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

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Research Article

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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 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.

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 antidiabatic[7], antimutagenic, anti-inflammatory[8], antimicrobial activities[9] of medicinal plants in a lesser or larger capacity[10]. Passiflora foetida L. popularly known as striking passionflower[11] 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[12–17].

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[18, 19]. This study screened phytoconstutuents 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[20]. The leaves were collected from Lonar Lake area Maharashtra, India.

Materials and Methods

Sample collection

The leaf samples were gathered 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[21, 22]. Water extract prepared by Maceration process, leaf powder soaked in water for 72 Hrs with occasional shaking filtered through whatman No.1 filter paper. Crude filtrate extract 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[23–26].

Test for Alkaloids (Wagner's Reagent Test)[27]

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 is confirmed by a reddish-brown precipitate.

Phenol Test (Ferric Chloride Test)

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)

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 indicates the presence of saponins.

Test for Terpenoids

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 indicates the presence of terpenoids.

Test for Tannins (Ferric Chloride Test)

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

Test for Steroids

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 indicates the presence of steroids.

Test for Flavonoids (Alkaline Reagent Test)

The extract was treated with 2–3 drops of Sodium hydroxide solution. Acute yellow color development shows the presence of flavonoids, which turn colorless when some drops of sulfuric acid were added.

Antimicrobial Assay

Microbial culture

Four bacterial strains were used for assay, includes two Gram positive bacteria viz. *Bacillus subtilis* (NCIM 2250), *Staphylococcus aureus* (NCIM 2079) and two Gram negative bacteria viz. *Salmonella typhimurium* (MTCC 3224), *Klebsiella pneumoniae* (NCTC 13368). Fungi were used as *Penicillium chrysogenum* (ATCC 10106), *Tricoderma viride* (ATCC 20476), *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[28].

Antifungal Activity

Antifungal activity was determined by disc diffusion method[29]. 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.[30]

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.

Results and Discussion

Qualitative Phytochemical Analysis

The preliminary phytochemical screening of the leaf extracts of *Passiflora foetida* L shows to contain 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 following results 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	+	+	-	+	+	-

Antibacterial Activity

Ethanol leaf extract exhibited remarkable antibacterial activity against *Staphylococcus aureus* (20.5 mm). 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 do not show positive activity against any bacteria. Whereas Acetone and methanol extract does not show any activity against *Klebsiella pneumonia*. The ethanol leaf extract exhibited its good activity against *Staphylococcus aureus, Bacillus subtilis, Salmonella typhimurium, Klebsiella pneumonia.* The antimicrobial activity of all solvents was compared with standard reference antibiotic vancomycin. (Table 2). Results of 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

Antifungal Activity

Each extracts antifungal properties were observed against three fungal pathogen 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

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).

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	10.68	1,2,3-Propanetriol, 1-acetate	Inhibitory effect on microorganisms
2	9.323	0.23	Pyranone	Antitumor activity
3	10.628	0.23	5-Octen-2-yn-4-ol	Antibacterial and anti-inflammatory effects
4	11.727	0.17	γ -Terpineol	Antibacterial and anti-inflammatory effects
5	12.116	2.59	1,2,3-Propanetriol, 1-acetate	Inhibitory effect on microorganisms
6	12.887	0.53	2,4-Dimethylfuran	Antibacterial and anti-inflammatory effects
7	18.810	0.33	Heneicosane	Exhibits antimicrobial activity
8	23.813	0.32	Eicosane	Exhibits antimicrobial activity
9	25.188	0.96	Tetradecanoic acid	Used as emulsifier.
10	26.102	0.89	cis-11-Hexadecenal	Antimelanogenic, antifungal properties.
11	26.497	0.62	Isopropyl myristate	Used in Cosmetics.
12	28.450	0.37	2-Hexyldecanol	Used as fungicide.
13	29.341	11.98	n-Hexadecanoic acid	Anti-inflammatory, Antihistaminic, anti-arthritic property.
14	29.911	0.37	Hexadecanoic acid	Anti-inflammatory, Antihistaminic, anti-arthritic property.
15	30.352	10.72	Hydnocarpic acid	Use to treat leprosy
16	30.923	1.11	Ethyl hydnocarpate	Antileprotic activity, antimicrobial and anticancer properties.
	31.781	0.52	9,12-Octadecadienoic acid	Anti-inflammatory, antihistaminic, anti-arthritic, and hepatoprotective activity.
17	31.918	0.45	9-Octadecenoic acid	Anti-inflammatory, antihistaminic, anti-arthritic, and

