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Original Research Article

Comparative Phytochemical Profiling and in Vitro Antimicrobial Efficacy of Selective Solvent Extracts of *Ziziphus xylopyrus* Bark and *Berberis aristata* Root Extracts

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Abstract:

Background: Plant-derived secondary metabolites serve as valuable lead compounds in the discovery of novel antimicrobial agents. This study evaluates the extraction yield, phytochemical constituents, and in vitro antibacterial potential of selective solvent extracts from the bark of *Ziziphus xylopyrus* and the root of *Berberis aristata*.

Methods: Dried plant materials (*Ziziphus xylopyrus* bark and *Berberis aristata* root) were sequentially extracted via the Soxhlet apparatus using solvents of increasing polarity, including petroleum ether and ethyl acetate (EA). Preliminary qualitative phytochemical screening was performed on all crude extracts to test for carbohydrates, alkaloids, flavonoids, glycosides, proteins, amino acids, and saponins using standard chemical assays. The antibacterial efficacy of the petroleum ether and EA extracts was assessed against *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive) using the agar disc/well diffusion method on prepared Nutrient Agar media.

Results: The extraction process yielded distinct variations in colour, physical appearance, and weight percentage across the selective solvents for both plants. Phytochemical analysis revealed a higher concentration of flavonoids, alkaloids, and glycosides in the ethyl acetate extracts, whereas petroleum ether extracts predominantly tested positive for non-polar constituents. In the antibacterial assay, both *Ziziphus xylopyrus* bark and *Berberis aristata* root ethyl acetate extracts demonstrated notable growth inhibition against both test strains. The *Berberis aristata* extract showed no inhibition at 100 µg/ml, while inhibition zones increased with concentration, reaching a maximum of 10.666 ± 0.577 mm at 400 µg/ml. In contrast, the *Ziziphus xylopyrus* extract exhibited antibacterial activity even at the lowest concentration (7 ± 0 mm at 100 µg/ml) and showed a stronger effect at higher concentrations, with a maximum inhibition zone of 15.333 ± 1.527 mm at 400 µg/ml. Notably, *Ziziphus xylopyrus* exhibited activity even at 100 µg/ml, whereas *Berberis aristata* was ineffective at this concentration.

Conclusion: The findings confirm that polar/medium-polar fractions (ethyl acetate extracts) of *Ziziphus xylopyrus* bark and *Berberis aristata* root possess significant phytochemical richness and promising broad-spectrum antibacterial activity against *E. coli* and *S. aureus*. These plant parts represent viable candidates for further isolation and structural characterisation of novel antimicrobial leads.

Keywords: Soxhlet extraction, Phytochemical screening, antimicrobial activity, *Escherichia coli, Staphylococcus aureus*, Ethyl acetate extract.

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Introduction

Antibiotics, first introduced in the twentieth century, have saved millions of lives worldwide and revolutionised the management of infectious diseases. Nevertheless, widespread and inappropriate use has led to antimicrobial resistance, in which pathogenic microorganisms develop the capacity to withstand previously effective treatments. This resistance has reduced the efficacy of standard therapies, particularly for respiratory, urinary tract, and tuberculosis infections. Furthermore, the rate of new antimicrobial agent development has slowed, limiting therapeutic options for clinicians. Addressing the growing crisis of antimicrobial resistance (AMR) requires the ongoing development of new drugs. Pharmacologists are therefore essential in identifying novel plant extracts with potential therapeutic applications. This study investigates the comparative phytochemical profiles and in vitro antimicrobial activities of root extracts from

Ziziphus xylopyrus and *Berberis aristata*, both recognised for their ethnomedicinal significance. *Ziziphus xylopyrus*, found in Pakistan, China, and North-Western India, is valued in Ayurvedic and folk medicine for treating diabetes, obesity, diarrhea, and insomnia. Contemporary scientific research has validated these traditional uses, confirming antiulcer efficacy and the use of fruit triturates with milk for diabetes management (Modi et al., 2014). Recent studies also indicate that Ziziphus species may serve as novel anti-inflammatory agents, with cyclopeptide alkaloids showing promise in the treatment of cardiovascular diseases, diabetes, and neurological disorders (Alsayari et al., 2021)[2]. Ethnobotanical evidence shows that various parts of the *Berberis* plant have important therapeutic applications; leaves are traditionally used for skin diseases and cholera, while root decoctions are used for wound healing (Bhaila et al., 2022). Scientific studies support these uses, demonstrating that *Berberis* extracts possess significant antimicrobial, antifungal, and hepatoprotective properties with broad medicinal relevance (Bhatt et al., 2018; Bhattarai et al., 2022; Singh et al., 2007)[3]. Therefore, this study aims to compare the phytochemical profiles of *Z. xylopyrus* bark and B. aristata root using selective solvents and to assess their in vitro antimicrobial efficacy.

