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GC MS analysis and antimicrobial activity of *Isopterygium albescens*

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Abstract

Plant extracts have traditionally been utilized as an alternate reservoir of antibacterial chemicals. The exploration of alternate, cost-effective sources became necessary due to the recent proliferation of multi-drug-resistant bacteria and the subsequent rise in treatment expenses. The *Hypnaceae* is a highly diverse and expansive family of pleurocarpous mosses, with over 60 taxa. The antibacterial activity of the ethanolic extract of *Isopterygium albescens* was investigated against two Gram-positive bacteria, *Staphylococcus aureus* and *Bacillus subtilis*, as well as two Gram-negative bacteria, *Escherichia coli* and *Pseudomonas aeruginosa*, using the Kirby Bauer Disc diffusion test. Discs made of paper were created, containing various concentrations of the extracts (150, 250, and 500 µg ML⁻¹), as well as an ethanol control and a standard antibiotic control. A GC/MS analysis was conducted to examine the phytochemical constituents found in the methanolic extracts of the plant. The plants were analyzed using GC/MS, and a total of 7 distinct phytochemical substances were discovered. Bryophyte-derived secondary metabolites exhibit diverse pharmacological actions, such as antibacterial, antioxidant, anti-inflammatory, and anticancer activities. The investigation utilized the methanolic extract. The extract underwent filtration and concentration by the utilization of a rotary evaporator. The antibacterial activity was assessed against four established bacterial strains. The ethanolic extract shown inhibitory effects on all four tested strains, encompassing both gram-positive and gram-negative bacteria. Two bacteria, one gram-positive and one gram-negative, were utilized. The following microbial strains were selected: *Staphylococcus aureus* MTCC 96, *Bacillus subtilis* MTCC 1133, *Escherichia coli* MTCC 452, *Pseudomonas aeruginosa* MTCC 3541, and *Candida albicans* MTCC 183, a fungal strain.

Keywords: GC-MS analysis, *Staphylococcus aureus* MTCC 96, *Bacillus subtilis* MTCC 1133, *Escherichia coli* MTCC 452, *Pseudomonas aeruginosa* MTCC 3541, *Candida albicans* MTCC 183

Introduction

Bryophytes are the second most diverse group of green land plants (Asakawa Y. 2007) ^[1]. Bryophytes are categorized into three distinct groups: liverworts (Marchantiophyta), which consist of both leafy and thalloid types, hornworts (Anthocerotophyta), and mosses (Bryophyta). Bryophytes possess numerous therapeutic qualities and contain bioactive substances. Bryophyta encompasses mosses, Marchantiophyta encompasses both leafy and thalloid liverworts, and Anthocerotophyta (Harris *et al.* 2020) ^[5] is another group. *Isopterygium albescens* is a monoecious moss, characterized by its slender form and yellow-green to green coloration. The stem has a creeping growth habit, with branches arranged in a pinnate pattern. The leaves are oval in shape and are oriented in an erectopatent manner. The leaf margin is nearly smooth, however it may have some tooth-like projections towards the upper portion. The sporophytes are found on sturdy stems, and the capsule is positioned horizontally or drooping. It has an ovate-cylindrical shape and is curled at the base. Seta was discovered in an upright and slim form, measuring 1-2 cm in length.

They are dispersed in tropical and temperate environments. Mosses are bryophytes that lack vascular tissue and serve as excellent indicators of environmental conditions. Bryophytes possess pharmacologically active substances and have been historically employed in European, Chinese, Indian, and North American medical practices for the treatment of cardiovascular ailments, bronchitis, tonsillitis, tympanites, and skin disorders. Madsen and Pates (1952) ^[6] found the presence of antibacterial compounds in the thalli of bryophytes. The antifungal activity of 18 species of bryophytes, including *Pellia epiphylla* and *Diplophyllum albicans*, as well as 16 additional mosses, was assessed by Wolter in 1964 ^[13].

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The methanolic extract derived from the liverworts *Targionia hyophylla* was discovered to have efficacy against five plant pathogenic fungi, namely *Aspergillus niger*, *Botrytis cineria*, *Trichoderma viridae*, *Penicillium chrysogenum*, and *Penicillium expansum* (Alam 2012) [12].