Peak No.	R.T.	Peak Area %	Name of compound	Activity*
				hepatoprotective activity.
18	32.134	0.65	Phytol	Precusor of Vitamin E and Vitamin K
	32.537	8.97	(9E,11E)-Octadecadienoic acid	Anti-inflammatory, antihistaminic, anti-arthritic activity.
19	32.654	12.41	9-Octadecenoic acid,	Anti-inflammatory, antihistaminic, anti-arthritic, and hepatoprotective activity.
20	33.093	3.71	Octadecanoic acid	Anti-inflammatory, antihistaminic, anti-arthritic, and hepatoprotective activity.
21	34.122	4.57	Chaulmoogric acid	Use to treat leprosy
22	34.652	0.69	2-Cyclopentene-1-tridecanoic acid	Antimicrobial, anti-inflammatory, and antioxidant properties.
23	38.288	1.31	9-Octadecenoic acid	Anti-inflammatory, antihistaminic, anti-arthritic, and hepatoprotective activity.
24	39.326	2.74	Tetradecahydrocyclododeca[c]furan	Antimicrobial and anti-inflammatory activity.
25	39.473	0.97	2-Eicosen-5-olide	Anti-inflammatory, antibacterial, and antioxidant Activity.
26	41.249	0.75	(9Z)-9-Tetradecenal	Pheromone
27	41.608	4.27	Shyobunol	Antimicrobial, antioxidant, and anti-inflammatory activity.
28	42.745	6.14	2-[(9Z,12Z)-9,12-Octadecadienyloxy]ethanol	Lipid metabolism
29	42.879	6.99	cis-13-Docosenamide	Plant metabolite
30	44.115	1.03	E,E,Z-1,3,12-Nonadecatriene-5,14-diol	Analgesic, anti-inflammatory, and antimicrobial properties
31	45.077	0.84	(R)-(-)-14-Methyl-8-hexadecyn-1-ol	Analgesic, anti-inflammatory, and antimicrobial properties
32	45.192	0.88	cis-4,4-Dimethylbicyclo(6.3.0)undecane-2,6-dione	Analgesic, anti-inflammatory, and antimicrobial properties

*Dr. Duke's Phytochemical and Ethnobotanical Databases.

Medicinally important Twenty-eight phytoconstituents were found in the Ethanol extract according to the GC-MS analysis. Plants containing these phytoconstituents have been shown to have pharmacological properties such as antimicrobial, anti-inflammatory, anticancer and antioxidant effects.

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	Antibacterial and antifungal activity.
2	18.500	0.71	1-(4-Ethoxyphenyl)propan-1-ol	Moderate Microbial activity.
3	19.139	0.35	Pentadecane	Alkane.
4	19.710	1.89	Benzoic acid	Antimicrobial and Antifungal Agent
5	25.200	4.54	Tetradecanoic acid	Used as emulsifier.
6	25.405	1.00	3-Methylheptadecane	Alkane.
7	26.111	2.49	cis-9-Hexadecenal	Antimelanogenic, antifungal properties.
8	26.507	0.94	Isopropyl myristate	Emollient as used in cosmetics.
9	29.322	9.67	n-Hexadecanoic acid	Hepatoprotective, anti-inflammatory,antihistaminic, and anti-arthritic activity.
10	29.512	1.17	3-Methylheptadecane	Alkane.
11	29.923	2.02	Hexadecanoic acid	Hepatoprotective, anti-inflammatory,antihistaminic, and anti-arthritic activity.
12	32.533	3.86	10E,12Z-Octadecadienoic acid	Anti-inflammatory, antihistaminic activity.
13	32.652	10.94	9-Octadecenoic acid	Anti-inflammatory, antihistaminic activity.
14	33.096	4.22	Octadecanoic acid	Anti-inflammatory, antihistaminic activity.
15	33.634	4.76	Octadecanoic acid	Anti-inflammatory, antihistaminic activity.
16	36.498	3.21	12-Hydroxystearic acid	Fatty acid surfactant.
17	38.084	3.70	Tricyclo[20.8.0.0(7,16)]triacontane	Antioxidant and anti-inflammatory effects
18	38.923	2.56	Hexadecanoic acid	Hepatoprotective, anti-inflammatory,antihistaminic, and anti-arthritic activity.

