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A study of Antimicrobial Activity of Berberis vulgaris (Zirishk) Aqueous Plant Extract using Pathogenic Isolates from Patients of Islamabad and Rawalpindi

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Abstract: *Berberis vulgaris (Barberries) have long been used as herbal remedy for the treatment of a variety of complaints. This study was conducted to determine the in vitro antimicrobial activity of plant extracts of the fruit of Berberis vulgaris against 09 clinically isolated pathogenic microbes. Diffusion and dilution methods were used to determine the antimicrobial activity of aforesaid extracts. In the diffusion method, the fruit extract of Berberis vulgaris exhibited some antimicrobial activity against Pseudomonas aeroginosa only but in the dilution method, the extract shows antibacterial impacts against all pathogens tested. On these bases, future prospects of plant medicines were discussed. In the new era, this type of drug research will open the field for scientists to develop safe drugs and industry to serve not only the nation rather the humanity.*

1. Introduction

Plants have been used during the ages for cure and treatment of diseases since the start of mankind. The seeds, roots, stems and leaves of different herbs, shrubs, and trees have been used for centuries to treat the diseases [11]. The Arabian physicians described the infections of the body and also mentioned several medicinal plant and vegetable substance against diseases such as rabies and hydrophobia in their pharmacopeias or medical formularies [7]. The traditional medicines are mostly of plant origin and are still widely used in the regions comprising countries of the third world and China. Plants have also been used to treat other diseases such as diabetes, cardiovascular diseases, thrush, giardiasis, heavy metal poisoning, jaundice, congestion of abdominal and pelvic cavities, rheumatism and scarlet fever etc. [9, 10, 13, and 16].

In developing countries, the World Health Organization estimates that about three-quarters of the population rely on plant-based preparations used in traditional medicine for primary health care as fundamental human needs. Therefore, some herbs have been evaluated for antimicrobial activity may be used to treat a variety of diseases and microbial origin [22]. During the past few years, a number of studies have been focused on the medicinal evaluation of plants used in traditional medicine. These include examples of Bonafousia species, Croton menthodorum and Heisteria acuminate which possess anti-inflammatory activity and are commonly used in pathologies related to inflammation [14]. Allium sativum not only possesses anti-thrombogenic activity but also contains anti-atherogenic effects along with antibacterial, antifungal and anticancer activity [9]. Studies also claimed that some plants, which are already used as traditional medicine, possess antimicrobial properties against bacteria, Virus, and fungi, and preparation from such plants considered to be effective against diseases of microbial etiology like Hepatitis B & C Tuberculosis, typhoid and diphtheria etc. [7]. Barberry fruits have been used in the traditional Austrian medicine internally as tea, jelly or syrup for treatment of disorders of the respiratory tract, fever, infections, cold, and flu [20]. When considering the antibacterial activity of preparations from plants, studies revealed that plants possess considerable antibacterial activity when compared with modern antibiotics like chloramphenicol and streptomycin [7]. Since diseases like typhoid fever and food poisoning are commonly treated with antibiotics like chloramphenicol and ampicillin, the extensive illogical use of these antibiotics have led to the problems of drug resistance [17].

In Pakistan and other third world countries where infectious diseases are prevalent, there is a need to develop some medicines of plant origin against these persisting infectious diseases, which may be comparable to modern medicines and antibiotics. Medicinal plants which are used in the traditional medicine, offer a great reservoir for the discovery of new plants having antimicrobial properties comparable to antibiotics used in modern medicine. Since almost all the antimicrobial agents are being imported and by considering the availability of medicinal plants in these countries, a lot of foreign exchange may be saved [7]. In addition to the cost of treatment is steadily increasing and it is becoming unaffordable by a common user. Therefore, the development of therapeutic agents from our own indigenous resources will be of a great help.
In traditional medicine, some of the indigenous medicinal plants including Berberis vulgaris has been claimed to exert curative effects in the diseases caused by Salmonella species [13, 16]. Berberis vulgaris L. Fruits are used for diseases and disorders of the kidney, urinary tract and gastrointestinal tract, liver disease, bronchial ailments, and as a stimulant for the circulatory system. The root and stem of this plants are used for the diseases and disorders of the gastrointestinal tract, liver, gallbladder, kidney and urinary tract, respiratory tract, heart and circulatory systems [4]. Berberine, the active ingredient in Berberis vulgaris (barberry), inhibits the growth of bacteria and has antioxidant properties in vitro [18].
This plant is very common in Rawalpindi and Islamabad and a very little work has been done as yet. It is commonly used in traditional and homeopathic medicines.

