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Antibacterial Sensitivity of Crude Extracts and Various Fractions of *Berberis vulgaris* L. Roots

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Abstract

The problem of antibiotic resistance is an increasing health challenge in the world and is requiring the search of natural sources of new antimicrobial agents. Plant-based phytochemicals have also been of much interest as alternative therapeutic agents. *Berberis vulgaris* L., also known as barberry, is a typical medicinal plant which has a broad range of secondary metabolites that have been reported to have antimicrobial ability. This study investigates the antibacterial activity of crude methanolic extract and solvent-partitioned fractions (n-hexane, chloroform, ethyl acetate, methanol and butanol) of *B. vulgaris* roots. Antibacterial activity was evaluated against five clinically significant bacterial strains (*E. coli*, *K. pneumoniae*, *S. epidermidis*, *S. aureus* and *B. subtilis*) using the disc diffusion assay. Streptomycin served as a standard reference drug. The results showed that antibacterial activity varied considerably among fractions, with methanol, butanol and ethyl acetate displaying the strongest inhibitory effects. The methanol fraction demonstrated the highest activity against *B. subtilis* (28.44 ± 1.60 mm), while chloroform fraction exhibited notable activity against *S. epidermidis* (22.76 ± 1.05 mm). Comparatively, non-polar fractions such as n-hexane showed limited antibacterial effects. The findings suggest that *B. vulgaris* root extracts, particularly polar fractions, contain potent antibacterial constituents that warrant further purification and pharmacological validation.

KEYWORDS

Berberis vulgaris, antibacterial activity, inhibition zone, medicinal plants, bioactive compounds, solvent fractions

1.0 INTRODUCTION

The global rise in antimicrobial resistance (AMR) has become a critical medical challenge, leading to treatment failures and increased morbidity and mortality[1-3]. Conventional antibiotics are losing their efficacy due to widespread misuse, rapid bacterial mutation and development of genetic resistance mechanisms[4-5]. As a result, researchers are increasingly turning toward medicinal plants as promising sources of novel antimicrobial compounds that may overcome current resistance patterns[6-7].

Plants manufacture a wide range of bioactive metabolites encompassing alkaloids, flavonoids, terpenoids, phenolics and glycosides that have a wide range of pharmacological activities[8-9]. The use of *Berberis vulgaris* L. (family Berberidaceae) plants in traditional medicine has been an earned focus of attention among medicinally important plants given its long history of application[10-12]. The plant is common in Asia, Europe with its roots, bark and fruits being commonly used to treat bacterial infections, fever, diabetes, digestive disorders and inflammation[13-14].

Phytochemical investigations of *B. vulgaris* have identified berberine, palmatine, berbamine and oxyacanthine as major alkaloids responsible for antimicrobial and therapeutic effects[15-17]. However, limited studies have evaluated the comparative antibacterial activity of its solvent-partitioned fractions. Solvent fractionation is crucial because compounds of varying polarity demonstrate different extraction efficiencies and biological responses.

This study aims to investigate the antibacterial potential of *B. vulgaris* root extracts against selected pathogenic bacteria. The findings provide baseline evidence supporting the plant's medicinal relevance and its potential as a natural antimicrobial agent.

2.0 MATERIALS AND METHODS

Fresh roots of *Berberis vulgaris* L. were collected from the District Dir,Khyber Pakhtunkhwa. The plant was authenticated by a botanist, and voucher specimens UOS, Bot 701 were preserved for record. The roots were washed thoroughly, shade-dried at room temperature, and ground to a fine powder.

2.1 Extraction and Fractionation

Approximately 250 grams of powdered root material was subjected to cold maceration using analytical grade methanol for 72 hours with periodic stirring. The extract was filtered and concentrated using a rotary evaporator under reduced pressure at a controlled temperature. The crude methanol extract was then dissolved in distilled water and fractionated sequentially using n-hexane, chloroform, ethyl acetate, methanol and butanol to obtain solvent-specific fractions representing a polarity gradient.

2.2 Antibacterial Assay

The disc diffusion method was employed to assess the antibacterial activity. Bacterial cultures (*E. coli, K. pneumoniae, S. epidermidis, S. aureus* and *B. subtilis*) were adjusted to 0.5 McFarland standard. Sterile discs impregnated with individual extracts were placed on pre-inoculated agar plates. Streptomycin served as a positive control. Plates were incubated at 37°C for 24 hours, and the inhibition zones were measured (mm).

2.3 Statistical Analysis

All tests were performed in triplicate and values were represented as mean ± standard deviation. Results were interpreted based on concentration-dependent activity patterns.

