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## *In-vitro* antibacterial activity of *Elytraria acaulis* roots against some gram-positive and gram-negative bacterial strains

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**Abstract**

*Elytraria acaulis* also known as Patharchatta in Hindi belongs to the family of Acanthaceae. *Elytraria acaulis* has been long utilized as medicine by some ethnic people of India to cure many diseases including skin diseases, ring worm, and leucorrhoea. In the present study, the aqueous, alcoholic and ether extracts of *Elytraria acaulis* roots were screened for their antibacterial activities against both Gram-positive (*Staphylococcus aureus* Methicillin Resistant *Staphylococcus aureus*, *Streptococcus agalactiae* and *Bacillus cereus*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*) bacterial strains and compared with the standard drug, Enrofloxacin (1.3mg/ml) using Zone of Inhibition (ZOI) with the help of agar well diffusion method and microtitre broth dilution assay (MIC). The data was analysed using SPSS programming, one-way ANOVA and post hoc multiple comparisons equal variance assumed by Tukey. Methanolic and ether extracts showed significant antibacterial activity than the aqueous extract but not up to the mark as compared to standard drug. It can be concluded that various extracts of *Elytraria acaulis* roots (EAR) might have potential chemical constituents that could be used in the future for the development of novel antibacterial agent.

**Keywords:** Agar well diffusion method, Antibacterial, *Elytraria acaulis*, Extracts, MIC, Roots

**Introduction**

*Elytraria acaulis* belongs to the family *Acanthaceae* locally called as Patharchatta in Hindi, Nilakadambai or Pumikatambu in Tamil, and also Asian Scalystem, is widely distributed, stemless perennial herb with unbranched flowering stem. *Elytraria acaulis* is widely distributed in tropical Africa and Peninsular India [1]. This plant is often found on rocky or sandy soils. This plant contains medicinally important bioactive compounds and it justifies their use in the traditional medicines for the treatment of different diseases. The phytochemical evaluation of *Elytraria acaulis* whole plant extracts (Ethanol, Methanol, Petroleum ether, Aqueous, Acetone) revealed the presence of glycosides, saponins, phytosterols, phenolic compounds, flavanoids, and tannins [2]. Methanolic extract of the *Elytraria acaulis* leaves showed the presence of alkaloids, amino acid, carbohydrates, flavonoids, glycosides, phenol, protein, saponins, steroids, and tannins; and it has exhibited anti-inflammatory, antioxidant, anti-hyperglycemic, anti-diarrhoeal, hepatoprotective, anthelmintic and anti-fertility activities [3]. Phytochemicals (total phenols, flavonoids, and tannin contents) from methanolic extract of *Elytraria acaulis* plant were analyzed quantitatively and presented as gallic acid equivalents/g (GAE/g) of dry weight and its antioxidant efficacy were tested in-vitro [4]. *Elytraria acaulis* is used in medicine for treating skin diseases, ring worm [5], leucorrhoea [6]. The roots of *Elytraria acaulis* have been used as various medication. Half-teaspoon root extract is given to children once a day for two days in asthma. Root paste is given once in a day for 21 days in leucorrhoea. Root paste with black pepper is applied on snake bite. Root paste is applied on piles in the evening and morning [7]. Although *Elytraria acaulis* roots are commonly used in traditional medicine, a literature survey showed a lack of studies on the antibacterial efficacy of its root extracts. The present study aims to validate the antibacterial potential of various extracts of *Elytraria acaulis* roots (EAR).

**Materials and methods****Plant Materials**

The fresh roots of *Elytraria acaulis* were collected in the month of July-August from Firozabad district, Uttar Pradesh, India. The plant species was taxonomically identified and authenticated by Department of Botany, Rashtrasant Tukadoji Maharaj Nagpur University,

Maharashtra, India and also by Central Institute of Medicinal and Aromatic Plants, (CIMAP), Lucknow, Uttar Pradesh, India with reference authentication number/herbarium accession number is 1007 and 8333, respectively.

### Extraction

The plants roots were dried under shade. Then the dried parts were powdered. The powder was passed through sieve plate no. 20 to collect the fine powder. This powder was used for preparation of *Elytraria acaulis* methanolic, petroleum ether, and aqueous extracts using Soxhlet hot extraction at 40-50°C.

### Antibacterial Activity of *Elytraria acaulis* Root Extract

The inhibitory effect of *Elytraria acaulis* root extracts were evaluated against nine bacterial strains and compared with standard antibacterial drug Enrofloxacin (1.3mg/ml), taken as a positive control and DMSO as the negative control. The *in-vitro* antimicrobial activity was determined using the agar well-diffusion method and microtitre broth dilution assay.