The antimicrobial screening of *Marchantia polymorpha* L. was conducted against three bacterial strains, namely *E.coli*, *P. mirabilis*, and *S.aureus*, as well as four fungal strains, namely *A niger*, *A flavus*, and *T mentagrophytes*, by Mewari and Kumar in 2008 [10]. The study conducted by Sawant and Karagade (2010) studied the antibacterial activity of liverworts *T hyophylla*, *H wallichiana*, and *P.intermedium* using in vitro methods. The GC-MS technique is a convenient method for identifying the chemical components and antibacterial characteristics of moss species *I. albescens*. This investigation facilitated the identification of the components of the methanolic plant extract through the utilization of GC-MS. The plant extract was assessed for its cytotoxic and antibacterial characteristics, taking into consideration the plant's traditional ethno pharmacological applications.

Materials and Methods

Plant specimens were gathered from several locations in Mount Abu, a region in the western state of Rajasthan, India, on the boundary with Gujarat. Situated atop a lofty rocky plateau within the Aravalli Range and encompassed by dense woodland. The location is situated at a latitude of 24°36' north and a longitude of 72°42' east in the Sirohi district. The plant material was carefully rinsed with water to eliminate all dirt particles and subsequently dried under ambient environmental conditions. Taxonomic identity was confirmed by comparing it with authenticated herbarium specimens at the Department of Botany Chandigarh, Panjab University, Chandigarh, and herbarium specimen (PAN) no.6457. Voucher specimens of the plants are kept in the herbarium of the Department of Botany Chandigarh. *I. albescens* was extracted using a methanolic solvent at a 1:5 ratio and subjected to agitation in an orbital shaker for a duration of 48 hours. The extracts were strained through Whatman no.1 filter paper. The produced extracts were utilized to conduct a preliminary screening of phytochemical substances and perform GC-MS analysis to identify the pharmacological components and assess the antibacterial and antifungal effectiveness. The GC-MS analysis was conducted using the Thermo TSQ8000 GC-MS instrument at CIL, Panjab University Chandigarh, with the methanolic extract as the sample. The material was analyzed using gas chromatography-mass spectrometry (GC-MS) with the Thermo TSQ8000 instrument for chemical separation. The chemical components of the plant extract were determined by comparing their retention time using NIST libraries.

Gas chromatography-mass spectrometry (GC-MS) analysis

Gas chromatography (GC) can be used to separate the volatile constituents in a sample. However, mass spectrometry (MS) assists in both analyzing the components and determining their identities based on their mass. In this work, the technique of Thermo-Trace 1300 GC coupled with Thermo TSQ 8000 Triple Quadrupole Mass Spectrometer was employed to evaluate the phytochemical contents found in *I. albescens*. The Thermo-Trace 1300 GC

was used for gas chromatography, while the Thermo TSQ 8000 was used for mass spectrometry. The mass spectrum was obtained within the 50-700 range, using an ionization voltage of 70 electron volts (eV). The temperature of the ion source was adjusted to 230°C.

Antimicrobial assay

Test Microorganisms. The ethanolic extract was further tested against bacterial strains including *Staphylococcus aureus* MTCC 96, *Bacillus subtilis* MTCC 1133, *Escherichia coli* MTCC 452, *Pseudomonas aeruginosa* MTCC 3541.