Materials and Methods

Plant Collection and Authentication: *Ziziphus xylopyrus* bark and *Berberis aristata* root were collected from Bhopal, M.P., on 14.08.24. The species was identified by the local people, and authentication was done at Pinnacle Biomedical Research Institute (PBRI), Shanti Marg, Shamla Hills, Bhopal, Madhya Pradesh 462003, India. To prepare the extract from *Zizyphus xylopyrus* bark and *Berberis aristata* root, the bark and roots were washed thoroughly with tap water, then repeatedly with distilled water to remove dirt and other impurities.

Preparation of Selective Solvent Extracts

Table 1: Chemical used

S. No	Reagents & Chemicals	Company
1.	Petroleum ether	Rankem
2.	Methanol	Rankem
3.	Sodium hydroxide	Merck
4.	Conc. Sulphuric acid	Merck
5.	Copper sulphate	Rankem
6.	Lead acetate	Merck
7.	Mercuric chloride	Merck
8.	Ammonium sulphate	Merck
9.	Ferric chloride	Merck
10.	Hydrochloric acid	Sunkem
11.	Sodium nitrite	Merck
12.	Ninhydrin	Fisher Scientific
13.	Potassium sodium tartrate	Himedia
14.	Sodium citrate	Fizmerck
15.	Sodium carbonate	Fizmerk
16.	Follin ciocalteu's reagent	Himedia
17.	AgNo3	Merck

Extraction of plant material using different solvents (polarity-wise)

The powdered-plant-sample was placed-in-a-thimble-of a Soxhlet-apparatus, and extraction-was performed utilising different organic-solvents, namely petroleum-ether and hydroalcoholic solvent, for-8–10-hrs. The heating-mantle temperature was maintained at 40–60 °C. After extraction, the sample extracts were filtered-&-concentrated to dryness. The dried extracts were then collected in airtight containers **(Rizvi et al., 2022).** The extraction-yield-of-all-extracts was-calculated utilising the formula-below:

$$\text{Formula of Percentage-yield} = \frac{\text{Actual-yield}}{\text{Theoretical yield}} \times 100$$

Qualitative Phytochemical Estimation of Extracts

Detailed phytochemical testing was performed to identify presence or absence of different phytoconstituents in extracts of Rhododendron griffithianum, Rhododendron wightii and Rhododendron rawatii by using standard procedures **(Kokate et al., 2006).** The extracts were subjected to the following tests:

Tests for carbohydrates:

- **Molisch test:** To 1ml of extract, 2-3 drops of alcoholic α-naphthol solution was added. Conc. sulphuric acid was added along the side of the test tube. The appearance of purple ring at the junction of two liquids was observed, which confirms the presence of carbohydrates in the test samples.
- **Fehling's test:** To 1 ml of extract, similar quantity of Fehling's solution A and B was added and heated on a water bath for few minutes. The development of brick red precipitate was observed.
- **Benedict's test:** Equal volume of Benedict's reagent and extract were mixed in a test tube and heated in the water bath for 5-10 minutes. Solution appears green, yellow or red depending on the amount of reducing sugar present in the test solution which indicated the presence of reducing sugar.
- **Barfoed's test:** 1 ml of extract and Barfoed's reagent were mixed in a test tube and heated on water bath for 2 minutes. Red colour due to formation of cupric oxide indicates the presence of monosaccharide.

Test for alkaloids:

All the test extracts were first treated with dil. hydrochloric acid separately and filtered. The filtrate of all the test extracts was exposed to following tests:

- **Mayer's test:** To 2-3 ml of filtrate, few drops of Mayer's reagent were added along sides of tube. Formation of white or creamy precipitate indicates the presence of alkaloids.
- **Hager's test:** To 1-2 ml of filtrate, few drops of Hager's reagent were added in a test tube. Formation of yellow color precipitate indicates the presence of alkaloids.
- **Wagner's test:** To 1-2 ml of filtrate, few drops of Wagner's reagent were added in a test tube. Formation of reddish-brown precipitate indicates the presence of alkaloids.

Test for flavonoids:

- **Lead acetate test:** The extract was treated with few drops of lead acetate solution. Formation of yellow precipitate may indicate the presence of flavonoids.
- **Alkaline reagent test:** The extract was treated with few drops of sodium hydroxide separately in a test tube. Formation of intense yellow color, which becomes color less on addition of few drops of dilute acid, indicate presence of flavonoids.
- **Shinoda test:**To the extract, 5 ml (95%) of ethanol was added. The mixture was treated with few fragments of magnesium turning, followed by drop wise addition of concentrated hydrochloric acid. Formation of pink color indicates presence of flavonoids.