Peak No.	R.T.	Peak Area %	Name of compound	Activity*
19	39.102	2.02	Undec-10-ynoic acid	Antifungal, anti-quorum sensing, and potential antimicrobial properties
20	39.341	3.41	Tetradecahydrocyclododeca[c]furan	antimicrobial and anti-inflammatory
21	39.490	1.41	2-Eicosen-5-olide	Anti-inflammatory, antibacterial, and antioxidant Activity.
22	40.923	1.31	9-Octadecenoic acid	Anti-inflammatory, antihistaminic activity.
23	41.623	3.24	Tricyclo[20.8.0.0(7,16)]triacontane	Antioxidant and anti-inflammatory effects
24	42.379	2.90	1,4-Benzenedicarboxylic acid	Anti-inflammatory, antimicrobial, and anticancer properties
25	42.764	5.56	E,E-3,13-Octadecadien-1-ol	Analgesic, anti-inflammatory, and antimicrobial properties
26	42.894	17.29	cis-13-Docosenamide	Antimicrobial and anticancer activity.
27	43.381	1.09	Squalene	Antioxidant, antitumor, antibacterial activity.
28	44.134	1.83	E,E,Z-1,3,12-Nonadecatriene-5,14-diol	Analgesic, anti-inflammatory, and antimicrobial properties

*Dr. Duke's Phytochemical and Ethnobotanical Databases.

Discussion

Pyranone dervatives shows remarkable antitumor activities[31]. Terpineols possess various pharmacological benefits such as anticancer, antimicrobial, anti-inflammatory, antioxidant, and antiallergic[32]. Tetradecane was reported to possess antimicrobial properties[33]. Hydnocarpic acid and Chaulmoogric acid are used to treat leprosy[34]. Heneicosane and eicosane are the pheromone exhibit microbial activity[35]. It was declared that pentadecane and hexadecanoic acid have antifungal, antibacterial and antioxidant activities[36]. Phytol showed antibacterial, anti-inflammatory, immune-modulating, cytotoxic, antioxidant, autophagy- and apoptosis-inducing, anxiolytic, and metabolism-modulating properties[37]. Octadecanoic acid, 9,12 hexadecadionic acid and 9,12 octadecadionic acid are the compounds that plays an important role in prostaglandin biosynthesis of cell membranes with several biological functions such as anti-inflammatory, antihistaminic, anti-arthritic, and hepatoprotective[38, 39]. It was found that 1, 2, 3-propanetriol 1-acetate exhibits an inhibitory effect on

microorganisms[40]. It was declared that hexadecanoic acid have antifungal, antibacterial and antioxidant activities[36].

Conclusion

In the present study, Thirty-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.* by (GC-MS) analysis. The major phytoconstituents identified in Ethyl acetate extract was Hydnocarpic acid and Chaulmorgic acid (15.29%) used to treat leprosy. Octadecanoic acid derivatives (27.4%) exhibited strong Anti-inflammatory, antihistaminic, anti-arthritic, and hepatoprotective activity. The major phytoconstituents observed in ethanol extract was hexadecanoic acid (14.25%) shows Hepatoprotective, anti-inflammatory, antihistaminic, and anti-arthritic activity and cis-13-docosenamide (17.29%) shows Antimicrobial and anticancer activity.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent for Publication Granted for publication.