With the help of the Zone Inhibition method, also known as the Kirby-Bauers Method, the antibacterial activity was evaluated. To determine whether or not the extract is effective against bacteria, a measured quantity of the standardized stock suspension of bacteria, which included 105-106 CFU per ml, was combined with 100 ml of Mueller-Hinton agar medium that was kept at a temperature of 45 °C. The Mueller-Hinton agar medium that had been inoculated as a consequence was divided into 20 ml portions and placed on plates that had been sterilized earlier. It was left to sit at room temperature for the agar medium to become solid. Following the removal of the agar discs, the hole was filled with ethanol extract at two different concentrations, 100 µg/mL and 500 µg/mL. The mixture was then allowed to diffuse at room temperature for a duration of two hours without any disturbances. The plates that were produced were left to incubate at 37 °C for an entire night. Following this, the plates were examined to determine whether or not there was any inhibition in the development of the bacteria, and the diameter of the zone of inhibition was assessed. The same methodology that was utilized for the investigation of antibacterial activity was also utilized for the testing of the extract's antifungal activity. There is only one modification in the approach, and that is that instead of using Mueller-Hinton agar medium, another medium called Sabouraud dextrose agar media was employed, and the temperature of the inoculation medium was kept at 25 degrees Celsius. In this study, we utilized two gram-positive and gram-negative bacteria, specifically *Staphylococcus aureus* MTCC 96, *Bacillus subtilis* MTCC 1133, *Escherichia coli* MTCC 452, and *Pseudomonas aeruginosa* MTCC 3541. Additionally, we utilized one fungal strain, *Candida albicans* MTCC 183, which was evaluated based on the presence or absence of inhibition zones, as well as the diameters of inhibition zones. In each plate, a single disc was loaded with solvent alone, which served as the vehicle control. Additionally, a Ciprofloxacin disc containing 10µg and an Amphotericin B disc containing 50µg were added as the positive control.

Result and Discussion

The moss *Isopterygium albescens* in GC-MS investigation showed a wide range of phytoconstituents compounds.

GC-MS chromatogram of a methanolic plant extract containing the chemicals shown. The active chemicals are listed with their RT, peak area percentage, and molecular formula. 9,12,15-Octadecatrienoic Acid, 2-[(Trimethylsilyl)oxy]-1-[(trimethylsilyl)oxy] methyl Ethyl ester (Z, Z, Z) is a fatty acid that is antioxidant, anti-inflammatory, antibacterial, cancer-preventive, hypocholesterolemic, hepatoprotective, and nematocide (Rajendran et al. 2017) [9]. Ethyl iso-allocholate has

antibacterial, anti-inflammatory, antiasthmatic, diuretic, and anti-diabetic properties. 17-Pentatriacontene is known to have anti-inflammatory, anticancer, antibacterial, and antiarthritic effects (Dinesh *et al.* 2018) ^[4]. Oleic acid and 3-(octadecyloxy) propyl ester are fatty acids with antifungal properties.

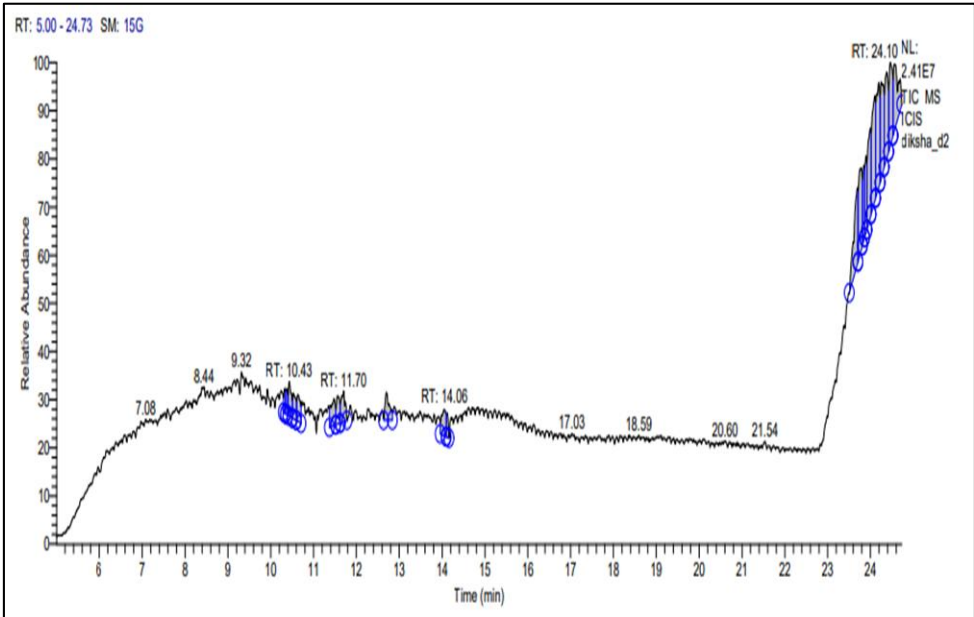


Fig 1: GC-MS chromatogram of phytoconstituents in aqueous methanolic fraction of *I. albescens*

