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EVALUATION OF PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL ACTIVITY OF *TYPHA ANGUSTIFOLIA* L. AND *ECHINOPS RITRO*

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ABSTRACT

Background: Medicinal plants have historically provided significant supplies of bioactive chemicals. The worrisome rise in antibiotic resistance has made it more important than ever to find natural alternatives to antibiotics. Typha angustifolia L. and Echinops ritro are ethnomedicinally significant species recognised for their varied phytoconstituents; nonetheless, comparable assessments of their antibacterial efficacy are scarce. **Objectives:** This study sought to investigate the phytochemical profiles and antibacterial activity of Typha angustifolia and Echinops ritro utilising extracts derived from various solvents and plant components. **Methods:** Plant specimens were gathered from the Aravali range (Rajasthan, India), shade-dried, and subjected to sequential solvent extraction utilising water, ethanol, chloroform, and petroleum ether. Standard qualitative and quantitative assays were used to do phytochemical screening. The disc diffusion method was used to test antibacterial activity against Escherichia coli, Enterobacter aerogenes, Agrobacterium tumefaciens, Bacillus subtilis, Staphylococcus aureus, Pseudomonas putida, and Pseudomonas syringae. We used GC–MS analysis to find bioactive chemicals. **Results:** Both plant species demonstrated a wide range of antibacterial activity, with petroleum ether extracts displaying the largest inhibitory zones. Echinops ritro (dry root extract) exhibited the most substantial inhibition (12.8 mm against P. syringae), but Typha angustifolia (dry root extract) displayed considerable inhibition (11.0 mm against S. aureus). Phytochemical analysis verified the existence of alkaloids, flavonoids, glycosides, saponins, tannins, and terpenoids. GC–MS profiling identified bioactive components including n-hexadecanoic acid, squalene, lupeol, stigmasterol, and phytol compounds recognised for their significant antibacterial, antioxidant, and anti-inflammatory properties. **Conclusions:** The results confirm the ethnomedicinal significance of T. angustifolia and E. ritro as effective antibacterial agents. The increased activity in petroleum ether and chloroform extracts suggests the involvement of lipophilic phytoconstituents in antibacterial effectiveness. These findings underscore the potential of both plants as viable sources for the development of plant-based antimicrobial agents, necessitating additional isolation and pharmacological assessment of active chemicals.

KEYWORDS: Typha angustifolia L., Echinops ritro, phytochemical analysis, GC–MS, antibacterial activity, natural products, antibiotic resistance, bioactive chemicals

INTRODUCTION

1.1 The History of Ethnopharmacology and Medicinal Plants

Since ancient times, medicinal plants have played a crucial role in human healthcare systems. For the treatment of chronic and infectious diseases, traditional medicine has mainly relied on plant-derived products, especially in Asia, Africa, and the Mediterranean. Nearly 80% of the world's population, according to the World Health Organisation (WHO), gets their primary medical care from plant-based medications (WHO, 2013). These plants' abundance of secondary metabolites, which include alkaloids, flavonoids, tannins, terpenoids, phenolics, and saponins, is thought to contribute to their therapeutic potential. These compounds are frequently in charge of antimicrobial, anti-inflammatory, antioxidant, and anticancer properties (Bora & Sharma, 2011; Petrovska, 2012).

The concerning increase in antibiotic resistance in recent decades has sparked a renewed interest in ethnomedicinal plants as supplementary or alternative sources of new antimicrobial medicines (Rather et al., 2021). Investigating phytochemicals in traditional plants paves the way for therapeutic development and discovery while also offering scientific support for traditional knowledge.

1.2 The Importance of Natural Products and Antimicrobial Resistance

The emergence of multi-drug-resistant (MDR) bacteria, including Pseudomonas aeruginosa, Escherichia coli, and Staphylococcus aureus, has become a global health concern. Since traditional antibiotics are becoming less and less effective, new methods of infection control are required. Natural products provide a range of chemical structures that are effective against resistant strains, particularly those made from ethnomedicinal plants (Aneja et al., 2012). Unlike synthetic medications, phytochemicals frequently operate through several channels, making it more difficult for organisms to build resistance (Newman & Cragg, 2020). Thus, it is pertinent and opportune to investigate the antibacterial capabilities of lesser-known species such as Typha angustifolia and Echinops ritro.

1.3 Typha angustifolia: A Quick Overview L. Typha angustifolia L., often known as narrowleaf cattail, is a long-lived aquatic plant that belongs to the Typhaceae family. Wetlands, marshes, and riverbanks are among the many locations in Asia, Europe, and North America where it can be found. The plant's leaves, rhizomes, pollen, and roots are traditionally used to cure wounds, burns, haemorrhages, diarrhoea, and infections, among other ailments (Sharma et al., 2014).

Flavonoids, phenolic acids, tannins, and sterols have been found by phytochemical studies in T. angustifolia, and these components have been linked to the plant's antibacterial, antioxidant, and wound-healing properties (Gao et al., 2017). Recent studies support its ethnomedicinal use by highlighting its ability to inhibit bacterial growth and reduce oxidative stress (Xu et al., 2025).

1.4 A quick overview of Echinops ritro L.

The perennial herb known as globe thistle, or Echinops ritro L., is a member of the Asteraceae family and grows in Central Asia and the Mediterranean. The herb has historically been used to treat pyrexia, gastrointestinal issues, inflammatory ailments, and microbial infections (Mohamed et al., 2021). The roots and aerial parts contain large concentrations of flavonoids, alkaloids, polyacetylenes, and sesquiterpene lactones. All of these have been shown to have cytotoxic, antimicrobial, and anti-inflammatory properties (Kharchoufa et al., 2023).

Extracts from Echinops species offer a broad spectrum of antibacterial and antifungal activities, according to recent pharmacological studies, making them promising candidates for natural antimicrobial medications.

1.5 The Value of Comparative Research

There is little scientific data on the relative antibacterial activity of Typha angustifolia and Echinops ritro, despite their ethnomedical relevance. When combined with antibacterial activity, an analysis of their phytochemical profiles can: • Verify conventional claims; • Identify bioactive phytochemicals; and • Provide information for potential drug discovery.

In addition to identifying which plant has the most promise for antibacterial development, this study aims to establish a pharmacological basis for the traditional uses of these two plants. **(Mishra et al, 2025).**

1.6 Research Deficit

Although Typha angustifolia and Echinops ritro have been the subject of independent research, their phytochemical composition and antibacterial activity have not yet been systematically compared. By integrating traditional antimicrobial testing with qualitative and quantitative phytochemical studies, this work seeks to remedy that shortcoming.