Test for glycosides:

- **Borntrager's test:** To 3 ml of extract, dilute sulphuric acid was added, boiled for 5 minutes and filtered. To the cold filtrate, equal volume of benzene or chloroform was added and shake it welled. The organic solvent layer was separated and ammonia was added to it. Formation of pink to red color in ammonical layer indicates presence of anthraquinone glycosides.
- **Legal's test:** 1 ml of extract was dissolved in pyridine. 1 ml of sodium nitropruside solution was added and made alkaline using 10% sodium hydroxide solution. Formation of pink to blood red color indicates the presence of cardiac glycosides.
- **Keller-Killiani test:** To 2 ml of extract, 3 ml of glacial acetic acid and 1 drop of 5% ferric chloride were added in a test tube. Add carefully 0.5 ml of concentrated sulphuric acid by the side of the test tube. Formation of blue color in the acetic acid layer indicates the presence of cardiac glycosides.

Test for protein and amino acids:

- **Biuret's test:** The extract was treated with 1 ml of 10% sodium hydroxide solution in a test tube and heated. A drop of 0.7% copper sulphate solution was added to the above mixture. The formation of violet or pink colour indicates the presence of proteins.
- **Ninhydrin test:** 3 ml of the extractwas heated with 3 drops of 5% Ninhydrin solution in a water bath for 10 minutes. Formation of blue colour indicates the presence of amino acids.

Test for saponins:

- **Foam test:** 1 ml of extract was dissolved in 20 ml of distilled water and shacked for 15min in a graduated cylinder. Formation of persistent foam around 1cm layer was observed.

Test for triterpenoids and steroids:

- **Salkowski's test:** The extract was treated with chloroform and filtered. The filtrate was added with few drops of concentrated sulphuric acid, shaken and allowed to stand. If the lower layers turn red, sterol are present. Presence of golden yellow layer at bottom indicates the presence of triterpenes.

- **Libermann-Burchard's test:** The extract was treated with chloroform. To this solution few drops of acetic anhydride were added, boiled and cooled. Concentrated sulphuric acid was added through the sides of the test tube. Formation of brown ring at the junction of two layers, if upper layer turned green, indicate presence of steroids and formation of deep red color indicate presence of triterpenoids.

Test for tannin and phenolic compounds:

- **Ferric chloride test:** Some amount of extract was dissolved in distilled water. To this solution 2 ml of 5% ferric chloride solution was added. Formation of blue, green or violet color indicates presence of phenolic compounds.
- **Lead acetate test:** Some amount of extract was dissolved in distilled water. To this solution few drops of lead acetate solution was added. Formation of white precipitate indicates the presence of phenolic compounds.

Extraction

Soxhlet Extraction:

Powdered sample were placed in a thimble of Soxhlet apparatus. The extraction was carried out using different organic solvents; petroleum ether, ethyl acetate and methanol for 8-10 hours and 40-60oC temperature of the heating mantle were adjusted. After the extraction process, the extract of sample were filtered and concentrated to dryness. Extracts were collected in air tight container (Rizvi et al., 2022). Extraction yield of all extracts were calculated using the following equation below:

Anti-bacterial Activity: Preparation of Dilutions of the Samples The dilutions of the samples were made for the concentration as 100µg/ml, 200µg/ml, 300µg/ml, and 400µg/ml respectively of the sample, after that volume makeup was done with distilled water till 1ml.

Preparation of Nutrient Agar Media

28 g of Nutrient Media was dissolved in 1 litre of distilled water. pH of media was checked before sterilization. Media was sterilized in autoclave at 121oC at 15 lbs pressure for 15 minutes. Nutrient media was poured into plates and placed in the laminar air flow until the agar was get solidified.

Well Diffusion Assay: The bacterial suspension (*E. coli, and S. aureus*) was standardized to 108 CFU/ml of bacteria and kept into the shaker. Then, 100µl of the inoculum from the broth (containing 108 CFU/ml) was taken with a micropipette and then transferred to fresh and sterile solidified Agar Media Plate. The agar plate was inoculated by spreading the inoculum with a sterile spreader, over the entire sterile agar surface. Four wells of 6 mm were bored in the inoculated media with the help of sterile cork-borer. Each well was filled with different concentration (100µg/ml, 200µg/ml, 300µg/ml, and 400µg/ml) of samples. It was allowed to diffuse for about 30 minutes at room temperature and incubated for 18-24 hours at 37o C. After incubation, plates were observed for the formation of a clear zone around the well which corresponds to the antimicrobial activity of tested compounds. The zone of inhibition (ZOI) was observed and measured in mm. Zones were measured to a nearest millimeter using a ruler, which was held on the back of the inverted Petri plate. The Petri plate was held a few inches above a black, non-reflecting background. The diameters of the zone of complete inhibition (as judge by unaided eye) were measured, including the diameter of the well (Manandhar et al., 2019).