### Bacterial Strains Used

A total of nine bacterial strain from Gram -positive, i.e., *Staphylococcus aureus* (ATCC 25923, ATCC 33591, ATCC 29213), *Streptococcus agalactiae* (ATCC 13813), *Bacillus cereus* (ATCC 10876) and Gram-negative, i.e., *E. coli* (ATCC 25922, ATCC 35218), *Pseudomonas aeruginosa* (ATCC 10145), *Klebsiella pneumoniae* (ATCC 700603) were used to assess the antibacterial properties of the test compounds.

### Concentrations of Test Drug and Standard

The stock solutions of aqueous, methanolic and ether extracts were prepared at a concentration of 0.5mg/ml, 1mg/ml, 2mg/ml, 10mg/ml, and 20mg/ml. 100µL/well was used from each stock solution for the study. Standard drugs enrofloxacin (1.3 mg/ml) was used as appositve control against all the bacterial strains.

### Agar Well Diffusion Method

The evaluation of antimicrobial activity of methanolic extract of EAR was performed using agar well diffusion method [8] with slight modifications [9].

Single pure colony of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus agalactiae*, *Klebsiella pneumoniae* and *Bacillus cereus* was grown in Muller Hunter broth (MHB) by overnight incubation at 37°C. Bacterial inoculums were prepared from 24-hour old cultures by taking 3-5 morphologically similar colonies of respective microorganisms and transferred into 5 ml sterile saline solution and adjusted to 0.5 McFarland (Hi-Media) turbidity standards equivalent to the cell density of  $1.5 \times 10^8$  CFU/ml. 100 µl of this mid-log growth culture each of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus agalactiae*, *Klebsilla pneumoniae* and *Bacillus cereus* were inoculated into separate MHA plate and spread evenly by sterilized cotton swabs. The plates were kept upright for an hour so that the culture gets absorbed onto the plates evenly. Wells of 8 mm diameter were punched on agar plates with a sterile gel puncher and 100 µl of extracts were loaded with a micropipette in the well, marked for different concentrations. The plates were allowed to stand for 1-2 hours at 4°C so that the extract get diffused into the agar medium

completely. The plates were incubated at 37°C for 16-18 hours and the zone of inhibition surrounding the well was measured with the scale. Enrofloxacin (1.3mg/ml) was used as positive controls to compare the zone of inhibition. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (mm) including the well diameter.

### Microtitre Broth Dilution Assay

Minimal inhibitory concentration (MIC) of extracts were determined to establish susceptibilities of bacteria to these roots extract using the broth microtitre dilution method [10]. This method was used to determine the lowest concentration of extracts that inhibits the growth of the bacterium under defined test conditions.

### Preparation of Inoculum

The bacterial inoculums were prepared from 24-hour old cultures by taking 3-5 morphologically similar colonies of respective microorganisms and transferred into 5 ml sterile saline solution and adjusted to 0.5 McFarland turbidity standards (Hi-Media) equivalent to the cell density of  $1.5 \times 10^6$  CFU/ml.

### Broth Dilution Assay

100µl of MHB was dispensed into 12 wells in a row of a sterile microtitre plate. 100µl of sample was added in a first well. 100µl of the mixture from first well was transferred to the second well, mixed by sucking up and down and the procedure was repeated up to 10<sup>th</sup> well and 100µl solution was discarded from 10<sup>th</sup> well. 5µl of the mid-logarithm phase of growing culture of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus agalactiae*, *Klebsiella pneumoniae* and *Bacillus cereus* each was added into the first well of the microtitre plates containing broth and mixed by sucking up and down for six times using a micropipette. 0.5 McFarland turbidity standards (HiMedia) equivalent to the cell density of  $1.5 \times 10^8$  CFU/ml were transferred into 1-11<sup>th</sup> wells. The 12<sup>th</sup> well served as the background control. Plates were incubated at 37°C. OD was recorded using ELISA microplate reader (Bio-Rad, USA) at 620 nm. A similar dilution was carried out for standard (Enrofloxacin 1.3 mg/ml).

### Statistical Analysis

Standard deviation of the mean was calculated using SPSS software. The means were compared by one-way ANOVA (Post hoc multiple comparisons equal variance assumed by Tukey, significant level ( $p < 0.05$ )) statistical package (Version-16.0).

