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Phytochemical Analysis and Antibacterial Activity of *Berberis vulgaris* Extract

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Abstract

Background: Plants are main source of various bioactive compounds that possess great medicinal importance and have gained much popularity for therapeutics due to their less side effects. A diverse category of plants is present to be explored and evaluated for the treatment of different bacterial infection. In the current work, *Berberis vulgaris* extract in various solvents was evaluated for its phytochemical properties and antimicrobial potential against six different pathogenic bacterial strains.

Methods: Different phytochemical tests were carried out to analyze the plant for active biocomponents. The disc diffusion method was used to screen the plant for different pathogenic bacterial strains. Phytochemical analysis revealed the presence of various plant bioactive compounds (alkaloids, saponins, tannins, flavonoids, terpenoids, carbohydrates, and proteins) in variable amount.

Results: Among all solvents extracts, butanoic and aqueous fraction showed abundant presence of bioactive compounds, while n-hexane showed least intensity of various phytochemicals. For antimicrobial potential, methanolic and butanoic fractions showed maximum growth inhibition against all strains tested at 1.5mg disc^{-1}. Ethyl acetate and n-hexane also showed better activity against all tested bacteria at all concentrations. The most susceptible microbe was *Bacillus subtilis*. These results further revealed that least activity was recorded by water extracted solvent and showed no activity against *Staphylococcus aureus* at all concentrations.

Conclusion: The current work highlights the apparent antimicrobial potential of extract derived from of *Berberis vulgaris*. This plant may be explored for further activities and can be used for the production of antibiotics.

Introduction

For thousands of years plants have been used as medicine for the very purpose of treatment [1]. About 60 to 80% of the world population still depends upon medicines which are derived from different plants [2]. The use of plants having medicinal impacts, which are traditionally used all across the world for healing mechanisms, became an important source for the discovery of novel antibiotics [3]. Recently, it has been reported that most of the pathogenic bacteria become resistant to various antibiotics [4]. *Staphylococcus aureus* (gram positive), *Pseudomonas aeruginosa* (gram negative) and Mycobacterium tuberculosis are the most important resistant bacteria [5]. These medicinal plants need very deep study so that their undiscovered abilities, safety and effects are better identified [6].

The barberry, botanically named as *Berberis vulgaris* represents the most significant Berberidaceae of Europe. Different species of Barberry have been found to be very active against various microbes and poisonous substances [7, 8]. Now-a-days, the bark and roots of barberry are mostly used in medicine and found to be very effective against malaria and leishmaniasis [9]. Many studies show that barberry acts faster than antibiotics [10]. The active chemical constituent present in barberry is berberine e.g., isoquinoline alkaloids [11]. Different constituents of berberine have been found to inhibit the growth of bacteria, amoebae, fungi, and protozoa [12]. This phytochemical constituent normally treats diarrhea caused by *Escherichia coli* [13].

Phytochemicals are basically divided into two categories, primary and secondary. Secondary metabolites hold great importance in medicinal purposes [14]. Alkaloids have been utilized as anesthetic agents and they are present in many medicinal plants [15]. It is generally noted that phenolic acids occur naturally and have been detected for various allelopathic activities [16]. Alkaloid, coumarin, flavonoids, saponins and volatile components of the essential oils have been discovered as allelopathic agents [17]. This study has been conducted to screen the bioactive components of *Berberis vulgaris* and to evaluate the antibacterial potentials in active solution of butanol, methanol, ethyl acetate, n-hexane and water extracted samples against six different pathogenic strains of bacteria (*Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumonia*e, *Pseudomonas aeruginosa*, *Xanthomonas campestris* and *Staphylococcus aureus*).

Methods

This study has been performed at Institute of Biotechnology and Genetic Engineering, The University Agriculture Peshawar KPK Pakistan.

Plant Collection and Sample Preparation

Berberis vulgaris (bark) was obtained from Mardan herbarium, Pakistan. The selected parts of the plant were washed with tap water and shade dried. After drying, samples were grinded finely into powdered form through tissue homogenizer (Infinigen™ Tissue Mixer Mill, ACTGene) and stored for further study.

