RESEARCH ARTICLE   **OPEN ACCESS**

Assessing the Antibacterial Effectiveness against MDR Strains and the Antioxidant Characteristics of *Ficus auriculata*

Namrata Singh[1] , **Rashmi Verma[1]*** , **Rabia Basri Aziz[1]** ,
Aayushi Bhakta[1] and **Pramod Rawat[2]**

[1]Department of Biotechnology, School of Basic and Applied Sciences, Shri Guru Ram Rai University, Dehradun, Uttarakhand, India.
[2]Department of Biotechnology, Graphic Era Deemed to be University, Dehradun, Uttarakhand, India.

Abstract

For millennia, people have successfully treated a wide range of illnesses, including bacterial infections, with different plant parts or extracts. "MDR strain" typically refers to a "multi-drug resistant strain" of a bacteria. In the context of infectious diseases, a multi-drug resistant strain refers to a strain of the pathogen that has acquired resistance to multiple drugs that are commonly used to treat infections caused by that specific pathogen. MDR strains can present significant challenges in healthcare settings as they limit the effectiveness of standard treatments and may require more aggressive or specialized approaches to manage the infection. The discdiffusion method was used in this investigation to test the antimicrobial properties of Petroleum ether, acetone, ethanol, methanol, and aqueous extracts of *Ficus auriculata* leaves against four bacterial strains namely *Salmonella enteric serovar typhi, Salmonella enteric ser Paratyphi* (MDR strain) *Pseudomonas aeruginosa* and *Escherichia coli.* The findings showed that all of the investigated organisms (zone of inhibition of 0.5 ± 0.15 & 18 ± 1.7 mm) were significantly inhibited by the petroleum ether, ethanol, and methanol extracts, with the exception of *Salmonella typhi* (an 18 mm inhibitory zone). The restricted area (≤5) indicated moderate activity in the aqueous extracts. It's crucial to remember that antimicrobial activity analysis of plant extracts is just one step in the process of identifying potential natural antimicrobial agents. Further studies, including the identification and isolation of specific bioactive compounds, toxicology assessments, and clinical trials, are required before any plant extract can be considered for use as a drug development.

Keywords: MDR Strain, *Ficus auriculata,* Disc Diffusion Method, Antimicrobial Activity, Drug Development

*Correspondence: rashverma26@gmail.com

INTRODUCTION

Ficus auriculata, referred to as the Roxburgh fig in common or elephant ear fig, is a type of flowering plant being a part of the family Moraceae.[1] Here are some key characteristics and information about *Ficus auriculata*:

Habitat

This species comes from Southeast Asia and is commonly found in nations like India, Bangladesh, Sri Lanka, Myanmar, and Thailand. It typically develops in a tropical and subtropical region.[2,3]

Tree Characteristics

Ficus auriculata is a large, evergreen tree that can reach heights of up to 30 meters. It has a spreading canopy and distinctive, large, ear-shaped leaves, which give it the common name "elephant ear fig."

Leaves

The leaves of *Ficus auriculata* are glossy, dark green, and can grow up to 30 centimeters in length. The shape of the leaves is somewhat reminiscent of an elephant's ear, hence the common name.[4]

Fruits

The figs produced by this species are small, green to yellowish-brown, and typically measure about 1 to 1.5 centimeters in diameter. The fruits are often used by birds and other wildlife as a food source.[5]

Ecological importance

Ficus species, in general, play a crucial role in various ecosystems. They are known for their mutualistic relationship with fig wasps, which are essential for the pollination of fig flowers.

Cultural significance

In some cultures, *Ficus auriculata* may have cultural or religious significance. *Ficus* trees, in general, are associated with sacred sites and are often planted near temples or other religious places.

Landscaping

Due to its large and attractive foliage, *Ficus auriculata* is sometimes used in landscaping for its ornamental value. However, its size needs to be considered when planting in smaller spaces.

Propagation

Ficus auriculata can be propagated through seeds or stem cuttings. The plant is relatively hardy and can adapt to a variety of soil types, but it prefers well-drained soil.

Always be sure to check local regulations and guidelines before introducing non-native plant species to new areas, as they can potentially become invasive and disrupt local ecosystems.

While *Ficus auriculata*, commonly known as the Roxburgh fig or elephant ear fig, is not as extensively documented in traditional medicine as some other *Ficus* species, certain plant parts have been utilised in customary practices in some regions. It's important to note that the information provided here is based on traditional knowledge, and scientific evidence may be limited. Always consult with healthcare professionals before using any plant for medicinal purposes.[6-8]

Leaves and latex

In traditional medicine, *Ficus auriculata* leaves and latex have been used for various purposes. The latex, in particular, is known for its potential wound-healing properties. In some cultures, it has been applied topically to wounds or skin conditions.

Antimicrobial properties

Some *Ficus* species, including *Ficus auriculata*, are believed to possess antimicrobial properties. The leaves and latex may be utilised in conventional medicine to treat skin infections or wounds.[9]

Anti-inflammatory effects

The plant's components, when applied externally, are thought to have anti-inflammatory effects. This may be relevant in traditional remedies for conditions such as skin inflammation or joint pain.

Digestive issues

In certain traditional systems, *Ficus* species, including *Ficus auriculata,* have been used to address digestive issues. This might include the use of specific parts of the plant to alleviate symptoms like indigestion.

Antioxidant potential

Some *Ficus* species are known to contain compounds with antioxidant properties. Antioxidants are believed to help combat oxidative stress in the body, and in traditional medicine, plants with such properties are sometimes used to promote overall health.

It's important to emphasize that while there may be anecdotal evidence supporting the use of *Ficus auriculata* in traditional medicine, scientific studies validating its efficacy and safety are often lacking. If you are considering using *Ficus auriculata* or any other plant for medicinal purposes, it's crucial to consult with healthcare professionals or traditional medicine practitioners who are knowledgeable about the specific uses and potential risks associated with the plant. Additionally, be aware that traditional practices can vary widely, and the effectiveness of traditional remedies may not be universally accepted or supported by scientific evidence. [6,13,14]

The term "MDR strain" typically refers to a strain of a pathogenic microorganism that has developed resistance to multiple drugs. MDR stands for multidrug-resistant. This phenomenon is a significant concern in the context of bacterial, viral, fungal, or parasitic infections where the microorganism evolves to withstand the effects of multiple drugs that were originally effective in treating the infection.