Table 1: Phytoconstituents identified from methanolic extract of *I. albescens*

Antibacterial and Antifungal Activity (Zone of Inhibition in mm)					
Amount (µg/disk)	<i>S. aureus</i>	<i>E. coli</i>	<i>B. subtilis</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>
PC	30.6±0.57	26±0	33±0	26±0	16.3±0.57
150	7.3±0.58	9±0	8.33±0.57	9±0	9±0
250	7±0	9±0	9±0	9±0	9±0
500	7±0	9.7±0.57	9.3±0.57	9.7±0.57	9±0

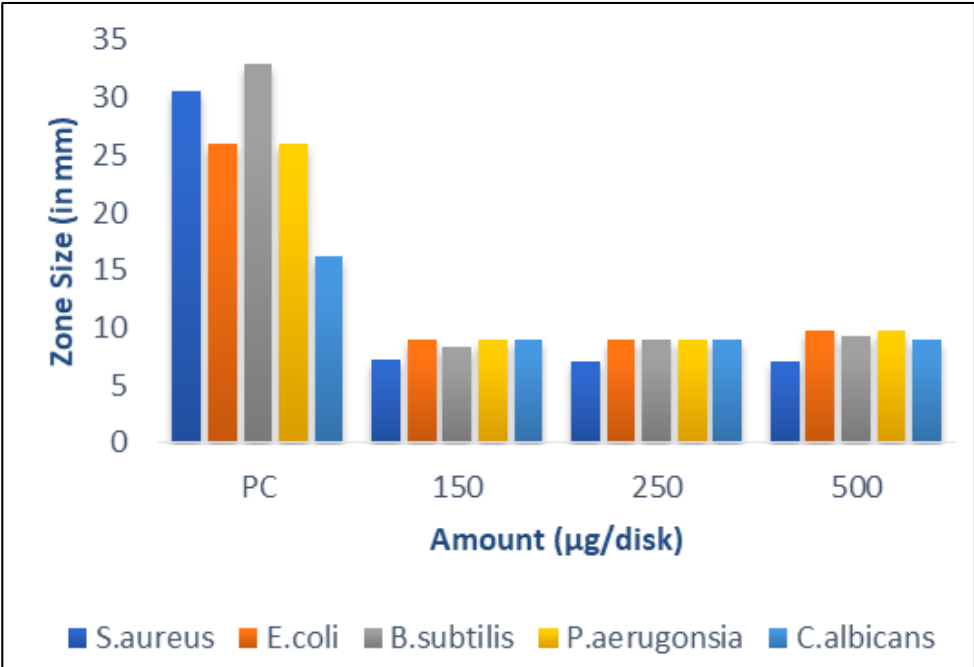


Table 2: Antibacterial and antifungal activity of *I. albescens*

Compound name	Retention time	Peak area %	Properties	Molecular formula
9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[[trimethylsilyl]oxy]methyl ethyl ester, (Z, Z, Z)	10.32	1.00	Antioxidant, anti-inflammatory, antimicrobial activity, cancer preventive, hypocholesterolemic, hepatoprotective, nematicide.	C27H52O4Si2
PSI. psi.Carotene, 1,1',2,2'-tetrahydro-1,1'-dimethoxy	10.43	2.42	Antioxidant	C42H64O2

1-Monolinoleoylglycerol trimethylsilyl ether	10.53	1.91	Antioxidant, antimicrobial, anti-inflammatory, antiarthritic, antiasthma, diuretic	C ₂₇ H ₅₄ O ₄ Si ₂
Ethyl iso-allocholate	11.70	2.97	Antimicrobial, anti-inflammatory, antiasthma, diuretic, antidiabetic	C ₂₆ H ₄₄ O ₅
1,2-Benzenedicarboxylic acid, butyl 2-Ethylhexyl ester	12.70	2.92	Antibacterial and antioxidant	C ₂₀ H ₃₀ O ₄
Oleic acid, 3-(octadecyloxy)propyl ester	23.75	7.47	Anti-inflammatory, antioxidant, and antimicrobial	C ₃₉ H ₇₆ O ₃
17-Pentatriacontene	24.10	9.20	Anti-inflammatory, anticancer, antibacterial, and antiarthritic properties	C ₃₅ H ₇₀