Preparation of Plant Extract

About 25g of each sample was taken in 400ml of different solvents i.e butanol, methanol, ethyl acetate, n-hexane and distilled water. All solutions were prepared separately in five beakers and kept at room temperature for 6 to 7 days until the color of the solvent changed. These different mixtures were then filtered using Whatman filter paper to get the filtrate and then kept at room temperature to air dry. After 4 to 5 days the final extract from each sample was obtained and used for phytochemical and antibacterial test.

Media Preparation

The media made up of agar technical (agar No. 3) and nutrient broth (CM0001) was used in this study for the growth and culturing of bacteria. The required number of media was prepared from agar technical (28g/liter) and nutrient broth (13g/liter) by dissolving it into distilled water in a conical flask. The media was sterilized in autoclave for 40 minutes at 20 psi and 120°C. The media was poured into petri dishes just after sterilization aseptically in a laminar flow hood to prevent contamination. The petri dishes were kept in laminar flow hood for 30 to 45 minutes to allow solidification of media and then these dishes were placed in incubator for 24 hours at 37°C. Next day contaminated plates were discarded and uncontaminated were used for culturing of bacteria.

Phytochemical analysis

Crude aqueous extract of *Berberis vulgaris* was tested for the presence of terpenoids, tannins [18], proteins, carbohydrates [19], alkaloids, flavonoids and saponins [20] with little modification.

Antimicrobial activity

Bacterial Strains

Different bacteria used in this study are shown in Table. 3Table. 1. All bacterial cultures were taken from different labs, then refresh by streaking with a sterile loop in a nutrient agar media inside laminar flow hood. Then they were kept at 37°C for 24 hours to incubate. Next day the cultures were again streaked on fresh plates and incubated at 37°C for 24 hours. The culture was taken from the sub-culture with the help of sterile loop and incubated in a nutrient broth media in a shaking incubator at 37°C (180 rpm) for 24 hours.

Bacterial species	Gram strain type	Detail of bacterial strains used
Escherichia coli	Gram negative	ATCC#25922
Pseudomonas aeruginosa	Gram negative	ATCC#9721
Xanthomonas campestris	Gram negative	ATCC#33913
Klebsiella pneumonia	Gram negative	ATCC#700603
Bacillus subtilis	Gram positive	ATCC#6051
Staphylococcus aureus	Gram positive	ATCC#6538

Table 1: Bacterial strain tested for susceptibility of *Berberis vulgaris* extracts.

Disc diffusion susceptibility assay

Bacterial strains were tested against different extracts of *Berberis vulgaris* which were obtained through different solvents by disc diffusion method as described by Bakht et al [21]. Plant extracts having concentrations of 0.5mg/disc, 1mg/disc, and 1.5gm/disc in 6µl, 12µl, and 18µl volume were applied on the disc respectively. Azithromycin (50µg/6µl) was used for gram positive bacteria as a control while ciprofloxacin (50µg/6µl) was used for negative bacteria. For negative control DMSO 6µl/disc was used. The diameter of inhibition zones was measured in mm after incubation at 37°C for 24 hours. The triplicate of this experiment was conducted, and the inhibition zones were determined by the formula.

Inhibition % = (Zone of sample)/(Zone of control) × 100

Statistical Analysis

The data was analyzed as mean of triplicate data and LSD test was used at $p<0.05$ through GraphPad Prism 5 [22].

Results

Phytochemical analysis of different extracts of *Berberis vulgaris*

In this study different extracts from bark of *Berberis vulgaris* were analyzed for phytochemical screening according to standard procedures of analysis (Table 2). The methanolic extract was found rich in tannins and carbohydrates whereas less concentration of saponins were detected. In case of butanoic extract, flavonoids were much higher in concentration. Phytochemical analysis of ethyl acetate and n-hexane showed negative result for saponins. All other constituents were also found in much lesser quantity. Alkaloids, saponins, and terpenoids were found in higher concentration in aqueous extract. For saponins aqueous extract showed negative result.

Antimicrobial potential of *Berberis vulgaris* extract against various bacterial strains

This work investigates the antibacterial potential of selected plant sample against different bacterial strains.

Escherichia coli

Plant extract of *Berberis vulgaris* was applied to *E. coli* at different concentrations i.e., 0.5, 01 and 1.5 mg/disc as described in material and methods. Among different extracts tested, methanolic extract was found to be more effective followed by butanol, while aqueous extract showed least effect against *E. coli*. At highest concentration (1.5 mg/disc), methanolic extract showed maximum zone of inhibition i.e., 59.8%, while aqueous extract showed minimum zone of inhibition of 37.92 % at 1.5 mg/disc. The effect of different solvent extracted sample was dose dependent i.e., higher the concentration of the plant extract showed higher zone of inhibition.

