/10.21203/rs.3.rs-6833796/v1

Additional Declarations: No competing interests reported.

Abstract

Lonar Lake Distinctive Saline Crater Lake Located in Maharashtra's Buldhana district, India, Crater Lake is encircled by thick woodlands containing numerous plants with medicinal properties. Passiflora foetida L. commonly known as stinking passion flower has been used as traditional medicine in treating diseases such as throat infection, giddiness, liver disorder, diarrhea, tumor, nervous disorder, anxiety, sleep disorders, skin infections, hysteria and asthma. The present study was carried out to assess phytoconstituents and in vitro pharmacological evaluation of Passiflora foetida L. The Ethyl acetate and ethanol extracts of Passiflora foetida L. leaves were subjected to GC-MS analysis. The Ethyl acetate extracts yielded thirty-four phytochemical compounds, while the ethanol extracts revealed twenty-eight phytochemical compounds upon analysis. The compounds were identified by comparing their retention time and peak area with that of the literature and by interpretation of mass spectra. Using the agar disc diffusion method, antibacterial and antifungal properties were evaluated for extracts obtained using n-hexane, ethyl acetate, acetone, ethanol, methanol, and water as solvents. The phytoconstituents of Passiflora foetida L. may have other medicinal uses beyond its anti-inflammatory, antibacterial, and antioxidant properties, such as lowering the use of synthetic medications.

Introduction

Meteor impact craters like Barringer Crater in Arizona United States and the Lonar Crater in Buldhana District of Maharashtra, India are rare on Earth‘s surface [1, 2]. Lonar Lake is a salt water lake created due to the impact of massive meteorites. It is a unique saline water lake in Asia. It is situated in Buldhana district of Maharashtra (India). The Lake is surrounded by the dense forest. It preserves innumerable valuable plants with medicinal values [3]. Plants are a great source of medications, especially in traditional medicine, that can be utilized to treat a wide range of diseases [4]. About 90% of prescribed medicines are plant-based in the traditional practice of Unani, Ayurveda, Homeopathy, and Siddha in India [5].It has been observed that the therapeutic impact of medicinal plants is related to the existence of secondary metabolites, such as alkaloids, terpenoids, glycosides, phenolic and other organic compounds, which are synthesized in all parts of the plant [6]. The presence of these compounds increases the potential for antidiabetic [7], antioxidant [8], anti-inflammatory [9, 10], antimicrobial activities [11, 12], anti-urease [13] of medicinal plants in a lesser or larger capacity [14]. *Passiflora foetida* L. popularly known as striking passionflower [15] is a particularly renowned species belonging to the genus *Passiflora*, with tremendous ethnobotanical applications. Various studies conducted on *Passiflora foetida* L. have revealed extracts of the plant to possess numerous promising bioactivities such as antidiarrhoeal, antiulcerogenic, analgesic, antidepressant anti-inflammatory, anti-hypertensive, hepaprotective, anticancer, antibacterial and antinociceptive [16–21]. The *Passiflora foetida* L. Leaf extracts holds significant pharmacological value. In order to encourage industrialization and modernization, this study focuses on the GC-MS study of the phytochemicals present in the leaves and their respective fractions. Subsequently, in vitro assays will be conducted to determine the different extracts that predominantly exhibits antimicrobial activity for the first time.

Gas chromatography-mass spectrometry (GC-MS) is a system that unites the characteristics of gas-liquid chromatography and mass spectrometry to determine the different substances present in a given test sample [22, 23].

This study screened phytoconstituents present in the ethyl acetate and ethanol extracts by GC-MS analysis. The antimicrobial potency of the n-hexane, ethyl acetate, acetone, ethanol, methanol and water was evaluated using the disc-diffusion assay assessing the antimicrobial efficiency [24]. The leaves were collected from Lonar Lake area Maharashtra, India.

Materials and Methods

Sample collection

The leaf samples were collected in rainy season July 2023 from the Lonar Lake Forest geographical locality (Latitude: 19.970386 and Longitude: 76.512862), District Buldhana, Maharashtra, India, identified and samples deposited at the Department of Botany Herbarium Dr. Babasaheb Ambedkar Marathwada, University Chh.Sambhajinagar (Accession no.-01156). The plant samples were shade-dried, ground to powder, and stored at −4°C for future use.

Preparation of Extracts

5g of leaf sample powder were sequentially extracted with solvents namely n-hexane, ethyl acetate, acetone, ethanol, methanol by soxhelt apparatus [25, 26]. Water extract was prepared by maceration process, leaf powder was soaked in water for 72 Hrs with occasional shaking filtered through Whatman No.1 filter paper. Crude filtrate extract was used for preliminary phytochemical study.

Phytochemical Analysis

Preliminary Phytochemical Screening of the Extracts

Phytochemical examination of n-hexane, Ethyl acetate, Acetone, Ethanol, Methanol extracts was performed using established protocols to detect the active components present in the extracts. Alkaloids, saponins, phenols, tannins, anthraquinones, terpenoids, flavonoids, and steroids were tested [27–30].

Test for Alkaloids (Wagner's Reagent Test) [31]

Plant extracts were diluted in HCl and filtered. Wagner's reagent (which is an iodine solution in potassium iodide) was applied to filtrates. The presence of alkaloids in the extracts was confirmed by a reddish-brown precipitate.

Phenol Test (Ferric Chloride Test) [32]

The extract (50 mg) was dissolved in 5 mL of purified water. A few drops of neutral 5% ferric chloride solution were added to these. The presence of phenolic compounds was indicated by bluish green or black coloration.

Test for Saponins (Foam Test) [33]

The crude extract was mixed with 5 mL of distilled water in a test tube and was shake vigorously for 30 seconds. The formation of stable foam for 10 min indicated the presence of saponins.

Test for Terpenoids [34]

The crude extract was dissolved in 2 mL of chloroform and dried by evaporation. To this, 2 mL of the concentrated sulfuric acid was added. Formation of a reddish-brown coloration at the interface indicated the presence of terpenoids.

Test for Tannins (Ferric Chloride Test) [35]

Each plant extract was stirred with 1 mL of distilled water, after being filtered, ferric chloride reagent was added to the filtrate. A blue-black, green, or blue-green precipitate indicated the presence of tannins.

Test for Steroids [36]

To each of the four plant extracts, 10 mL of chloroform was added. 1 mL of acetic anhydride was added to these extracts, followed by 2 mL of concentrated sulfuric acid along the walls of the test tube. The appearance of blue green color at the junction indicated the presence of steroids.

Test for Flavonoids (Alkaline Reagent Test) [37]

The extract was treated with 2–3 drops of Sodium hydroxide solution. Acute yellow color development showed the presence of flavonoids, which turned colorless when some drops of sulfuric acid were added. Preliminary phytochemical screening shown in Table 1.