MATERIALS AND METHODS

Plant Sample collection and authentication

The foliage of *F. auriculata* were collected from Tehri region of Uttarakhand, India. The BSI (Botanical Survey of India) validated and taxonomically identified the plant samples. A voucher specimen (872) of *F. auriculata* Lour were accessioned at herbarium BSI, Dehradun. Wiped the sample to get rid of dust. Cut the leaves and stem into small pieces after washing. Plant stems and leaves were air dried for two weeks in shaded area before being packaged in paper bags and kept in storage. To prevent any contamination from growing, samples were constantly monitored. To create a fine powder sample, dried leaves and stems were ground up in an electric grinder.

Preparation of plant extracts

The sequential Soxhlet extraction technique was used for the extraction.

Soxhlet extraction

This method was applied to get extracts for pharmaceutical and biological testing, as well as phytochemical screening. To obtain a uniformly sized powder, the leaves and stem were ground together in a grinder. A homogenous 25 gm of powered plant substance was placed in a thimble, and 250 mL of numerous solvents were extracted from each separately. After that, the thimble was situated inside the Soxhlet apparatus, where extraction was carried out using petroleum ether, acetone, ethanol, methanol, as well as water as solvents in a sequential order from non-polar to polar. The extraction process lasts for a whole day, or until the solvent in the extractor's siphon tube turns colorless.[10] After the petroleum ether extract was collected, acetone was extracted in a further step using powder made from the thimble, and the yield was estimated. The same process was used for drying, and thimbles were filled with powder that was utilized for ethanol, methanol, and water extraction. Finally, soluble fractions in water, methanol, ethanol, acetone, and petroleum ether were obtained. Crude extracts were then left behind as the extract was allowed to concentrate in a vacuum using a rotating evaporator. The dehydrated mixture was stored in a refrigerator at 4°C for their future use in different analysis.[11,12] All extracts obtained from Tehri regions of Uttarakhand.

Test organism collection: used microorganisms and growth conditions

For the experiment, MTCC 735 for *Salmonella enterica ser. Paratyphi* and MTCC 733 for *Salmonella enterica serovar Typhi* were provided by the "Chandigarh Institute of Microbial Technology (IMTECH)". Human harmful bacterial strains, such as *Escherichia coli* and *Pseudomonas aeruginosa*, were gathered for antimicrobial

screening and antibacterial research purposes from Medical & Health Sciences department, SGRR University Dehradun, India. Bacteria were cultivated using "Mueller-Hinton broth medium (MHB) as well Mueller-Hinton agar medium (MHA)" from Liofilchem, Italy.

Preparation of stock solutions

To avoid sterile filtration, the samples' stock solutions were made by dissolving the raw desiccated extracts in (DMSO) pure dimethyl sulfoxide (Sigma-Aldrich) concentrate. Meanwhile, sterile distilled water was used to dilute the aqueous extracts and filtered using a Millipore 0.22 µm sterile filter.[15]

Preparation of bacterial inoculums

Bacterial inoculation for *Escherichia coli*, *Typhi* and *Paratyphi* are two types of *Salmonella enterica* and *Pseudomonas aeruginosa* made using the Luria-Bertani broth (HIMEDIA). After thoroughly dissolving nutrient agar in distilled water, after mixing it was autoclaved regarding 30 minutes at 120°C as well 15 to 20 psi pressure for inoculate bacterial colonies. After being recovered in BHI, pathogenic cultures were cultured for an entire night at 36°C. Once the cultures were maintained at 0.5 of the McFarland scale which is equivalent to 108 CFU.ml^{-1}, they were diluted to 10^5 CFU.ml^{-1} using casein peptone water.[16]

Preparation of Agar plates

For the purpose of cultivating bacteria, nutrient agar medium was prepared and then put into sterile plates. It ought to be carried out in an undisturbed, laminar flow environment to prevent medium contamination. Agar medium covered the sterile plates evenly. Agar nutrition plates were now positioned within an incubator set at 37°C for the entire night, or until the agar nutrient solidified in the sterile plates.[17]

Placements of plates

Following the even distribution of agar on sterile plates, a 5 mm sterile paper disc was immersed in a mixture of 50-100 µL of petroleum ether, acetone, ethanol, methanol, and water extracted from the leaves of *Ficus auriculata*. These plates are now placed back into the incubator to develop bacterial cultures overnight at 37°C. The following day, measure the inhibition zone at various concentrations and compare it to the conventional streptomycin (10 µg) zone of inhibition. Inhibition zone diameter as measured on a scale (mm).[18]

Disc diffusion

The isolates were inoculated onto Müller-Hinton Agar media using swabs. Following that, each plate received three sterile discs of Whatman filter paper, measuring 6 mm in diameter. 15 µl of the extracts were put to the paper discs. The negative control was sterile distilled water. For 24 hours, the plates were cultivated at 35 ± 1°C. Using a pachymeter, the inhibitory zone' diameters were determined, and the outcomes was given in millimetres (mm). The extract's antibacterial efficacy against the studied pathogens increases in the inhibition zone. Every test was run in triplicates.[19,21]

Minimum inhibitory concentration (MIC)

The plant extract's minimum inhibitory concentration, or "MIC", at which no growth emerged, was ascertained against two harmful and two multidrug-resistant strains of Salmonella were ascertained by the use of a macrobroth dilution experiment. In well plates, extracts were serially diluted using Mueller-Hinton Broth in a 2-fold range in accordance with the findings of the disc diffusion method. The same controls, both positive and negative were used in the MIC. Following a 20 µl inoculation with freshly made bacterial suspension (5×10^8 CFU/ml), the plates were retained at 37°C for an entire day. It was determined what the minimal amount that inhibits growth "MIC" of the plant extract was at which no growth appeared.[22]

MBC value determination

Samples (5 µl) collected from the wells that did not show growth during the MIC determination were cultivated on agar media in order to determine the minimum bactericidal "MBC" of the plant crude extracts. The least amount of crude extracts that did not show signs of growth following a 24-hour incubation period at 37°C was designated as MBC.[23]

Determination of the antioxidant or Free radical scavenging activity of *Ficus auriculata* different extracts by DPPH Scavenging for Free Radicals

The activity of the antioxidant was tested with some minor modifications of method of Jothi and Jebamalar.[22] Various sample concentrations (20%, 40%, 60%, 80%, and 100%) were combined with 0.1 mM of working DPPH reagent. Keep the mixture for incubation at ambient temperature for 2 hours. Discoloration of sample was predicted by spectrophotometer at 517 nm. Utilising the standard ascorbic acid, the outcomes were stated as mg ASC/g of extract.[20]

Hydrogen peroxide scavenging activity

0.1 ml of dilutions of the sample (20%, 40%, 60%, 80% and 100%) was added in 0.4 ml of 50 mM of phosphate buffer to which 2 mM of 0.6 ml of H_2O_2 was added, vortex the sample for 10 minutes, absorbance was taken through spectrophotometer at 230 nm. The results were articulated as mg ASC/gm extract, using ascorbic acid as the benchmark.[24]

Data processing

The three replicates' mean ± standard deviation was provided as the result. To examine the experimental data, SPSS version 16.0 was utilised (SPSS Inc. Chicago, IL). The mean differences were assessed utilising an ANOVA in one way.