2. Resources and Procedures

2.1 Gathering and Verifying Plant Material

Fresh plant fragments of Typha angustifolia L. and Echinops ritro were collected from the Aravali range in Rajasthan's Pali district. Books from the Department of Botany at J.N.V. University in Jodhpur were used to verify their identity. Using flowing tap water and then distilled water, we cleaned various plant parts, including the leaves, blossoms, pods, stems, seeds, and bark, to remove any dirt or debris that might have adhered to them. After that, we let them dry for about ten days at 28±2°C in the shade. The dried plant samples were ground into a fine powder using a mixer grinder, and the resulting particles, which ranged in size from 50 to 150 mm, were then sieved. The powder was stored at room temperature in airtight polythene bags before extraction. 25 grammes of dry powder were retained on Whatman filter paper No. 1, and 100 millilitres of solvent were used to extract the powder in a Soxhlet system. Water, ethanol, petroleum ether, and chloroform were used as extraction solvents. The extracts were then dried. The dried extracts were stored at 4°C in a refrigerator. Finally, 5 mg of each extract was applied to each disc.

2.2 Susceptibility Test for Antibacterials

The pathogenic bacterial strains Pseudomonas putida, Agrobacterium tumefaciens, Bacillus subtilis, Enterobacter aerogenes, E. coli, and Pseudomonas aeruginosa were tested against all plant extracts. The plant extracts' antibacterial effectiveness was assessed using the disc diffusion method (Bauer et al., 1966). Twenty millilitres of sterile nutrient agar medium for pathogens were added to each sterile petri plate. After five minutes of hardening, the plates were equally

swabbed with a 0.1% inoculum suspension. Each plate's entire agar surface was infected with a swab, initially horizontally and subsequently vertically. This guarantees that the organism is dispersed uniformly across the surface of the agar. After the agar plates were seeded with bacteria, we placed the filter paper discs (5 mm in diameter) containing 5 mg of dry extract on top and allowed the substance to diffuse for 5 minutes. After that, we incubated the plates for 24 hours at 37°C. After a day, we measured the inhibition zones that developed around the disc using a clear ruler. These investigations were carried out twice.

2.3 Phytochemical Analysis

Collecting plant matter

The field provided us with fresh plant resources. After that, the plants were brought inside the lab and thoroughly cleaned with running tap water and then distilled water to remove any dirt or debris that might have adhered to them. After that, they spent ten to fifteen days drying in the shade. These dehydrated plant components were then stored in an airtight container until they were needed again.

Preparing for extracts from plants

For 48 to 72 hours, dissolve 10 grammes of dried plant powder in 100 millilitres of distilled water and methanol. The samples were filtered via a muslin fabric. Using a water bath, the solvent—distilled water and methanol—was transformed into a semisolid (Mahida and Mohan 2007).

First Phytochemical Evaluation

Using standard methods, we qualitatively screened the plant extract using the supernatant from various solvents to identify several chemical classes (Harborne and Harborne, 1973).

Check for alkaloids

Dragendroff's test: Two millilitres of extract and two millilitres of 1% hydrochloric acid were put into a test tube. The solution was then gradually supplemented with a few drops of freshly prepared Dragendroff reagent. The orange-brown precipitate indicated the presence of alkaloids.

Test for glycosides

Keller-Kilani test: We mixed 3 millilitres of plant extract with 2 millilitres of glacial acetic acid, followed by 1-2 drops of a 2% ferric chloride solution. The liquid was then put into a test tube that contained two millilitres of sulphuric acid that had been concentrated. The presence of glycosides was shown by the reddish-brown ring that developed at the contact.

Check for flavonoids

Two millilitres of plant extract were mixed with a few drops of a 2% sodium hydroxide solution in water. The presence of flavonoids was shown by the intense yellow colour that went colourless when more diluted hydrochloric acid was applied.

Look for saponins.

Ten millilitres of distilled water and two millilitres of plant extract were combined in a test tube and vigorously shaken for fifteen minutes. The formation of a 1 cm thick layer of foam demonstrated the presence of saponins.

Check for Tannins

0.5g of the dried powdered samples were boiled in 20 millilitres of water in a test tube before being filtered. After adding a few drops of 0.1% ferric chloride, we checked for a blue-black or brownish green colour.

Look for terpenoids

The Salkowski test Three millilitres of chloroform solution were added to a test tube containing two millilitres of plant extract. After a few seconds, we added two millilitres of strong sulphuric acid along the test tube's walls. Terpenoids were evident from the reddish-brown ring that developed at the two liquids' contact.

2.4 GC-MS analysis

Preparing the extract for GC-MS examination: 100 millilitres of HPLC-grade methanol were used to extract 10 grammes of dried plant powder, which was then kept in the dark for 48 hours while being stirred occasionally. The extract was then centrifuged for 15 minutes at 2500 rpm after being filtered through Whatman filter paper No. 1. The supernatant was evaporated at 40°C in a water bath to get the crude syrupy extract. For GCMS analysis, we made a stock solution by dissolving the crude extract again in methanol. One microlitre of the stock solution was used in the GCMS analysis.

Identifying chemical compounds: We analysed the crude extract using the QP 2010 Shimadzu, Japan, GCMS apparatus. The GC-MS experiment was conducted under the following circumstances. Helium served as the carrier gas, flowing at a constant 16.3 ml/min and a column flow rate of 1.21 ml/min. The mass transfer line and injector had temperatures of 260 and 280 degrees Celsius for ten minutes. The GC-MS ran for fifty minutes. The injection had a volume of 1 µl. A mass spectrum graph, which is a molecule's fingerprint, was created as each chemical exited the GC column after being struck by a stream of electrons that broke them apart. The samples were run at a range of 50/650 m/z. The NIST library and the Wiley spectral library search program were compared to the discovered compounds.

3. Findings and Insights

3.1 Antimicrobial activity

Secondary metabolites found in the genus Echinops, such as tannins, saponins, and phenolic compounds, have been shown to improve the antimicrobial activity of crude medications against specific pathogenic microbes, promoting health (Alizadeh Behbahani et al., 2020). Oral microbiome has been shown to be suppressed by piper

betel leaves (Bissa et al. 2007). The antibacterial efficacy against E. aerogenes was investigated by Bissa and Bohra (2015). Because of its antibacterial properties, religious plants can combat a number of harmful illnesses (Bissa 2018). In a variety of solvents, such as aqueous, alcoholic, chloroform, and petroleum ether, Echinops ritro extracts from fresh leaves, flowers, stems, and roots showed notable antibacterial activity against Escherichia coli, Enterobacter aerogenes, Agrobacterium tumefaciens, Staphylococcus aureus, Bacillus subtilis, Pseudomonas putida, and Pseudomonas syringae.

Echinops ritro root extracts in petroleum ether and fresh chloroform showed the strongest zone of inhibition against P. putida, P. syringae, S. aureus, and E. aerogenes (Table 3.1). On the other hand, extracts from stems, leaves, and flowers exhibited a moderate level of effectiveness against each of the seven bacterial strains.

The biggest zone of inhibition for E. aerogenes, E. coli, and P. syringae is observed in dry extracts of Echinops ritro roots and flowers (Table 3.2). Aqueous extracts of all plant components show a modest zone of inhibition against all seven bacterial strains, whereas petroleum ether, alcoholic, and chloroform extracts of leaves and stems show a moderate zone of inhibition.