I have studied the in-vitro antibacterial effects of aqueous fruit extract of Berberis vulgaris (Zirishk). The plant extract was used in aqueous form as it is used in traditional medicine. Antibacterial assays were performed on a variety of clinically isolated Gram-positive and Gram-negative bacteria.

2. Materials and Methods

This research work was conducted in the bacteriology department of Public Health Division at National Institute of Health, Islamabad.

2.1 Materials

PLANT USED IN THIS RESEARCH:
Berberis vulgaris plant was used in the study. It is found in the mountains of the Himalayas in Nepal, Tibet, and Afghanistan. This plant is also common on Margalla Hills from where its fruits were collected and processed to determine its antimicrobial activity. The plant was identified by the herbarium of National Agriculture Research Council, Islamabad prior to the start of work.

2.1.1 Extraction of Active Ingredients

Extraction is an important process in the preparation of medicine from plants. This process removes constituents from one phase bringing into contact with a second immiscible liquid phase [8]. In this experiment ”Soxhlet extractor” was used. This extractor comprised of the flat bottom flask, chamber to which side arm and siphon tube are attached, along with condenser [8].

2.1.2 Procedure of Extraction

20 grams of finely ground fruits of Berberis vulgaris were placed in the chamber. Distilled water that was used as a solvent, was boiled in the flask. The vapors produced entered into the condenser through the side arm of Soxhlet extractor. After condensation, the condensed water entered into the chamber containing the crude fruits until the level of the liquid in the chamber reached to the top of the siphon then passed through the siphon and collected into the flask [8]. The process of extraction of active ingredients from fruit was completed in four hours. The extracted material was kept in the refrigerator until use.

2.2 Sterilization of Extract:

Extracts were sterilized by 0.22 µ membrane filters (Millipore) under positive pressure and kept at 4°C until use [5].

2.3 Media and Reagents

Nutrient Agar (Difco.U.K), Nutrient Broth (Difco.U.K), Antibiotic Discs(Oxoid U.K)

2.4 Microbial Isolates

Microorganisms used in this study were provided by the Bacteriology Laboratory, Public Health Division at National Institute of Health, Islamabad. These organisms were isolated from human blood, urine, throat, and pus, in the Bacteriology Laboratory of Public Health Division, National Institute of Health Islamabad. These bacteria were re-identified and their antibiogram activity was determined. Streptomycin was found most suitable to be used as a control.

The following table shows the sources of isolated pathogens:

S. #	Bacteria	Sample
1	Staphylococcus aureus	Pus
2	Proteus mirabilis	Pus
3	Salmonella typhi	Blood
4	Salmonella para-typhi A	Blood
5	Salmonella para-typhi B	Blood
6	Klebsiella pneum.	Throat
7	Strep. β	Throat
8	E. coli	Urine
9	Pseudomonas aeroginosa	Urine

Table 2.1, Bacterial Isolates and their sources of Isolation

2.5 Anti-Microbial Activity of Plant Extracts

In past research on the antimicrobial activity of medicinal plants has been encountering several problems because of the diversity of criteria and techniques employed for testing. The lipophilic properties of some extracts such as oils make it very difficult to use an aqueous medium for the study of antimicrobial activity [1]. Among the several methods, which were employed in the plant research, following two conventional methods were adapted for this study:

Diffusion method and Dilution method, these two methods are being described one by one:

2.6 Diffusion Method

In this method, microbial culture is inoculated on the surface of agar medium using disk or hole as a reservoir of extracts or antibiotics. The same to be tested, present in the reservoir comes into contact with an inoculated medium, and after overnight incubation at 37ºC, the plates are observed for the zone of inhibition surrounding the reservoirs. The zone of inhibition is the clear area around the reservoir, shows the inhibition of growth of microorganism by the diffused substance through the agar. The diameter of the clear zone around the reservoir (zone of inhibition) is measured [15, 2]. Well method was used in this study.

2.6.1 Material

Nutrient agar plates, Streptomycin as control (15µg/ 100µl) and Crude fruits extract of Berberis vulgaris and Borer.