Authors Contributions Both authors contributed equally to the study.

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

Availability of data and Materials All the data obtained during this study are included in this article.

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

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Figures

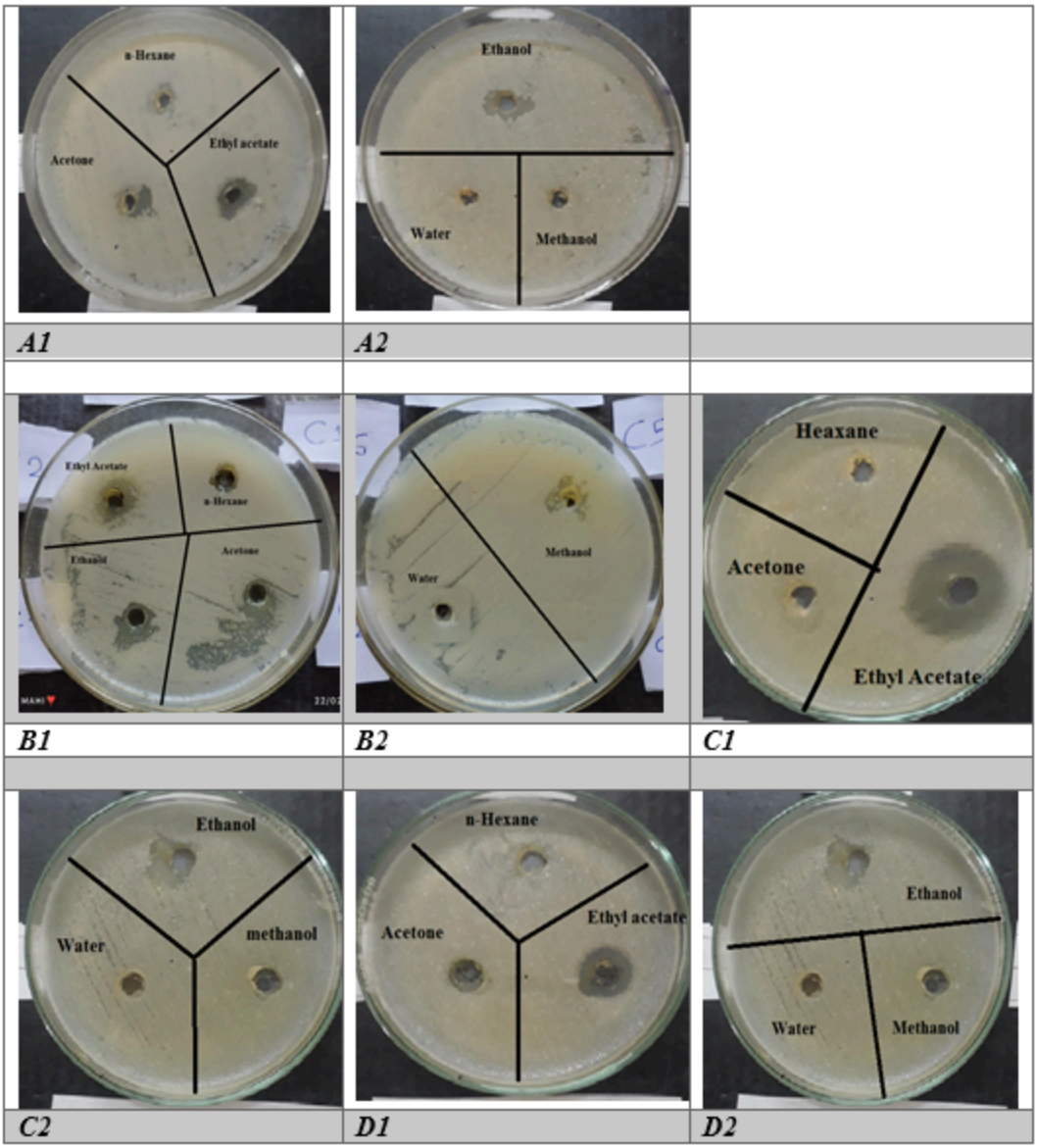

Figure 1

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

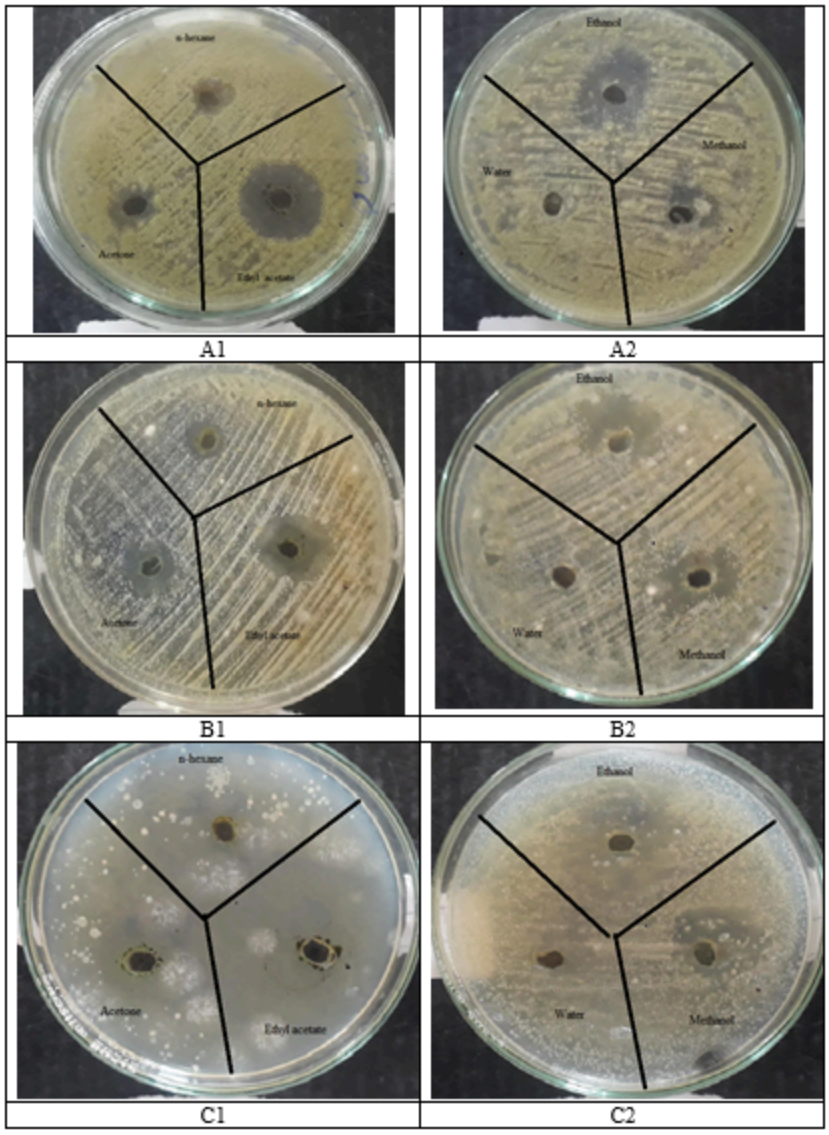

Figure 2