Results

Plant Extraction: The plant material of *Ziziphus xylopyrus* and *Berberis aristata* was extracted by the maceration extraction method, and the percentage yield was calculated by the following formula:-

$$\% \text{ yield} = \frac{\text{Actual Yield}}{\text{Theoretical yield}} \times 100$$

Table 2: Percentage yield

S. No.	Plant name	Solvent	Color of extract	Theoretical weight (gm)	Yield (gm)	% Yield
1.	*Berberis aristata*	Petroleum Ether	Dark yellow to brown	38.76 gm	0.56	1.44
2.	*Berberis aristata*	EA	Brown	60.36 gm	4.36	7.22
3.	*Berberis aristata*	MeOH	Dark brown	76.36 gm	9.64	12.62
4.	*Ziziphus xylopyrus*	Petroleum Ether	Brown	41.16 gm	0.64	1.55
5.	*Ziziphus xylopyrus*	EA	Dark yellow to brown	70.00 gm	7.84	11.2
6.	*Ziziphus xylopyrus*	MeOH	Brown	72.00 gm	12.1	16.80

Source: Own Work: The percentage yield obtained from the extraction of *Berberis aristata* and *Ziziphus xylopyrus* using different solvents is summarised in Table 2 In the case of *Berberis aristata*, extraction with petroleum ether yielded a dark yellow to brown extract with a yield of 0.56 g from 38.76 g of plant material, corresponding to a percentage yield of 1.44%. Extraction with ethyl acetate produced a brown-colored extract with a significantly higher yield of 4.36 g, resulting in the maximum percentage yield of 7.22%, indicating superior extraction efficiency of moderately polar constituents in this solvent. In contrast, methanolic extraction of *Berberis aristata* yielded a dark brown extract weighing 9.64 g from 76.36 g of plant material, corresponding to the percentage yield of 12.62%. For *Ziziphus xylopyrus*, petroleum ether extraction resulted in a brown-colored extract with a yield of 0.64 g from 193.36 g of plant material, giving a percentage yield of 1.55%, suggesting a high content of non-polar phytoconstituents. Ethyl acetate extraction of *Ziziphus xylopyrus* produced a dark yellow to brown extract with a yield of 7.84 g from 70.00 g of plant material, corresponding to a comparatively lower percentage yield of 11.2%. Methanolic extraction of *Ziziphus xylopyrus* resulted in a brown-colored extract with a yield of 12.1 g from 72.00 g of plant material, yielding 16.80%, indicating effective extraction of polar phytoconstituents. Overall, the variation in percentage yield across different solvents reflects the influence of solvent polarity on the extraction efficiency and phytochemical composition of the selected plant materials.

Table 3: Phytochemical analysis of *Berberis aristata* Extract

S. No.	Experiment	Result	
		Petroleum ether	EA
Test for Carbohydrates			
1.	Molisch's Test	-	+
2.	Fehling's Test	-	+
3.	Benedict's Test	-	+
4.	Bareford's Test	-	+
Test for Alkaloids			
1.	Mayer's Test	-	-
2.	Hager's Test	-	-
3.	Wagner's Test	-	-
4.	Dragendroff's Test	-	-
Test for Terpenoids			
1.	Salkowski Test	-	+
2.	Libermann-Burchard's Test	-	+
Test for Flavonoids			
1.	Lead Acetate Test	-	+
2.	Alkaline Reagent Test	-	+
3.	Shinoda Test	-	+
Test for Tannins and Phenolic Compounds			
1.	$FeCl_3$ Test	+	+
2.	Lead Acetate Test	+	+
3.	Gelatine Test	+	+
4.	Dilute Iodine Solution Test	+	+
Test for Saponins			
1.	Froth Test	+	+
Test for Protein and Amino acids			
1.	Ninhydrin Test	-	+
2.	Biuret's Test	-	+
3.	Million's Test	-	+
Test for Glycosides			
1.	Legal's Test	-	-
2.	Keller Killani Test	-	-
3.	Borntrager's Test	-	-

