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# Phytochemical analysis and evaluation of antibacterial activity of various extracts from leaves, stems and roots of *Thalictrum foliolosum*

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## Abstract

**Background** *Thalictrum foliolosum*, a member of the Ranunculaceae family, is recognized for its therapeutic potential in addressing gastric issues, dyspepsia, tooth pain, abdominal colic pain, and piles. The diverse array of secondary metabolites present in the plant contributes to these therapeutic applications. This study aims to uncover and quantify the bioactive secondary metabolites found in the unexplored leaves, stems, and roots of *T. foliolosum*. Additionally, we also aimed to evaluate the antibacterial activity and MIC values of these extracts against a panel of pathogenic bacteria, such as pathogenic strains, including *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus mutant* and *Staphylococcus aureus*.

**Result** HPLC analysis suggested all examined compounds were found significantly more in root parts of plant. To determine the potential antimicrobial activity of different plant parts result suggested chloroform fraction of root most effective with variable potency against each examined pathogen at 25–100 µg/ml extracts which indicated rich content of berberine in this fraction. Minimum MIC (121.26 µg/mL) of the chloroform fraction of the root was also supported the results. Fatty acid methyl ester analysis by gas chromatography revealed that the stem contained high levels of fatty acids, such as palmitic acid, stearic acid, and linolenic acid, all of which have antibacterial properties.

**Conclusion** The potential antimicrobial activity of extracts of various plant parts strongly supports the *T. foliolosum* plant's widespread use in folk medicine for the treatment of various chronic diseases and adulterants with various associated medicinal plant species.

**Keywords** *Thalictrum foliolosum*, Ranunculaceae, Magnoflorine, Berberine, Disc diffusion assay, MIC values

## Background

In nature, plants are the richest resource of bioactive natural products, such as alkaloids, phenolics, flavonoids, carotenoids, triterpenoids and other secondary

metabolites for thousands of years [1]. Extensive studies in ancient pharmaceutical practices and contemporary clinical findings consistently affirm that plant-based medicines exhibit a higher safety profile compared to their synthetic counterparts, maintaining a crucial role in healthcare. Derived from botanical sources, these medicinal compounds serve as chemical entities in traditional medicine systems, modern pharmaceuticals, dietary supplements, pharmaceutical intermediates, and synthetic drug formulations [1, 2]. *Thalictrum*, a genus encompassing approximately 220 species, exhibits a diverse array

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of secondary metabolites including flavonoids, alkaloids, steroids, triterpenoids, triterpenoid glycosides, carotenoids, lignins, tannins, cardiac glycosides with high molecular weight, fatty acids, phenolic acids and sterol across its various species, highlighting the pharmacological significance of this genus [3–5]. While there isn't extensive research specifically on *Thalictrum foliolosum*'s, although this species has been used in traditional medicine for various purposes. Given the relevance of phytopharmaceuticals in today's society, the current study concentrated on *Thalictrum foliolosum* DC, an endemic herb that has received little attention.

*T. foliolosum* is a potential medicinal herb belonging to the Ranunculaceae family that is found widely in the Northern Hemisphere region in India and the China Pacific [6–8]. Previous studies have shown that *T. foliolosum* roots are used locally as a tonic, antipyretic, diuretic, laxative, and collyrium for the improvement of eyesight as well as in the treatment of gastric problem, dyspepsia, tooth pain, abdominal colic pain and piles [8, 9]. The combination of the dried root of *T. foliolosum* with *Thymus linearis* was very effective in treating colic and gastric problems [10]. Whole-plant extracts of *T. foliolosum* also showed inhibitory effects against cancer cells as well as the progression of malignant malarial fever [11, 12]. *T. foliolosum* plants may be regarded as belonging to an elite group of medicinal plants due to the presence of benzyloquinoline alkaloids (BIQ), which include berberine, protoberberine, magnoflorine, palmatine, jatrorrhizine, and other considered isoquinoline alkaloids along with several groups of polyphenolic compounds that demonstrated strong bioactivity [13–15]. Previously published reports showed the roots and rhizome parts of *T. foliolosum* have alleviative properties. Therefore, unrestricted collection of roots and rhizome parts resulted in the decline of their population in their habitats [6, 7]. Generally, it is observed that complete knowledge about the chemical composition and potential medicinal uses of various parts of a plant will enable systematic harvesting and conservation of declining plant populations. Considering the therapeutic importance of *T. foliolosum*, there were no more reports of bioactive compounds in aerial parts of this plant. The current study used reverse phase HPLC to identify and quantify four therapeutically important BIQ alkaloids, namely magnoflorine, berberine, palmatine, and thalicarpine, in different parts of the plant (leaves, stem, and roots). Additionally, fatty acids were identified using gas–liquid chromatography. Moreover, a comparative study of antimicrobial activity against human pathogenic bacteria, such as *E. coli*, *Pseudomonas aeruginosa*, *Streptococcus mutans*, and *Staphylococcus aureus*, was conducted using extracts or fractions of various parts of the plant. Thus, the present study was

undertaken to analyze the alkaloids content and major fatty acids in *T. foliolosum* leaves, stem and roots and to understand the relation between with antimicrobial activities.