2.6.2 Procedure

Dehydrated nutrient agar (23 grams) was mixed with one liter distilled water and boiled to dissolve the contents of the medium. It is sterilized by autoclaving at 121ºC for 20 minutes at 15 Lbs. pressure. When the temperature reached between 50 and 60ºC the medium was poured in the Petri plates which were already washed and sterilized before the preparation of medium. The medium was poured aseptically in 30ml quantity in each plate; plates were allowed to solidify for 30 minutes and after solidification, all plates were incubated at 37ºC for overnight to check for contamination.

Borer, which was comprised of 6mm stainless steel tube attached to the arm of the conical flask and suction pump, which was attached to the mouth of the armed conical flask with a glass tube, assembled the above-mentioned components of the borer aseptically. Total 2 holes were cut on the surface of agar medium in each of the 09 plates, those were used in each experiment-one plate for each bacterium. The holes were marked for Berberis vulgaris, and one for Control (Streptomycin). Bacterial cultures were inoculated using cotton swabs after standardization with McFarland standard solution. Each hole was filled with 100ul corresponding product. Plates were kept in the refrigerator for one hour to allow the content of each hole to absorb in the medium. Plates were incubated at 37ºC for 18-20 hours. After incubation, the diameter of each zone of inhibition was measured at two different places and the mean value was taken for the record. This procedure was repeated 03 times to confirm the size of zone of inhibition and antibacterial effect of extracts on each bacterium used in this study and to evaluate the results [5].

2.7 Dilution Method

This method is generally used for quantitative estimation of antimicrobial activities. It is also used in the preliminary screening purpose. In this method turbidity is the indication of growth, which is estimated by the colorimetric/spectrophotometric method for quantitative estimation whereas when there is no growth, the medium remains clear, due to antimicrobial activity of samples incorporated into the medium [15, 19]. The standard plate count method was adopted in the study.

2.7.1 Materials

Glass test tubes, Media: Nutrient broth tubes and Nutrient Agar plates

Controls: 1) Control 37ºC is the test tube containing 1 ml distilled water instead of plant extract and kept at 37ºC to compare the growth with the treated sample tubes. 2) Control +4ºC: Control +4ºC is the initial load of bacteria used in the test and during the test it was kept at +4ºC to compare the level of growth in the treated sample tubes.

2.7.2 Procedure

Nutrient broth tubes and Nutrient agar plates were prepared and checked for contamination and finally refrigerated until use. 24 hours before the start of experiment, the bacterial culture was freshly prepared by inoculating 9ml nutrient broth with 1ml bacterial culture and incubated at 37ºC.

After overnight incubation at 37ºC the nutrient broth was distributed in 20ml quantity into 100ml flasks. McFarland solution was used for standardi-zation purpose. One ml bacterial culture freshly prepared was inoculated to this flask.

After inoculation medium was distributed in 4ml amount to three tubes. Tubes were marked for Berberis vulgaris, Control 37ºC and Control +4ºC.

In tube marked for extract, 1ml of extract was added. Similarly, 1ml distilled water was added to each control tubes.

All tubes were incubated at 37ºC for 18-20 hours except the tube marked +4ºC, which was kept in the refrigerator at +4ºC.

After overnight incubation turbidity in each tube was checked. Serial dilutions from turbid tubes were prepared from each tube upto 10^{-5}.

From each dilution 3 plates of Nutrient Agar were inoculated for plate count and incubated for overnight. Colonies on each plate were counted and recorded.

Same method was used to test other 8 bacterial cultures. Each experiment was repeated three times.

3. RESULTS

Physical Characters and pH of Crude Extact of Berberis vulgaris:

Following are the physical features of the crude extract of Berberis vulgaris (Table 3.1).

Extract	Parameters		
	Color	Turbidity	pH
Berberis vulgaris fruit extract	Dark brown	Turbid	4.14

Table 3.1 Physical features and pH of extracts.

3.1. Diffusion Method

In order to show the effectiveness of extracts against each microbe data of 06 experiments has been presented statistically in table 3.2 and3.3 in the form of most commonly used measures of position in statistics. These are mean, Median, and mode [12, 21]. The table 3.2 shows that fruit extract of Berberis vulgaris inhibited the growth of Pseudomonas aeroginosa only and the other microorganisms grew normally. Whereas Streptomycin showed significant inhibition of growth of all 9 Bacteria (table 3.3).