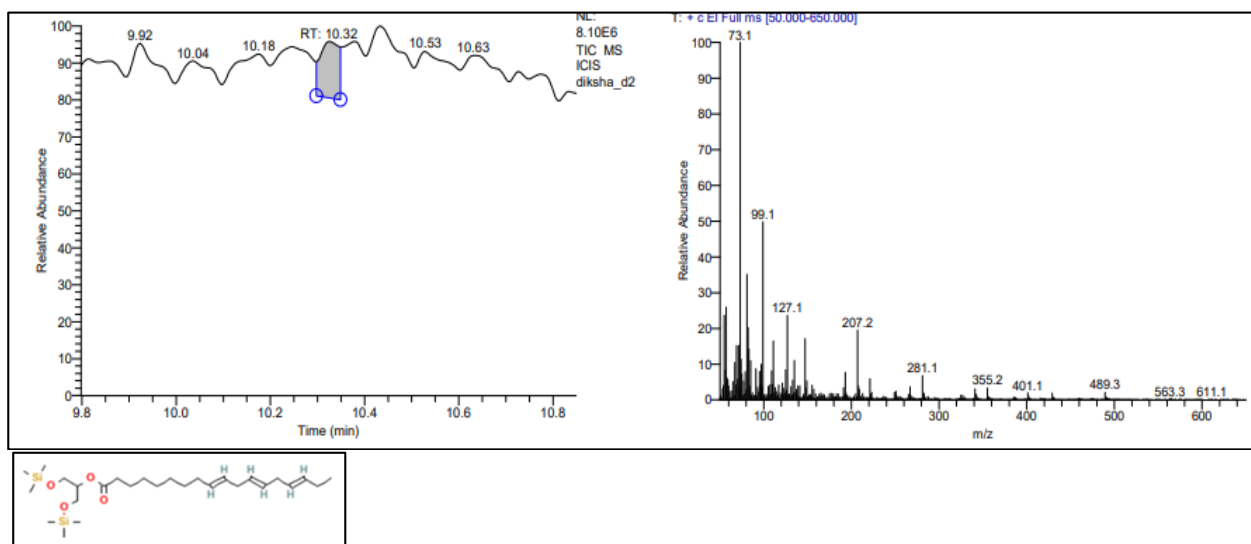


Fig 1: GC-MS Chromatogram of 9, 12, 15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[[[(trimethylsilyl)oxy]methyl]ethyl] ester, (Z, Z, Z)