RESULTS AND DISCUSSION

Extract preparation

Ficus auriculata leaf extracts were made using the soxhlet method. After the extracts

Table 1. Physical properties and Yield of *Ficus auriculata* leaf extract[31]

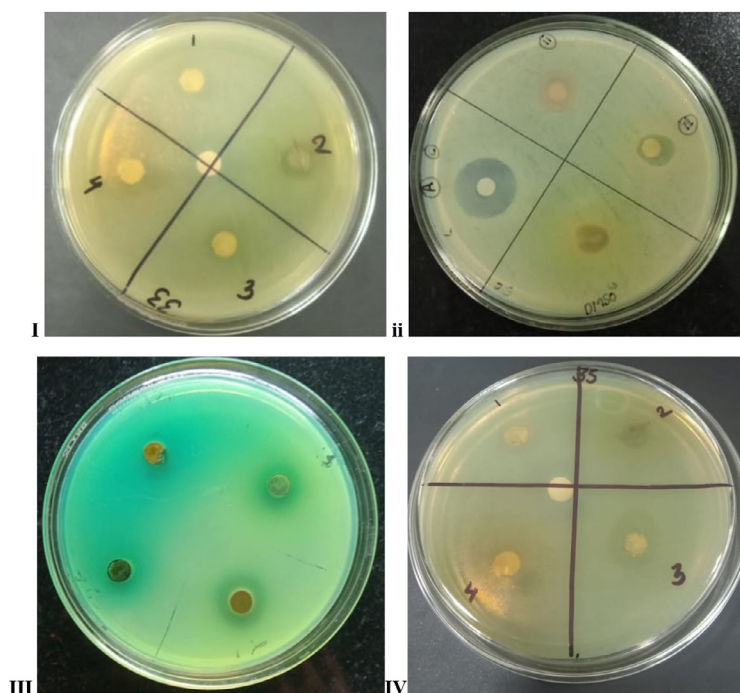| No.	Solvent used	*Ficus auriculate*		
		Yield	Colour	State
1	Petroleum ether	1.0	Light green	Liquid
2	Acetone	2.34	Dark green	Viscous
3	Ethanol	2.91	Dark green	Viscous
4	Methanol	2.84	Dark green	Viscous
5	Water	5.04	Light green	Liquid

Figure 1. Showing antimicrobial activity of: i. *Salmonella enterica typhi,* ii. *Escherichia coli,* iii. *Pseudomonas aeruginosa,* iv. *Salmonella enterica paratyphi*

Table 2. Antibacterial activity of *Ficus auriculata* leaves for various extract determined by disc diffusion[24]

No.	Microbial culture	Concentration of Various Extracts									
		Petroleum ether		Acetone		Ethanol		Methanol		Water	
		0.5 mg/ 0.1 mL	1 mg/ 0.1 mL	0.5 m/ 0.1 mL	1 mg/ 0.1 mL	0.5 m/ 0.1 mL	1 mg/ 0.1 mL	0.5 mg/ 0.1 mL	1 mg/ 0.1 mL	0.5 mg/ 0.1 mL	1 mg/ 0.1 mL
1	*S. enterica ser typhi*	1.5 ± 0.1	3 ± 2	5 ± 0.5	11 ± 1.1	4 ± 1	11 ± 2	6 ± 0.5	14 ± 1.5	1.5 ± 0.1	4 ± 2
2	*S. enterica paratyphi*	1.2 ± 0.1	5 ± 1.5	5 ± 0.5	10 ± 2	5.2 ± 0.1	12 ± 2	9 ± 1	18 ± 1	1.2 ± 0.1	2.8 ± 0.2
3	*E. coli*	0.6 ± 0.1	0.8 ± 0.6	10 ± 1	15 ± 1.5	8 ± 1	12 ± 1.5	4 ± 0.5	16 ± 1	1.6 ± 0.2	10 ± 1
4	*P. aeruginoa*	0.8 ± 0.11	7 ± 0.57	7 ± 1	18 ± 0.5	7 ± 0.57	17 ± 1.1	6.3 ± 0.1	18 ± 1.7	2.5 ± 0.05	2.6 ± 0.2

were ready, Table 1 tallied their yield, colour, and condition. The extracts containing water and ethanol had the highest yield, followed by those containing methanol, acetone, and P. ether.