## Methods

### Sample collection of *T. foliolosum* plants and processing

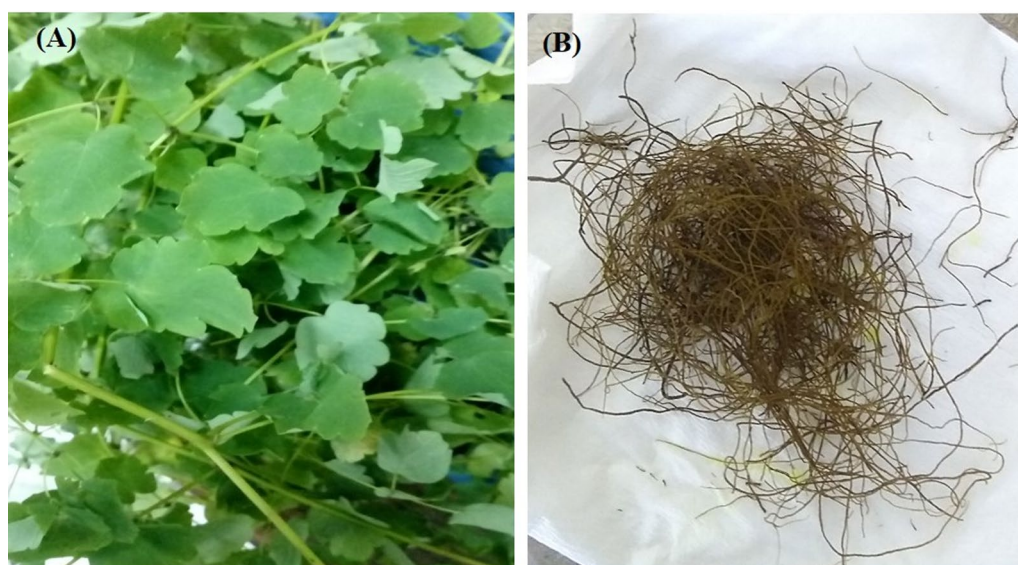
*T. foliolosum*, a perennial herb, was obtained in September month from the forest areas of the Nanital district (2290-m asl), Kumaon region of Uttarakhand state in India (Fig. 1). Nanital is located at the GPS coordinates of 29° 22' 49.0944" N and 79° 27' 48.8520" E. This region has a humid subtropical climate with an average annual temperature of 17.1 °C (62.7 °F) and 1903 mm (74.9 in) of rainfall. The plants were identified by Dr. Harsh Singh (Taxonomist scholar) and a voucher specimen number 257717(LWG) was deposited at CSIR-National Botanical Research Institute (India). The collected plant materials (leaves, stem and roots) were initially carefully washed with saline water, then the samples were placed under running tap water for further washing. After some time, plant materials are rinsed again with autoclave water for half an hour and then put on blotting paper for 48 h to dry. The plant materials were blotted dry before being chopped into small pieces and dried in the shade for 15–20 days. Finally, the dried plant material was ground into a fine powder using an electric grinder (Mill CT 293 Cyclotec TM) and stored in sterilized glass bottles [5, 6].

### Chemicals

All the standards of BIQ alkaloids (Magnoflorine chloride, berberine chloride, palmatine chloride and thalicarpine) were procured from Sigma-Aldrich (USA). Solvent such as methanol, hexane, ethyl acetate, chloroform and acetonitrile and other chemicals were purchased from Merck (India).

### Extraction of plant materials for HPLC analysis

For quantitative analysis, fine powders of different plant materials (1 g) were extracted with 25 ml of acidic (1% HCl) methanol using ultrasonication method (Aczet, Ultrasonic cleaner CUB) at room temperature for 40 min. After ultrasonication, plant materials in extracted solvents were kept at room temperature for overnight. The next day, all sonicated extracts were filtered through a Whatman No 1 filter paper in a round bottle flask, and fresh extracted solvent was added to the same plant materials for additional ultra-sonification. Three times, the same procedure was followed. Later all extracted materials were pooled in round bottom flask and evaporated at 50 °C using a rotary evaporator (Buchi, USA) under low pressure. Finally, the dried extracted plant materials were dissolved in methanol and filtered through a 0.45 µm



**Aerial parts of *T. foliolosum***

**Roots of *T. foliolosum***

**Fig. 1** *Thalictrum foliolosum* plant parts **a** aerial parts (Leaf and stem), **b** Roots of *T. foliolosum*

filter and stored at 4 °C for subsequent HPLC analysis [6, 7]. The acidic-methanol extract of *T. foliolosum* had a yield of 27.42%, 21.10% and 16.24% of the extracted root, leaves and stem respectively. Reference standard solutions (i.e. berberine, palmatine, magnoflorine and thalicarpine) (Sigma-Aldrich) were prepared in methanol. All of the extracted solutions as well as the standard solutions were maintained at 4 °C in the refrigerator.

#### HPLC method

Plant samples were extracted and subjected to further analysis using High-Performance Liquid Chromatography (HPLC) with a gradient method for identifying bioactive compounds, following the protocol established by Mishra et al. [6, 7]. The analysis was performed using a Shimadzu (Japan) HPLC Prominence system comprising a 20 µL sample loop, a PDA SPD M 20 A photodiode array system, an LC-20AD dual pump system, and a SIL-20 AC Autoinjector with a cooler. Compounds were separated on a Shimadzu RP-C18 column (250×4.6, 5 µm pore size) with a guard column of the same packing material. A gradient mobile phase was employed consisting of component A (0.3% formic acid + 0.3% triethylamine) and component B (acetonitrile). The mobile phase gradient program was as follows: 0–25 min, 5–25% B; 25–35 min, 25–35% B; 35–45 min, 35–45% B; 45–60 min, 45–100% B, at a flow rate of 1 mL/min. Data integration and compound identification were performed using Shimadzu Lab Solution software at a wavelength of 265 nm [6].

Results were compared against standards obtained from Sigma-Aldrich, USA. Quantitative analysis was conducted by averaging the results of three independent analyses of the same sample.