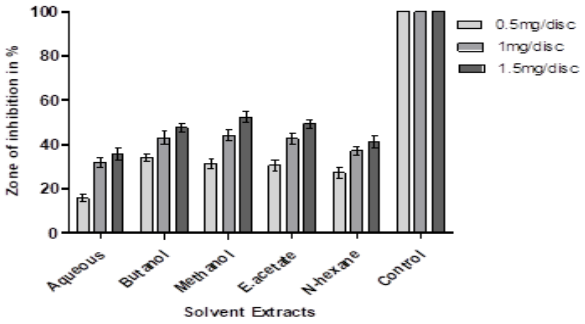

Figure 1: Antibacterial activities of aqueous, ethyl acetate, methanolic and butanoic extracts of *Berberis vulgaris* bark against *Escherichia coli*.

Pseudomonas aeruginosa

Results in Fig. 2 indicated that butanoic and methanolic fractions inhibited growth almost at the same level at all concentrations except slight differences (48.59% by butanoic extract and 47.98% by methanolic extract at 1.5mg $disc^{-1}$). The lowest zone of inhibition (ZI) was formed by aqueous extract (37.04%) at 1.5mg $disc^{-1}$. It showed that the activity of ethyl acetate and n-hexane extracted sample was normal. These results revealed that zone of inhibition is dependent upon concentration of the extracts.

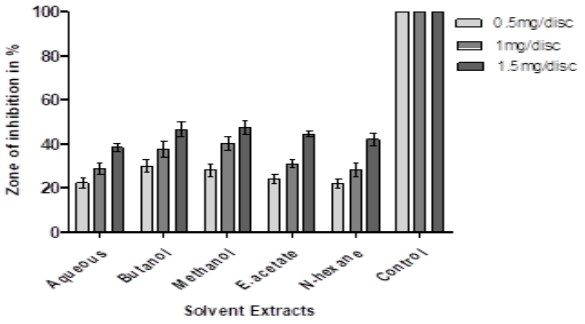

Figure 2: Antibacterial activities of aqueous, ethyl acetate, methanolic and butanolic extracts of *Berberis vulgaris* bark against *Pseudomonas aeruginosa*.

Solvents	PHTO-CHEMICALS CONSTITUENTS						
	ALKALOIDS	SAPONINS	TANNINS	FALVONOIDS	TERPENOIDS	CARBOHYDRATE	PROTEINS
METHANOL	++	+	+++	++	++	+++	++
BUTANOL	++	++	++	+++	++	++	+++
E. ACETATE	+	_	++	+	+	+	++
AQUEOUS	+++	+++	+	++	+++	++	++
N-HEXANE	_	_	+	_	+	++	+

Table 2: Phytochemical profile of solvent extracted samples from shoots of *Berberis vulgaris*.

Staphylococcus aureus

Aqueous fraction of *Berberis vulgaris* did not inhibit the growth of *Staphylococcus aureus* at any concentration (Fig. 3). Methanolic extract at high concentration (1.5mg disc^{-1}) showed maximum activity (49.67%) against *S. aureus* when compared with the positive control. Butanol, ethyl acetate and n-hexane extracts showed significant activities at 1mg disc^{-1} and 1.5mg disc^{-1} while at 0.5mg disc^{-1} they showed less activity. distribution of other reasons for not taking a Pap smear test from the participants' point of view is shown in detail in Table 4.

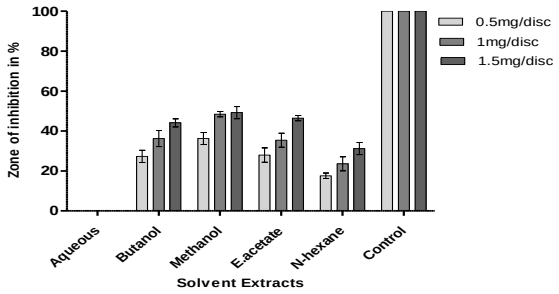

Figure 3: Antibacterial activities of aqueous, ethyl acetate, methanolic and butanolic extracts of *Berberis vulgaris* bark against *Staphylococcus aureus*.