Antibacterial activity

All leaf extracts, including petroleum ether, acetone, ethanol, methanol as well as water, were exposed to an antibacterial assay using the disc diffusion method against four different microorganisms. Of these, two were multidrug-resistant (MDR) bacteria, the two strains of *Salmonella enterica* are *Typhi* and *Paratyphi* and the other two were gram-negative bacteria, *E. coli* as well as *Pseudomonas aeruginosa.* ZOI was measured in order to determine microbial proliferation. Regarding Ficusauriculata, it was determined that at a dose of 1 mg/100 µl of methanolic extract, *S. typhi* was the most susceptible, followed by *S. typhi, S. paratyphi, E. coli*, and *Pseudomonas aeruginosa*, with ZOIs of 14 ± 1.5 mm, 18 ± 1 mm, 16 ± 1 mm, and 18 ± 1.7 mm, respectively.[5,9,25] At a dose of 0.5 mg/100 µl, the highest ZOI was recorded by *S. typhi, S. para typhi, E. coli*, and *Pseudomonas aeruginosa*, measuring 6.0 ± 0.5 mm, 9.0 ± 1 mm, 4.0 ± 0.57 mm, and 6.1 ± 0.1 mm, respectively.[7] *S. typhi, S. paratyphi, E. coli,* and *Pseudomonas aeruginosa* demonstrated the highest susceptibility to ethanol extract at 1 mg/100 µl dose, with ZOI values of 11 ± 2 mm, 12 ± 2 mm, 12 ± 1.5 mm, and 17 ± 1.1 mm, respectively; in contrast, at 0.5 mg/100 µl dose, the highest ZOI values were 4.0 ± 1 mm, 5.0 ± 0.17 mm, 8.1 ± 1 mm, and 7.1 ± 0.57 mm for *S. typhi, S. paratyphi, E. coli*, and *Pseudomonas aeruginosa*. The highest ZOI for *S. typhi, S. paratyphi, E. coli*, and *Pseudomonas aeruginosa* in the case of Acetone extract for a 1 mg/100 µl dose was found to be 11 ± 1.1 mm, 10 ± 2 mm, 15 ± 1.5 mm, and 18 ± 0.5 mm, respectively.[3] In contrast, the most susceptible bacteria in the case of a 0.5 mg/100 µl dose were *S. typhi*, *S. paratyphi, E. coli*, and *Pseudomonas aeruginosa*, with ZOIs of 5 ± 0.5 mm, 5.2 ± 0.5 mm, 10.1 ± 1 mm, and 7 ± 1 mm.[15,26] *S. typhi*, *S. paratyphi*, *E. coli*, and *Pseudomonas aeruginosa* demonstrated the highest susceptibility to *P. ether* extract at 1 mg/100 µl dose, with ZOI values of 3.0 ± 2 mm, 5.0 ± 1.5 mm, 0.8 ± 0.6 mm, and 7.0 ± 0.57 mm, respectively; meanwhile, at 0.5 mg/100 µl dose, the highest ZOI values were 1.5 ± 0.15 mm,

Table 3. *Ficus auriculata* MIC and MBC extracts, as well "MIC/MBC" values in the index against MTCC 733 (*Salmonella enterica ser. Typhi*) bacterial strains[19]

Extract	Range (mg/mL)	Control of MIC (mg/mL)	MIC extract (mg/mL)	MBC control (mg/mL)	MBC (mg/mL) extract	Index of MIC (control)	Index of MIC (extract)
P. ether	0.5 to 0.0156	0.0156	0.125	0.0312	0.25	2	2
Acetone	0.5 to 0.0156	0.0156	0.125	0.0312	0.25	2	2
Ethanol	0.5 to 0.0156	0.0156	0.0625	0.0312	0.125	2	2
Methanol	0.5 to 0.0156	0.0156	0.0625	0.0312	0.125	2	2
Water	0.5 to 0.0156	0.0156	0.25	0.0312	0.50	2	2

Table 4. *Ficus auriculata* MIC and MBC extracts, as well "MIC/MBC" values in the index against MTCC 735 (*Salmonella enterica ser. Paratyphi*) bacterial strains

Extract	Range (mg/mL)	Control of MIC (mg/mL)	MIC extract (mg/mL)	MBC control (mg/mL)	MBC (mg/mL) extract	Index of MIC (control)	Index of MIC (extract)
P. ether	0.5 to 0.0156	0.0156	0.125	0.0312	0.5	2	2
Acetone	0.5 to 0.0156	0.0156	0.125	0.0312	0.25	2	2
Ethanol	0.5 to 0.0156	0.0156	0.0625	0.0312	0.125	2	2
Methanol	0.5 to 0.0156	0.0156	0.0625	0.0312	0.012	2	2
Water	0.5 to 0.0156	0.0156	0.25	0.0312	0.50	2	2

Table 5. *Ficus auriculata* MIC and MBC extracts, as well "MIC/MBC" values in the index against *E. coli* bacterial strains[22,23]

Extract	Range (mg/mL)	Control of MIC (mg/mL)	MIC extract (mg/mL)	MBC control (mg/mL)	MBC (mg/mL) extract	Index of MIC (control)	Index of MIC (extract)
P. ether	0.5 to 0.0156	0.0156	0.125	0.0312	0.25	2	2
Acetone	0.5 to 0.0156	0.0156	0.125	0.0312	0.125	2	2
Ethanol	0.5 to 0.0156	0.0156	0.0156	0.0312	0.125	2	2
Methanol	0.5 to 0.0156	0.0156	0.0156	0.0312	0.125	2	2
Water	0.5 to 0.0156	0.0156	0.25	0.0312	0.5	2	2

Table 6. *Ficus auriculata* MIC and MBC extracts, as well "MIC/MBC" values in the index against *Pseudomonas aeruginosa* bacterial strains[19,21]

Extract	Range (mg/mL)	Control of MIC (mg/mL)	MIC extract (mg/mL)	MBC control (mg/mL)	MBC (mg/mL) extract	Index of MIC (control)	Index of MIC (extract)
P. ether	0.5 to 0.0156	0.0156	0.25	0.0312	0.5	2	2
Acetone	0.5 to 0.0156	0.0156	0.125	0.0312	0.25	2	2
Ethanol	0.5 to 0.0156	0.0156	0.0156	0.0312	0.125	2	2
Methanol	0.5 to 0.0156	0.0156	0.0625	0.0312	0.0312	2	2
Water	0.5 to 0.0156	0.0156	0.25	0.0312	0.5	2	2

1.2 ± 0.1 mm, 0.6 ± 0.15 mm, and 0.8 ± 0.11 mm for *S. typhi, S. paratyphi, E. coli* as well as *Pseudomonas aeruginosa*. *S. typhi, S. paratyphi, E. coli*, as well as *Pseudomonas aeruginosa* demonstrated the highest susceptibility to water extract at a dose of 1 mg/100 µl, with ZOIs of 4 ± 2 mm, 2.8 ± 0.25 mm, 10 ± 1 mm, and 2.6 ± 0.2 mm, respectively; at a dose of 0.5 mg/100 µl, the highest ZOIs were 1.5 ± 0.17 mm, 1.2 ± 0.1 mm, 1.6 ± 0.2 mm, and

2.5 ± 0.05 mm for *S. typhi, S. paratyphi, E. coli*, and *Pseudomonas aeruginosa*[6] (Table 2, Figure 1-5).