Fresh Typha angustifolia L. roots, flowers, and leaves showed a significant zone of inhibition against each of the seven bacterial strains in Table 3.3. The biggest inhibition zone against A. tumefaciens was shown by floral extracts.

The strongest antibacterial action against E. aerogenes, S. aureus, B. subtilis, and P. syringae is found in dry leaves and roots (Table 3.4). In contrast, all seven bacterial strains were strongly inhibited by the entire plant.

Table 3.1: Zone of inhibition of Fresh extracts of *Echinops ritro*.

Plant Part	Extract Type	*E. coli*	*E. aerogenes*	*A. tumefaciens*	*B. subtilis*	*S. aureus*	*P. putida*	*P. syringae*
Leaves	Aqueous	0	1.5±0.6	0	0	1.5±1.0	1.5±1.2	2.5±2.0
	Ethanol	4.0±0.6	6.8±1.0	3.8±1.5	5.0±0.6	2.5±1.0	4.5±0.5	2.2±0.9
	Chloroform	1.5±0.5	2.5±1.0	5.0±0.5	4.2±1.8	4.5±2.0	3.5±1.0	5.2±1.0
	Petroleum ether	3.5±1.0	7.5±2.0	7.5±1.2	2.5±1.0	2.5±0.5	4.5±1.0	7.2±1.5
Flowers	Aqueous	0	2.5±0.5	0	2.5±0.5	0	0	2.2±0.9
	Ethanol	4.5±1.0	2.5±0.6	1.5±0.5	4.2±1.0	3.5±0.5	0	3.5±1.5
	Chloroform	6.2±1.5	4.5±1.0	4.2±1.0	6.5±1.5	5.5±1.2	2.5±1.0	5.2±1.8
	Petroleum ether	7.2±0.9	8.5±1.0	6.2±1.5	6.2±1.0	4.2±0.9	3.5±2.0	4.5±0.5
Stem	Aqueous	1.5±0.5	5.5±2.0	2.5±0.5	2.2±1.5	0	1.5±0.5	2.5±0.5
	Ethanol	2.5±2.0	3.5±1.5	3.5±1.5	4.2±1.0	6.2±1.0	3.5±0.5	5.2±1.5
	Chloroform	6.5±2.0	5.2±1.0	3.2±1.5	7.5±0.5	4.5±1.0	5.2±1.0	5.2±1.0
	Petroleum ether	7.5±1.0	7.2±1.5	6.2±0.5	6.5±1.0	4.5±0.5	4.5±0.5	7.5±1.5
Root	Aqueous	2.2±1.0	3.2±0.5	2.5±0.5	1.5±0.5	2.5±1.0	3.5±2.0	3.0±1.8
	Ethanol	3.8±1.0	3.5±0.5	4.5±1.0	5.0±0.8	5.5±1.0	5.2±1.0	4.5±2.5
	Chloroform	5.5±0.5	5.2±1.5	4.5±0.5	4.5±1.0	6.5±0.5	7.2±0.8	5.5±1.5
	Petroleum ether	5.2±1.5	8.2±2.0	6.2±1.0	5.5±1.5	7.2±1.0	9.2±0.5	8.5±1.2

Table 3.2: Zone of inhibition of Dry extracts of *Echinops ritro*.

Plant Part	Extract Type	*E. coli*	*E. aerogenes*	*A. tumefaciens*	*B. subtilis*	*S. aureus*	*P. putida*	*P. syringae*
Leaves	Aqueous	0	0	1.5±0.6	0	0	3.2±1.5	3.5±1.5
	Ethanol	3.5±2.0	3.5±1.5	6.0±1.5	4.2±1.5	3.5±1.0	4.5±1.0	4.2±1.5
	Chloroform	7.5±1.5	6.2±1.0	9.0±2.5	6.2±1.0	5.5±0.6	5.2±1.0	7.5±2.0
	Petroleum ether	10.2±1.0	9.5±1.5	10.5±2.0	9.5±0.6	8.5±1.5	7.5±1.0	9.5±1.0
Flowers	Aqueous	1.5±0.5	3.2±1.0	3.2±1.0	3.5±1.5	3.2±1.0	3.2±0.5	2.2±1.0
	Ethanol	5.2±1.5	4.5±0.6	2.5±1.0	5.2±1.5	5.2±1.0	7.8±1.5	5.2±0.6
	Chloroform	9.5±2.0	9.5±1.5	7.5±0.6	10.2±1.0	6.5±0.6	10.5±0.6	3.5±1.0
	Petroleum ether	11.5±1.5	10.2±0.5	5.2±1.0	11.8±1.0	9.5±1.0	10.8±1.8	6.2±1.0
Stem	Aqueous	5.2±0.5	3.5±1.0	4.2±1.5	0	0	4.2±1.0	3.5±0.5
	Ethanol	7.5±1.0	4.5±1.0	7.2±1.0	3.5±1.0	1.5±0.5	7.5±2.0	5.5±1.0
	Chloroform	8.2±1.5	7.5±1.5	7.2±1.5	4.2±0.5	5.5±2.0	6.2±1.5	6.5±1.0
	Petroleum ether	9.5±0.5	7.2±2.5	8.5±0.5	6.5±2.5	8.2±0.5	5.2±1.5	6.2±1.0
Root	Aqueous	4.5±1.0	1.5±0.5	2.5±0.5	3.5±1.0	4.5±0.5	3.5±2.0	3.2±1.0
	Ethanol	6.2±0.5	7.8±1.5	5.2±1.0	5.5±0.5	7.2±1.0	4.2±0.5	3.5±0.5
	Chloroform	5.2±1.5	9.2±1.0	7.5±0.5	6.5±0.5	9.5±1.0	7.5±2.0	10.2±1.0
	Petroleum ether	8.2±1.0	12.5±1.5	9.2±1.8	6.2±2.5	9.5±0.5	9.5±0.5	12.8±1.8

Table 3.3: Zone of inhibition of Fresh extracts of *Typha angustifolia*.