### Results and Discussion

The zone of inhibition produced by various extracts of *Elytraria acaulis* roots against bacterial strains has been presented in Table 1, Figure 1, 2, & 3. *Staphylococcus aureus* seems to be most inhibited by the methanolic extract. The maximum zone of inhibition (18mm) was produced by the hot methanolic extract against the *Staphylococcus aureus* (ATCC 25923) at a concentration of 2 mg/ml. Increase in concentration further did not show any improvement in bacterial inhibition. MIC of all three extracts of

**Table 1:** Agar well diffusion assay of crude extracts of *Elytraria acaulis* roots in different concentration against nine bacterial strains

S. No.	Extracts	Conc <sup>a</sup>	Zones of Inhibition (mm)								
			I	II	III	IV	V	VI	VII	VIII	IX
	Ether extract	0.5mg/ml	13.0 <sup>B,c</sup> ±0.36	12.0 <sup>B,ab</sup> ±0.36	12.0 <sup>B,a,b</sup> ±0.36	11.0 <sup>B,b</sup> ±0.36	12.0 <sup>B,b</sup> ±0.36	11.0 <sup>B,b</sup> ±0.36	12.0 <sup>B,C,a,b</sup> ±0.36	12.0 <sup>B,a,b</sup> ±0.36	8.0 <sup>A,a</sup> ±0.00
		1mg/ml	15.5 <sup>C,D,b,c</sup> ±0.18	13.0 <sup>B,C,c</sup> ±0.36	12.0 <sup>B,b,c</sup> ±0.36	12.0 <sup>B,d</sup> ±0.36	9.0 <sup>A,a</sup> ±0.36	11.0 <sup>B,b</sup> ±0.36	12.0 <sup>B,C,b,c</sup> ±0.36	12.0 <sup>B,b,c</sup> ±0.36	11.0 <sup>B,b</sup> ±0.36
		2mg/ml	16.0 <sup>C,D,c</sup> ±0.36	15.0 <sup>D,E,c</sup> ±0.36	12.0 <sup>B,b</sup> ±0.36	12.0 <sup>B,b</sup> ±0.36	9.0 <sup>A,a</sup> ±0.36	12.0 <sup>B,b</sup> ±0.36	12.0 <sup>B,C,b</sup> ±0.36	15.0 <sup>C,c</sup> ±0.36	12.0 <sup>B,C,b</sup> ±0.36
		10mg/ml	16.0 <sup>C,D,a</sup> ±0.36	15.0 <sup>D,E,d</sup> ±0.36	12.0 <sup>B,d</sup> ±0.36	12.0 <sup>B,b,c</sup> ±0.36	9.0 <sup>A,b,c</sup> ±0.36	12.0 <sup>B,b,c</sup> ±0.36	13.0 <sup>B,C,c</sup> ±0.36	11.0 <sup>B,b</sup> ±0.36	13.0 <sup>B,C,c</sup> ±0.36
		20mg/ml	17.0 <sup>D,d</sup> ±0.36	18.0 <sup>F,d</sup> ±0.36	13.0 <sup>B,a,b</sup> ±0.36	12.0 <sup>B,a</sup> ±0.36	12.0 <sup>B,a</sup> ±0.36	12.0 <sup>B,a</sup> ±0.36	14.0 <sup>C,b</sup> ±0.36	14.0 <sup>C,b</sup> ±0.36	14.0 <sup>C,b</sup> ±0.36
	Methanol extract	0.5mg/ml	15.5 <sup>C,D,c</sup> ±0.18	14.0 <sup>B,C,c</sup> ±0.36	11.0 <sup>B,b</sup> ±0.36	11.0 <sup>B,b</sup> ±0.36	11.0 <sup>B,b</sup> ±0.36	11.0 <sup>B,b</sup> ±0.36	11.0 <sup>B,C,b</sup> ±0.36	11.0 <sup>B,b</sup> ±0.36	9.0 <sup>A,B,a</sup> ±0.36
		1mg/ml	16.0 <sup>D,E,c</sup> ±0.36	15.0 <sup>C,D,c</sup> ±0.36	9.0 <sup>A,a</sup> ±0.36	8.0 <sup>A,a</sup> ±0.00	11.0 <sup>B,b</sup> ±0.36	12.0 <sup>B,b</sup> ±0.36	12.0 <sup>C,b</sup> ±0.36	12.0 <sup>B,C,b</sup> ±0.36	11.0 <sup>B,C,b</sup> ±0.36