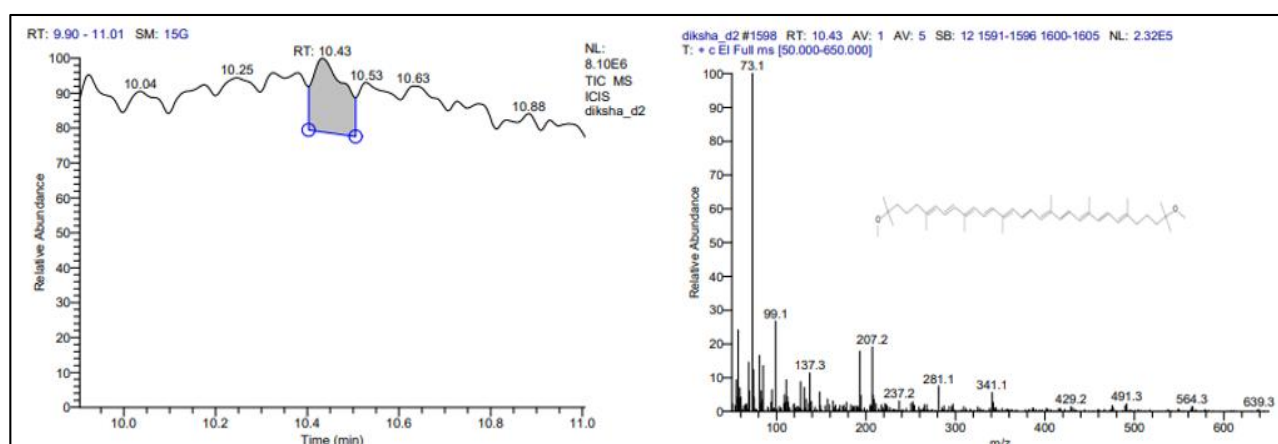


Fig 2: GC-MS Chromatogram of PSI, PSI. Carotene, 1, 1', 2, 2'-tetrahydro-1, 1'-dimethoxy