Table 1. Preliminary phytochemical screening of *Passiflora foetida* L leaves

Sr.No	Phytoconstituents	n-hexane	Ethyl Acetate	Acetone	Ethanol	Methanol	Water
1	Alkaloids	+	+	-	+	+	+
2	Carbohydrates	-	+	+	+	+	+
3	Glycosides	-	-	-	-	-	-
4	Flavonoids	+	+	+	+	-	+
5	Phenols & Tannins	+	+	+	+	+	-
6	Steroids	+	-	-	+	-	-
7	Terpenoids	-	+	+	+	+	-
8	Saponins	-	-	-	+	-	-
9	Proteins	+	+	+	+	-	+
10	Amino Acids	+	+	-	+	+	-

Antimicrobial Assay

Microbial culture

Four bacterial strains were used for assay, including two Gram positive bacteria, viz*. Bacillus subtilis* (NCIM 2250) and *Staphylococcus aureus* (NCIM 2079) and two Gram negative bacteria, viz. *Salmonella typhimurium* (MTCC 3224) and *Klebsiella pneumoniae* (NCTC 13368). Fungi were used as *Penicillium chrysogenum* (ATCC 10106), *Tricoderma viride* (ATCC 20476) and *Aspergillus niger* (NCIM 1196).The Nutrient agar slants were used for microbial culture. Antibiotic vancomycin, was used as a standard reference antibiotic and the concentrations are prepared according to NCCLS[38].

Antifungal Activity

Antifungal activity was determined by disc diffusion method [39]. The fungal spores were adjusted to the concentration of 1 × 108 CFU mL−1 as per McFarland standard and suspension was swabbed on the potato dextrose agar. Then, 50 µL of each extract dilution was impregnated into sterile discs of 90 mm in diameter. Nystatin (50 µL) was used as a positive control and respective solvents loaded discs were used as negative control. The plates were incubated at 37°C for 8 h. The zone of inhibition around the discs was measured after the incubation. Each experiment was conducted in triplicates and the average inhibition zone values were calculated [40].

GC-MS Analysis

The extract was directly used for GC-MS analysis. GC-MS analysis was carried out on a GCMS-QP2010 Plus (Shimadzu, Kyoto, Japan) system with head space sampler (AOC-20s) and auto-injector (AOC-20i),

equipped with mass selective detector, having ion source temperature of 230°C, interface temperature of 260°C, a solvent cut time of 2.50 min. threshold of 1,000 eV and mass range of 40 to 650 m/z.

Statistical section

All experimental work carried out in this study were performed on triplicate. Results were expressed as mean (±) standard deviation (SD).

Results and Discussion

Qualitative Phytochemical Analysis

The preliminary phytochemical screening of the leaf extracts of *Passiflora foetida* L. showed the containment of flavonoids, steroids, alkaloids, terpenoids, saponins, phenols, carbohydrates, amino acids, tannin and cardiac glycosides in all the extracts. Phytochemical tests for the presence of secondary phytoconstituents showed the following results shown in (Table 1)

Antibacterial Activity

Ethanol leaf extract exhibited remarkable antibacterial activity against *Staphylococcus aureus* (20.5 mm). Phytochemical screening of ethanol extract showed (17.29%) of cis-13-Docosenamide responsible for the activity. Ethyl acetate extract exhibited good activity against *Salmonella typhimurium* (24.5 mm). n-hexane extract shows remarkable activity against *Klebsiella pneumonia* (23.5mm). Water extract did not show positive activity against any bacteria. Whereas acetone and methanol extract did not show any activity against *Klebsiella pneumonia*. The ethanol leaf extract exhibited its good activity against *Staphylococcus aureus, Bacillus subtilis, Salmonella typhimurium* and *Klebsiella pneumonia.* The antimicrobial activity of all solvents was compared with standard reference antibiotic vancomycin. (Table 2). Results of the anti-bacterial activities are provided visually in (Fig.1). Zones of inhibitions were measured in mm (millimeters).

Table 2. Antibacterial activity of leaf extract of *Passiflora foetida* L.

Sr. No.	Solvent	Zone of inhibition in (mm)			
		Staphylococcus aureus	*Bacillus subtilis*	*Salmonella typhimurium*	*Klebsiella pneumoniae*
1	n-hexane	16.5	11.5	18	23.5
2	Ethyl acetate	18.5	15.5	24.5	19.5
3	Acetone	16.5	26.5	15.5	NA
4	Ethanol	20.5	16.5	21.5	17.5
5	Methanol	NA	15	20.5	NA
6	Water	NA	NA	NA	NA
7	DMSO	NA	NA	NA	NA
8	Vancomycin	19	24	28	23

Fig.1 showing antibacterial assays of crude extract of *Passiflora foetida L.* against human pathogens [(A1,A2,A3)- *Staphylococcus aureus*,(B1,B2)- *Bacillus subtilis*,(C1,C2)- *Salmonella typhimurium*, (D1,D2)- *Klebsiella pneumonia*

Antifungal Activity

Each extract antifungal properties were observed against three fungal pathogens listed in (Table 3). Ethyl acetate and methanol extracts showed higher zone of inhibition against *Aspergillus niger* with a 26 ± 0.50 mm inhibition zone at 50µL compared to other extracts. Ethanol extract showed a higher zone of inhibition against *Penicillium chrysogenum* with a 24mm inhibition zone at 50µL. Ethyl acetate and ethanol extract showed higher zone of inhibition against *Trichoderma viride* with a 25 mm inhibition zone at 50µL compared to other extracts. Out of all the fungi examined, the aqueous extract exhibited the least antifungal activity. Results of antifungal activities are provided visually in (Fig.2). Zones of inhibitions were measured in mm (millimeters).

Table 3. Antifungal activity of leaf extract of *Passiflora foetida L.*

Sr. No.	Solvent	Zone of inhibition in (mm)		
		Penicillium chrysogenum	*Trichoderma viride*	*Aspergillus niger*
1	n-hexane	14	14.5	20
2	Ethyl acetate	15.5	25	26
3	Acetone	16	17	24
4	Ethanol	24	25	25.5
5	Methanol	21	17	26.5
6	Water	NA	NA	NA
7	Nystatin	26	46	36

Fig. 2 Antifungal activity of crude extracts of *Passiflora foetida L.*against (A1,A2) *Tricoderma viride; (B1,B2) Aspergillus niger; (C1,C2) Penicillium chrysogenum*

Chemical Characterization via (GC-MS) Analysis

Medicinally important thirty-four phytoconstituents were found in the ethyl acetate extract according to the GC-MS analysis. Plants containing these phytoconstituents have been shown to have pharmacological properties such as antimicrobial, anti-inflammatory, and antioxidant effects. The chromatogram is shown in (Fig. 3) and phytochemical constituents with activities are shown in the (Table 4).

Fig. 3 GC-MS chromatogram showing relative abundance and retention time of the Ethyl acetate leaf extracts of *Passiflora foetida* L.

Table 4. Phytochemical constituents identified in the ethyl acetate leaf extracts of *Passiflora foetida* L. using gas chromatography-mass spectrometry (GC-MS) and their activities