Bacillus subtilis

Methanolic fraction was observed as more potent to control the growth of *B. subtilis* at all concentrations followed by butanolic extract then other tested samples (Fig. 4). Methanolic extract inhibited the growth of *B. subtilis* by 59.89% at 1.5mg disc^{-1} and its activity was reduced with the decrease in concentration i.e., 50.4% at 1mg disc^{-1} and 37.34% at 0.5mg disc^{-1}. Butanolic fraction exhibited slightly less activity than methanol (54.67%) at 1.5mg disc^{-1}. In case of ethyl acetate at higher concentration (1.5mg disc^{-1}) the zone of inhibition formed was 49.4% which was recorded much lower than the methanolic fraction. N-hexane and aqueous extracts showed same activity at low concentration while the activity of aqueous extracts was increased with increase in concentration.

Klebsiella pneumonia

The data also revealed that methanolic fraction inhibited the growth of *K. pneumonia* at maximum level then it was followed by butanolic extract (Fig. 5). Methanolic fraction controlled the activity up to 47.65 % at a concentration of 1.5mg disc^{-1} which was recorded as highest. Meanwhile in case of butanolic extract the zone of inhibition was 46.02 % slightly less than methanolic fraction. Ethyl acetate and aqueous sample extract showed some resistance to *K. pneumoniae*, but n-hexane fraction was recorded less-resistant fraction as it did not stop the growth level effectively.

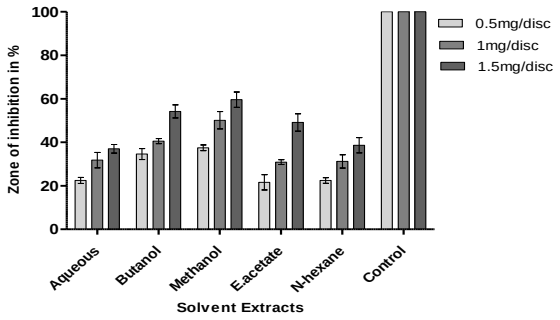

Figure 4: Antibacterial activities of aqueous, ethyl acetate, methanolic and butanoic extracts of *Berberis vulgaris* bark against *Bacillus subtilis*

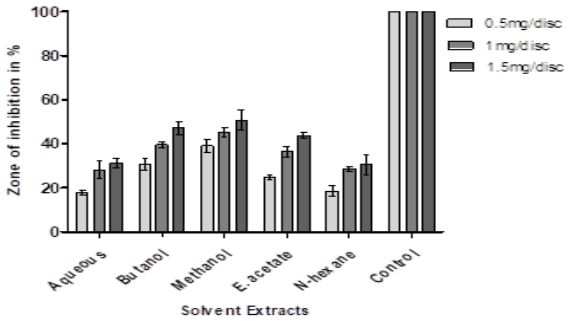

Figure 5: Antibacterial activities of aqueous, ethyl acetate, methanolic and butanolic extracts of *Berberis vulgaris* bark against *Klebsiella pneumonia*.

Xanthomonas campestris

Data regarding different extracted samples against *Xanthomonas campestris* is shown in Fig. 6. Butanolic and ethyl acetate fractions presented a similar activity i.e., 48.03% at highest concentration 1.5mg disc^{-1} while the highest zones were recorded in methanolic extracts (57.17%) at 1.5mg disc^{-1}. At high concentration n-hexane and aqueous samples revealed similar activity i.e 38 %. At lower concentration n-hexane showed slightly higher activity than aqueous fraction (21.32%).

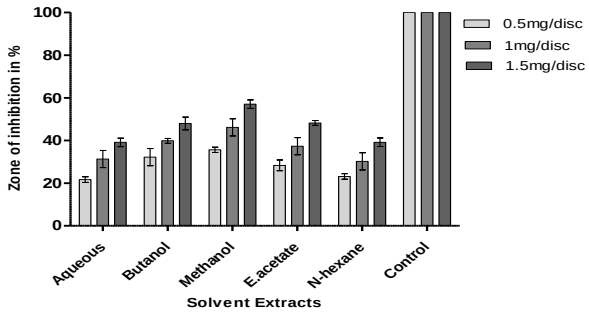

Figure 6: Antibacterial activities of aqueous, ethyl acetate, methanolic, n-hexane and butanoic extracts of *Berberis vulgaris* bark against *Xanthomonas campestris*.