Microorganisms	Mean	Median	Mode
E. Coli	0	0	0
Pseudomonas aerogino.	13	13	13
S.typhi	0	0	0
S.typhi A	0	0	0
S.typhi B	0	0	0
S.aureus	0	0	0
Kl.Pneumoniae	0	0	0
Proteus mirabilis	0	0	0
β Streptococci	0	0	0

Table 3.2 shows the antimicrobial activity of Berberis vulgaris against Pseudomonas aeroginosa only in Diffusion Method.

Microorganisms	Mean	Median	Mode
E. Coli	39	39	39
Pseudomonas	38	39	39
S.typhi	40	40	40
S.typhi A	40	40	40
S.typhi B	40	40	40
S.aureus	30	31	32
Kl.Pneumoniae	29	30	30
Proteus mirabilis	28	29	29
β Streptococci	28	29	30

Table 3.3 showing the results of Control in the form of zones of inhibition (m.m.),.

3.2. DILUTION METHOD

In this method, all the selected microorganisms were tested separately and the sensitivity of each microorganism against the plant extract was checked thrice. But it was observed that the extract of Berberis vulgaris inhibited the growth of almost all pathogens as shown in table 3.4. Although it was observed that it is more inhibitory to E.cli, Salmonella typhi A, Staphylococcus aureus and Proteus mirabilis. and in other cases, the extract promoted the growth of all eight bacteria. Table 3.5 exhibited that it is growth promoting as well as slightly growth inhibitiing in the case of

Pseudomonas aeroginosa, Klebsiella pneumonaei and β Streptococci.

Microorganisms	Plant Extract & Controls	Bacterial Count of 3 Replicates (Mean)	Inhibitory Index*
E. coli	Extract	9.07 x 10^7	0.086936
	¬Control 37^{0}C	10.433 x 10^8	
	¤Control 4^{0}C	1.33 x 10^6	
Pseudo. aeroginosa	Extract	7.3 x 10^7	0.123041
	Control 37^{0}C	5.933 x 10^8	
	Control 4^{0}C	2.733 x 10^8	
Salmonella typhi	Extract	1.33 x 10^8	0.86928
	Control 37^{0}C	1.53 x 10^8	
	Control 4^{0}C	1.83 x 10^6	
Salmonella para typhi A	Extract	4.2 x 10^6	0.077777
	Control 37^{0}C	5.4 x 10^7	
	Control 4^{0}C	1.7 x 10^6	
Salmonella para typhi B	Extract	5.33 x 10^7	0.82893
	Control 37^{0}C	6.43 x 10^7	
	Control 4^{0}C	14 x 10^5	
Staphylococcus aureus	Extract	2.6 x 10^7	0.278671
	Control 37^{0}C	9.33 x 10^7	
	Control 4^{0}C	8.6 x 10^5	
Klebsiella pneumoniae	Extract	2.33 x 10^8	1.89431
	Control 37^{0}C	1.23 x 10^8	
	Control 4^{0}C	1.833 x 10^7	
Proteus mirabilis	Extract	8.5 x 10^7	0.10494
	Control 37^{0}C	8.1 x 10^8	
	Control 4^{0}C	2.4 x 10^6	
β Streptococci	Extract	1.033 x 10^8	2.9514
	Control 37^{0}C	3.5 x 10^7	
	Control 4^{0}C	1.433 x 10^6	

Table 3.4A showing actual count and inhibitory index.
*Inhibitory Index: Ratio of bacterial count in treated and untreated samples and calculated by dividing the bacterial count in extract from bacterial count in control 37^{0}C.
¬Control 37^{0}C: The tube that contained distilled water instead of plant extract and kept at 37^{0}C in the incubator to compare the growth with the treated samples.
¤Control 4^{0}C: The initial load of bacterial used in the test and during the test it was kept in the refrigerator to compare the level of growth in the treated samples.

S. #	Bacteria	Growth Inhibition
01	E. Coli	0.086936
02	Pseudo. Aeroginosa	0.123041
03	S.typhi	0.86928
04	S.typhi A	0.077777
05	S.typhi B	0.82893
06	S.aureus	0.27867
07	Kl.Pneumoniae	1.89431
08	Proteus mirabilis	0.10494
09	β Streptococci	2.9514

Table 3.4B Growth inhibitions of bacteria by the fruit extract of Berberis vulgaria in dilution method.