Peak No.	R.T.	Peak Area %	Name of compound	Activity
1	7.861	10.6	1,2,3-Propanetriol, 1-acetate	Dermatological agent used as an antiseptic [41].
2	9.323	0.23	Pyranone	Antitumor activity [42].
3	10.628	0.23	5-Octen-2-yn-4-ol	Antibacterial and anti-inflammatory effects [43].
4	11.727	0.17	γ -Terpineol	Antibacterial and anti-inflammatory effects [44].
5	12.116	2.59	1,2,3-Propanetriol, 1-acetate	Dermatological agent used as an antiseptic [41].
6	12.887	0.53	2,4-Dimethylfuran	Antibacterial and anti-inflammatory effects [45].
7	18.810	0.33	Heneicosane	Exhibits antimicrobial activity [46].
8	23.813	0.32	Eicosane	Exhibits antimicrobial activity [46].
9	25.188	0.96	Tetradecanoic acid	Antimicrobial and anticancer activity [47].
10	26.102	0.89	cis-11-Hexadecenal	Antimelanogenic, antifungal properties [48].
11	26.497	0.62	Isopropyl myristate	Used in cosmetics [49].
12	28.450	0.37	2-Hexyldecanol	Anti-inflammatory activity [50].
13	29.341	11.98	n-Hexadecanoic acid	Anti-inflammatory, Antihistaminic, anti-arthritic property [51].
14	29.911	0.37	Hexadecanoic acid	Anti-inflammatory, Antihistaminic, anti-arthritic property [51].
15	30.352	10.7	Hydnocarpic acid	Use to treat leprosy [52].
16	30.923	1.11	Ethyl hydnocarpate	Antileprotic activity, antimicrobial and anticancer properties [53].
17	31.781	0.52	9,12-Octadecadienoic acid	Anti-inflammatory, antihistaminic, anti-arthritic, and hepatoprotective activity [54].
18	31.918	0.45	9-Octadecenoic acid	Anti-inflammatory, antihistaminic, anti-arthritic, and hepatoprotective activity [54].
19	32.134	0.65	Phytol	Cytotoxic, antioxidant, autophagy- and apoptosis-inducing,

				antinociceptive, anti-inflammatory [55].
20	32.537	8.97	(9E,11E)-Octadecadienoic acid	Anti-inflammatory, antihistaminic, anti-arthritic activity [54].
21	32.654	12.4	9-Octadecenoic acid,	Anti-inflammatory, antihistaminic, anti-arthritic, and hepatoprotective activity [54].
22	33.093	3.71	Octadecanoic acid	Anti-inflammatory, antihistaminic, anti-arthritic, and hepatoprotective activity [54].
23	34.122	4.57	Chaulmoogric acid	Use to treat leprosy [52].
24	34.652	0.69	2-Cyclopentene-1-tridecanoic acid	Antimicrobial, anti-inflammatory, and antioxidant properties [56]
25	38.288	1.31	9-Octadecenoic acid	Anti-inflammatory, antihistaminic, anti-arthritic, and hepatoprotective activity [54].
26	39.326	2.74	Tetradecahydrocyclododeca[c]furan	Antimicrobial and anti-inflammatory activity [45].
27	39.473	0.97	2-Eicosen-5-olide	Anti-inflammatory, antibacterial, and antioxidant activity
28	41.249	0.75	(9Z)-9-Tetradecenal	Pheromone
29	41.608	4.27	Shyobunol	Antioxidant, antimicrobial, antiviral, and anti-tumor activities [57]
30	42.745	6.14	2-[(9Z,12Z)-9,12-Octadecadienyloxy]ethanol	Used as surfactant.
31	42.879	6.99	cis-13-Docosenamide	Antimicrobial, antioxidant, and anticancer properties [58].
32	44.115	1.03	E,E,Z-1,3,12-Nonadecatriene-5,14-diol	Analgesic, anti-inflammatory, and antimicrobial properties [59]
33	45.077	0.84	(R)-(-)-14-Methyl-8-hexadecyn-1-ol	Long chain fatty alcohol.
34	45.192	0.88	cis-4,4-Dimethylbicyclo(6.3.0)undecane-2,6-dione	Analgesic, anti-inflammatory, and antimicrobial properties [60]

Medicinally important twenty-eight phytoconstituents were found in the ethanol extract according to the GC-MS analysis.

Fig.4 GC-MS chromatogram showing relative abundance and retention time of the Ethanol leaf extracts of *Passiflora foetida* L.

The chromatogram is shown in (Fig. 4) and phytochemical constituents with activities are shown in the (Table 5).

Table 5. Phytochemical constituents identified in the ethanol leaf extracts of *Passiflora foetida* L. using gas chromatography-mass spectrometry (GC-MS) and their activities

Peak No.	R.T.	Peak Area %	Name of compound	Activity
1	16.567	1.89	Tetradecane	Antimicrobial properties.[61].
2	18.500	0.71	1-(4-Ethoxyphenyl)propan-1-ol	Antimicrobial, anti-inflammatory, neuroprotective, and anticancer properties [62]
3	19.139	0.35	Pentadecane	Alkane.
4	19.710	1.89	Benzoic acid	Antimicrobial and Antifungal Agent [63].
5	25.200	4.54	Tetradecanoic acid	Antimicrobial and Anticancer activity[47].
6	25.405	1.00	3-Methylheptadecane	Alkane.
7	26.111	2.49	cis-9-Hexadecenal	Antimelanogenic, antifungal properties [48].
8	26.507	0.94	Isopropyl myristate	Used in Cosmetics [49].
9	29.322	9.67	n-Hexadecanoic acid	Anti-inflammatory, Antihistaminic, anti-arthritic property [51].
10	29.512	1.17	3-Methylheptadecane	Alkane.
11	29.923	2.02	Hexadecanoic acid	Anti-inflammatory, antihistaminic, anti-arthritic property [51].
12	32.533	3.86	10E,12Z-Octadecadienoic acid	Anti-inflammatory, antihistaminic, anti-arthritic, and hepatoprotective activity [54].
13	32.652	10.94	9-Octadecenoic acid	Anti-inflammatory, antihistaminic, anti-arthritic, and hepatoprotective activity [54].
14	33.096	4.22	Octadecanoic acid	Anti-inflammatory, antihistaminic, anti-arthritic, and hepatoprotective activity [54].
15	33.634	4.76	Octadecanoic acid	Anti-inflammatory, antihistaminic, anti-arthritic, and hepatoprotective activity [54].
16	36.498	3.21	12-Hydroxystearic acid	Fatty acid surfactant.
17	38.084	3.70	Tricyclo[20.8.0.0(7,16)]triacontane	No activity.
18	38.923	2.56	Hexadecanoic acid	Anti-inflammatory, antihistaminic, anti-arthritic property [51].
19	39.102	2.02	Undec-10-ynoic acid	Fatty acid.

20	39.341	3.41	Tetradecahydrocyclododeca[c]furan	Antimicrobial and anti-inflammatory activity [45].
21	39.490	1.41	2-Eicosen-5-olide	Anti-inflammatory, antibacterial, and antioxidant Activity [64]
22	40.923	1.31	9-Octadecenoic acid	Anti-inflammatory, antihistaminic, anti-arthritic, and hepatoprotective activity [54].
23	41.623	3.24	Tricyclo[20.8.0.0(7,16)]triacontane	No activity.
24	42.379	2.90	1,4-Benzenedicarboxylic acid	Antimicrobial, antifungal, and cytotoxic effects [65].
25	42.764	5.56	E,E-3,13-Octadecadien-1-ol	Pheromone.
26	42.894	17.29	cis-13-Docosenamide	Antimicrobial, antioxidant, and anticancer properties [58].
27	43.381	1.09	Squalene	antioxidant, anti-inflammatory, and immune-modulating effects[66].
28	44.134	1.83	E,E,Z-1,3,12-Nonadecatriene-5,14-diol	Analgesic, anti-inflammatory, and antimicrobial properties [59]