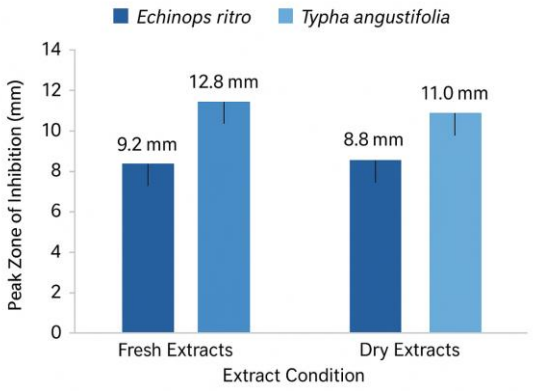
Plant Part	Extract Type	*E. coli*	*E. aerogenes*	*A. tumefaciens*	*B. subtilis*	*S. aureus*	*P. putida*	*P. syringae*
Leaves	Aqueous	2.0±1.0	0	2.5±0.6	1.5±0.5	0	0	3.5±1.5
	Ethanol	4.2±1.0	3.2±1.0	3.8±1.5	3.5±1.2	2.5±1.0	2.5±0.6	3.8±1.5
	Chloroform	5.5±1.5	4.5±2.0	4.5±1.0	5.5±0.5	3.5±1.0	6.2±1.0	7.2±0.6
	Petroleum ether	6.2±1.5	4.5±1.0	5.5±2.0	8.5±0.5	5.5±1.5	4.5±1.5	8.2±1.0
Flowers	Aqueous	2.5±1.5	2.5±0.5	2.0±1.5	3.2±1.5	2.5±0.5	3.2±1.5	2.5±2.0
	Ethanol	4.5±0.5	3.5±1.0	4.5±0.5	3.5±1.0	4.5±1.0	2.5±1.0	3.5±2.0
	Chloroform	6.5±1.0	4.5±1.0	6.2±1.0	6.5±1.5	5.2±1.0	4.2±0.5	7.0±2.0
	Petroleum ether	6.5±1.5	6.0±1.5	8.8±3.5	6.0±1.5	6.2±1.0	7.5±0.5	6.5±1.0
Root	Aqueous	1.2±0.5	0	0	1.5±0.5	1.5±1.2	2.5±0.5	1.5±0.5
	Ethanol	3.5±2.0	2.5±1.0	0	2.2±1.0	5.5±2.0	4.2±1.0	3.5±1.5
	Chloroform	4.5±1.5	4.5±3.5	3.5±1.0	7.5±0.5	6.0±1.0	8.0±1.5	5.2±0.5
	Petroleum ether	4.5±2.0	5.2±2.0	4.5±0.5	5.0±1.5	6.0±1.5	7.5±2.0	6.5±1.0

Table 3.4: Zone of inhibition of Dry extracts of *Typha angustifolia*.

Plant Part	Extract Type	*E. coli*	*E. aerogenes*	*A. tumefaciens*	*B. subtilis*	*S. aureus*	*P. putida*	*P. syringae*
Leaves	Aqueous	3.5±1.0	2.5±1.5	2.8±1.5	2.5±1.0	2.5±0.5	3.5±0.5	3.2±1.0
	Ethanol	5.5±2.0	5.2±1.5	5.5±2.0	3.2±1.5	4.5±1.5	4.5±1.0	8.5±1.0
	Chloroform	8.2±1.0	9.0±2.0	8.8±1.5	3.2±0.5	7.5±2.0	10.2±2.0	9.0±1.5
	Petroleum ether	9.8±1.0	12.2±1.5	9.5±1.0	4.0±1.0	8.5±2.0	6.0±1.5	10.5±2.0
Flowers	Aqueous	3.0±1.5	2.2±1.0	2.5±0.5	0	3.5±1.0	2.5±1.0	3.8±2.0
	Ethanol	5.5±0.5	5.5±1.5	0	4.5±0.5	5.5±1.5	3.5±1.0	7.8±1.5
	Chloroform	7.5±1.0	4.2±1.0	3.5±0.5	6.5±2.0	6.5±1.5	4.2±0.5	9.5±1.0
	Petroleum ether	6.5±2.0	8.0±2.0	5.2±1.0	5.5±1.5	7.8±2.0	5.2±1.5	9.2±1.5
Root	Aqueous	3.5±1.0	2.0±1.0	2.8±1.5	3.0±2.0	3.5±1.5	3.0±1.0	3.5±1.0
	Ethanol	5.5±1.0	6.2±1.0	6.5±0.5	6.5±0.5	6.2±1.0	6.0±1.0	4.5±1.0
	Chloroform	6.0±1.5	5.2±2.0	8.5±2.0	7.5±1.0	8.0±1.0	8.0±0.5	7.2±2.0
	Petroleum ether	7.5±1.0	5.2±1.0	6.5±1.0	10.2±1.0	11.0±2.0	10.5±2.0	7.5±0.5

Table 3.5 Comparative Summary of Antibacterial Activity of *Echinops ritro* and *Typha angustifolia*

	Extract Condition	Most Active Solvent	Most Active Part	Peak Zone of Inhibition (mm)	General Trend (Activity Order)
Echinops ritro	Fresh Extracts	Petroleum ether	Stem / Root	9.2 mm (*P. putida*)	Petroleum ether > Chloroform > Ethanol > Aqueous
Echinops ritro	Dry Extracts	Petroleum ether	Root	12.8 mm (*P. syringae*)	Petroleum ether > Chloroform > Ethanol > Aqueous
Typha angustifolia	Fresh Extracts	Petroleum ether	Flowers	8.8 mm (*A. tumefaciens*)	Petroleum ether > Chloroform > Ethanol > Aqueous
Typha angustifolia	Dry Extracts	Petroleum ether	Root	11.0 mm (*S. aureus*)	Petroleum ether > Chloroform > Ethanol > Aqueous



Key Comparative Insights

- ✓ The petroleum ether extracts inhibited both plants the most, which means that the non-polar fraction had considerable antibacterial properties.
- ✓ Echinops ritro (dry extracts) exhibited the highest overall inhibition (12.8 mm against P. syringae), indicating a concentration of active metabolites post-drying.
- ✓ The petroleum ether extract of Typha angustifolia dry root was especially good in killing S. aureus (11.0 mm).
- ✓ Aqueous extracts exhibited minimal action in both species, corroborating that the majority of phytochemicals of relevance exhibit low water solubility.
- ✓ Chloroform extracts exhibited significant broad-spectrum action, particularly in the roots and stems of Echinops ritro.

Preliminary Phytochemical Results

In *Echinops ritro* and *Typha angustifolia* various phytochemical present such as alkaloids, glycosides, flavonoids, saponins, tannins and terpenoids are present in leaves, stem, flower, immature pods and gum (Fig. 1, Fig. 2 and Table 3.5, Table 3.6, Table 3.7, Table 3.8, Table 3.9).

Table 3.5: Quantitative Phytochemical analysis of *Echinops ritro.*

Name of Plant Part	Alkaloids	Glycosides	Flavonoids	Saponins	Tannins	Terpenoids
Leaves	-	+	-	+	+	+
Flower	+	+	+	+	-	+
Root	+	+	+	+	-	-
Stem	-	+	-	+	-	+

Table 3.6: Quantitative Phytochemical Analysis of *Typha angustifolia.*

Name of Plant Part	Alkaloids	Glycosides	Flavonoids	Saponins	Tannins	Terpenoids
Leaves	+	-	+	-	+	+
Flower	+	-	+	+	+	+
Root	-	+	+	-	-	-