#### Fatty acid analysis using gas liquid chromatography

500 mg dry weight of plant samples (leaf, stem and roots) were used for FAME preparation. Fatty acid methyl esters (FAMES) were prepared as per method followed by Bureau of Indian Standards IS: 548 (Part-III), 1976 reaffirmed 1994. Prepared FAME dissolved in hexane were analyzed using GC system (7890B GC System, Agilent Technologies) equipped with a flame ionization detector (FID). FAMES were separated on DB-225 column (30 m×0.25 mm ID×0.25 µm film thickness). Nitrogen was used as carrier gas and hydrogen and air as ignition gases. The conditions used for GC analysis were: injector temperature of 230 °C, detector (FID) temperature of 260 °C with split mode injection (1:20). The oven temperature program was started from 90 (2 min hold) to 130 °C at ramping rate of 3 °C min<sup>-1</sup>. Temperature from 130° to 230° (5 min hold) was achieved with ramp of 2 °C min<sup>-1</sup>. The peaks were identified by comparing with standards and the results are presented as mean of triplicates. Data were integrated by Open LAB CDS Chem Station Edition software. Reference standards of methyl palmitate, methyl stearate, methyl oleate, methyl linoleate, methyl arachidate and methyl behenate, methyl linoleate were procured by M/s Sigma-Aldrich.

### Extracts preparation for antimicrobial assay

For antimicrobial activity various plant parts materials (20 g) were extracted in aqueous methanol (50%) by sonication method. Then extracted solvents were further fractionated by various organic solvents, such as ethyl acetate, chloroform and hexane [5]. All extracted samples were filtered using Whatman filter paper No. 1. The filtrate was then dried using a low-pressure rotary evaporator (Buchi, USA) and lyophilized. All samples were stored at 4 °C in an airtight tube until further analysis was completed.

### Microorganism used in study

In vitro antimicrobial activity was determined against four different human pathogenic bacteria i.e. *E. coli* (ATCC 25922), *S. mutans* (ATCC 25175), *S. aureus* (ATCC 4944) and *P. aeruginosa* (MTCC 424). *E. coli* is a gram-negative, rod-shaped bacterium that is commonly found in the lower intestine and most of these strains are harmless. However, pathogenic *E. coli* varieties cause serious food poisoning, septic shock, meningitis, or urinary tract infections in humans [16]. *S. mutans* is a gram-positive coccus bacterium, primarily found in mouth, pharynx, and intestine and significantly involved in tooth decay [17]. *Staphylococcus aureus* is a gram positive, round shape, facultative anaerobe bacterium. *S. aureus* are frequently found in the upper respiratory tract and on the skin and being a common cause of respiratory infections including skin infections [18]. *P. aeruginosa* is encapsulated rod-shaped, gram-negative bacterium. The important characteristic of this bacteria is multidrug resistance. Usually, *P. aeruginosa* found in medical equipment's hence these bacteria associated hospital acquired infections, i.e. ventilator associated pneumonia and other sepsis syndrome the general symptoms of these bacteria are inflammation and sepsis but if their colonization occurs in lungs, kidneys and urinary tract it may be fatal [19].

### Screening of antimicrobial activity

In vitro antimicrobial activity was determined against four different human pathogenic bacteria using disk-diffusion method with slight modification [20]. In short, 18–24 h old bacteria culture (0.5 OD<sub>600</sub> nm) was spreading over the entire surface of nutrient agar plates (90 mm size) using autoclaved steel spreader and sterile paper disks (approx. 6 mm diameter) were placed on them. Then different concentrations of test samples (25, 50 and 100 µg) were loaded on disks. Streptomycin (25 µg) was utilized as a positive control and respective solvents to which extract dissolved was used as a negative control. Then, plates were allowed to incubate at 37 ± 1 °C for 18–24 h. The diameter of the inhibition zone (ZOI) was

measured after 24 h of incubation to assess antibacterial activity (measured in mm including disk size). All experiments were carried out in triplicate, and the observed ZOI values are expressed as a mean with standard error of the mean (SEM).

### Minimum inhibitory concentration (MIC)

MIC was determined of various extract of leaves, stems and roots parts of *T. foliolosum* against the different pathogenic bacteria by using the previously reported serial dilution method using 96-well microtiter plates. In brief, all pathogenic bacteria were grown in nutrient broth for 6 h before being inoculated, followed by 10<sup>6</sup> cells/mL bacterial culture was inoculated in 200 µL nutrient broth containing tube. Now each extract (leaf, stem, and root) has been added separately in bacterial broth with concentrations ranging from 100 to 800 µg/ml. All tubes were incubated at 37 °C for 24 h and further examined for visible turbidity. The minimum inhibitory concentration (MIC) was determined to be the lowest concentration that inhibited visible growth of the tested bacteria. MIC of berberine, magnoflorine and streptomycin were determined with similar procedure.