Discussion

The phytochemical analysis showed that methanolic extract was found rich in tannins and carbohydrates whereas saponins were present in less concentration. In case of butanolic extract, flavonoids were much higher in concentration. Phytochemical analysis of ethyl acetate and n-hexane showed negative result for saponins. All other constituents were found in much lesser quantity. Alkaloids, saponins and terpenoids were found in higher concentration in aqueous extract. For saponins aqueous extract showed negative result. Same findings were also previously reported [12, 23, 24].

The antibacterial potential of *Berberis vulgaris* extracts revealed that methanolic extracts were more effective against *E. coli* than other tested extracts. At highest concentration of 1.5 mg/disc, methanolic extract showed maximum zone of inhibition i.e., 59.8%, while aqueous extract showed minimum zone of inhibition at 1.5 mg/disc i.e., 37.92%. Similar results were also previously reported [25, 26]. The data further suggested that butanolic and methanolic fractions inhibited the growth of *P. aeruginosa* almost at the same level at all concentrations except slight differences at 1.5mg $disc^{-1}$. The lowest zone of inhibition (ZI) was formed by aqueous extract (37.04%) at 1.5mg $disc^{-1}$. These results agree with the results previously reported [27]. It was observed that aqueous fraction of *Berberis vulgaris* did not inhibit the growth of *S. aureus* at any concentration. Methanolic extract at high concentration (1.5mg $disc^{-1}$) showed maximum activity against *S. aureus* when compared with the positive control. Butanolic, ethyl acetate and n-hexane showed significant activities at 1mg $disc^{-1}$ and 1.5mg $disc^{-1}$, while at 0.5mg $disc^{-1}$ they showed less activity. Similar results were also reported earlier [28, 29].

Our results also showed that methanolic fraction was observed as more potent to control the growth of *B. subtilis* at all concentrations followed by butanolic than other solvents. Methanolic extract stopped the growth of *B. subtilis* highly at 1.5mg $disc^{-1}$ and its activity was reduced with decrease in concentration. Butanolic fraction exhibited slightly less activity than methanolic at 1.5mg $disc^{-1}$. In case of ethyl acetate, at higher concentration (1.5mg $disc^{-1}$) the ZI formed, which was lesser than methanolic fraction. N-hexane and aqueous extracted samples showed same activity at low concentration while the activity of aqueous extracts was increased with increase in concentration. These findings are in correspondence with previous reports [30, 31]. Methanolic fraction inhibited the growth of *K. pneumonia* at higher level then followed by butanolic fraction. Methanolic fraction stopped the growth upto 47.65% at a concentration of 1.5mg $disc^{-1}$ which was recorded as highest. Meanwhile in case of butanolic extract zone of inhibition was slightly less than methanolic extract. Ethyl acetate and aqueous extract showed some resistance to K. pneumonia, but n-hexane was being recorded less-resistant fraction as it did not stop the growth level effectively. These results resemble with previous reports [22]. Our results also showed that butanolic and ethyl acetate extracts of *X. campestris* showed similar activity at highest concentration 1.5mg $disc^{-1}$ while the highest zones were recorded in methanolic extracts at 1.5mg $disc^{-1}$. At high concentration n-hexane and aqueous samples revealed similar activity while at lower concentration n-hexane showed slightly higher activity than aqueous fraction. These results are also supported by previous reports [24].

This study revealed the presence of different important phytochemical compounds in plant extract of Berrberis vulagaris. Plant extract of Berrberis vulagaris showed antimicrobial potential against different bacterial strains i.e., *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumonia* and Xanthomonas compestris. Further research is required to use these extracts for antibiotic production for treatment of various diseases.

Competing Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Author Contribution

Mian Afaq Ahmad (Conceptualization, Supervision of project), Attequr Rahman (Experimental work, Writing of manuscript and submission), Abdur Rauf & Hamza Iqbal (Analysis), Bakhtiar Ali & Farman Ullah (Interpretation of results), Mohib Ullah & Sidra Ahmad (review & editing), Murad Ali & Maaz Iqbal (Data curation).

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