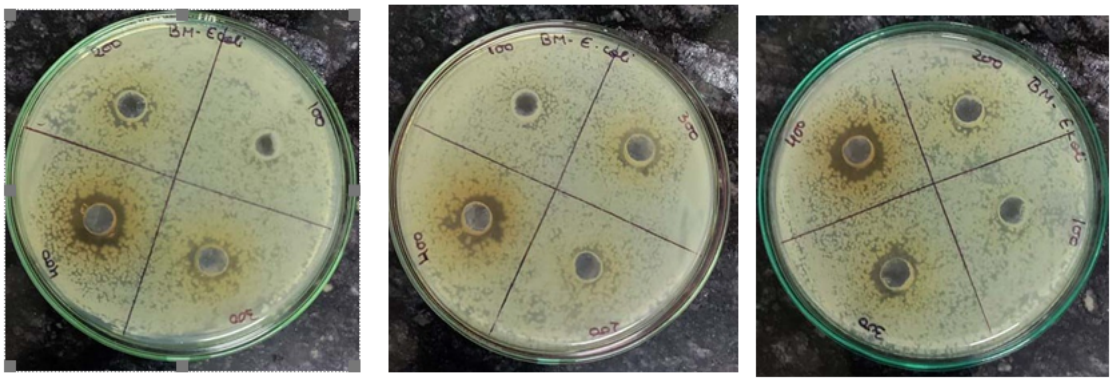
Table 4: Phytochemical analysis of *Ziziphus xylopyrus* Extract

S. No.	Experiment	Result	
		Petroleum ether	EA
Test for Carbohydrates			
1.	Molisch's Test	-	+
2.	Fehling's Test	-	+
3.	Benedict's Test	-	+
4.	Bareford's Test	-	+
Test for Alkaloids			
1.	Mayer's Test	-	-
2.	Hager's Test	-	-
3.	Wagner's Test	-	-
4.	Dragendroff's Test	-	-
Test for Terpenoids			
1.	Salkowski Test	-	+
2.	Libermann-Burchard's Test	-	+
Test for Flavonoids			
1.	Lead Acetate Test	-	+
2.	Alkaline Reagent Test	-	+
3.	Shinoda Test	-	+
Test for Tannins and Phenolic Compounds			
1.	$FeCl_3$ Test	+	+
2.	Lead Acetate Test	+	+
3.	Gelatine Test	+	+
4.	Dilute Iodine Solution Test	+	+
Test for Saponins			
1.	Froth Test	+	+
Test for Protein and Amino acids			
1.	Ninhydrin Test	-	+
2.	Biuret's Test	-	+
3.	Million's Test	-	+
Test for Glycosides			
1.	Legal's Test	-	-
2.	Keller Killani Test	-	-
3.	Borntrager's Test	-	-

Antibacterial activity

Table 5: Antibacterial activity of *Berberis aristata* methanolic extract against E. coli

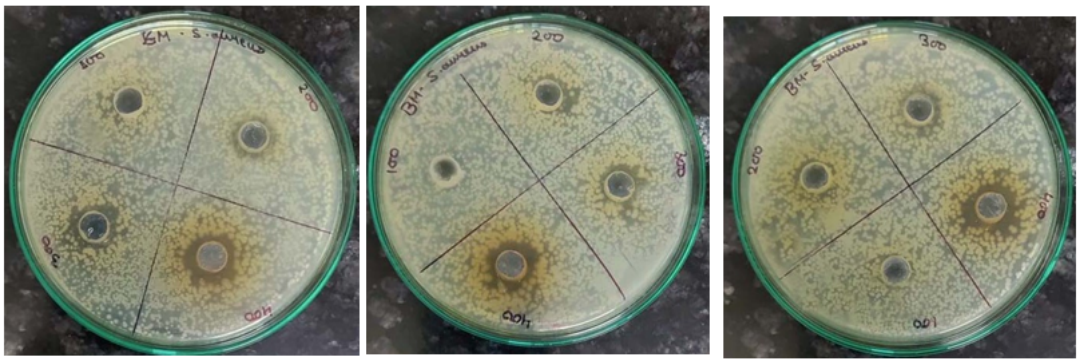
Concentration (µg/ml)	Zone of Inhibition in mm			
	Plate 1	Plate 2	Plate 3	Mean ±SD
100	0	0	0	0±0
200	7	7	7	7±0
300	8	8	9	8.333±0.577
400	11	10	11	10.666±0.577



The antibacterial activity of the methanolic extract of *Berberis aristata* was evaluated against *E. coli* using the agar diffusion method. The extract exhibited a concentration-dependent increase in antibacterial activity, as evidenced by the progressive enlargement of the zone of inhibition with increasing concentrations. At a concentration of 100 µg/ml, the extract did not produced any zones of inhibition on all three different plates, so the observed mean inhibition zone of 0±0 mm. When the concentration was increased to 200 µg/ml, a consistent zone of inhibition of 8 mm was observed across all plates, resulting in a mean value of 7 ± 0 mm. A further increase in concentration to 300 µg/ml enhanced the antibacterial effect, producing inhibition zones of 8,8 and 9 mm, with a mean value of 8.33 ± 0.577 mm. The highest antibacterial activity was observed at 400 µg/ml, where zones of inhibition of 11, 10, and 11 mm were recorded, yielding a mean inhibition zone of 10.666±0.577 mm. These findings clearly indicate that the methanolic extract of *Berberis aristata* possesses antibacterial activity against E. coli, with efficacy increasing proportionally with concentration.