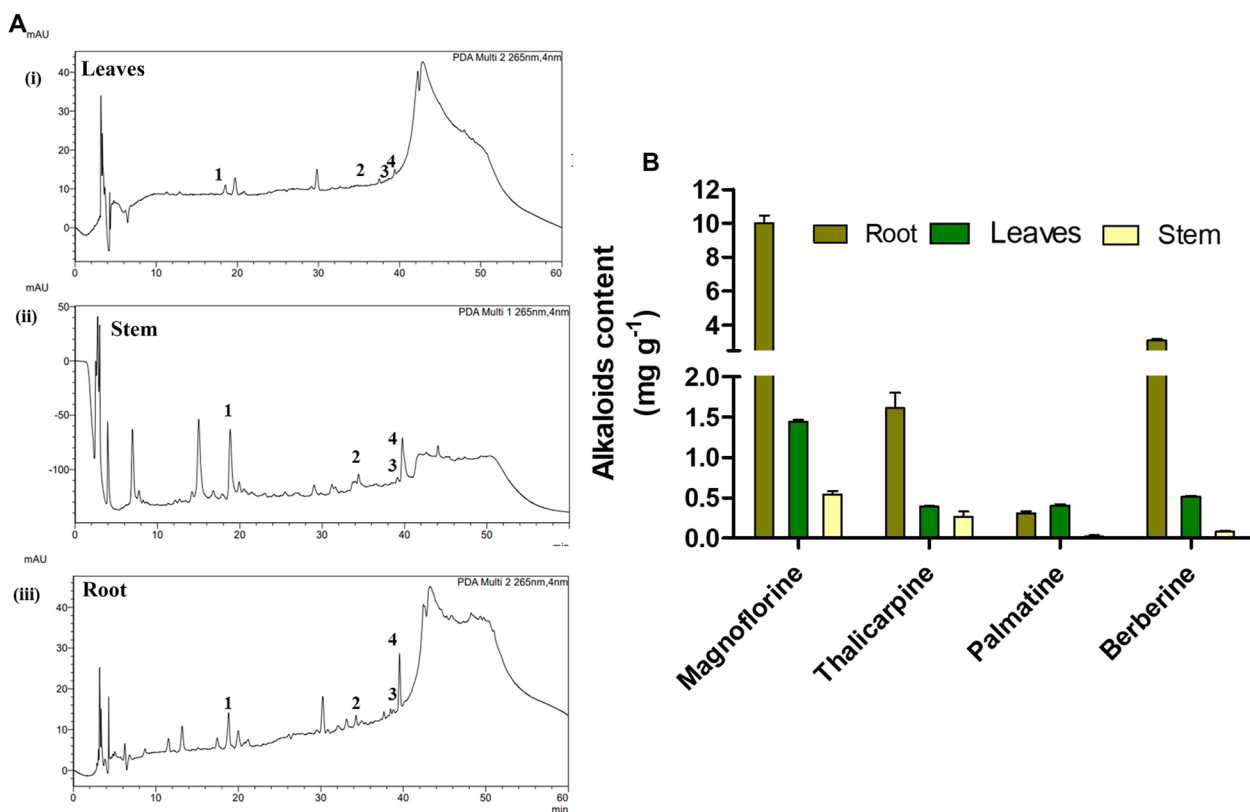
## Results

### Identification and quantification of BIQ alkaloids contents in leaves, stems and roots

Chromatographic analysis aimed to quantify biologically active BIQ alkaloids, namely magnoflorine, berberine, palmatine, and thalicarpine, in extracts obtained from leaves, stems, and roots. Identification of all significant peaks in the chromatogram was accomplished using authentic reference standards of the respective alkaloids. (Fig. 2A) (Supplementary Fig. 1). In terms of quantitative analysis, the root extracts of *T. foliolosum* exhibited the highest total alkaloid content (15.03 mg g<sup>-1</sup>), followed by the leaves (2.75 mg g<sup>-1</sup>), while the stem extracts contained a comparatively lower amount (Fig. 2B). Examining individual alkaloids in the root part revealed the following order of content: magnoflorine (10.02 mg g<sup>-1</sup>) > berberine (3.09 mg g<sup>-1</sup>) > thalicarpine (1.60 mg g<sup>-1</sup>) > palmatine (0.31 mg g<sup>-1</sup>). Similar pattern in alkaloid content were observed in the leaves, with stem-derived extracts displaying lower alkaloid levels compared to roots and leaves.

### Antimicrobial activity of extracted plant materials (leaf root and stem) and standard compounds berberine and magnoflorine

Various extract fractions obtained from different plant parts exhibited a range of antimicrobial activities against the microbial strains employed in the experiment, as detailed in Table 1. The antimicrobial assay indicated that



**Fig. 2** HPLC analysis of antimicrobial responsive alkaloids. **a** Identified BIQ alkaloids i.e. magnoflorine, thalicarpine, palmatine and berberine peaks in HPLC chromatogram of different parts (leaf, stem and root) of *T. foliolosum*. **b** Bar graph represents the comparative observation of identified benzylisoquinoline alkaloids in different plant parts of *Thalictrum foliolosum* plants. The results are expressed as means  $\pm$  SD of three replicates

the chloroform fractions of roots and leaves were particularly effective, demonstrating broad-spectrum growth inhibition across all concentration ranges (25–100  $\mu\text{g/ml}$ ) against all pathogenic bacteria. The ethyl acetate fractions of leaves and roots displayed remarkable antimicrobial activity at a concentration of 50  $\mu\text{g/ml}$  against all pathogenic bacterial cultures. However, the ethyl acetate fractions from the stem part did not inhibit the growth of gram-negative *P. aeruginosa* (Table 1). Additionally, aqueous methanolic extracts of leaves and roots exhibited impressive growth inhibition against *E. coli*, *P. aeruginosa*, and *S. mutans* at concentrations  $\geq$  100  $\mu\text{g/ml}$ . Hexane extracts of leaves and stems demonstrated antimicrobial activity against all tested microorganisms except *P. aeruginosa*. However, in our report, hexane root extracts did not exhibit a zone of inhibition against both gram-negative and gram-positive bacteria at concentrations ranging from 25 to 100  $\mu\text{g/ml}$ . Comparatively, when standard compounds berberine chloride and magnoflorine chloride were employed at similar concentrations against the same pathogenic bacterial cultures, berberine emerged as the most effective compound at each concentration. While magnoflorine did not exhibit effective

growth inhibition against various pathogens at various concentrations, a slight zone of inhibition was observed against *E. coli* at 100  $\mu\text{g/ml}$  (Fig. 3).