MIC and MBC

All extracts were subjected to the MIC assay utilising a two-fold serial dilution method. The "minimum inhibitory concentration" (MIC) of an antibiotic or test sample needed in order to avoid or slow the rise of germs is known as

Table 7. *Ficus auriculata* (Leaf) DPPH scavenging activity in various solvent extracts[27,29]

Concen. (µg/ml)	Methanol	Ethanol	Acetone	Petroleum ether	Water
20	25.71 ± 0.15	22.14 ± 0.012	23.80 ± 0.12	24.76 ± 0.12	27.14 ± 0.11
40	30.95 ± 0.13	31.19 ± 0.009	33.33 ± 0.11	35.23 ± 0.10	33.80 ± 0.10
60	46.66 ± 0.10	44.2 ± 0.014	45.47 ± 0.09	47.61 ± 0.08	47.14 ± 0.08
80	62.85 ± 0.06	58.33± 0.021	56.90 ± 0.06	59.52 ± 0.06	60.71 ± 0.05
100	72.38 ± 0.05	71.9 ± 0.007	71.19 ± 0.04	69.04 ± 0.04	70 ± 0.04
IC 50	3.18	3.35	3.32	3.24	3.2

Table 8. *Ficus auriculata* (Leaf) H_2O_2 activity in various solvent extracts[28,29]

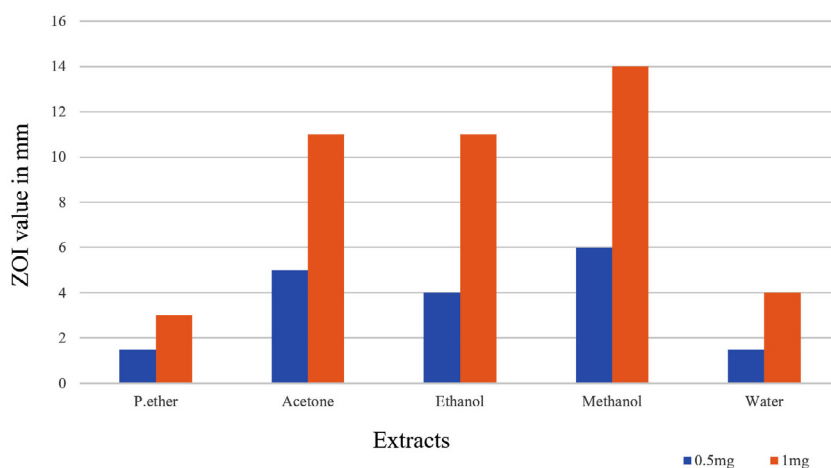| Concen. (µg/ml)	Methanol	Ethanol	Acetone	Petroleum ether	Water
20	13.22 ± 0.19	6.44 ± 0.20	24.06 ± 0.24	11.69 ± 0.01	10 ± 0.19
40	25.25 ± 0.16	21.86 ± 0.16	30.84 ± 0.18	34.74 ± 0.05	16.94 ± 0.16
60	41.01 ± 0.12	41.01 ± 0.13	39.66 ± 0.16	44.57± 0.008	38.64 ± 0.12
80	62.54 ± 0.09	59.32 ± 0.10	46.10 ± 0.12	49.83 ± 0.04	51.01 ± 0.09
100	72.37 ± 0.06	70.84 ± 0.06	65.93 ± 0.07	64.23 ± 0.04	67.62 ± 0.06

Figure 2. Shows antibacterial activity of *F. auriculata* extracts at quantities of 0.5 mg and 1 mg/100 mL against *Salmonella enterica ser typhi* cultures

this. The MIC and MBC control extracts were discovered to be 0.0156 mg/mL and 0.0312 mg/mL, in that order. All of the bacterial strains had MIC Indices of 2.0 for both the extracts and the control. The concentration of ZOI was highest in methanol plant extract, then in ethanol, acetone, petroleum ether, and aqueous. MBC levels varied from 0.5 to 0.0312 mg/mL, based on the MBC data (Table 3-6). The extract's bactericidal properties were evaluated using the MIC Index.[25,26]

Free radical scavenging activity

The primary cause of plant's antioxidant activity is the presence of bioactive compounds within them. The reason for this may not solely be attributed to the large proportion of primary elements, but also to the existence of other constituents in minimal amounts.[28] The hydrogen peroxide assay and the "DPPH" assay were utilised to measure antioxidant activity. Methanolic extract of *Ficus auriculata* shows a strong free radical activity shown in Figure 6. DPPH assay and H_2O_2 assay show strong activity at FP 100% whereas FP 80% shows strong activity for hydrogen peroxide assay[13,27] (Table 7 and 8) (Figure 6 and 7).

The IC_{50} values for each extract in DPPH are given in Table 6, where the methanol extract had 3.18 µg/mL, the petroleum ether extracts 3.24
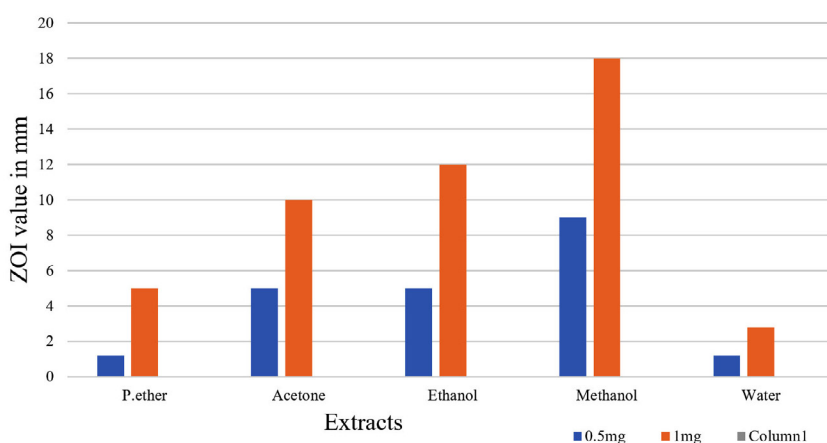