		2mg/ml	18.0 <sup>F,c</sup> ±0.36	16.0 <sup>D,E,b</sup> ±0.36	11.0 <sup>B,a</sup> ±0.36	11.0 <sup>B,a</sup> ±0.36	11.0 <sup>B,a</sup> ±0.36	15.0 <sup>C,b</sup> ±0.36	15.0 <sup>D,b</sup> ±0.36	15.0 <sup>D,b</sup> ±0.36	12.0 <sup>C,D,a</sup> ±0.36
		10mg/ml	17.5 <sup>E,F,c</sup> ±0.18	15.5 <sup>C,D,E,b</sup> ±0.18	11.8 <sup>B,a</sup> ±0.40	11.8 <sup>B,a</sup> ±0.40	12.0 <sup>B,C,a</sup> ±0.36	15.0 <sup>C,b</sup> ±0.36	15.0 <sup>D,b</sup> ±0.36	16.0 <sup>D,b,c</sup> ±0.36	12.0 <sup>C,D,a</sup> ±0.36
		20mg/ml	17.5 <sup>E,F,c</sup> ±0.18	15.5 <sup>C,D,E,b</sup> ±0.18	11.8 <sup>B,a</sup> ±0.40	11.8 <sup>B,a</sup> ±0.40	12.0 <sup>B,C,a</sup> ±0.36	15.0 <sup>C,b</sup> ±0.36	15.0 <sup>D,b</sup> ±0.36	16.0 <sup>D,b,c</sup> ±0.36	12.0 <sup>C,D,a</sup> ±0.36
	Aqueous extract	0.5mg/ml	11.0 <sup>B,b</sup> ±0.36	11.0 <sup>A,a</sup> ±0.36	8.0 <sup>A,a</sup> ±0.36	8.0 <sup>A,b</sup> ±0.00	8.0 <sup>A,b</sup> ±0.00	8.0 <sup>A,a</sup> ±0.00	9.0 <sup>A,B,a</sup> ±0.36	10.0 <sup>B,b</sup> ±0.36	11.0 <sup>C,D,a</sup> ±0.36
		1mg/ml	11.0 <sup>B,C,b</sup> ±0.36	12.0 <sup>A,a</sup> ±0.36	8.0 <sup>A,a</sup> ±0.36	8.0 <sup>A,b</sup> ±0.00	8.0 <sup>A,b</sup> ±0.00	8.0 <sup>A,a</sup> ±0.00	9.0 <sup>A,B,b</sup> ±0.36	10.8 <sup>B,C,b</sup> ±0.40	12.0 <sup>C,D,a</sup> ±0.36
		2mg/ml	13.0 <sup>C,D,b</sup> ±0.36	13.0 <sup>A,b</sup> ±0.36	9.0 <sup>A,a</sup> ±0.36	8.0 <sup>A,c,d</sup> ±0.00	8.0 <sup>A,b,c</sup> ±0.00	8.0 <sup>A,a</sup> ±0.00	11.0 <sup>B,C,b</sup> ±0.36	12.0 <sup>B,C,d</sup> ±0.36	12.0 <sup>C,D,a</sup> ±0.36
		10mg/ml	14.0 <sup>C,D,b</sup> ±0.36	13.0 <sup>A,b</sup> ±0.36	9.0 <sup>A,a</sup> ±0.36	9.0 <sup>A,b</sup> ±0.36	9.0 <sup>A,b</sup> ±0.36	9.0 <sup>A,b</sup> ±0.36	11.0 <sup>B,C,b</sup> ±0.36	13.0 <sup>C,c</sup> ±0.36	12.0 <sup>C,D,b</sup> ±0.36
		20mg/ml	14.5 <sup>D,E,b</sup> ±0.18	14.0 <sup>B,a,b</sup> ±0.36	11.0 <sup>B,a</sup> ±0.36	11.0 <sup>B,d</sup> ±0.36	11.0 <sup>B,b</sup> ±0.36	12.0 <sup>B,a</sup> ±0.36	12.0 <sup>C,b</sup> ±0.36	15.0 <sup>D,d</sup> ±0.36	13.0 <sup>D,b</sup> ±0.36
	DMSO	50%	8.0 <sup>A</sup> ±0.00	8.0 <sup>A</sup> ±0.00	8.0 <sup>A</sup> ±0.00	8.0 <sup>A</sup> ±0.00	8.0 <sup>A</sup> ±0.00	8.0 <sup>A</sup> ±0.00	8.0 <sup>A</sup> ±0.00	8.0 <sup>A</sup> ±0.00	
	Enrofloxacin	1.3mg/ml	31.1 <sup>F,a</sup> ±0.83	31.5 <sup>D,a</sup> ±0.80	30.8 <sup>D,a</sup> ±0.54	30.6 <sup>D,a</sup> ±0.66	31.1 <sup>D,a</sup> ±0.74	30.8 <sup>E,a</sup> ±0.54	31.6 <sup>E,a</sup> ±1.05	30.3 <sup>F,a</sup> ±0.33	31.5 <sup>E,a</sup> ±0.95

Data presented are mean ± SE of six extracts samples. Different capital superscripts (A,B,C,D..) in the columns indicates significant and small superscripts (a,b,c,d..) in the row indicate (p<0.05) differences between the extracts and different bacterial strains groups.