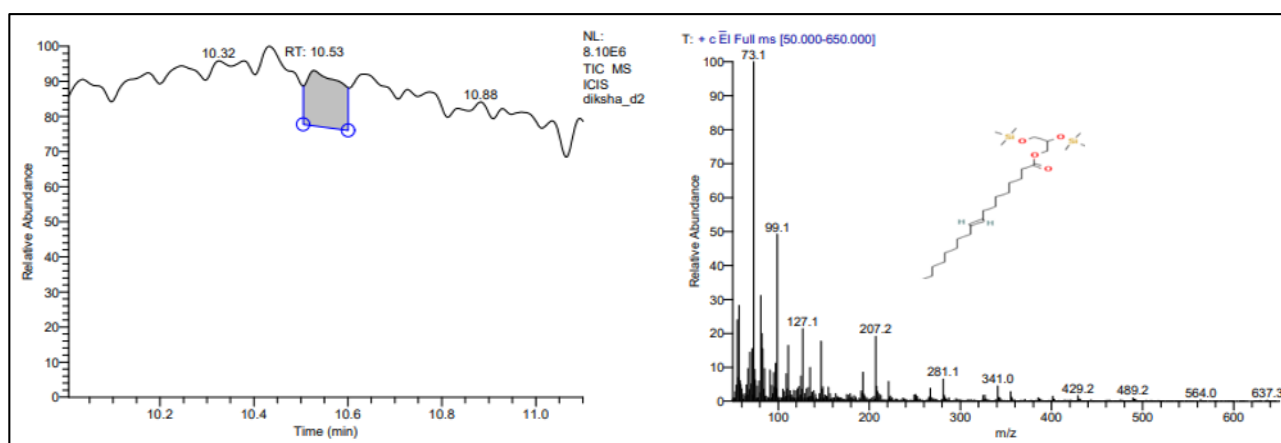


Fig 3: GC-MS Chromatogram of 1-Monolinoleoylglycerol trimethylsilyl ether

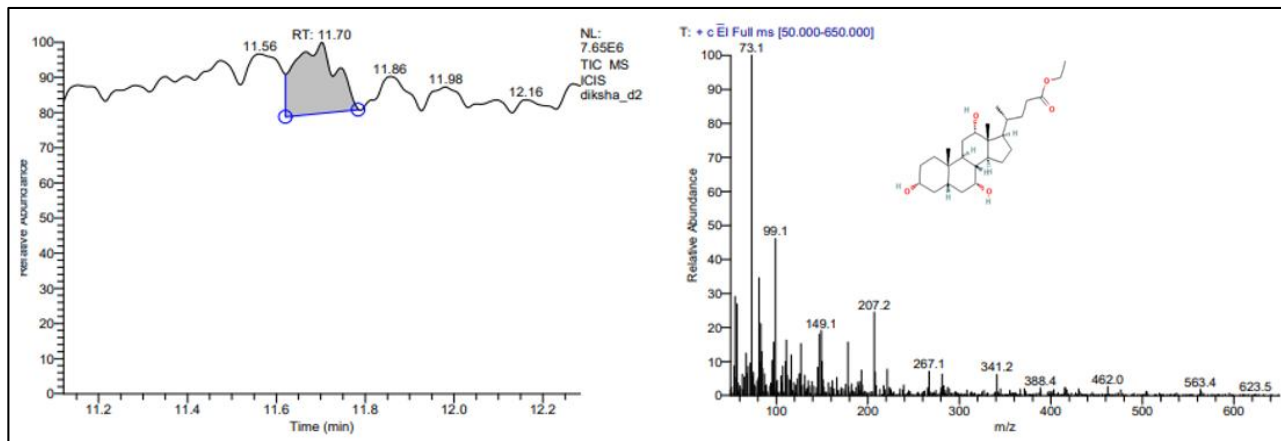


Fig 4: GC-MS Chromatogram of Ethyl iso-allocholate

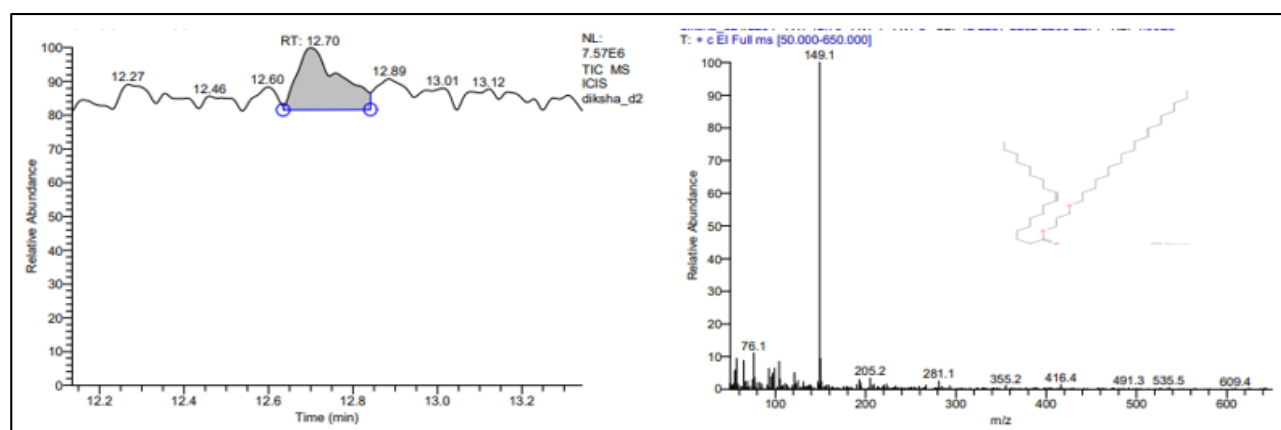


Fig 5: GC-MS Chromatogram of 1, 2-Benzenedicarboxylic acid, butyl 2-Ethylhexyl ester