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

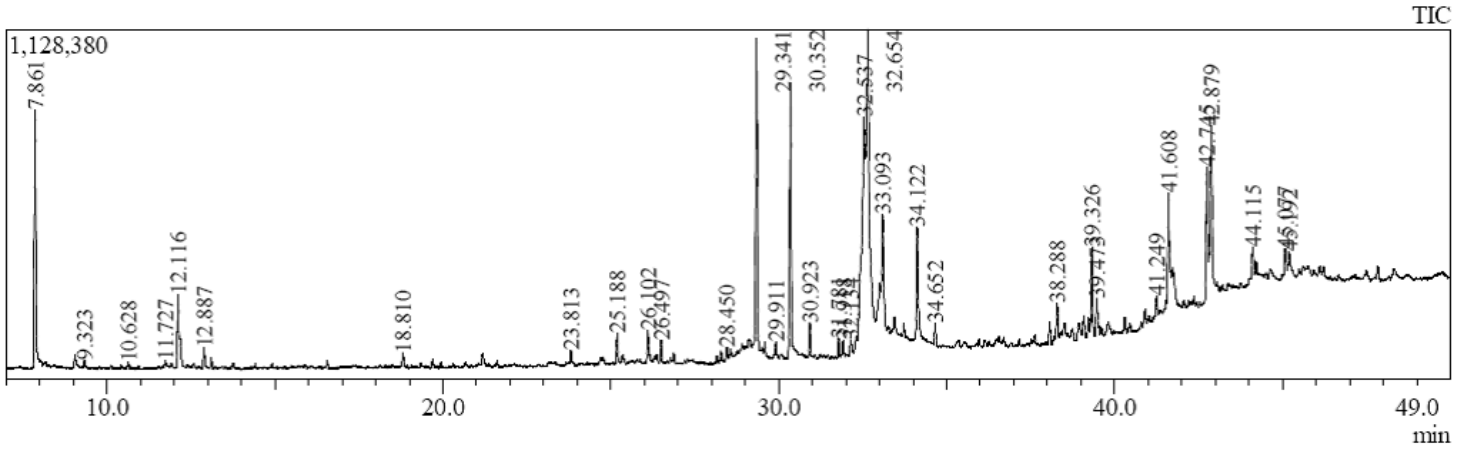

Figure 3

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

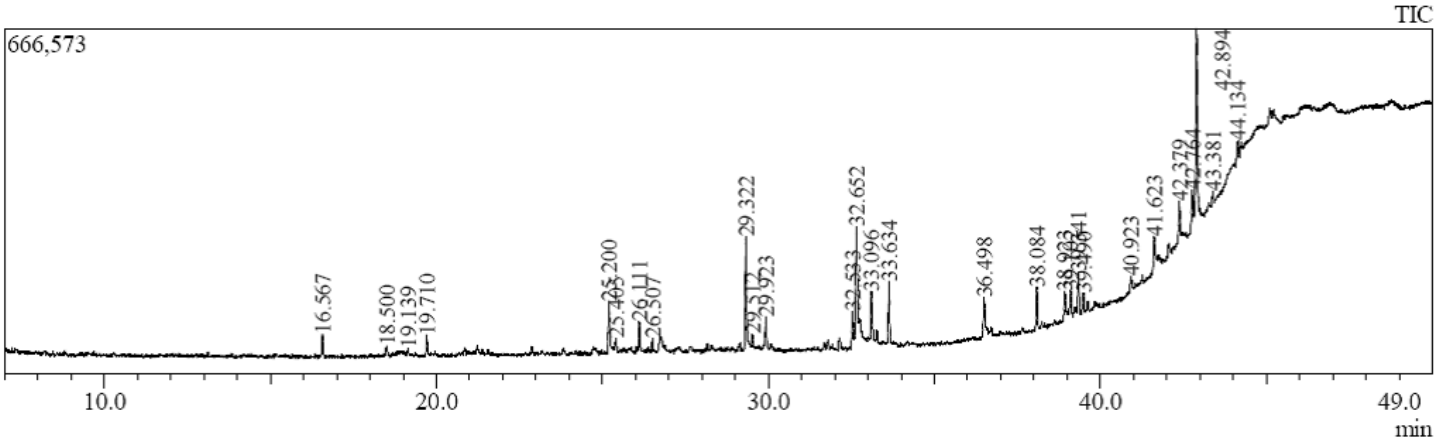

Figure 4

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