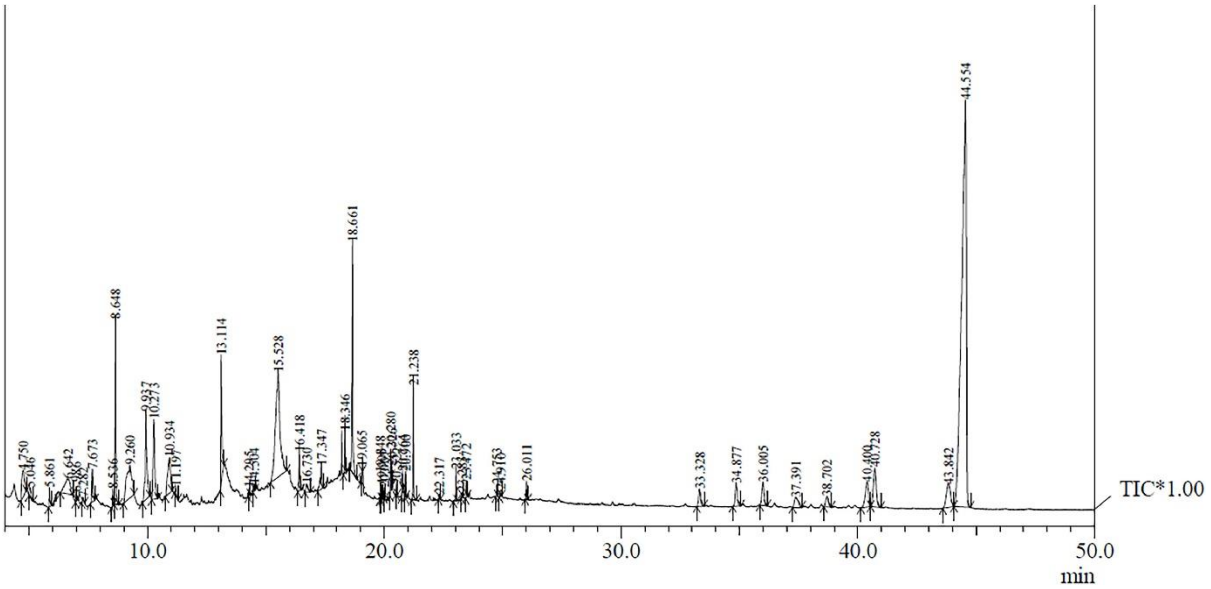


Fig. 1: Chromatogram of methanolic root extract of *Echinops ritro.*

Table 3.7: List of compounds present in methanolic root extracts of *Echinops ritro*.

Peak#	R. Time	Area%	Molecular weight	Molecular Formula	Name
1.	4.750	1.65	180	$C_6H_{12}O_6$	dl-Glyceraldehyde dimer
2.	5.046	0.33	98	$C_5H_6O_2$	2(3H)-FURANONE, 5-METHYL-
3.	5.861	0.35	144	$C_6H_8O_4$	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one
4.	6.642	1.89	92	$C_3H_8O_3$	Glycerin
5.	6.986	0.13	166	$C_5H_8O_3$	4-OXOPENTANOIC ACID
6.	7.282	0.13	128	$C_6H_8O_3$	2,5-ANHYDRO-1,6-DIDEOXYHEXO-3,4-DIULOSE
7.	7.673	0.80	126	$C_7H_{10}O_2$	Cyclopentane, 1-acetyl-1,2-epoxy-
8.	8.536	0.31	144	$C_6H_8O_4$	2-ACETYL-2-HYDROXY-.GAMMA.-BUTYROLACTON

9.	8.648	3.37	144	$C_6H_8O_4$	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
10.	9.260	3.71	92	$C_3H_8O_3$	1,2,3-PROPANETRIOL
11.	9.937	3.24	126	$C_6H_6O_3$	5-Hydroxymethylfurfural
12.	10.273	3.13	134	$C_5H_{10}O_4$	1,2,3-Propanetriol, 1-acetate
13.	10.934	2.16	144	$C_7H_{12}O_3$	Heptanoic acid, 6-oxo-
14.	11.197	0.18	144	$C_7H_{12}O_3$	4-PENTENOIC ACID, 3-HYDROXY-, ETHYL ESTER
15.	13.114	2.83	136	$C_8H_8O_2$	2.83 2,4-CRESOTALDEHYDE
16.	14.295	0.12	256	$C_{16}H_{32}O_2$	HEXADECANOIC ACID
17.	14.504	0.13	168	$C_{11}H_{20}O$	1-Cyclohexene-1-ethanol, 2,6,6-trimethyl-
18.	15.528	10.58	192	$C_7H_{12}O_6$	1,3,4,5-TETRAHYDROXY-CYCLOHEXANECARBOXY
19.	16.418	0.89	180	$C_{10}H_{12}O_3$	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol
20.	16.730	0.39	192	$C_7H_{12}O_6$	1,3,4,5-TETRAHYDROXY-CYCLOHEXANECARBOXY
21.	17.347	0.78	336	$C_{19}H_{32}N_2O_3$	1,5-Dimethyl-3,7-bis-(3-methylbutyryl)-3,7-diazabicyclo [3
22.	18.346	0.75	216	$C_{12}H_8S_2$	5-(But-3-ene-1-ynyl)-2,2′-bithienyl
23.	18.661	4.71	256	$C_{16}H_{32}O_2$	n-Hexadecanoic acid
24.	19.065	0.08	287	$C_{15}H_{29}NO_4$	l-Isoleucine, N-ethoxycarbonyl-, isohexyl ester
25.	19.848	0.33	294	$C_{19}H_{34}O$	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
26.	19.900	0.16	296	$C_{19}H_{36}O_2$	9-Octadecenoic acid (Z)-, methyl ester
27.	20.007	0.12	296	$C_{20}H_{40}O$	2-HEXADECEN-1-OL,3,7,11,15-TETRAMETHYL-
28.	20.280	0.42	280	$C_{18}H_{32}O_2$	9,12-Octadecadienoic acid (Z,Z)-
29.	20.326	0.16	238	$C_{16}H_{30}O$	cis-9-Hexadecenal
30.	20.522	0.13	284	$C_{18}H_{36}O_2$	Octadecanoic acid
31.	20.764	0.25	246	$C_{18}H_{30}$	Chrysene, octadecahydro-
32.	20.900	0.31	248	$C_{15}H_{20}O_3$	Santamarine
33.	21.238	1.73	248	$C_{12}H_8S_3$	[2,2′;5′,2"]TERTHIOPHENE
34.	22.317	0.09	256	$C_{13}H_{21}C_lOsi$	4-Chlorobenzyl alcohol, TBDMS derivative
35.	23.033	0.47	232	$C_{15}H_{20}O_2$	5-Isopropyl-2-methylphenyl 2-methylbut-2-enoate
36.	23.283	0.07	326	$C_{22}H_{46}O$	Behenic alcohol
37.	23.472	0.22	330	$C_{19}H_{38}O_4$	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl este
38.	24.753	0.18	272	$C_{15}H_{12}O_5$	7-(3,4-Methylenedioxy)-tetrahydrobenzofuranone
39.	24.910	0.10	266	$C_{18}H_{34}O$	9-Octadecenal, (Z)-
40.	26.011	0.30	410	$C_{30}H_{50}$	Squalene
41.	33.328	0.75	412	$C_{29}H_{48}O$	Stigmasterol
42.	34.877	1.09	414	$C_{29}H_{50}O$	STIGMAST-5-EN-3-OL, (3.BETA.,24S)-
43.	36.005	1.15	426	$C_{30}H_{50}O$	.beta.-Amyrin
44.	37.391	0.76	470	$C_{31}H_{50}O_3$	METHYL COMMATE B
45.	38.702	0.63	468	$C_{32}H_{52}O_2$	Olean-12-en-3-ol, acetate, (3.beta.)-
46.	40.400	1.78	442	$C_{30}H_{50}O_2$	Betulin
47.	40.728	2.53	426	$C_{30}H_{50}O$	Lupeol
48.	43.842	2.12	468	$C_{32}H_{52}O_2$	Lup-20(29)-en-3-ol, acetate, (3.beta.)-
49.	44.554	41.51	468	$C_{32}H_{52}O_2$	LUP-20(29)-EN-3-YL ACETATE