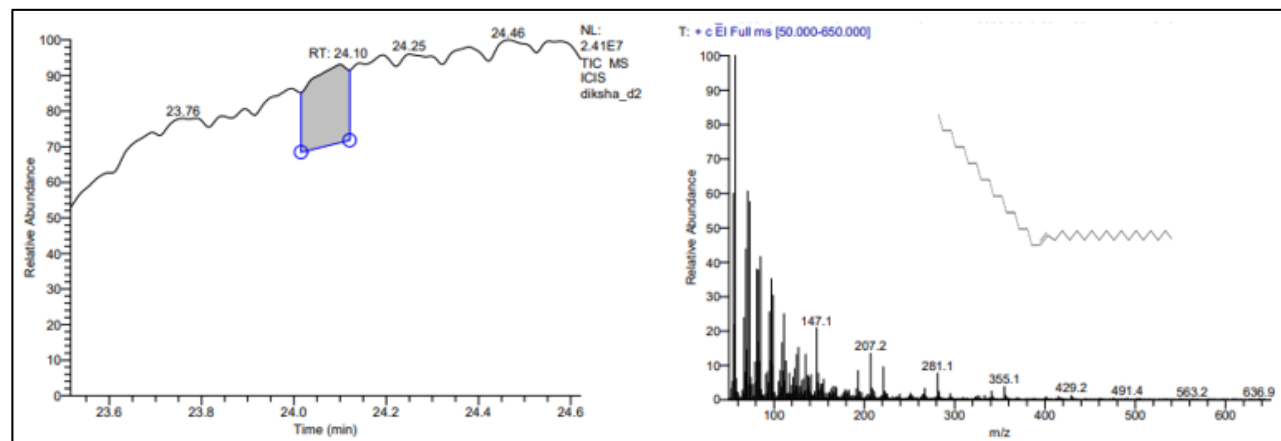


Fig 6: GC-MS chromatogram of oleic acid, 3-(octadecyloxy) propyl

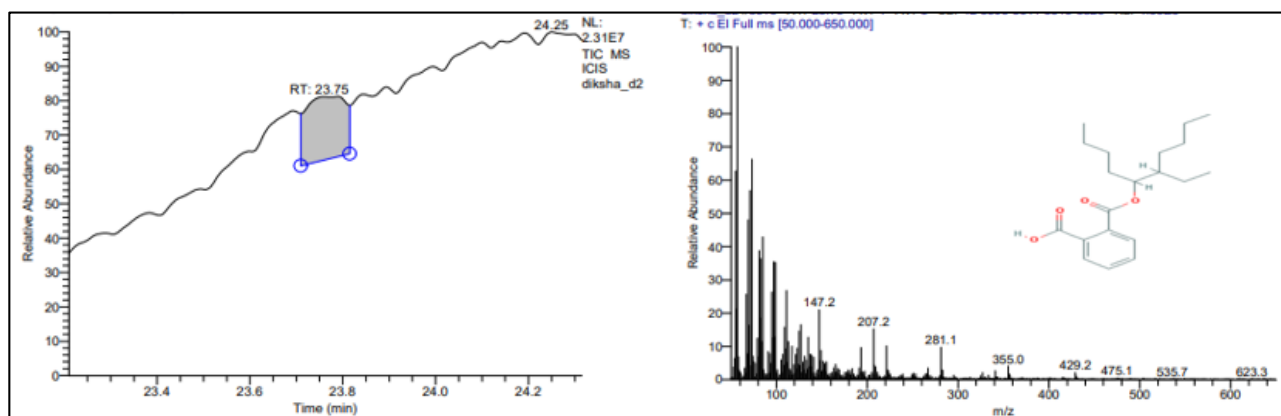


Fig 7: GC-MS Chromatogram of 17-Pentatriacontene

The diameter of the inhibition zones of antimicrobial agents demonstrated the antibacterial capacity of both conventional antibiotics and ethanol plant extract at a range of doses. As a consequence of this, the extract samples were found to possess antibacterial properties and were able to inhibit the growth of all of the selected bacterial strains. These strains included Gram-positive bacteria, which included *Staphylococcus aureus* MTCC 96, *Bacillus subtilis* MTCC 1133, *Escherichia coli* MTCC 452, and *Pseudomonas aeruginosa* MTCC 3541, as well as one fungal strain, *Candida albicans* MTCC 183. Ciprofloxacin, at a concentration of 10µg, was employed as the positive control for every bacterial strain, whereas Amphotericin B, at a concentration of 50µg, was utilized together with the fungal strain as the positive control. When typical antibiotics were present in varying quantities, the widths of the inhibition zones of antimicrobial drugs demonstrated their ability to prevent bacterial growth.

Conclusion

The GC MS study of *Isopterygium albescens* reveals a significant abundance of bioactive chemicals and a wide range of pharmacological properties, including antibacterial, antifungal, anti-inflammatory, anticancer, antioxidant, and hepatoprotective activity. The current research demonstrates the ability of *Isopterygium albescens* to act as an antibiotic against *S. aureus*, *E. coli*, *B. subtilis*, and *P. aeruginosa*. Plant extract exhibits substantial potential for inhibiting the proliferation of bacterial strains. However, it is necessary to conduct a comprehensive examination of the plant and its Phytochemistry through a systematic process of isolating and purifying its bioactive chemicals. It will ultimately contribute to ensuring the verification. *Isopterygium* is a bryophyte of significant ethnomedicinal importance due to its strong efficacy in treating both bacterial and fungal illnesses, making it a viable option for incorporation into current healthcare practices.

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