Discussion

This study conducted phytochemical screening tests to substantiate its traditional applications and offer scientific support. The phytochemical analysis with ethyl acetate and ethanol extracts revealed the existence of several major groups of phytoconstituents (Fig.3; Table 4) and (Fig.4; Table 5) respectively bolstering the potential of the plant as an herbal remedy against certain diseases. Ethyl acetate extract contains (10.6%) 1, 2, 3-Propanetriol, 1-acetate showed dermatological and antiseptic activity [41]. n-hexadecanoic acid (11.98%) exhibited anti-inflammatory, antihistaminic, anti-arthritic property [51]. Octadecanoic acid and its derivatives in high abundance (27.36%) exhibited anti-inflammatory, antihistaminic, anti-arthritic, and hepatoprotective activity [54]. Compounds like hydnocarpic acid (10.7%) and chaulmoogric acid (4.57%) exhibited activity against leprosy [52]. Heneicosane and eicosane are pheromones that exhibited microbial activity [46]. Ethanol extract contains tetradecane (1.89%) was reported to possess antimicrobial properties [61]. Hexadecenoic acid (12.15%) and octadecanoic acid (8.98%) exhibits anti-inflammatory, antihistaminic, anti-arthritic, and hepatoprotective activity [54]. It was declared that hexadecanoic acid (14.25%) had anti-inflammatory, Antihistaminic, anti-arthritic property [51]. cis-13-Docosenamide (17.29%) showed Antimicrobial, antioxidant, and anticancer properties [58]. Plant material extraction and analysis are crucial to the creation, modernization, and quality assurance of herbal medicines [67]. Intense pharmacological research has been conducted over the past few decades to increase the value of different medicinal plants as possible sources of novel therapeutics and top prospects for drug development [68].

Conclusion

In the present study, GC–MS analysis were performed to explore the phytochemical constituents profile of this *Passiflora foetida* L collected from the dense forest near Lonar Lake:A Distinctive Saline Crater Lake in IndiaThirty-four phytochemical constituents have been identified from the ethyl acetate extract and twenty-eight phytoconstituents identified in the ethanol extract of leaves of *Passiflora foetida* L. This study supports the use of leaf as traditional medicine. The compounds identified in the present study may be extracted and quantified to develop a new drug to use as an antioxidant, anti-inflammatory, anticancer, fungicide, insecticide and antibiotic in the field of pharmacy. As a result, *Passiflora foetida* L. may be used as an essential candidate for pharmacological activity testing. Thus, further pharmacological research are required to confirm the exact compound/compounds responsible for health benefits.

Declarations

Acknowledgments

We are thankful to Management, Principal and Head Dept. of Botany, M.S.P. Mandal's Balbhim Arts, Science and Commerce College Beed, Maharashtra, India for availing all necessary facilities and infrastructure for carrying out the research. Also we would like to thank Centre For Analytical Instrumentation, Kerala Forest Research Institute, Peechi, Thrissur, Kerala, India for providing GC-MS facility.

Authors Contributions Conceptualization, Methodology, GC-MS analysis, Investigation, Writing- original draft preparation- Sunita Bhosle, Sharmishtha Doifode, Experimentation-Sharmishtha Doifode, Writing—review and editing-Sunita Bhosle, Sharmishtha Doifode.

Funding This work was supported by the Mahatma Jyotiba Phule Research and Training Institute [MAHAJYOTI] [An autonomous institute of the OBC welfare Department, Govt. of Maharashtra]

Data availability All data generated or analysed during this study are included in this published article [and its supplementary information files].

Ethical Approval Not applicable [Research not involving human research participants or live vertebrates]

Consent to Participate Not applicable.

Consent for Publication Granted for publication.

Competing Interests The authors have no relevant financial or non-financial interests to disclose.

Conflict of Interest The authors declare that they have no conflict of interest.

References

1. Iqbal, N., Vahia, M. N., Masood, T., & Ahmad, A. (n.d.). A Probable Meteor Impact Crater in Kashmir Valley (India).
2. Lyttleton, R. A. (1964). The Moon, Meteorites, and Comets.
3. Surve, R. R., Shirke, A. V., Athalye, R. R., & Sangare, M. M. (2021). A Review on Chemical and Ecological Status of Lonar Lake. *Current World Environment*, *16*(1), 61–69. https://doi.org/10.12944/CWE.16.1.07
4. Ahmad Khan, M. S., & Ahmad, I. (2019). Herbal Medicine. In *New Look to Phytomedicine* (pp. 3–13). Elsevier. https://doi.org/10.1016/B978-0-12-814619-4.00001-X
5. Shukia, R., Sharma, S. B., Puri, D., Prabhu, K. M., & Murthy, P. S. (2000). Medicinal plants for treatment of diabetes mellitus. *Indian Journal of Clinical Biochemistry*, *15*(S1), 169–177. https://doi.org/10.1007/BF02867556
6. A. Hussein, R., & A. El-Anssary, A. (2019). Plants Secondary Metabolites: The Key Drivers of the Pharmacological Actions of Medicinal Plants. In P. F. Builders (Ed.), *Herbal Medicine*. IntechOpen. https://doi.org/10.5772/intechopen.76139
7. Shokeen, P., Anand, P., Murali, Y. K., & Tandon, V. (2008). Antidiabetic activity of 50% ethanolic extract of Ricinus communis and its purified fractions. *Food and Chemical Toxicology*, *46*(11), 3458–3466. https://doi.org/10.1016/j.fct.2008.08.020
8. Sharifı Rad, J., Hoseini Alfatemi, S. M., Sharifi Rad, M., & Iriti, M. (2014). Free Radical Scavenging and Antioxidant Activities of Different Parts of*Nitraria schoberi*L. *Journal of Biologically Active Products from Nature*, *4*(1), 44–51. https://doi.org/10.1080/22311866.2014.890070
9. Saini, A. K., Goyal, R., Gauttam, V. K., & Kalia, A. N. (2010). Evaluation of anti-inflammatory potential of Ricinus communis Linn leaves extracts and its flavonoids content in Wistar rats. *Journal of Chemical and Pharmaceutical Research*, *2*(5), 690–695.
10. Sharifi-Rad, M., Panda, J., Mohanta, Y. K., Pohl, P., Zengin, G., & Moloney, M. G. (2025). Essential oil of Cleome coluteoides (Boiss.): phytochemical constituents, antioxidant, antimicrobial, antiproliferative, anti-inflammatory, enzymatic inhibition, and xanthine oxidase inhibitory properties. *Journal of Herbal Medicine*, *52*, 101036. https://doi.org/10.1016/j.hermed.2025.101036
11. Rao, N., Mittal, S., & Menghani, E. (2013). Assessment of phytochemical screening, antioxidant and antibacterial potential of the methanolic extract of Ricinus communis L. *Asian Journal of Pharmacy and Technology*, *3*(1), 20–25.
12. Sharifı-Rad, M., Mohanta, Y. K., Pohl, P., Jaradat, N., Aboul-Soud, M. A. M., & Zengin, G. (2022). Variation of phytochemical constituents, antioxidant, antibacterial, antifungal, and anti-inflammatory properties of Grantia aucheri (Boiss.) at different growth stages. *Microbial Pathogenesis*, *172*, 105805. https://doi.org/10.1016/j.micpath.2022.105805
13. Benmohamed, M., Guenane, H., Messaoudi, M., Zahnit, W., Egbuna, C., Sharifı-Rad, M. Yousfı, M. (2023). Mineral Profile, Antioxidant, Anti-Inflammatory, Antibacterial, Anti-Urease and Anti-α-Amylase

Activities of the Unripe Fruit Extracts of Pistacia atlantica. *Molecules*, *28*(1), 349. https://doi.org/10.3390/molecules28010349