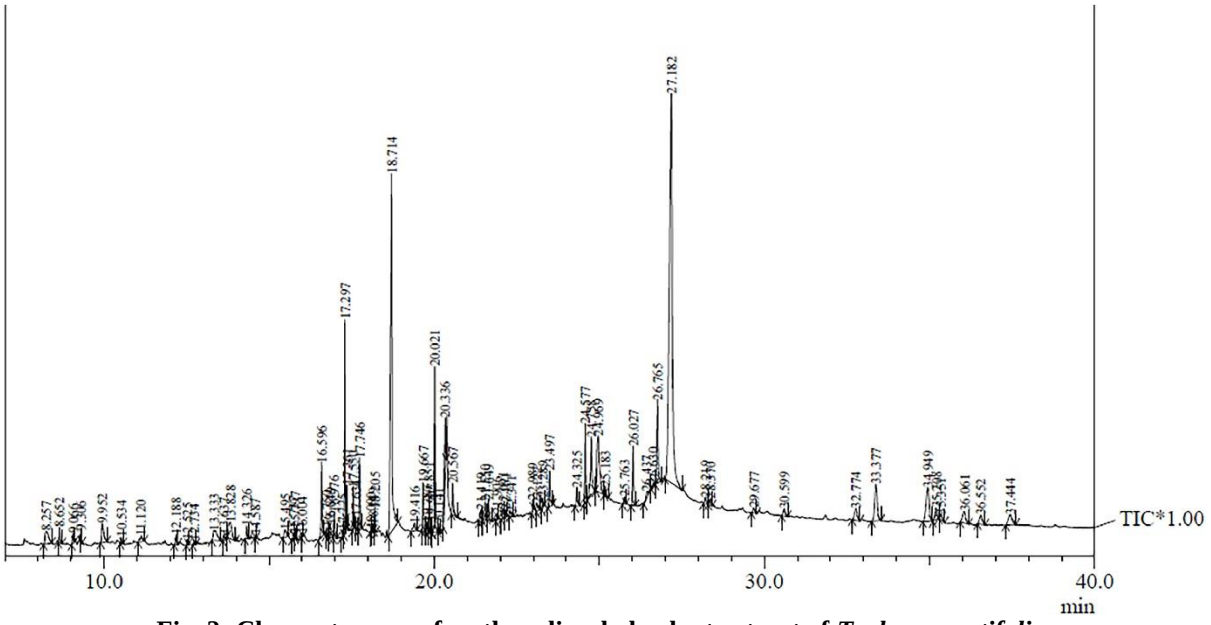


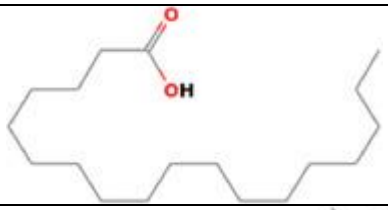
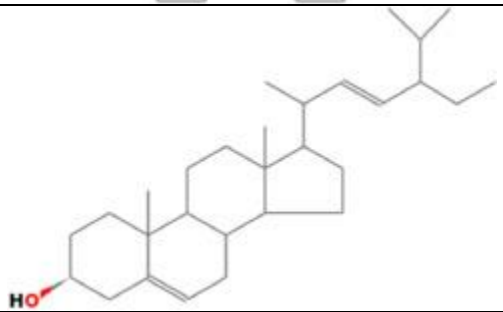
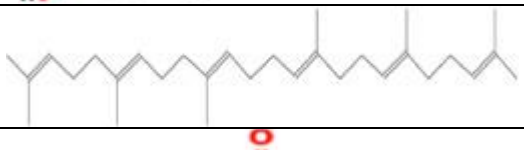
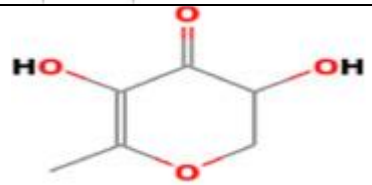
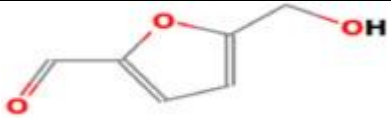
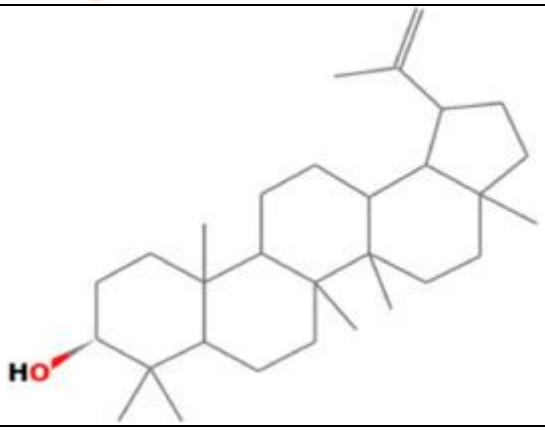
Fig. 2: Chromatogram of methanolic whole plant extract of *Typha angustifolia*.

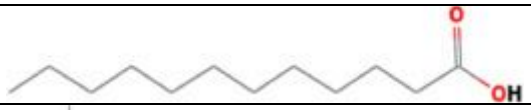
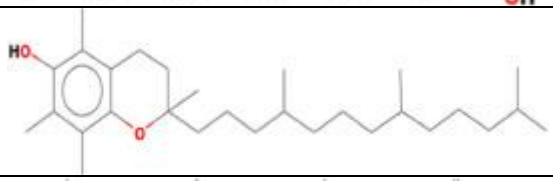
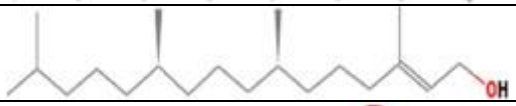
Table 7.8: List of compounds present in the methanolic whole plant extract of *Typha angustifolia.*