I: *Staphylococcus aureus* (ATCC 25923) II: Methicillin

Resistant *Staphylococcus aureus* (ATCC 33591) III: *Staphylococcus aureus* (ATCC 29213) IV: *Escherichia coli* (ATCC 25922) V: *Escherichia coli* (ATCC 35218) VI: *Pseudomonas aeruginosa* (ATCC 10145) VII: *Streptococcus agalactiae* (ATCC 13813) VIII: *Klebsiella pneumoniae* (ATCC 700603) IX: *Bacillus cereus* (ATCC 10876)

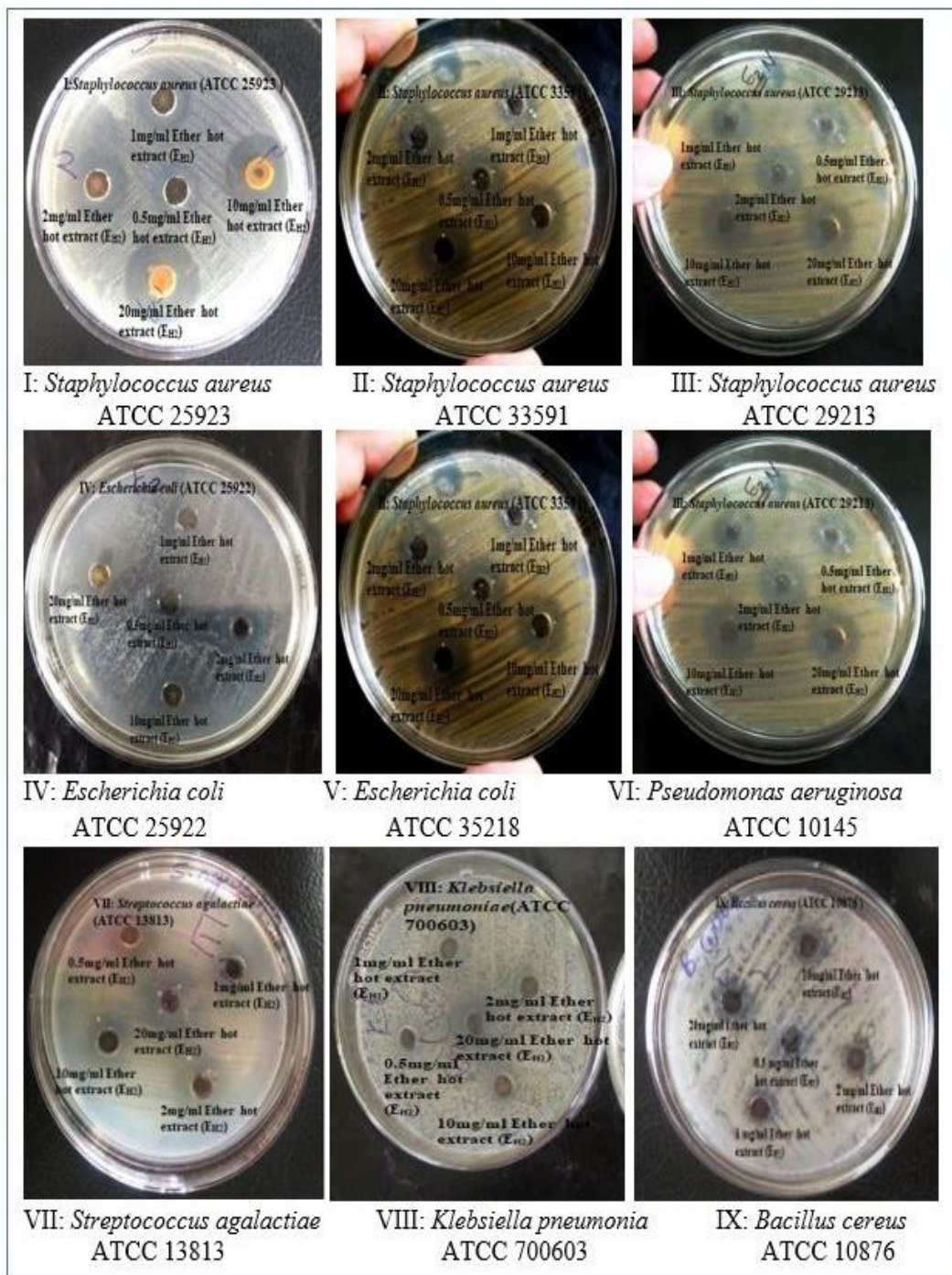


Fig. 1: Agar well diffusion assay of ether extract of *Elytraria acaulis* roots

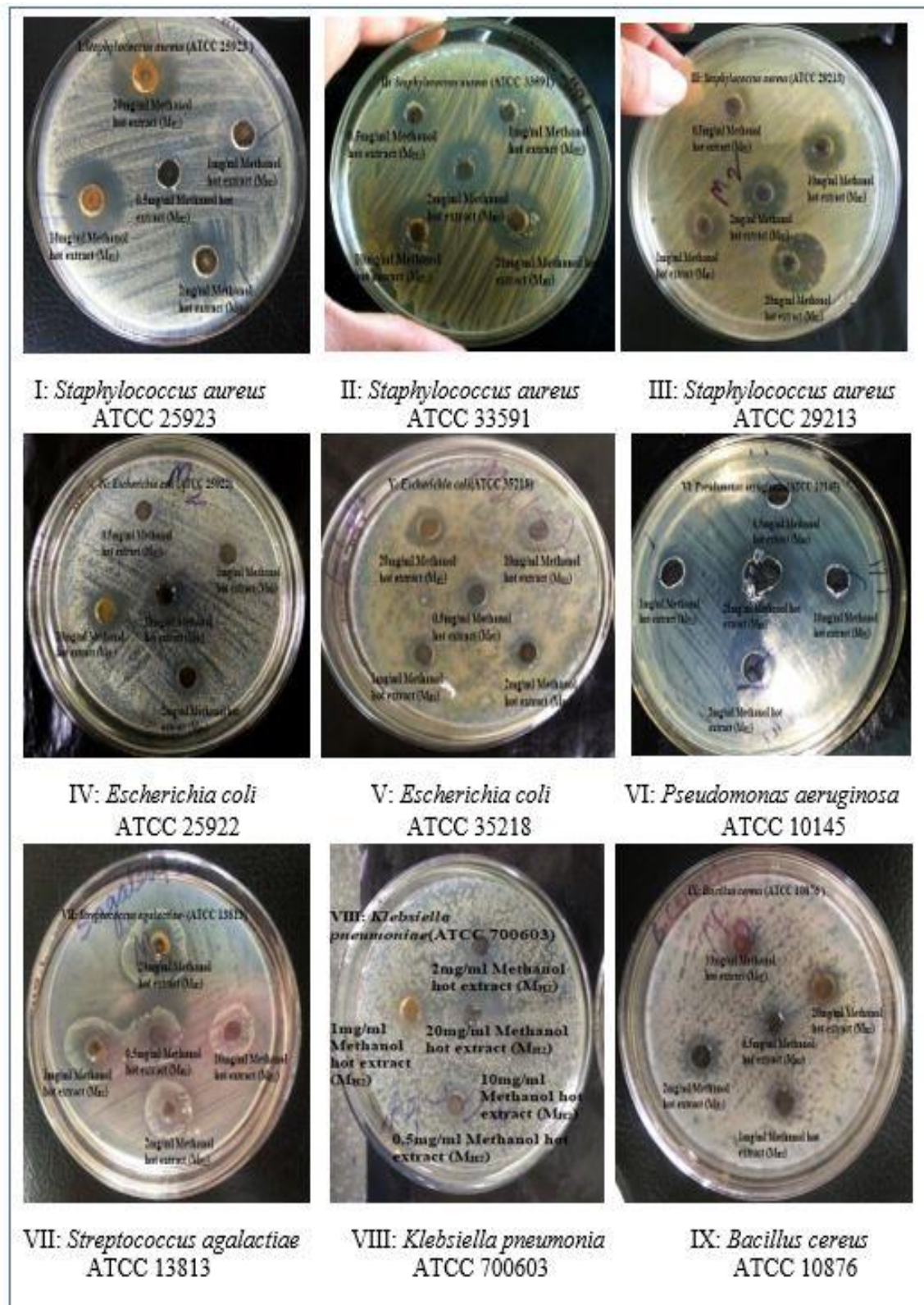


Fig. 2: Agar well diffusion assay of methanolic extracts of *Elytraria acaulis* roots