14. Shaiq Ali, M., Ahmed, F., Kashif Pervez, M., Azhar, I., & Ibrahim, S. A. (2005). Parkintin: A new flavanone with epoxy-isopentyl moiety from *Parkinsonia aculeata* linn. (Caesalpiniaceae). *Natural Product Research*, *19*(1), 53–56. https://doi.org/10.1080/14786410310001643812
15. Chiavaroli, A., Di Simone, S. C., Sinan, K. I., Ciferri, M. C., Angeles Flores, G., Zengin, G., … Orlando, G. (2020). Pharmacological Properties and Chemical Profiles of Passiflora foetida L. Extracts: Novel Insights for Pharmaceuticals and Nutraceuticals. *Processes*, *8*(9), 1034. https://doi.org/10.3390/pr8091034
16. Patil, A. S., Paikrao, H. M., & Patil, S. R. (2013). Passiflora foetida Linn: a complete morphological and phytopharmacological review. *International Journal of Pharma and Bio Sciences*, *4*(1), 285–296.
17. Sasikala, V., Saravanan, S., & Parimelazhagan, T. (2011). Analgesic and anti–inflammatory activities of Passiflora foetida L. *Asian Pacific journal of tropical medicine*, *4*(8), 600–603.
18. Sutar, V., & Bhosale, U. P. (2013). Screening of Passiflora foetida extracts as anticancer agents on MCF 7 cell line. *BIOINFOLET-A Quarterly Journal of Life Sciences*, *10*(3a), 808–810.
19. Anandan, R., Jayakar, B., Jeganathan, S., Manavalan, R., & Kumar, S. R. (2009). Effect of ethanol extract of fruits of Passiflora foetida Linn. on CCl. *J. Pharm. Res*, *2*, 413–415.
20. Sathish, R., Sahu, A., & Natarajan, K. (2011). Antiulcer and antioxidant activity of ethanolic extract of Passiflora foetida L. *Indian Journal of Pharmacology*, *43*(3), 336. https://doi.org/10.4103/0253-7613.81501
21. Anandan, R., Jayakar, B., Jeganathan, S., Manavalan, R., & Kumar, S. R. (2009). Effect of ethanol extract of fruits of Passiflora foetida Linn. on CCl. *J. Pharm. Res*, *2*, 413–415.
22. Kell, D. B., Brown, M., Davey, H. M., Dunn, W. B., Spasic, I., & Oliver, S. G. (2005). Metabolic footprinting and systems biology: the medium is the message. *Nature Reviews Microbiology*, *3*(7), 557–565. https://doi.org/10.1038/nrmicro1177
23. Robertson, D. G. (2005). Metabonomics in Toxicology: A Review. *Toxicological Sciences*, *85*(2), 809–822. https://doi.org/10.1093/toxsci/kfi102
24. Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*, *6*(2), 71–79. https://doi.org/10.1016/j.jpha.2015.11.005
25. Torres-Rodriguez, A., Darvishzadeh, R., Skidmore, A. K., Fränzel-Luiten, E., Knaken, B., & Schuur, B. (2024). High-throughput Soxhlet extraction method applied for analysis of leaf lignocellulose and non-structural substances. *MethodsX*, *12*, 102644. https://doi.org/10.1016/j.mex.2024.102644
26. Stanojević, L. P., Stanković, M. Z., Cvetković, D. J., Cakić, M. D., Ilić, D. P., Nikolić, V. D., & Stanojević, J. S. (2016). The effect of extraction techniques on yield, extraction kinetics, and antioxidant activity of aqueous-methanolic extracts from nettle (*Urtica dioica* L.) leaves. *Separation Science and Technology*, *51*(11), 1817–1829. https://doi.org/10.1080/01496395.2016.1178774

27. Rao, U. M. (2016). Phytochemical Screening, Total Flavonoid and Phenolic Content Assays of Various Solvent Extracts of Tepal of Musa paradisiaca. *Malaysian Journal of Analytical Science*, *20*(5), 1181–1190. https://doi.org/10.17576/mjas-2016-2005-25
28. Nortjie, E., Basitere, M., Moyo, D., & Nyamukamba, P. (2022). Extraction Methods, Quantitative and Qualitative Phytochemical Screening of Medicinal Plants for Antimicrobial Textiles: A Review. *Plants*, *11*(15), 2011. https://doi.org/10.3390/plants11152011
29. Ramaswamy, N., Samatha, T., Srinivas, P., & Chary, R. S. (2013). International Journal Of Pharmacy & Life Sciences. *Life Sci.*, *4*(1).
30. Mensah Donkor, A. (2016). In Vitro Bacteriostatic and Bactericidal Activities of Senna alata, Ricinus communis and Lannea barteri extracts Against Wound and Skin Disease Causing Bacteria. *Journal of Analytical & Pharmaceutical Research*, *3*(1). https://doi.org/10.15406/japlr.2016.03.00046
31. Shaikh, J. R., & Patil, M. (2020). Qualitative tests for preliminary phytochemical screening: An overview. *International Journal of Chemical Studies*, *8*(2), 603–608. https://doi.org/10.22271/chemi.2020.v8.i2i.8834
32. Gibbs, H. D. (1927). Phenol Tests. *Journal of Biological Chemistry*, *72*(2), 649–664. https://doi.org/10.1016/s0021-9258(18)84338-1
33. Chen, C., Li, R., Li, D., Shen, F., Xiao, G., & Zhou, J. (2021). Extraction and purification of saponins from *Sapindus mukorossi*. *New Journal of Chemistry*, *45*(2), 952–960. https://doi.org/10.1039/d0nj04047a
34. Rahman, A., Sitepu, I. R., Tang, S.-Y., & Hashidoko, Y. (2010). Salkowski's Reagent Test as a Primary Screening Index for Functionalities of Rhizobacteria Isolated from Wild Dipterocarp Saplings Growing Naturally on Medium-Strongly Acidic Tropical Peat Soil. *Bioscience, Biotechnology, and Biochemistry*, *74*(11), 2202–2208. https://doi.org/10.1271/bbb.100360
35. Falcão, L., & Araújo, M. E. M. (2011). Tannins characterisation in new and historic vegetable tanned leathers fibres by spot tests. *Journal of Cultural Heritage*, *12*(2), 149–156. https://doi.org/10.1016/j.culher.2010.10.005
36. Cook, R. P. (1961). Reactions of steroids with acetic anhydride and sulphuric acid (the Liebermann-Burchard test). *The Analyst*, *86*(1023), 373. https://doi.org/10.1039/an9618600373
37. Chen, M., He, X., Sun, H., Sun, Y., Li, L., Zhu, J. Zang, H. (2022). Phytochemical analysis, UPLC-ESI-Orbitrap-MS analysis, biological activity, and toxicity of extracts from Tripleurospermum limosum (Maxim.) Pobed. *Arabian Journal of Chemistry*, *15*(5), 103797. https://doi.org/10.1016/j.arabjc.2022.103797
38. Ginocchio, C. C. (2002). Role of NCCLS in antimicrobial susceptibility testing and monitoring. *American Journal of Health-System Pharmacy*, *59*(suppl_3), S7–S11. https://doi.org/10.1093/ajhp/59.suppl_3.S7
39. Lai, H. Y., Lim, Y. Y., & Tan, S. P. (2009). Antioxidative, Tyrosinase Inhibiting and Antibacterial Activities of Leaf Extracts from Medicinal Ferns. *Bioscience, Biotechnology, and Biochemistry*, *73*(6), 1362–1366. https://doi.org/10.1271/bbb.90018