Figure 3. Shows antibacterial activity of *F. auriculata* extracts at quantities of 0.5 mg and 1 mg/100 mL against *Salmonella enterica para typhi* cultures
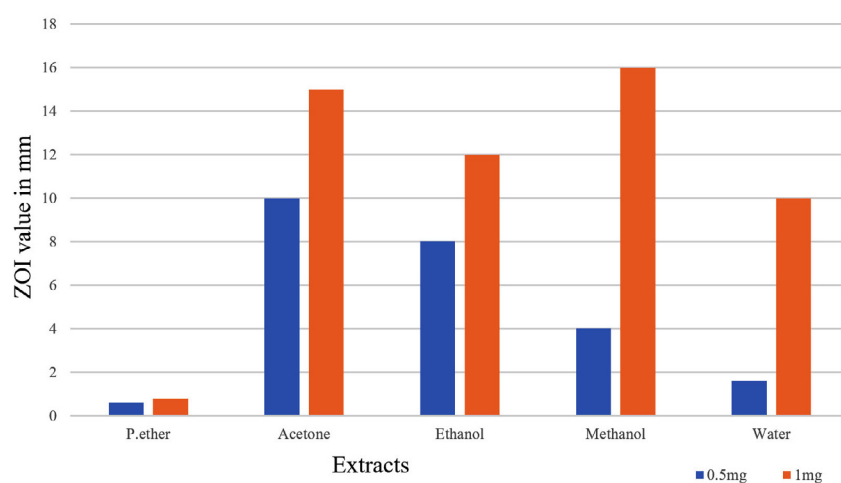

Figure 4. Shows antibacterial activity of *F. auriculata* extracts at quantities of 0.5 mg and 1 mg/100 mL against *E. coli* cultures
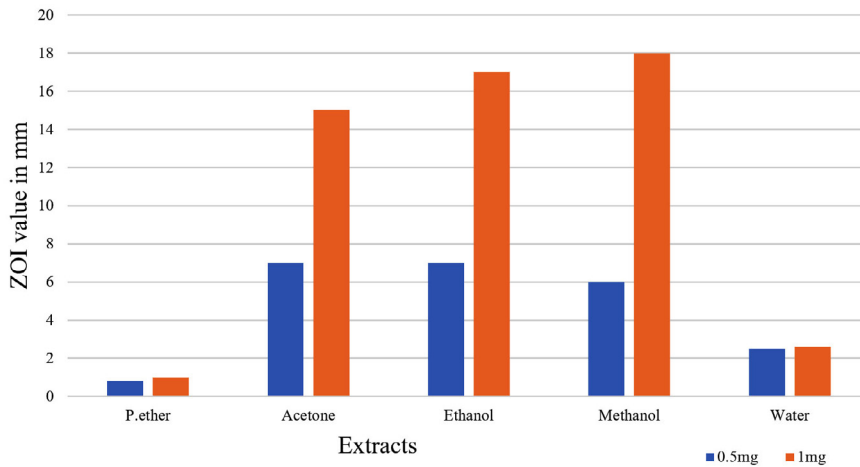
Figure 5. Shows antibacterial activity of *F. auriculata* extracts at quantities of 0.5 mg and 1 mg/100 mL against *Pseudomonas aeruginosa* cultures
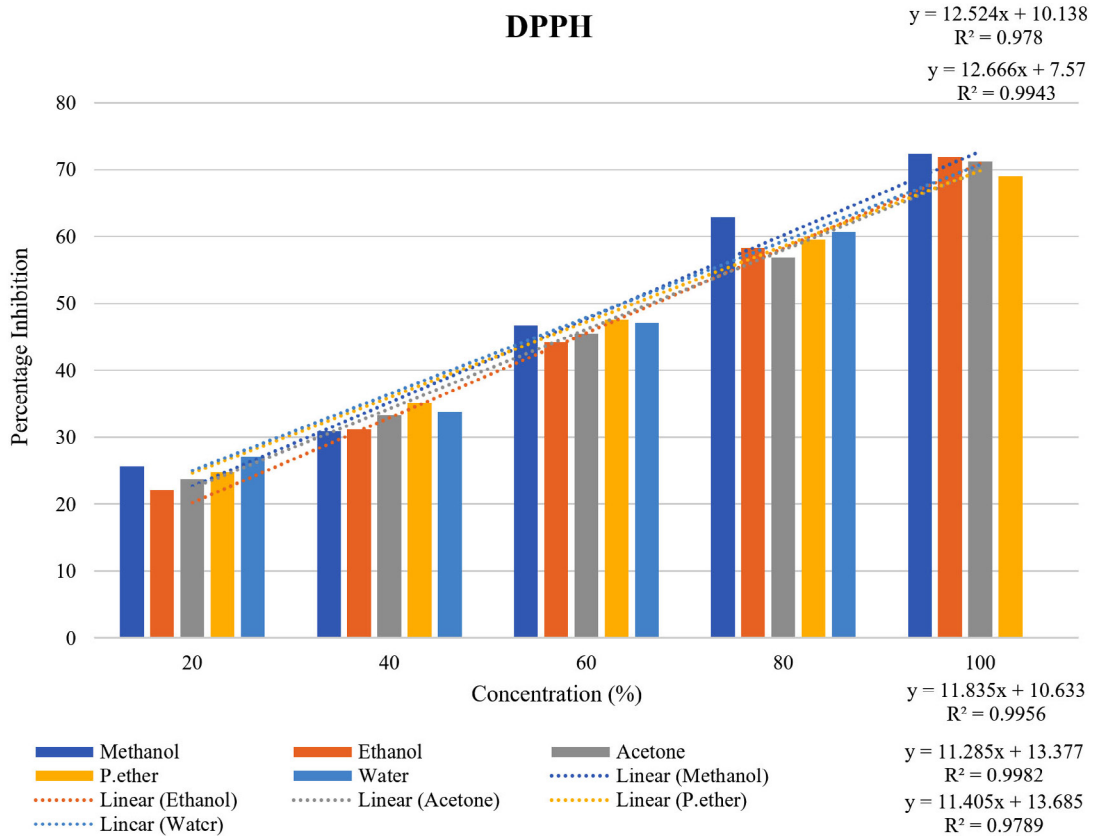