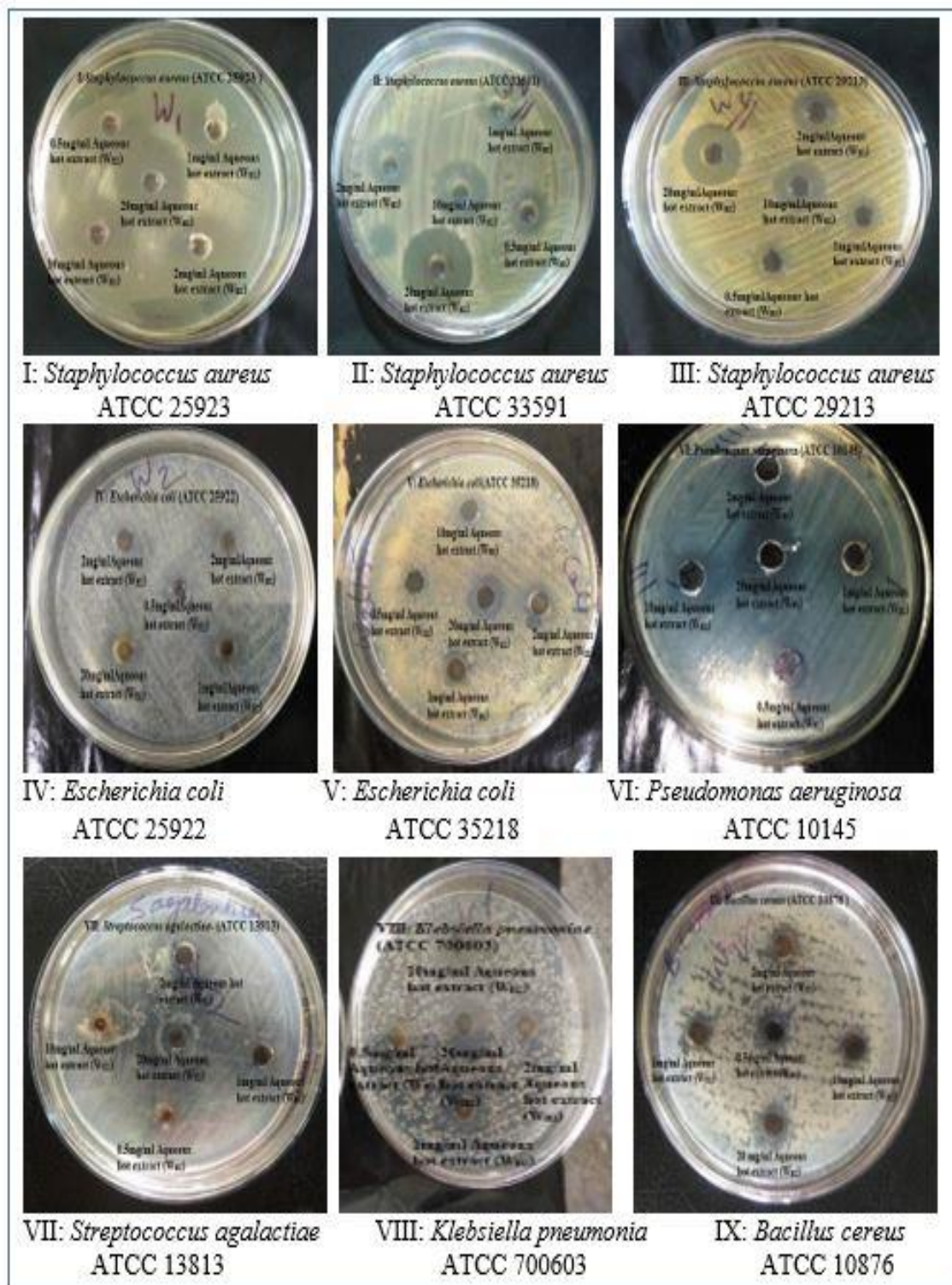


Fig. 3: Agar well diffusion assay of aqueous extracts of *Elytraria acaulis* roots

*Elytraria acaulis* roots is presented in Table 2. Aqueous extract showed moderate activity against all bacterial strains while methanolic and ether extracts showed moderate to

significant activity against all pathogens. Minimum inhibitory concentration (MIC) were found to be in the range of 0.089 to 7.29 mg/ml.