40. Mostafa, A. A., Al-Askar, A. A., Almaary, K. S., Dawoud, T. M., Sholkamy, E. N., & Bakri, M. M. (2018). Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases. *Saudi Journal of Biological Sciences*, *25*(2), 361–366. https://doi.org/10.1016/j.sjbs.2017.02.004
41. Claesen, J. (2018). Topical Antiseptics and the Skin Microbiota. *Journal of Investigative Dermatology*, *138*(10), 2106–2107. https://doi.org/10.1016/j.jid.2018.06.001
42. Yu, C., Nian, Y., Chen, H., Liang, S., Sun, M., Pei, Y., & Wang, H. (2022). Pyranone Derivatives With Antitumor Activities, From the Endophytic Fungus Phoma sp. YN02-P-3. *Frontiers in Chemistry*, *10*, 950726. https://doi.org/10.3389/fchem.2022.950726
43. Halappanavar, V., Teli, S., Sannakki, H. B., & Teli, D. (2025). Quinazoline scaffold as a target for combating microbial resistance: Synthesis and antimicrobial profiling of quinazoline derivatives. *Results in Chemistry*, *13*, 101955. https://doi.org/10.1016/j.rechem.2024.101955
44. Chen, Y., Zhang, L.-L., Wang, W., & Wang, G. (2023). Recent updates on bioactive properties of α-terpineol. *Journal of Essential Oil Research*, *35*(3), 274–288. https://doi.org/10.1080/10412905.2023.2196515
45. Sridhar, S., Dinda, S. C., & Prasad, Y. R. (2011). Synthesis and Biological Evaluation of Some New Chalcones Containing 2,5-Dimethylfuran Moiety. *Journal of Chemistry*, *8*(2), 541–546. https://doi.org/10.1155/2011/582603
46. Vanitha, V., Vijayakumar, S., Nilavukkarasi, M., Punitha, V. N., Vidhya, E., & Praseetha, P. K. (2020). Heneicosane—A novel microbicidal bioactive alkane identified from Plumbago zeylanica L. *Industrial Crops and Products*, *154*, 112748. https://doi.org/10.1016/j.indcrop.2020.112748
47. Sokmen, B. B., Hasdemir, B., Yusufoglu, A., & Yanardag, R. (2014). Some Monohydroxy Tetradecanoic Acid Isomers as Novel Urease and Elastase Inhibitors and as New Antioxidants. *Applied Biochemistry and Biotechnology*, *172*(3), 1358–1364. https://doi.org/10.1007/s12010-013-0595-2
48. Hoda, S., Gupta, L., Shankar, J., Gupta, A. K., & Vijayaraghavan, P. (2020). *cis*-9-Hexadecenal, a Natural Compound Targeting Cell Wall Organization, Critical Growth Factor, and Virulence of *Aspergillus fumigatus*. *ACS Omega*, *5*(17), 10077–10088. https://doi.org/10.1021/acsomega.0c00615
49. Bardhan, S., Kundu, K., Das, S., Poddar, M., Saha, S. K., & Paul, B. K. (2014). Formation, thermodynamic properties, microstructures and antimicrobial activity of mixed cationic/non-ionic surfactant microemulsions with isopropyl myristate as oil. *Journal of Colloid and Interface Science*, *430*, 129–139. https://doi.org/10.1016/j.jcis.2014.05.042
50. Li, L., Yang, B., Yang, S., Tian, X., Gao, Y., Song, J., Xing, F. (2024). Inhibitory effects of Lactobacillus brevis on Aspergillus westerdijkiae and antifungal compounds identification. *Postharvest Biology and Technology*, *214*, 112980. https://doi.org/10.1016/j.postharvbio.2024.112980
51. Kim, D. H., Park, M. H., Choi, Y. J., Chung, K. W., Park, C. H., Jang, E. J., … Chung, H. Y. (2013). Molecular Study of Dietary Heptadecane for the Anti-Inflammatory Modulation of NF-kB in the Aged Kidney. *PLoS ONE*, *8*(3), e59316. https://doi.org/10.1371/journal.pone.0059316

52. Walker, E. L., & Sweeney, M. A. (1920). The chemotherapeutics of the chaulmoogric acid series and other fatty acids in leprosy and tuberculosis: I. Bactericidal action; active principle; specificity. *Journal of Infectious Diseases*, *26*(3), 238–264. https://doi.org/10.1093/infdis/26.3.238
53. Ganesh, M., Lee, S. G., Jayaprakash, J., Mohankumar, M., & Jang, H. T. (2019). Hydnocarpus alpina Wt extract mediated green synthesis of ZnO nanoparticle and screening of its anti-microbial, free radical scavenging, and photocatalytic activity. *Biocatalysis and Agricultural Biotechnology*, *19*, 101129. https://doi.org/10.1016/j.bcab.2019.101129
54. Ajanaku, C., Echeme, J., Mordi, R., Bolade, O., Okoye, S., Jonathan, H., & Ejilude, O. (2018). In-Vitro Antibacterial, Phytochemical, Antimycobacterial Activities And Gc-Ms Analyses Of Bidens Pilosa Leaf Extract. *Journal of microbiology, biotechnology and food sciences*, *8*(1), 721–725. https://doi.org/10.15414/jmbfs.2018.8.1.721-725
55. Islam, M. T., Ali, E. S., Uddin, S. J., Shaw, S., Islam, M. A., Ahmed, M. I., … Atanasov, A. G. (2018). Phytol: A review of biomedical activities. *Food and Chemical Toxicology*, *121*, 82–94. https://doi.org/10.1016/j.fct.2018.08.032
56. Yang, W.-S., Kim, I., Seu, Y.-B., Jeong, Y. J., Han, H. S., Ryu, K. O., & Kang, S. C. (2016). Ethanolic Extract of Radish Sprout (*Raphanus Sativus* L.) Prevents Bisphenol A-Induced Testicular Dysfunction in Male Rats. *Journal of Food Biochemistry*, *40*(4), 490–498. https://doi.org/10.1111/jfbc.12245
57. St-Gelais, A., Mathieu, M., Levasseur, V., Ovando, J. F., Escamilla, R., & Marceau, H. (2016). Preisocalamendiol, Shyobunol and Related Oxygenated Sesquiterpenes from Bolivian *Schinus molle* Essential Oil. *Natural Product Communications*, *11*(4). https://doi.org/10.1177/1934578x1601100432
58. Zain, M. E., Awaad, A. S., Al-Othman, M. R., Alafeefy, A. M., & El-Meligy, R. M. (2013). WITHDRAWN: Biological activity of fungal secondary metabolites. Elsevier.
59. Zhang, Z., & Tang, W. (2018). Drug metabolism in drug discovery and development. *Acta Pharmaceutica Sinica B*, *8*(5), 721–732. https://doi.org/10.1016/j.apsb.2018.04.003
60. Qin, G.-F., Liang, H.-B., Liu, W.-X., Zhu, F., Li, P.-L., Li, G.-Q., & Yao, J.-C. (2019). Bicyclo [6.3.0] Undecane Sesquiterpenoids: Structures, Biological Activities, and Syntheses. *Molecules*, *24*(21), 3912. https://doi.org/10.3390/molecules24213912
61. Nasr, Z., El-shershaby, H., Sallam, K., Abed, N., Abd- El Ghany, I., & Sidkey, N. (2021). Evaluation of Antimicrobial Potential of Tetradecane Extracted from Pediococcus acidilactici DSM: 20284 - CM Isolated from Curd Milk. *Egyptian Journal of Chemistry*, *0*(0), 0–0. https://doi.org/10.21608/ejchem.2021.92658.4385
62. Gevorgyan, G. A., Gasparyan, N. K., Papoyan, O. A., Tumadzhyan, A. E., Tatevosyan, A. A., & Panosyan, G. A. (2017). Synthesis and Various Biological Properties of 3-(4-Benzylpiperizino)-1-(4-Ethoxyphenyl)-1-Alkyl(Aryl)-2-Phenylpropan-1-OL Dihydrochlorides. *Pharmaceutical Chemistry Journal*, *51*(1), 30–34. https://doi.org/10.1007/s11094-017-1552-0
63. Food additives. (2020). In *Introduction to the Chemistry of Food* (pp. 251–311). Elsevier. https://doi.org/10.1016/b978-0-12-809434-1.00007-4