Table 6: Antibacterial activity of *Berberis aristata* methanolic extract against *S. aureus*

Concentration (µg/ml)	Zone of Inhibition in mm			
	Plate 1	Plate 2	Plate 3	Mean ±SD
100	0	0	0	0±0
200	8	8	7	7.666±0.577
300	10	9	10	9.666±0.577
400	11	11	12	11.333±0.577

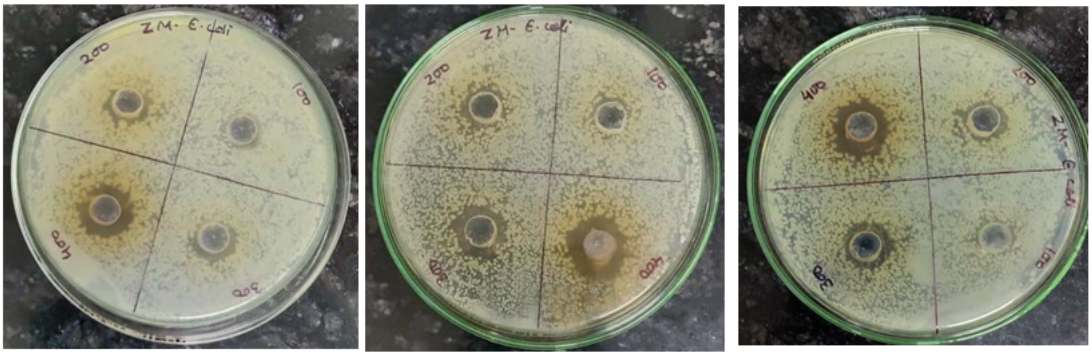


The antibacterial activity of the methanolic extract of *Berberis aristata* against *Staphylococcus aureus* was evaluated using the agar diffusion method, and the results are presented in Table 6. The extract exhibited a concentration-dependent increase in antibacterial activity, as evidenced by the progressive enlargement of the zone of inhibition with increasing extract concentration. At a concentration of 100 µg/ml, no zones of inhibition recorded for all plates. When the concentration was increased to 200 µg/ml, the zones of inhibition observed as 8 mm, 8 mm, and 7 mm, yielding a mean value of 7.666±0.577 mm. Further enhancement in antibacterial activity was observed at 300 µg/ml, where the zones of inhibition measured 10, 9, 10 mm across the three plates, with a corresponding mean of 9.666±0.577 mm. The maximum antibacterial effect was observed at 400 µg/ml, producing zones of inhibition of 11 mm, 11 mm, and 12 mm, with a mean value of 11.333±0.577 mm. These findings clearly indicate that the methanolic extract of *Berberis aristata* possesses antibacterial activity

against *S. aureus*, and the effect increases proportionally with concentration.

Table 7: Antibacterial activity of *Ziziphus xylopyrus* methanolic extract against *E. coli*

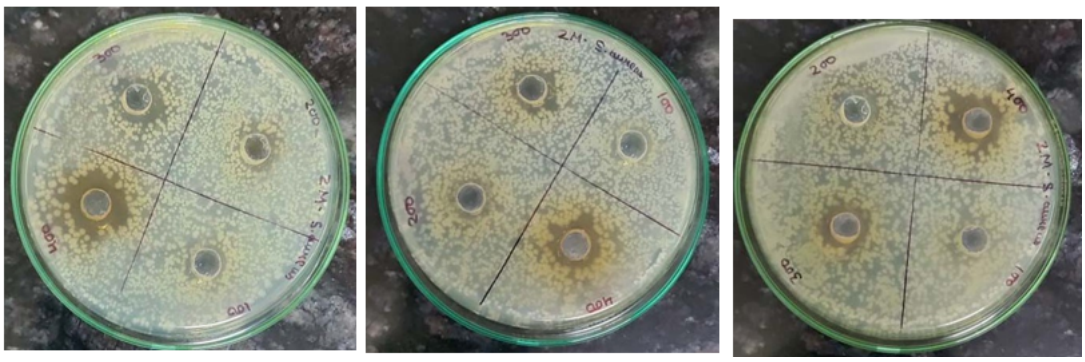
Concentration (µg/ml)	Zone of Inhibition in mm			
	Plate 1	Plate 2	Plate 3	Mean ±SD
100	7	7	7	7±0
200	8	8	8	8±0
300	10	12	11	11±1
400	13	16	14	14.333±1.527