3.0 RESULTS

The antibacterial activity of crude methanolic extract and solvent fractions of *Berberis vulgaris* roots showed different inhibition patterns against the selected bacterial strains.For *Escherichia coli*, the methanol fraction showed an inhibition zone of 22.38 ± 1.87 mm, followed by the butanol fraction (24.50 ± 1.29 mm) and n-hexane fraction (13.54 ± 1.29 mm). While no activity was observed for the chloroform and ethyl acetate fractions. Streptomycin, used as the positive control, recorded 27.76 ± 1.05 mm.Against *Klebsiella pneumoniae*, the methanol and butanol fractions were effective, showing inhibition zones of 20.29 ± 1.40 mm and 23.56 ± 1.50

mm, respectively. No antibacterial activity was observed for n-hexane, chloroform or ethyl acetate fractions. Streptomycin exhibited the highest inhibition (31.23 ± 1.49 mm).For *Staphylococcus epidermidis*, only two fractions showed activity: chloroform (22.76 ± 1.05 mm) and butanol (16.34 ± 1.34 mm). Similarly, no activity was observed in ethyl acetate, methanol, or n-hexane fractions. Streptomycin showed the highest inhibition (35.11 ± 1.23 mm).

In the case of *Staphylococcus aureus*, all fractions showed inhibitory effects, with methanol (27.99 ± 2.01 mm) and ethyl acetate (26.87 ± 1.77 mm). The methanol extract exhibited the highest activity (27.99 ± 2.01 mm), followed closely by ethyl acetate (26.87 ± 1.77 mm) and butanol (20.65 ± 1.65 mm). Chloroform demonstrated moderate inhibition (18.30 ± 1.54 mm), while n-hexane showed the weakest effect (8.09 ± 1.40 mm). Streptomycin again produced the highest activity (29.60 ± 1.20 mm).Against *Bacillus subtilis*, methanol showed the strongest response (28.44 ± 1.60 mm), followed by butanol (26.89 ± 1.00 mm) and ethyl acetate (18.30 ± 1.54 mm). Chloroform demonstrated moderate activity (19.56 ± 1.00 mm), while n-hexane showed a smaller inhibition zone (12.45 ± 1.60 mm). The standard antibiotic streptomycin recorded 34.39 ± 1.77 mm. The antibacterial activity results is given in the inset of **Table 1.**

Table 1. Antibacterial activity of crude extracts and various fractions of *Berberis vulgaris*Lroots

Bacterial strain	*n*-Hexane	Chloroform	Ethyl Acetate	Methanol	Butanol	Streptomycin
E. coli	13.54±1.29	-	-	22.38±1.87	24.50±1.29	27.76±1.05
K. pneumonia	-	-	-	20.29±1.40	23.56±1.50	31.23±1.49
S. epidermis	-	22.76±1.05	-	-	16.34±1.34	35.11±1.23
S. aureus	8.09±1.40	18.30±1.54	26.87±1.77	27.99±2.01	20.65±1.65	29.60±1.20
B. subtilis	12.45±1.60	19.56±1.00	18.30±1.54	28.44±1.60	26.89±1.00	34.39±1.77

4. DISCUSSION

The antibacterial screening demonstrated that *B. vulgaris* extracts possess varying extent of antibacterial activity depending on the solvent fraction and bacterial strain. The methanol and butanol fractions exhibited the strongest inhibitory activity, suggesting that active antibacterial constituents of *B. vulgaris* are more soluble in polar solvents. These findings align with previous studies reporting that alkaloids and phenolic compounds of *B. vulgaris* are efficiently extracted using polar extraction systems.Methanol fraction displayed the highest inhibition activities against B. subtilis and S. aureus, indicating strong bactericidal effeciency. Butanol fraction also showed promising results against Gram-negative (E. coli and K.

pneumoniae) bacteria, which are generally more resistant due to their outer lipid membrane barrier. Ethyl acetate extract exhibited moderate activity, while chloroform extract was particularly effective against S. epidermidis. In contrast, n-hexane fraction demonstrated weak or no inhibition, suggesting limited antibacterial phytochemicals in non-polar solvent layers.

Comparing activity with streptomycin revealed that although synthetic antibiotics exhibited higher inhibition, plant extracts showed noteworthy biological action, indicating their potential role as supplementary or alternative antibacterial agents. Such findings reinforce the pharmaceutical relevance of B. vulgaris and highlight its potential for isolation of lead antimicrobial compounds.

5.0 CONCLUSION

This study demonstrates that *Berberis vulgaris* root extracts possess significant antibacterial activity, particularly in polar solvent extracts such as methanol, butanol and ethyl acetate. The variability in antibacterial response suggests selective phytochemical distribution across fractions. The methanol extract exhibited the highest overall activity, indicating the presence of potent antibacterial compounds. Although streptomycin remained superior, the results provide strong justification for further purification, compound isolation, MIC studies and in vivo validation. The plant may serve as a potential source of natural antibacterial agents for pharmaceutical applications.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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