64. Maslova, O., Iskakova, Z., Doskaliyev, A., & Adekenov, S. (2023). Cytotoxicity and antitumor activity of sesquiterpene lactones. Structure, activity. *ScienceRise: Pharmaceutical Science*, (6(46)), 53–63. https://doi.org/10.15587/2519-4852.2023.295228
65. Aroonrerk, N. (2016). Anti-cariogenic activity of garcinone B from Garcinia mangostana Linn. *Journal of Pharmaceutical Care & Health Systems*, *03*(02). https://doi.org/10.4172/2376-0419.c1.012
66. Cheng, L., Ji, T., Zhang, M., & Fang, B. (2024). Recent advances in squalene: Biological activities, sources, extraction, and delivery systems. *Trends in Food Science & Technology*, *146*, 104392. https://doi.org/10.1016/j.tifs.2024.104392
67. Gomathi, D., Kalaiselvi, M., Ravikumar, G., Devaki, K., & Uma, C. (2015). GC-MS analysis of bioactive compounds from the whole plant ethanolic extract of Evolvulus alsinoides (L.) L. *Journal of Food Science and Technology*, *52*(2), 1212–1217. https://doi.org/10.1007/s13197-013-1105-9
68. Katiyar, C., Kanjilal, S., Gupta, A., & Katiyar, S. (2012). Drug discovery from plant sources: An integrated approach. *AYU (An International Quarterly Journal of Research in Ayurveda)*, *33*(1), 10. https://doi.org/10.4103/0974-8520.100295

Figures

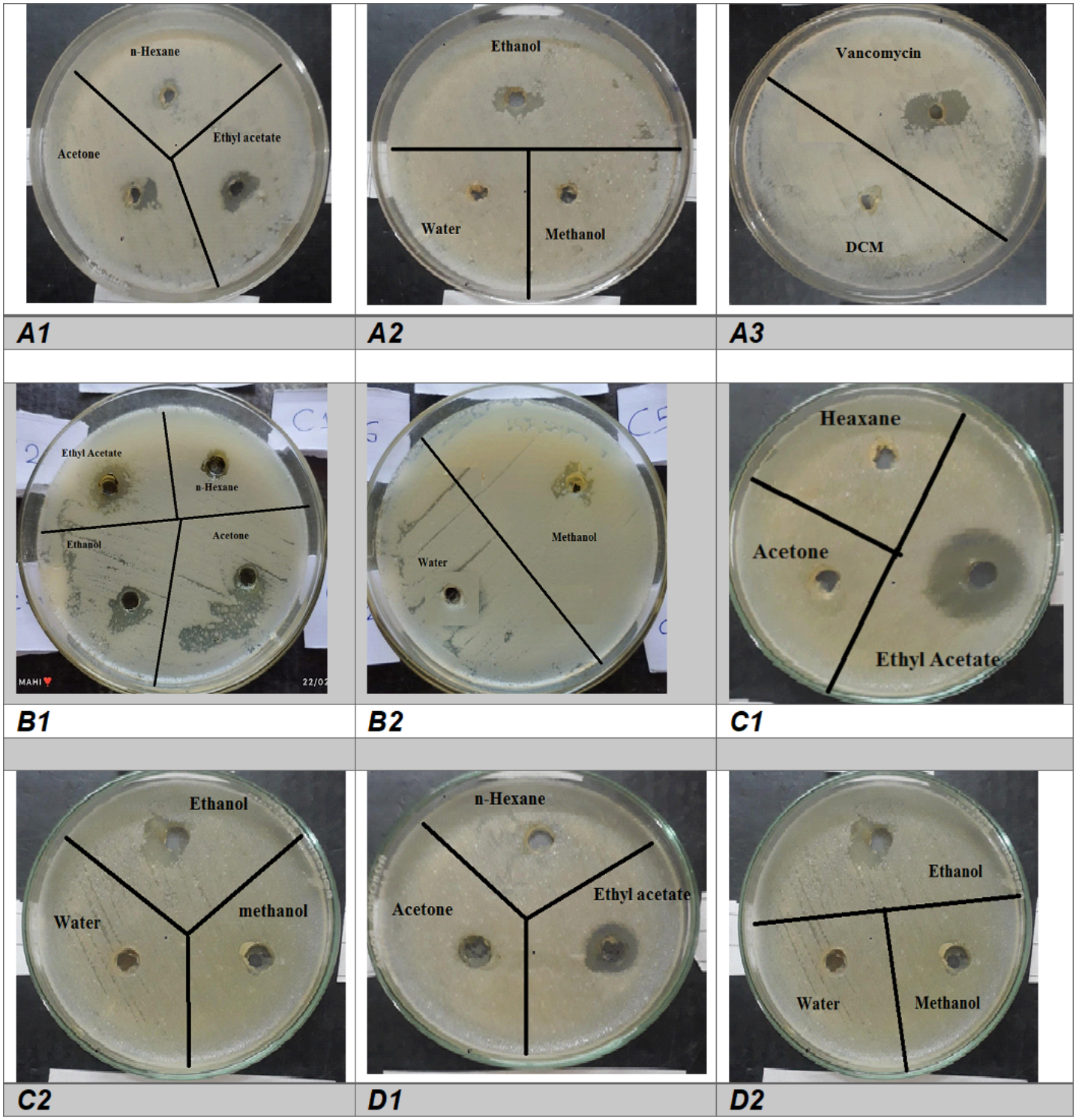

Figure 1

Showing antibacterial assays of crude extract of *Passiflora foetida L.* against human pathogens [(A1,A2,A3)- *Staphylococcus aureus*,(B1,B2)- *Bacillus subtilis*,(C1,C2)- *Salmonella typhimurium*, (D1,D2)- *Klebsiella pneumonia*