The antibacterial activity of the methanolic extract of *Ziziphus xylopyrus* against *Escherichia coli* was evaluated at different concentrations using the agar diffusion method, and the results are summarized in Table 7. The extract exhibited a concentration-dependent increase in antibacterial activity, as evidenced by the gradual enlargement of the zone of inhibition with increasing extract concentration. At a concentration of 100 µg/ml, the zones of inhibition recorded across three plates were 7 mm, with a mean value of 7.00± 0.00 mm, indicating mild antibacterial activity. When the concentration was increased to 200 µg/ml, the zones of inhibition increased to 8 mm, yielding a mean of 8.00± 0.00 mm. A further enhancement in antibacterial effect was observed at 300 µg/ml, where inhibition zones of 10, 12, and 11 mm were recorded, resulting in a mean zone of inhibition of 11.00 ± 1.00 mm. The highest antibacterial activity was observed at 400 µg/ml, showing zones of inhibition of 13, 16, and 14 mm, with a mean value of 14.33 ± 1.527 mm. These findings demonstrate that the methanolic extract of *Ziziphus xylopyrus* possesses significant antibacterial activity against E. coli, which increases proportionally with concentration, suggesting its potential as a natural antibacterial agent.

Table 8: Antibacterial activity of *Ziziphus xylopyrus* methanolic extract against *S. aureus*

Concentration (µg/ml)	Zone of Inhibition in mm			
	Plate 1	Plate 2	Plate 3	Mean ±SD
100	7	7	7	7±0
200	8	8	8	8±0
300	12	11	11	11.333±0.577
400	17	14	15	15.333±1.527



The antibacterial activity of the methanolic extract of *Ziziphus xylopyrus* was evaluated against *Staphylococcus aureus* using the agar diffusion method, and the results are presented in Table 8. The

extract exhibited a concentration-dependent antibacterial effect, as evidenced by a gradual increase in the zone of inhibition with increasing extract concentration. At a concentration of 100 µg/ml, the mean zone of inhibition was 7.00 ± 0.00 mm, indicating mild antibacterial activity. When the concentration was increased to 200 µg/ml, the extract produced a uniform zone of inhibition of 8.00 ± 0.00 mm, suggesting improved antibacterial efficacy. Further enhancement in antibacterial activity was observed at 300 µg/ml, where the mean zone of inhibition increased to 11.33 ± 0.577 mm. The highest antibacterial activity was recorded at 400 µg/ml, with a mean zone of inhibition of 15.33 ± 1.527 mm, demonstrating strong inhibitory action against *S. aureus*. These findings clearly indicate that the methanolic extract of *Ziziphus xylopyrus* possesses significant antibacterial potential against *Staphylococcus aureus*, with effectiveness increasing proportionally to the concentration of the extract.

Discussion

The antibacterial activity of *Berberis aristata* and *Ziziphus xylopyrus* methanolic extracts was evaluated against *Escherichia coli* and *Staphylococcus aureus* at different concentrations (100–400 µg/ml). The results indicate variations in the efficacy of both extracts against the tested bacterial strains. The *Berberis aristata* extract showed no inhibition at 100 µg/ml, while inhibition zones increased with concentration, reaching a maximum of 10.666 ± 0.577 mm at 400 µg/ml. In contrast, the *Ziziphus xylopyrus* extract exhibited antibacterial activity even at the lowest concentration (7 ± 0 mm at 100 µg/ml) and showed a stronger effect at higher concentrations, with a maximum inhibition zone of 15.333 ± 1.527 mm at 400 µg/ml. These results suggest that *Ziziphus xylopyrus* methanolic extract is more effective against *S. aureus* than *Berberis aristata*, as it demonstrated consistent inhibition at all tested concentrations and exhibited a larger zone of inhibition at higher doses.

Both extracts exhibited antibacterial activity against *S. aureus* from 200 µg/ml onwards. At 400 µg/ml, *Berberis aristata* showed an inhibition zone of 11.333 ± 0.577 mm, whereas *Ziziphus xylopyrus* demonstrated a slightly higher inhibition of 15.333 ± 1.527 mm. Notably, *Ziziphus xylopyrus* exhibited activity even at 100 µg/ml, whereas *Berberis aristata* was ineffective at this concentration.

Both extracts exhibit promising antibacterial potential, with *Ziziphus xylopyrus* demonstrating stronger and more consistent activity across all tested concentrations

Conclusion

The present study was undertaken to systematically investigate the pharmacognostical, phytochemical, antibacterial, of *Berberis aristata* root extract and *Ziziphus xylopyrus* bark extract, with the aim of scientifically validating their traditional medicinal claims and identifying bioactive constituents responsible for their pharmacological effects. The experimental design followed a logical sequence beginning with authentication and standardization of plant material, progressing through biological evaluation, and culminating in chromatographic isolation and spectroscopic characterization of the active principle.