#### Minimum inhibitory concentrations (MIC's) analysis

In our investigation, we determined the Minimum Inhibitory Concentration (MIC) of potent plant extracts using a 96-well microtiter plate. The MIC values for aqueous methanol, ethyl acetate, and chloroform fractions are detailed in Table 2. Notably, the chloroform fraction of the root exhibited the lowest MIC against all tested strains, with a lesser value of 121.26  $\mu\text{g/ml}$  observed against *S. aureus* (refer to Table 3). The ethyl acetate fraction from various plant parts demonstrated effective MIC values ranging from 158.15 to 448.78  $\mu\text{g/ml}$  against all tested microbial pathogens. Particularly, the ethyl acetate root fraction displayed the minimum MIC against *S. mutans*. Additionally, the aqueous methanolic extracts showed MIC values ranging from 224.78 to 482.67  $\mu\text{g/ml}$  (refer to Table 3). Furthermore, we analyzed the MIC values of reference alkaloid compounds, as presented in Table 4. The findings suggested that berberine chloride showed less MIC values (123.95  $\mu\text{g/ml}$ ) compared to

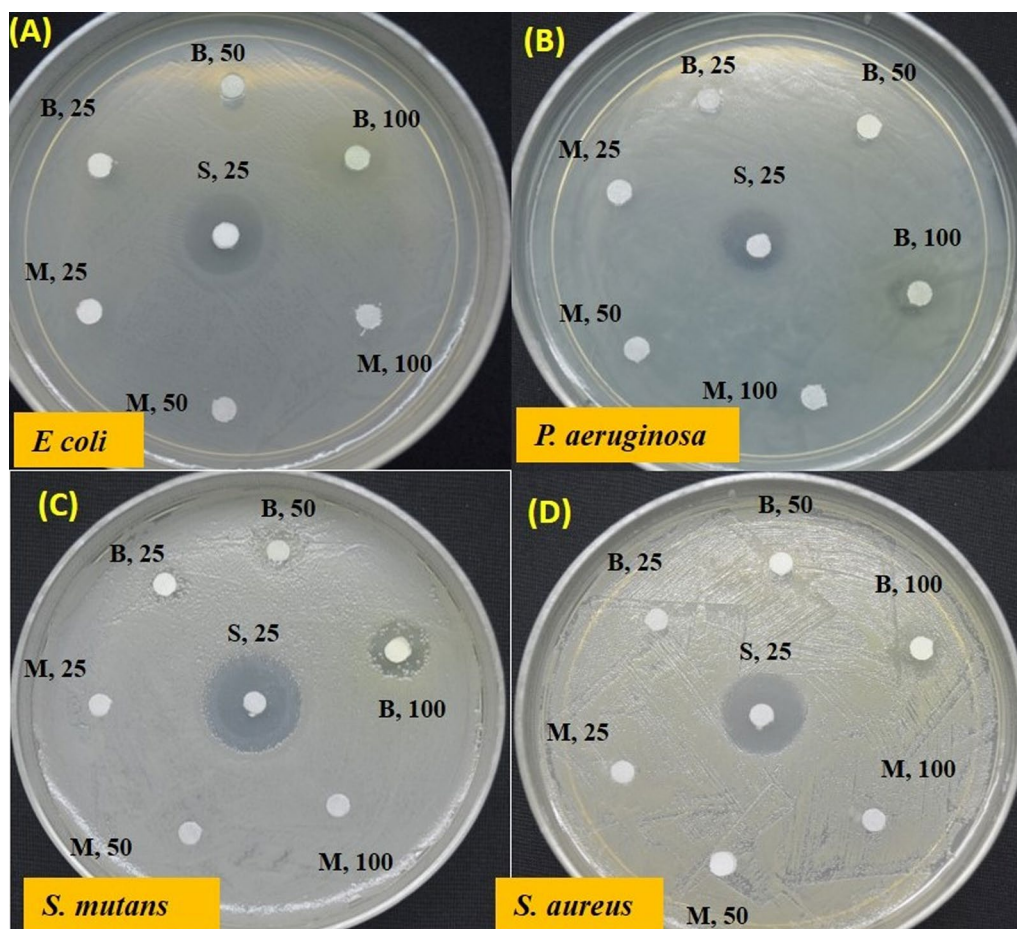
**Table 1** Antimicrobial activity of various plant part extracts of *T. foliolosum* against different pathogenic gram-positive and gram-negative microbes