Table 2: Minimum inhibitory concentration (MIC) of ether, methanolic and aqueous crude extracts of *Elytraria acaulis* roots

S. No.	Test Strain	MIC (mg/μl)				
		Ether extract	Methanolic extract	Aqueous extract	50% DMSO	Enrofloxacin
1	<i>Staphylococcus aureus</i> (ATCC 25923)	2.864	0.089	0.716	50.0	0.087
2	Methicillin Resistant <i>Staphylococcus aureus</i> (ATCC 33591)	2.864	0.227	2.864	45.833	0.750
3	<i>Staphylococcus aureus</i> (ATCC 29213)	0.227	0.227	0.715	29.166	0.087
4	<i>Streptococcus agalactiae</i> (ATCC 13813)	0.357	0.455	1.430	45.833	0.29
5	<i>Bacillus cereus</i> (ATCC 10876)	0.716	0.455	1.430	45.833	0.75

6	<i>Escherichia coli</i> (ATCC 25922)	0.716	2.864	7.291	29.166	0.75
7	<i>Escherichia coli</i> (ATCC 35218)	0.260	7.291	7.291	45.833	0.29
8	<i>Pseudomonas aeruginosa</i> (ATCC 10145)	0.716	0.455	1.430	45.833	1.18
9	<i>Klebsiella pneumoniae</i> (ATCC 700603)	0.047	1.430	2.864	45.833	0.75

The ether, methanolic and aqueous extracts of the roots of *Elytraria acaulis* were tested against Gram-positive and Gram-negative bacterial strains by using five different concentrations of 0.5mg/ml, 1mg/ml, 2mg/ml, 10mg/ml and 20mg/ml. Although all the three extracts, i.e., aqueous, alcoholic and ether extracts of the *Elytraria acaulis* roots inhibited the growth of various bacterial pathogens but the maximum degree of activity was observed by alcoholic and ether extracts as depicted by zone of inhibition (Table 1). It was observed that among all the five concentrations of the drug extracts, inhibitory effects were exhibited most against the methanolic extract at 2 mg/ml (Table 1).

Antibiotic resistance is becoming a global problem and majority of the antibiotics has developed or gradually developing resistance against these bacterial pathogens. To thwart these problem, we have to keep on exploring new source of antibacterial agents and in these scenario plant based drugs and antibacterial agents are being explored at an accelerated pace. Phytochemicals are naturally present in the medicinal plants, leaves, vegetables and roots that have defense mechanism and provide protection from various diseases. In the present study, various extracts of *Elytraria acaulis* roots (EAR) revealed the presence of carbohydrates, alkaloids, phenols, saponins, tannins, flavonoids, glycosides and phytosterols. Findings from the current study suggested that methanol and ether extracts have potential inhibitory effects on all tested bacterial pathogens while the aqueous extract showed lower inhibitory effects. This may be due to the presence of compounds like tannins, flavonoids, alkaloids and glycosides. The hydroxylated phenolic substances, flavonoids, have been found to be antimicrobial against a broad range of microorganisms. The antimicrobial nature of flavonoids may probably be due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall [11]. Alkaloids exerts its inhibitory effect by inhibiting nucleic acid production, by repressing cell division and perturbing the Z-ring, by trading off external layer and cytoplasmic integrity [12]. Glycosides may exert its antibacterial action by inhibiting the RNA nucleic acid synthesis [13]. In a study with various extracts of the aerial parts of the *Elytraria acaulis*, methanolic extract exhibited significant antimicrobial activity against various pathogenic bacterial infections [14]. The antibacterial activity of *Elytraria acaulis* Lindau against the multidrug resistant bacteria like *Pseudomonas aeruginosa*, *E. coli*, and methicillin resistant *Staphylococcus aureus*, isolated from burnt wound sepsis proved to be more efficacious than the honey and antibiotics [15]. The methanolic extracts of *Elytraria acaulis* showed potent antimicrobial activity against all the selected microorganisms [16, 17]. Based on some earlier studies and this study it may be concluded that the various parts like roots, leaves, flowers of the plant possess varying degree of antibacterial potential depending upon the presence of phytoconstituents in it.

### Conclusion

The present study confirms that the alcoholic and ether extracts of the *Elytraria acaulis* roots possess significant antibacterial action against both Gram-positive and Gram-negative pathogens when compared with aqueous extract. The

antibacterial efficacy might be because of existence of various bioactive components in these extracts. It was concluded that the test substances have potent antibacterial activity and may be utilized as a novel antibacterial agent to treat various infections related ailments.

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### Conflict of Interest

All the authors confirm that this article content has no conflicts of interest.

### Author contributions

**Simmi Singh:** Conceptualization, Methodology **Rajesh Nigam:** Supervision **Ambika Sharma:** Data curation **Ashish Kumar:** Writing-Original draft preparation, Reviewing and Editing **Vijay Laxmi:** Statistics

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