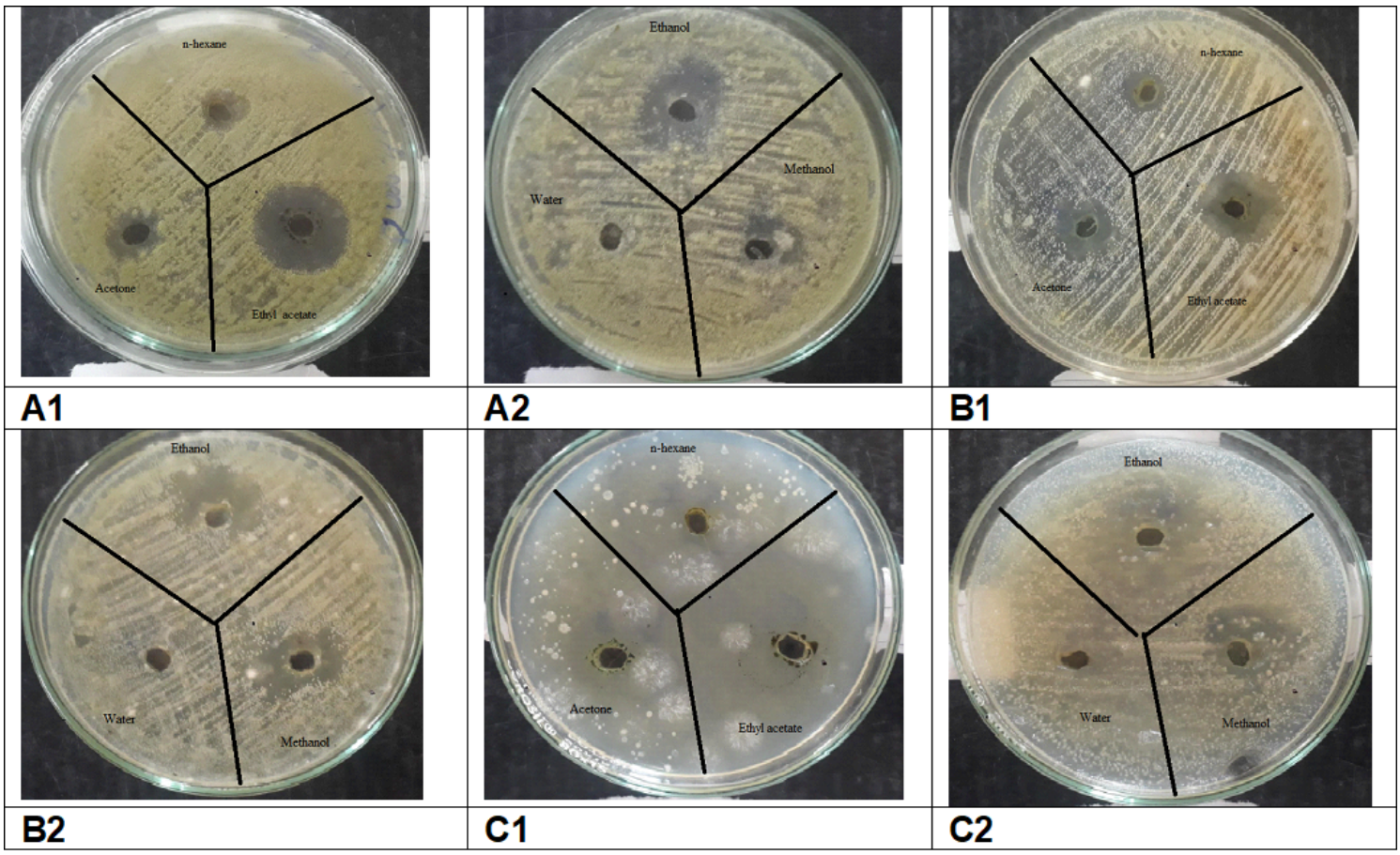

Figure 2

Antifungal activity of crude extracts of *Passiflora foetida L.*against (A1,A2) *Tricoderma viride; (B1,B2) Aspergillus niger; (C1,C2) Penicillium chrysogenum*

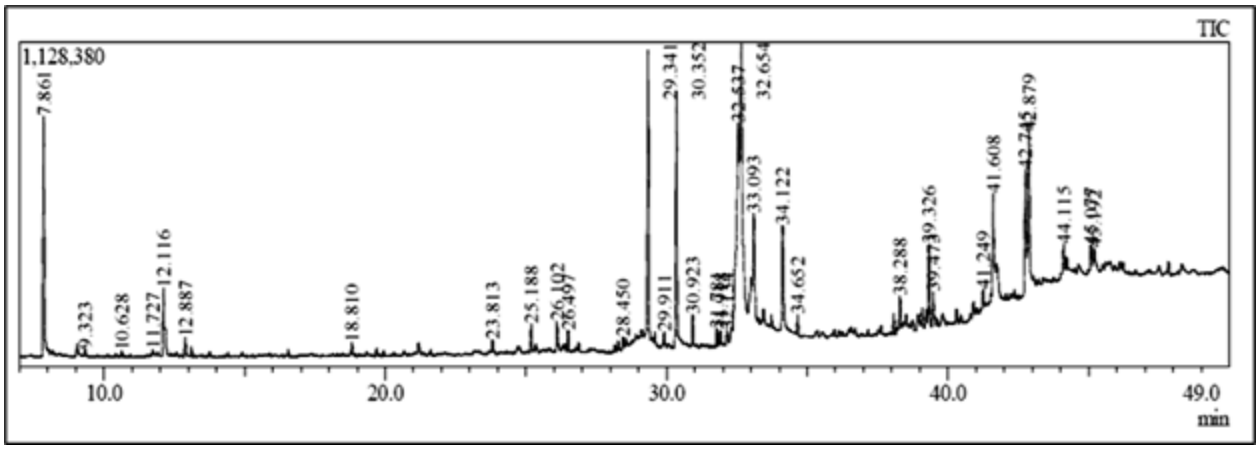

Figure 3

GC-MS chromatogram showing relative abundance and retention time of the Ethyl acetate leaf extracts of *Passiflora foetida* L.

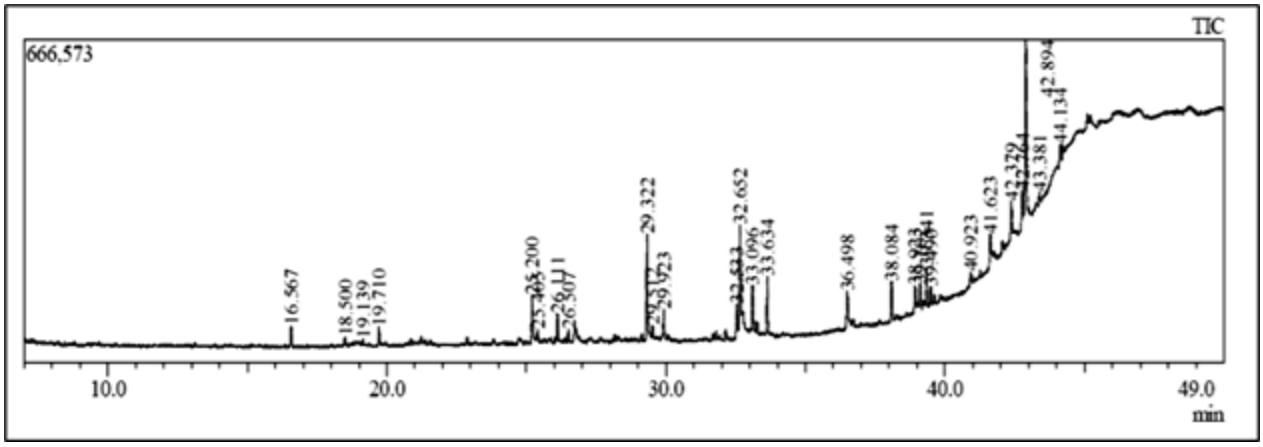

Figure 4

GC-MS chromatogram showing relative abundance and retention time of the Ethanol leaf extracts of *Passiflora foetida* L.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryData.pdf