Plant Parts	Extracts	Inhibition Zone diameter at 50 µg/ml and 100 µg/ml plant extracts in various organic solvents									
		<i>Staphylococcus aureus</i>		<i>Streptococcus mutans</i>		<i>Pseudomonas aeruginosa</i>		<i>Escherichia coli</i>			
		50	100	50	100	50	100	50	100		
(a) Leaf	Aqueous methanol	-	-	9.2±0.14 <sup>b</sup>	14.4±0.16 <sup>f</sup>	++	-	10.1±0.11 <sup>c</sup>	+	13.5±0.12 <sup>e</sup>	++
	Ethyl acetate	9.6±0.56 <sup>d</sup>	13.7±0.16 <sup>b</sup>	++	9.4±0.34 <sup>c</sup>	++	++	11.5±0.15 <sup>d</sup>	++	13.1±0.41 <sup>f</sup>	++
	Hexane	9.4±0.21 <sup>d</sup>	10.3±0.78 <sup>d</sup>	++	9.1±0.45 <sup>b</sup>	11.9±0.67 <sup>cde</sup>	++	-	-	12.2±0.18 <sup>ed</sup>	++
	Chloroform	11.8±0.35 <sup>c</sup>	13.1±0.43 <sup>b</sup>	++	8.3±0.45 <sup>a</sup>	15.4±0.56 <sup>g</sup>	+++	9.6±0.12	12.3±0.10 <sup>e</sup>	++	14.3±0.43 <sup>h</sup>
(b) Stem	Aqueous methanol	-	-	-	9.2±0.19 <sup>a</sup>	+	-	-	-	11.4±0.15 <sup>b</sup>	++
	Ethyl acetate	9.4±0.14 <sup>d</sup>	12±0.34 <sup>c</sup>	++	9.8±0.14 <sup>e</sup>	11.9±0.79 <sup>cde</sup>	++	-	-	10.2±0.45 <sup>a</sup>	+
	Hexane	13.5±0.31 <sup>a</sup>	14.3±0.89 <sup>a</sup>	++	-	9.8±0.42 <sup>b</sup>	+	-	-	12±0.21 <sup>d</sup>	++
	Chloroform	9.2±0.23 <sup>d</sup>	10.1±0.11 <sup>d</sup>	++	-	-	-	-	-	0	0
(c) Root	Aqueous methanol	-	-	-	11.5±0.56 <sup>cd</sup>	++	-	-	-	10.3±0.11 <sup>b</sup>	++
	Ethyl acetate	-	-	9.3±0.21 <sup>bc</sup>	11.2±0.31 <sup>c</sup>	++	-	9.5±0.13 <sup>a</sup>	+	9.6±0.24 <sup>a</sup>	++
	Hexane	-	-	-	-	-	-	-	-	-	-
	Chloroform	12.7±0.18 <sup>b</sup>	13.6±0.35 <sup>b</sup>	++	9.7±0.34 <sup>d</sup>	14.1±0.17 <sup>f</sup>	++	-	9.6±0.25 <sup>ab</sup>	+	15.7±0.57 <sup>j</sup>

Positive control streptomycin (25 µg/ml) showed inhibition diameter (16.4±0.12) (*S. aureus*), (18.12±0.23) (*S. mutans*), (15.6±0.18) (*P. aeruginosa*) and (16.2±0.16) (*E. coli*) respectively. Zone of inhibition (mm) is expressed as mean of triplicates with standard error of means (SEM). Inhibition zones include the circular paper disk diameter (6 mm), (-), negative control (respected solvent)

"-" = < 8 mm, "+" = 9 mm, "++" = 9 mm < 14 mm and "+++" = 15 mm to < 18 mm

The data are presented as the mean ± the standard deviation. The values are presented as the mean of three replicates ± the standard deviation. The data marked with different letters share significance at p < 0.05 (based on the Duncan test)



**Fig. 3** Growth inhibition assay. Antibacterial activity of magnoflorine (M) and berberine (B) reference compounds against human pathogenic bacteria (*E. coli*, *P. aeruginosa*, *S. mutans* and *S. aureus*) using 25–100 µg concentration (B). Streptomycin used as standard antibiotic drug 25 µg

**Table 2** Minimum inhibitory concentration (MIC) analysis of various plant part extracts against pathogenic bacteria

	Plant parts	Extracts	MIC (µg/ml)			
			<i>Staphylococcus aureus</i>	<i>Streptococcus mutans</i>	<i>Pseudomonas aeruginosa</i>	<i>Escherichia coli</i>
(a)	Leaf	Aqueous methanol	474.96 ± 3.25 <sup>h</sup>	264.91 ± 2.14 <sup>f</sup>	324.56 ± 2.42 <sup>e</sup>	298.76 ± 3.12 <sup>f</sup>
		Ethyl acetate	158.15 ± 1.56 <sup>d</sup>	250.54 ± 2.43 <sup>e</sup>	245.67 ± 2.65 <sup>b</sup>	216.25 ± 2.78 <sup>c</sup>
		Chloroform	121.26 ± 1.01 <sup>a</sup>	235.19 ± 2.13 <sup>d</sup>	194.54 ± 1.54 <sup>a</sup>	209.84 ± 2.87 <sup>b</sup>
(b)	Stem	Aqueous methanol	482.67 ± 3.76 <sup>i</sup>	350.73 ± 3.65 <sup>h</sup>	450.34 ± 3.56 <sup>h</sup>	324.54 ± 3.04 <sup>g</sup>
		Ethyl acetate	146.28 ± 1.23 <sup>c</sup>	225 ± 2.24 <sup>c</sup>	448.78 ± 3.46 <sup>j</sup>	345.12 ± 2.96 <sup>h</sup>
		Chloroform	168.67 ± 1.76 <sup>e</sup>	402.65 ± 3.42 <sup>i</sup>	389.45 ± 3.65 <sup>g</sup>	399.98 ± 3.78 <sup>i</sup>
(c)	Root	Aqueous methanol	400 ± 2.65 <sup>g</sup>	279.98 ± 2.45 <sup>g</sup>	364.98 ± 3.32 <sup>f</sup>	224.78 ± 2.12 <sup>d</sup>
		Ethyl acetate	345.56 ± 2.34 <sup>f</sup>	189.91 ± 1.76 <sup>b</sup>	278.14 ± 2.34 <sup>d</sup>	278.43 ± 2.76 <sup>e</sup>
		Chloroform	132.56 ± 0.97 <sup>b</sup>	164.56 ± 1.34 <sup>a</sup>	271.82 ± 2.12 <sup>c</sup>	181.23 ± 1.89 <sup>a</sup>