Peak No.	Retention Time (min)	Area (%)	Molecular Weight	Molecular Formula	Compound Name
1	8.257	1.11	130	$C_7H_{14}O_2$	1-Butanol, 3-methyl-, acetate
2	8.652	0.38	144	$C_6H_8O_4$	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
3	9.066	0.23	142	$C_8H_{14}O_2$	7-Octenoic acid
4	9.306	0.14	170	$C_{12}H_{26}$	Dodecane
5	9.952	0.95	126	$C_6H_6O_3$	5-Hydroxymethylfurfural
6	10.534	0.13	158	$C_9H_{18}O_2$	Nonanoic acid
7	11.120	0.32	158	$C_9H_{18}O_2$	2-Heptanol, acetate
8	12.188	0.25	206	$C_{13}H_{18}O_2$	1-(3,6,6-Trimethyl-1,6,7,7a-tetrahydro-cyclo)-
9	12.525	0.11	188	$C_{13}H_{16}O$	Ethanone, 1-(2,3-dihydro-1,1-dimethyl-1H-inden-4-yl)-
10	12.734	0.11	150	$C_{10}H_{14}O$	2,2,6-Trimethyl-4-methylene-1-oxo-5-cyclohexene
11	13.333	0.98	144	$C_8H_{16}O_2$	2-Pentanol, 3-methyl-, 2-acetate
12	13.637	0.14	188	$C_{13}H_{16}O$	3-Buten-2-one, 1-(2,3,6-trimethylphenyl)-
13	13.828	1.41	180	$C_6H_{12}O_6$	D-Allose
14	14.326	0.32	200	$C_{12}H_{24}O_2$	Dodecanoic acid
15	14.587	0.04	222	$C_{12}H_{14}O_4$	1,2-Benzenedicarboxylic acid, diethyl ester
16	15.495	0.43	194	$C_7H_{14}O_6$	β-D-Glucopyranoside, methyl
17	15.737	0.12	226	$C_{14}H_{26}O_2$	1-Isobutyl-7,7-dimethyl-octahydro-isobenzofuran-3a-ol
18	15.797	0.16	224	$C_{13}H_{20}O_3$	3-Buten-2-one, 4-(4-hydroxy-2,2,6-trimethyl-7-oxabicyclo-)
19	16.004	0.08	210	$C_{13}H_{22}O_2$	2-Cyclohexen-1-one, 4-(3-hydroxybutyl)-3,5,5-trimethyl-
20	16.596	1.85	228	$C_{14}H_{28}O_2$	Tetradecanoic acid
21	16.750	0.16	224	$C_{14}H_{24}O_2$	2-Pentanone, 4-(1,3,3-trimethyl-7-oxabicyclo[4.1.0]hept-2-yl)-
22	16.849	0.49	196	$C_{11}H_{16}O_3$	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-6-
23	16.976	0.70	222	$C_{13}H_{18}O_3$	(S,E)-4-Hydroxy-3,5,5-trimethyl-4-(3-oxobut-1-en-1-yl)cyclohex-2-en-1-one
24	17.225	0.13	280	$C_{20}H_{40}$	2-Hexadecene, 3,7,11,15-tetramethyl-, [R-(E)]-
25	17.297	3.78	278	$C_{20}H_{38}$	Neophytadiene
26	17.361	0.60	268	$C_{18}H_{36}O$	2-Pentadecanone, 6,10,14-trimethyl-
27	17.550	0.73	278	$C_{20}H_{38}$	Neophytadiene
28	17.634	0.16	282	$C_{18}H_{34}O_2$	9-Octadecenoic acid (Z)-
29	17.746	1.44	278	$C_{20}H_{38}$	Neophytadiene
30	18.090	0.06	382	$C_{24}H_{30}O_4$	Phthalic acid, 3-ethylphenyl octyl ester

31	18.149	0.10	268	$C_{18}H_{36}O$	Oxirane, hexadecyl-
32	18.205	0.40	270	$C_{17}H_{34}O_2$	Hexadecanoic acid, methyl ester
33	18.714	**15.37**	256	$C_{16}H_{32}O_2$	**n-Hexadecanoic acid**
34	19.416	0.44	282	$C_{18}H_{34}O_2$	9-Octadecenoic acid (Z)-
35	19.667	1.00	280	$C_{18}H_{32}O_2$	13-Hexyloxacyclotridec-10-en-2-one
36	19.787	0.21	296	$C_{20}H_{40}O$	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-
37	19.851	0.54	294	$C_{19}H_{34}O_2$	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
38	19.907	0.16	298	$C_{18}H_{31}$	(9E,12E)-9,12-Octadecadienoyl chloride
39	20.021	3.39	296	$C_{20}H_{40}O$	Phytol
40	20.141	0.13	298	$C_{19}H_{38}O_2$	Octadecanoic acid, methyl ester
41	20.336	2.07	280	$C_{18}H_{32}O_2$	9,12-Octadecadienoic acid (Z,Z)-
42	20.567	0.83	284	$C_{18}H_{36}O_2$	Octadecanoic acid
43	21.419	0.38	250	$C_{12}H_{26}O_3S$	Sulfurous acid, isohexyl hexyl ester
44	21.550	0.71	187	$C_{10}H_{21}NO_2$	Hexanoic acid, 2-dimethylaminoethyl ester
45	21.649	0.53	312	$C_{19}H_{36}O_3$	9-Octadecenoic acid, 12-hydroxy-, methyl ester
46	21.906	0.14	296	$C_{19}H_{36}O_2$	Methyl dihydromalvalate
47	22.091	0.31	352	$C_{22}H_{40}O_3$	3-Octadecyldihydro-2,5-furandione
48	22.161	0.14	324	$C_{21}H_{40}O_2$	4,8,12,16-Tetramethylheptadecan-4-olide
49	22.341	0.17	404	$C_{25}H_{40}O_4$	2-[2-Carboxyethyl]-3-methyl-tetrahydrofurano[4,5-a]androstane
50	22.989	0.20	251	$C_{15}H_{25}NO_2$	2-(Dimethylamino)ethyl 1-adamantanecarboxylate
51	23.122	0.27	254	$C_{15}H_{26}O_3$	2,6-Dimethyl-8-(tetrahydro-2H-pyran-2-yloxy)-
52	23.289	0.28	274	$C_{16}H_{31}ClO$	Palmitoyl chloride
53	23.497	0.95	330	$C_{19}H_{38}O_4$	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester
54	24.325	0.53	280	$C_{18}H_{32}O_2$	13-Hexyloxacyclotridec-10-en-2-one
55	24.577	1.75	313	$C_{17}H_{31}NO_4$	Fumaric acid, 2-dimethylaminoethyl nonyl ester
56	24.758	2.49	338	$C_{21}H_{38}O_3$	Glycidyl oleate
57	24.969	3.65	300	$C_{18}H_{33}ClO$	Oleoyl chloride
58	25.183	0.24	358	$C_{21}H_{42}O_4$	Octadecanoic acid, 2,3-dihydroxypropyl ester
59	25.763	0.19	281	$C_{18}H_{35}NO$	9-Octadecenamide
60	26.027	1.57	410	$C_{30}H_{50}$	Squalene
61	26.437	0.36	686	$C_{34}H_{38}O_{15}$	β-D-Glucopyranoside, 5-(acetyloxy)-7-[(acetyloxy)methyl]-
62	26.630	0.17	462	$C_{29}H_{50}O_4$	α-Tocospiro A
63	26.765	3.13	298	$C_{18}H_{34}O_3$	9-Octadecenoic acid, 12-hydroxy-
64	27.182	**28.87**	280	$C_{18}H_{32}O_2$	**13-Hexyloxacyclotridec-10-en-2-one**
65	28.219	0.28	368	$C_{24}H_{48}O_2$	Hexanoic acid, octadecyl ester
66	28.370	0.27	266	$C_{15}H_{22}O_4$	1,5,5-Trimethyl-6-[(1E)-3-oxo-1-butenyl]-7-oxabicyclo[4.1.0]heptane
67	29.677	0.24	454	$C_{31}H_{50}O_2$	Stigmasta-5,22-dien-3-ol, acetate (3β)-
68	30.599	0.40	430	$C_{29}H_{50}O_2$	Vitamin E (α-Tocopherol)
69	32.774	0.84	400	$C_{28}H_{48}O$	Ergost-5-en-3-ol (3β)-
70	33.377	2.56	412	$C_{29}H_{48}O$	Stigmasterol
71	34.949	2.62	414	$C_{29}H_{50}O$	Stigmast-5-en-3-ol (3β,24S)-
72	35.208	0.70	424	$C_{30}H_{48}O$	β-Amyrone
73	35.351	0.28	412	$C_{29}H_{48}O$	Fucosterol
74	36.061	0.94	426	$C_{30}H_{50}O$	β-Amyrin
75	36.552	0.69	424	$C_{30}H_{48}O$	Lup-20(29)-en-3-one
76	37.444	0.98	426	$C_{30}H_{50}O$	**Lupeol**