Figure 6. Percentage inhibition of DPPH for different solvent of *Ficus auriculata*
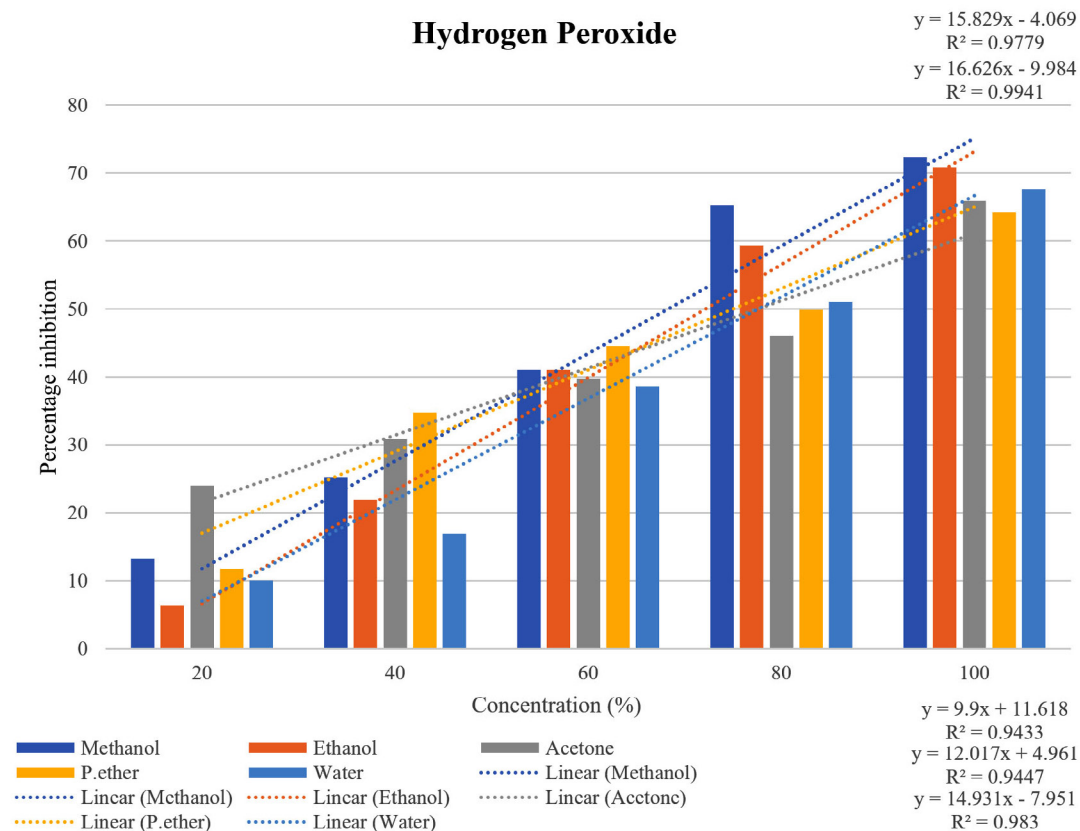
Hydrogen Peroxide

$y = 15.829x - 4.069$
$R^2 = 0.9779$
$y = 16.626x - 9.984$
$R^2 = 0.9941$

$y = 9.9x + 11.618$
$R^2 = 0.9433$
$y = 12.017x + 4.961$
$R^2 = 0.9447$
$y = 14.931x - 7.951$
$R^2 = 0.983$

Figure 7. Percentage inhibition of Hydrogen peroxide for different solvent of *Ficus auriculata*

μg/mL, the aqueous 3.32 μg/mL, the acetone 3.32 μg/mL, and the *P. ether* 3.24 μg/mL.[30]

CONCLUSION

Research on medicinal plants is crucial for the creation of new drugs and study on pharmaceuticals. They are employed directly as a medicinal agent, as well as a modern pharmacologically active chemical or as a raw material for drug synthesis. Compared to *F. auriculata* (leaf), a large portion of contemporary medications are depending on or coming from secondary metabolites of medicinal plants that have antibacterial activity against all four organisms. The highest ZOI found for several *F. auriculata* extracts was for *E. coli, P. aeruginosa, S.* (*serovar*) *typhi,* and *S. paratyphi.* While using antibiotics has brought about instant relief, MDR poses a major risk. Therefore, using different extracts from medicinal plants as an alternate form of therapy is the best way to cure illnesses caused by bacteria. The current investigation expandable to incorporate *in vivo* examinations to be able to determine the mechanism of the extracts' activity. India is home to several medicinal plants with the potential to be used in a variety of medication formulations.

ACKNOWLEDGMENTS

The authors would like to thank SGRR University Dehradun, Uttarakhand, India, for providing instrumentation facilities for the investigation of phytochemicals.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

NS, RV, RBA, AB and PR contributed to the study's conceptualization, design, data collection and analysis. NS and RV verify the integrity of the raw data. NS, RV, RBA, AB and PR drafted the manuscript. NS wrote the manuscript. NS, RV, RBA, AB and PR reviewed and edited the manuscript. All authors read and approved the final manuscript for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

REFERENCES

1. Al-Fishawy A, Zayed R, Afifi S. Phytochemical and pharmacological studies of *Ficus auriculata* Lour. (Moraceae) cultivated in Egypt. *Journal of Natural Products*.2011;4:184-95. doi: 10.1055/s-0031-1282668

2. Rekka R. Ethnobotanical investigation of yercaud hills eastern ghatstamilnadu and phytochemical and pharmacological studies on *ficus auriculata* roxb. 2016. http://hdl.handle.net/10603/244234

3. Gaire BP, Lamichhane R, Sunar CB, Shilpakar A, Neupane S, Panta S. Phytochemical screening and analysis of antibacterial and antioxidant activity of *Ficus auriculata* (Lour.) *Pharmacognosy Journal*.2011;3(21):49-55. doi: 10.5530/pj.2011.21.8

4. Kumari A, Verma R, Sharma M, Chauhan P, Kumar A. Evaluation of phytochemical, antioxidant, antibacterial and anti-cancerous activity of *Ficus auriculata* Lour. and *Osyris wightiana* Wall. ex Wight. *Bull Environ Pharmacol Life Sci.* 2018;7:64-70.

5. Khatun MJ, Rahman MM, Rahim MA, Jakariya M, Mirdah MH. Study on the ethnobotany and nutritional status of three edible *Ficus* species in hill district of Bangladesh. *Int J Min Fruits Med Arom Plants.* 2016;2(1):35-40.

6. Saklani S, Chandra S. *In vitro* antimicrobial activity, nutritional profile and phytochemical screening of wild edible fruit of Garhwal Himalaya (*Ficus auriculata*). *Int J Pharm Sci Rev Res.* 2012;12(2):61-66.

7. Shilpakar A, Gaire BP, Bahadur SC, Lamichhane R, Neupane S. Phytochemical screening and analysis of antibacterial and antioxidant activity of *Ficus auriculata*, Lour. Stem bark. Pokhara University, Pokhara, Nepal. 2009. doi: 10.5530/pj.2011.21.8

8. Malesh B, Satish S. Antimicrobial Activity of some important medicinal plant against plant and human pathogen. *World J Agric Sci.* 2008;4(5):839-843.