The data are presented as the mean ± the standard deviation. The values are presented as the mean of three replicates ± the standard deviation

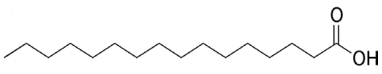
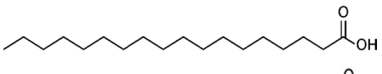
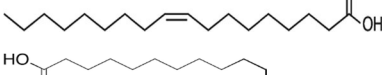
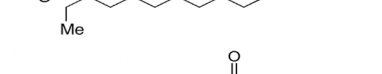
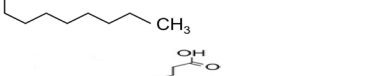

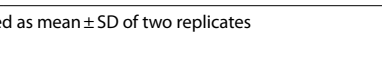
The data marked with different letters share significance at  $p < 0.05$  (based on the Duncan test)

**Table 3** MIC analysis of Berberine, Magnoflorine and Streptomycin against studied pathogens

		MIC ( $\mu\text{g/ml}$ )			
		<i>Staphylococcus aureus</i>	<i>Streptococcus mutans</i>	<i>Pseudomonas aeruginosa</i>	<i>Escherichia coli</i>
(a)	Berberine chloride	113.95 $\pm$ 1.45	115.45 $\pm$ 1.87	140.78 $\pm$ 1.21	150.36 $\pm$ 1.45
(b)	Magnoflorine	350 $\pm$ 2.41	310.25 $\pm$ 3.56	325.67 $\pm$ 3.87	254.65 $\pm$ 2.76
(c)	Streptomycin	15 $\pm$ 0.95	32.64 $\pm$ 2.14	31.45 $\pm$ 1.67	25 $\pm$ 1.20

The data are presented as the mean  $\pm$  the standard deviation. The values are presented as the mean of three replicates  $\pm$  the standard deviation

**Table 4** Identification and quantification of fatty acids in *T. foliolosum* by gas liquid chromatography

Identified fatty acid in <i>T. foliolosum</i>		Quantity of FAME in leaf (%)	Quantity of FAME in stem (%)	Structural characteristics
1	Palmitic acid 	5.72 $\pm$ 0.83	7.81 $\pm$ 1.65	Hexadecenoic acid. It is a fatty acid with a 16-carbon chain. It is the most common saturated fatty acid found in animals, plants and microorganisms
2	Stearic acid 	0.99 $\pm$ 0.06	2.98 $\pm$ 0.26	octadecanoic acid, saturated fatty acid with an 18-carbon chain
3	Oleic acid 	1.81 $\pm$ 0.13	3.42 $\pm$ 0.19	Long-chain carboxylic acid; its molecule contains one double bond between C9 and C10 with the cis configuration
4	Behenic acid 	1.28 $\pm$ 0.26	0.76 $\pm$ 0.04	Behenic acid (also docosanoic acid) is a carboxylic acid, saturated fatty acid
5	Arachidic acid 	0.98 $\pm$ 0.025	1.29 $\pm$ 0.11	Naturally occur in <i>Staphisagria macrosperma</i> , <i>Dipteryx lacunifera</i> . saturated long-chain fatty acid with a 20-carbon backbone
6	Linoleic acid 	1.30 $\pm$ 0.09	2.72 $\pm$ 0.12	An octadecadienoic acid in which the two double bonds are at positions 9 and 12 and have cis stereochemistry
7	Linolenic acid 	1.42 $\pm$ 0.151	3.27 $\pm$ 0.09	carboxylic acid with an 18-carbon chain and three cis double bonds

The results are expressed as mean  $\pm$  SD of two replicates

magnoflorine chloride against all pathogenic bacterial strains (refer to Table 4).

#### Fatty acid analysis

Analysis of fatty acids in various parts of the *T. foliolosum* plant (leaves, stems, and roots) revealed higher levels of FAME in the stems compared to other plant parts. Trace amounts of FAME were recorded in root, in our observation. Table 4 provides detailed information on the identified fatty acids and their respective quantities in different plant parts. Notably, fatty acid esters such as palmitic acid (16:0) (7.81%), stearic acid (2.98%), oleic acid (C18:1) (3.42%), linoleic acid (C18:2) (2.72%), and linolenic acid (C18:3) (3.27%) were found in significant amounts in the stem parts.

#### Discussion

Plant extracts have been used for centuries as natural medicines due to the diverse array of bioactive compounds they contain. These compounds can have therapeutic effects on the human body. Herbal remedies are often passed down through generations and used to treat various ailments. Extracts of various plant, i.e. *Andrographis paniculata*, *Withania somnifera*, *Panax ginseng*, *Panax quinquefolium*, *Coptis japonica* and *Carica papaya* have been widely used in natural medicine to treatment of various chronic disorders [5, 21]. Similarly, a lesser explored medicinal herb *T. foliolosum* widely used as traditional medicine in gastric problem, bloating, toothache, abdominal colic pain and piles (hemorrhoids) [7, 22]. Plants contain a variety of bioactive compounds, such as alkaloids, flavonoids, terpenoids, sterols and



polyphenols. These compounds possess medicinal properties and can have antioxidant, anti-inflammatory, antiviral, antibacterial, and antifungal effects. Previous reports and contemporary studies have shown that the therapeutic potential of *T. foliolosum* plants may be due to the presence of bioactive BIQ alkaloids and other non-alkaloidal compounds such as flavonoids, saponins, polyphenolic compounds and steroids, etc. [23, 24].