Table 3.9: List of important phytoconstituents identified in the investigated plant sample and their known biological activities.

S. No.	Compound name	Chemical class	Chemical Structure	Biological Activities
1.	n-Hexadecanoic acid	Fatty acid		Anti-inflammatory, antioxidant, anti-androgenic, hypocholesterolemic. **Kumar et al., (2010); Aparna et al., (2012)**
2.	Hexadecanoic Acid, Methyl ester	Fatty acid ester		Antioxidant, antimicrobial, hypocholesterolemic, nematicide, hemolytic **Duke's Phytochemical and Ethanobotanical Databases, (2016); Aleryani, (2005)**
3.	Octadecanoic acid	Fatty acid		Octadecanoic acid; Antimicrobial activity **Duke's Phytochemical and Ethanobotanical Databases, (2016)**
4.	Stigmasterol	Phytosterol		Anti-osteoarthritic, cholesterol-lowering activity, Anticancerous, thyroid-inhibiting, antiperoxidative, hypoglycemic. **Chen et al., (2012); Ghosh et al., (2012); Panda et al., (2009).**
6.	Squalene	Triterpene		Anti-inflammatory, cytotoxic, anticancer, antimicrobial **Spanova and Daum (2011)**
7.	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	Flavonoids		Treat osteoporosis, diabetes, and cardiovascular disease. **Chen et al., (2021), Yu et al., (2013)**
8.	5 Hydroxymethylfurfural	Furans		antimicrobial properties. **Zhang et al (2009)**
9.	Lupeol	Triterpenoid		antimicrobial activity, Antioxidant, Hepatoprotective and anticancer properties. **Siddique and Saleem (2011); Ghani, U (2011); Geetha and Varalakshmi (2001); Prasad and Kalra (2010)**.

10.	Dodecanoic acid	Saturated fatty acids		Antibacterial properties **Nakatsuji et al (2009).**
11.	Vitamin E (α-Tocopherol)	Tocopherols		Antioxidant, antimicrobial, Cardioprotective Properties. **Serafini et al (2002); Traber (2007).**
12.	Neophytadiene	Diterpene hydrocarbon		Antibacterial activity. **Balouiri et al (2016).**
13.	Phytol	Terpenoids		Antimicrobial and antiparasitic properties. **Nogueira et al (2017).**
14.	Hexanoic Acid	Carboxylic acids		Antimicrobial activity. **Desbois and Smith (2010).**

4. DISCUSSION

The current study reveals that both Echinops ritro and Typha angustifolia exhibit substantial antibacterial properties, hence validating their traditional therapeutic applications in the treatment of infectious illnesses. Phytochemical analysis validated the existence of alkaloids, flavonoids, tannins, saponins, terpenoids, and glycosides substances well-known for their antibacterial properties.

In Echinops ritro, petroleum ether and chloroform extracts from roots and flowers exhibited the most significant zones of inhibition against E. aerogenes, S. aureus, and P. syringae. This indicates that non-polar phytoconstituents, including terpenoids and thiophenes, may significantly contribute to its antibacterial properties. Prior research has underscored the antibacterial properties of thiophenes and sesquiterpene lactones derived from Echinops species (**Gohari et al., 2013; Kharchoufa et al., 2023).**

Typha angustifolia extracts, particularly the petroleum ether and chloroform fractions derived from its roots and leaves, have shown extensive antibacterial efficacy against E. coli, S. aureus, and B. subtilis. These results corroborate previous studies that emphasised the antibacterial and wound-healing properties of Typha angustifolia attributed to its flavonoid and phenolic constituents **(Gao et al., 2017; Wang et al., 2020).** The GC–MS analysis corroborated the existence of bioactive chemicals, including n-hexadecanoic acid, squalene, lupeol, and stigmasterol, recognised for their ability to compromise bacterial cell membranes and impede microbial enzymes.

Interestingly, aqueous extracts exhibited very low activity, potentially attributable to the inadequate solubility of non-polar bioactive chemicals in water. This shows how important the polarity of the solvent is for getting antimicrobial phytochemicals out. The comparison results indicate that both plants are effective; however, Typha angustifolia may provide broader antibacterial efficacy, whereas Echinops ritro has more effectiveness against certain diseases.

These findings underscore the pharmacological significance of both species and endorse their utilisation as prospective candidates for the manufacture of plant-derived antibacterial agents. However, additional bioassay-directed isolation and in vivo validation are necessary to pinpoint individual active compounds and assess their safety and efficacy.

5. CONCLUSION

The research demonstrates that Echinops ritro and Typha angustifolia exhibit significant antibacterial properties against both Gram-positive and Gram-negative bacteria, confirming their traditional therapeutic uses. Both plants have a lot of different phytoconstituents, such as flavonoids, terpenoids, and fatty acids, which are probably what make them antibacterial. Petroleum ether and chloroform fractions were the most effective of the extracts that were evaluated. This suggests that lipophilic chemicals may play a role in antibacterial activity.

In general, Typha angustifolia showed a wider range of antibacterial activity, but Echinops ritro extracts were quite effective at stopping some strains. The GC–MS profiling revealed important bioactive compounds such as n-hexadecanoic acid, lupeol, squalene, and stigmasterol that could be used to make new antimicrobial drugs.

This study not only confirms the ethnopharmacological significance of these plants but also highlights their potential for the creation of standardised herbal products. Subsequent research should concentrate on the isolation of pure substances, the clarification of their mechanisms of action, and the execution of toxicity and pharmacological assessments to promote their integration into contemporary medicine.

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