9. de Oliveira GF, Furtado NAJC, da Silva Filho AA, et al. Antimicrobial activity of Syzygiumcumini (Myrtaceae) leaves extract. *Braz J Microbiol.* 2007;38(2):381-384. doi: 10.1590/S1517-83822007000200035

10. Salem MZM, Salem AZM, Camacho LM, Ali HM. Antimicrobial activities and phytochemical composition of extracts of *Ficus* species: An over view. *Afr J Microbiol. Res.* 2013;7(33):4207-4219.

11. Manandhar S, Luitel S, Dahal RK. *In vitro* antimicrobial activity of some medicinal plants against human pathogenic bacteria. *J Trop Med.* 2019. doi: 10.1155/2019/1895340

12. Murugan R, Parimelazhagan T. Comparative evaluation of different extraction methods for antioxidant and anti-inflammatory properties from *Osbeckia parvifolia* Arn.- An *in vitro* approach. *J King Saud Univ Sci.* 2014;26(4):267-275. doi: 10.1016/j.jksus.2013.09.006

13. Shi YX, Xu YK, Hu HB, Na Z, Wang WH. Preliminary assessment of antioxidant activity of young edible leaves of seven Ficus species in the ethnic diet in Xishuangbanna, Southwest China. *Food Chem.* 2011;128(4):889-894. doi: 10.1016/j.foodchem.2011.03.113

14. Ostrosky EA, Mizumoto MK, Lima MEL, Kaneko TM, Nishikawa SO, Freitas BR. Methods for evaluation of the antimicrobial activity and determination of minimum inhibitory concentration (MIC) of plant extracts. *Braz J Pharmacogn.* 2008;18(2):301-3017. doi: 10.1590/S0102-695X2008000200026

15. Pa R, Mathew L. Antimicrobial activity of leaf extracts of *Justicia adhatoda* L. in comparison with vasicine. *Asian Pac J Trop Biomed.* 2012;2(3):S1556-S1560. doi: 10.1016/S2221-1691(12)60452-3

16. Oliveira BD, Rodrigues AC, Cardoso BMI, et al. Antioxidant, antimicrobial and anti-quorum sensing activities of *Rubus rosaefolius* phenolic extract. *Industrial Crops and Products.* 2016;84:59-66. doi: 10.1016/j.indcrop.2016.01.037

17. Singh M, Khatoon S, Singh S, Kumar V, Rawat AKS, Mehrotra S. Antimicrobial screening of ethnobotanically important stem bark of medicinal plants. *Pharmacognosy Res.* 2010;2(4):254-257. doi: 10.4103/0974-8490.69127

18. Parekh J, Chanda S. *In vitro* antimicrobial activity and phytochemical analysis of some Indian medicinal plants. *Turk J Biol.* 2007;31(1):53-58.

19. Bauer AW, Kirby WMM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol.* 1966;45(4_ts):493-496. doi: 10.1093/ajcp/45.4_ts.493

20. Oldoni TLC, Melo PS, Massarioli AP, et al. Bioassay-guided isolation of proanthocyanidins with antioxidant activity from peanut (*Arachis hypogaea*) skin by combination of chromatography techniques. *Food Chem.* 2016;192:306-312. doi: 10.1016/j.foodchem.2015.07.004

21. Shahzad A, Ishtiaq M, Tanveer H, et al. Analysis of antimicrobial potential of some Ficus taxa from district Bhimber Azad Jammu and Kashmir, Pakistan. *Appl Ecol Environ Res.* 2016;14(5):159-176. doi: 10.15666/aeer/1405_159176

22. Abraham J, Thomas TD. Antibacterial activity of medicinal plant *Cyclea peltata* (Lam) Hooks & Thoms. *Asian Pac J Trop Dis.* 2012;2(suppl 1):S280-S284. doi: 10.1016/S2222-1808(12)60166-2

23. Preethi R, Devanathan VV, Loganathan M. Antimicrobial and antioxidant efficacy of some medicinal plants against food borne pathogens. *Adv Biol Res.* 2010;4(2):122-125.

24. Bertoletti LL, Skoronski E, Schittler L, Kempka AP. Extracts of Leaves of *Ficus auriculata* Lour.: antioxidant, antimicrobial and phytotoxic activity. *Agriculturae Conspectus Scientificus.* 2018;83(4):321-328.

25. Gupta C, Garg AP, Uniyal RC, Kumari A. Antimicrobial activity of some herbal oils against common food-borne pathogens. *Afr J Microbiol Res.* 2008;2(10):258-261.

26. Kuete V, Ngameni B, Simo CCF, et al. Antimicrobial activity of the crude extracts and compounds from *Ficus chlamydocarpa* and *Ficus cordata* (Moraceae). *J Ethnopharmacol.* 2008;120(1):17-24. doi: 10.1016/j.jep.2008.07.026

27. Annan K, Houghton PJ. Antibacterial, antioxidant and fibroblast growth stimulation of aqueous extracts of *Ficus asperifolia* Miq. and *Gossypium arboreum* L., wound-healing plants of Ghana. *J Ethnopharmacol.* 2008;119(1):141-144. doi: 10.1016/j.jep.2008.06.017

28. Tchinda CF, Voukeng IK, Beng VP, Kuete V. Antibacterial activities of the methanol extracts of *Albizia adianthifolia, Alchornea laxiflora, Laportea ovalifolia* and three other Cameroonian plants against multi-drug resistant Gram-negative bacteria. *Saudi J Biol Sci.* 2017;24(4):950-955. doi: 10.1016/j.sjbs.2016.01.033

29. Zhu W, Liu J, Ye J, Li G. Effects of phytotoxic extracts from peach root bark and benzoic acid on peach seedlings growth, photosynthesis, antioxidance and ultrastructure properties. *Scientia Horticulturae.* 2017;215:49-58. doi: 10.1016/j.scienta.2016.12.004

30. Thingbaijam R, Dutta BK, Paul SB. *In vitro* antioxidant capacity, estimation of total phenolic and flavonoid content of *Ficus auriculata* lour. *Int J Pharm Pharm Sci.* 2012;4(4): 518-521.

31. Singh N, Verma R, Rawat P, Aziz RB, Kala A. *Ficus palmata* and *Ficus auriculata* Phytochemical Screening in Different Solvents by HPLC and FTIR Spectroscopic Analysis. *Scope.* 2023;13(4):459-472.