The plant materials selected for the study were carefully collected and documented to ensure authenticity, reproducibility, and consistency throughout the experimental procedures. The root extract of *Berberis aristata* and the bark of *Ziziphus xylopyrus* were chosen based on their extensive traditional use in indigenous medicine for the treatment of inflammatory disorders, infections, and immune-related conditions. Accurate documentation of the collected plant material weights ensured proper standardization prior to extraction, which is critical in pharmacognostical and phytochemical studies to minimize batch-to-batch variability and to maintain experimental reliability.

Pharmacognostical evaluation of *Berberis aristata* revealed acceptable physicochemical parameters that are essential indicators of drug quality and purity. The total ash value reflects the total inorganic content of the plant material, and the low value obtained suggests minimal contamination with extraneous inorganic matter. The water-soluble ash value indicated the presence of physiologically relevant inorganic salts, while the low acid-insoluble ash value confirmed minimal contamination with siliceous materials such as sand or soil. These parameters collectively indicate that the crude drug was of good quality and suitable for further investigation. Similarly, extractive values provide insight into the solubility of phytoconstituents in different solvents. The higher water extractive value compared to the alcoholic extractive value suggests that *Berberis aristata* contains a greater proportion of polar, water-soluble constituents, which may include glycosides, alkaloids, and phenolic compounds known for their biological activities.

The pharmacognostical assessment of *Ziziphus xylopyrus* similarly demonstrated favorable quality attributes. The total ash and acid-insoluble ash values were within acceptable limits, indicating minimal inorganic contamination. The relatively high water-soluble ash value suggested that a significant fraction of inorganic constituents were water soluble. The higher water extractive value compared to alcoholic extractive value indicated the abundance of polar phytochemicals, supporting the

selection of polar solvents for extraction. These findings provide essential baseline data for the standardization and authentication of *Ziziphus xylopyrus* as a crude drug and reinforce its suitability for pharmacological evaluation.

The present study revealed that the total weight of *Berberis aristata* (Root extract) was 167.61 gm and *Ziziphus xylopyrus* (Bark extract) was 180.16 gm. The pharmacognostical evaluation of these plants reveal that in plant *Berberis aristata* the total ash value was 4.13, water soluble ash was 2.56, acid soluble ash was 1.19. the extractive values in *Berberis aristate*, water extractive value was 3.22 and alcoholic extractive value was 2.45%. Similarly, the pharmacognostical evaluation of these plants reveal that in plant *Ziziphus xylopyrus* (root) the total ash value was 3.83, water soluble ash was 3.31, acid soluble ash was 1.00. the extractive values in *Ziziphus xylopyrus*, water extractive value was 3.09 and alcoholic extractive value was 1.11%.

After performing extraction of *Berberis aristata* (Root), the percentage yield of bark extracts in petroleum ether was 1.44, ethyl acetate was 7.22 and methanolic was 12.62%. In *Ziziphus xylopyrus* (bark), the percentage yield of root extracts in petroleum ether was 1.55, ethyl acetate was 11.2 and methanolic extract was 16.80.

Extraction studies conducted using solvents of varying polarity demonstrated a clear dependence of extractive yield on solvent type. In *Berberis aristata*, ethyl acetate produced the highest yield among the solvents tested, suggesting the presence of a substantial amount of moderately polar constituents. In contrast, methanol extraction yielded comparatively lower amounts in certain batches, which may reflect variability in phytochemical distribution or extraction conditions. For *Ziziphus xylopyrus*, petroleum ether and methanol yielded relatively higher extractive values, indicating the presence of both non-polar and polar constituents. The observed variation in extractive yield highlights the chemical diversity of the plant materials and underscores the importance of solvent polarity in phytochemical extraction. The maceration method employed ensured gentle extraction, preserving thermolabile compounds while allowing efficient solubilization of bioactive constituents.

Phytochemicals like carbohydrates, alkaloids, flavonoids, terpenoids, steroid, glycosides, protein, amino acid, tannins, phenolic, saponins was identified in ethyl acetate and methanolic extract by phytochemical investigation with respect to chemical tests. Extraction studies using solvents of varying polarity demonstrated that solvent selection plays a critical role in determining extractive yield and phytochemical composition. Methanol emerged as the most effective solvent for extracting polar bioactive constituents from both plant materials, while petroleum ether showed comparatively lower extraction efficiency, reflecting the lower abundance of non-polar compounds. These findings underscore the importance of solvent polarity in optimizing extraction strategies and maximizing recovery of pharmacologically active constituents. Phytochemical screening was done using reference method. Several kinds of secondary metabolites, including alkaloids, polyphenols, flavonoids, anthraguinones, coumarins, saponins, tannins, triterpenes, and steroids, were found by phytochemical screening.

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