Quantitative and qualitative analysis of BIQ alkaloids—specifically, berberine, magnoflorine, palmatine, and thalicarpine—was conducted using High-Performance Liquid Chromatography (HPLC) across different plant parts of *T. foliolosum*. The order of alkaloid abundance was consistently observed as magnoflorine > berberine > thalicarpine > palmatine in both the underground and leaf components of *T. foliolosum* plants. Magnoflorine is a quaternary aporphine alkaloid with numerous pharmacological properties, including anti-diabetic, anti-inflammatory, neuropsychopharmacology, immunomodulatory, hypotensive, antioxidant, and antifungal properties [25]. Berberine has long history of medicinal use as folk medicine in Chinese, Indian and Native American. Berberine has potential therapeutic use in metabolic syndrome, type 2 diabetes and dyslipidemia as well as antimicrobial activity [26]. The palmatine alkaloids are also used in traditional Asian medicine for the treatment of jaundice, liver infection, high blood pressure, inflammation and dysentery. Palmatine has recently been shown to be beneficial in the treatment of central nervous system problems [27]. Thalicarpine is a hypotensive alkaloid that has been recognized as being involved in the binding of novel antitumor agents [23, 24, 28].

In addition to biochemical analysis, antimicrobial activity showed that the chloroform fractions of root and leaves have significant activity against different pathogenic micro-organism. It was reported that berberine is the most active compound in the chloroform fractions and has been identified as the most important antibacterial component in various *Thalictrum* species of all the BIQ compounds identified [7, 14, 22, 24]. Similar reports were also found in other species of *Thalictrum* (*T. delavayi*, *T. minus*, *T. orientale*, *T. fortune* and *T. avanicum*) further supporting the antimicrobial potential of berberine [29]. Furthermore, our antimicrobial assay using berberine and magnoflorine standard compounds against pathogenic bacteria provided valuable insights into the potential bioactivity of these compounds. The observed superior effectiveness of berberine compared to other reference compounds highlights its promising antimicrobial properties (Table 4). The potential mechanisms of berberine antimicrobial activity may be mediated by the suppressing cell adhesion and migration as well as inhibiting the microbial enzymes [22]. Besides,

magnoflorine an aporphine BIQ (usually soluble in water, methanol, and ethanol, and insoluble in low polar organic solvents such as petroleum ether and chloroform) was occurred significant amount in root and leaves but this alkaloid has been showed poor antibacterial activity against various pathogenic bacteria (Fig. 3). However, it was reported that magnoflorine had potent antifungal activity against *Penicillium avellaneum* and *Candida* strain, which may account for the potential antifungal activity in *T. foliolosum* [25].

Additionally, ethyl-acetate extracts of leaves were found to have impressive antimicrobial activity than other parts (roots and stem) of ethyl acetate extract against *E. coli*, *S. aureus*, and *S. mutans*. It has been reported the ethyl-acetate fraction contained significant amount of polyphenolic or flavonoids compounds, which have strong antimicrobial activity and antioxidant activity [5, 30]. Moreover, aqueous methanolic extracts (50%) of leaves and roots also shows the effective antibacterial activity against the *E. coli*, *P. aeruginosa* and *S. mutans*. Inhibition activity in aqueous methanolic extract fraction may be due to soluble fraction of alkaloids, phenol and flavonoids compounds [14, 22]. Furthermore, the non-polar hexane fraction, particularly from stem parts, demonstrated effective antimicrobial activity against both gram-negative and gram-positive bacteria. Fatty Acid Methyl Ester (FAME) analysis revealed significant amounts of fatty acid esters including palmitic acid, stearic acid, and linoleic acid in *T. foliolosum* stem parts, contributing to their potential antibacterial activity [31, 32]. The comprehensive analysis of *Thalictrum foliolosum* extracts highlights the presence of various bioactive compounds with significant antimicrobial potential.

## Conclusion

In conclusion, our study provides valuable insights into the medicinal properties of *T. foliolosum*, a lesser-explored herb with a rich traditional history of use in treating various ailments. Through quantitative and qualitative analysis, we identified a diverse array of bioactive compounds in different plant parts, particularly BIQ alkaloids such as magnoflorine, berberine, palmatine, and thalicarpine, alongside substantial presence of both unsaturated and saturated fatty acids. Our findings underscore the potential therapeutic significance of *T. foliolosum* extracts, with particular emphasis on their antimicrobial activity against a range of pathogenic microorganisms. Berberine emerged as a key antimicrobial compound, exhibiting potent activity against various bacteria. Additionally, ethyl acetate and aqueous methanolic extracts displayed significant antibacterial effects, likely attributed to their polyphenolic or flavonoid content. Furthermore, our study highlights the importance

of understanding the composition and pharmacological properties of medicinal plants to harness their therapeutic potential effectively. *T. foliolosum* shows promise as a natural remedy for combating microbial infections, and further research into its mechanisms of action and clinical applications is needed.

#### Abbreviations

BIQ	Benzylisoquinoline
HPLC	High Performance Liquid Chromatography
FAME	Fatty acid methyl esters
GC	Gas chromatography
ZOI	Zone of inhibition
MIC	Minimum inhibitory concentration

#### Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s43094-024-00643-z>.

Supplementary Material 1

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#### Author contributions

Manoj Kumar Mishra; Conception and design the study, performed the experiments, analyzed the data and interpretation of data and wrote the manuscript.

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#### Availability of data and materials

Yes.

#### Declarations

#### Ethics approval and consent to participate

Not applicable.

#### Consent for publication

Not applicable.

#### Competing interests

The authors declare that they have no conflict of interest.

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