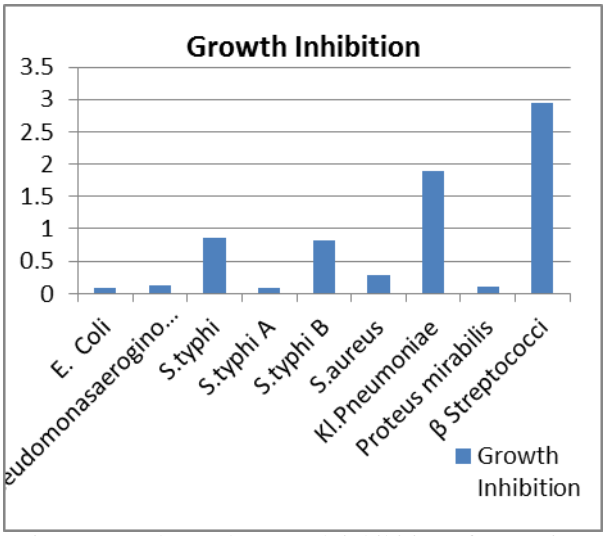

Figure 3.1. shows the growth inhibition of Bacteria by the fruit extract of Berberis vulgaris

S. #	Bacteria	Growth Promotion in Extract	Growth Promotion in Control 37ºC
01	E. Coli	68.1955	784.436
02	Ps. aerog.	267.10574	2170.8745
03	S.typhi	72.677596	83.60656
04	S.typhi A	2.470588	31.76471
05	S.typhi B	38.0714286	45.9286
06	S.aureus	29.988466	10.761245
07	Kl.pneum.	1.27114	6.710311
08	Pr. mirab	35.4167	337.5
09	β Strept.	72.086	24.4242

Table 3.5 shows the comparison of growth in Extract along with Control 37°C

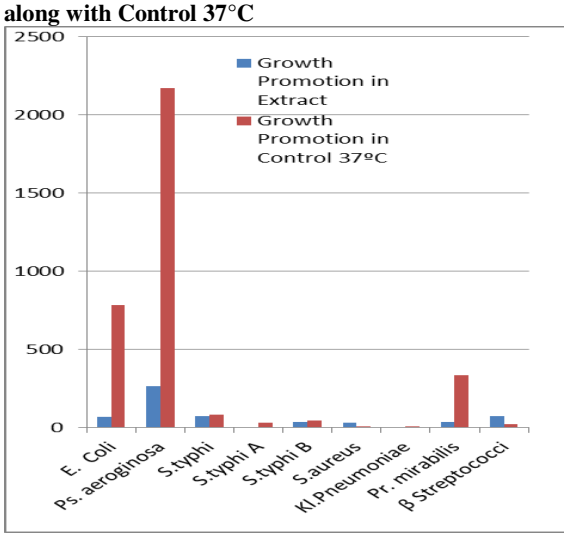

Fig. 3.2 shows the comparison of growth in Extract along with Control 37°C.

S. #	Bacteria	Growth Inhibition	Growth Promotion in Extract	Growth in Control 37ºC
01	E. Coli	0.086936	68.1955	784.436
02	Ps. Aerog.	0.123041	267.10574	2170.8745
03	S.typhi	0.86928	72.677596	83.60656
04	S.typhi A	0.077777	2.470588	31.76471
05	S.typhi B	0.82893	38.071428	45.9286
06	S.aureus	0.27867	29.988466	10.761245
07	Kl.pneum.	1.89431	1.27114	6.710311
08	Pr. mirab.	0.10494	35.4167	337.5
09	β Strep.	2.9514	72.086	24.4242

Table 3.6 shows the comparison of growth Inhibition, growth promotion in Extract along with growth in Control 37°C

3.3 SUMMARY OF RESULTS IN DILUTION METHOD

S. #	Bacteria	**Results**
01	E. Coli	***Growth inhibition***
02	Ps. aeroginosa	***Growth inhibition***
03	S.typhi	***Growth inhibition***
04	S.typhi A	***Growth inhibition***
05	S.typhi B	***Growth inhibition***
06	S.aureus	***Growth inhibition***
07	Kl.Pneumoniae	***Less growth inhibition***
08	Prot. mirabilis	***Growth inhibition***
09	β Streptococci	***Less growth inhibition***

Table 3.7 summarizes the results i.e., antibacterial activity of fruit extract of Berberis vulgaris in the dilution method.

4. DISCUSSION:

In the diffusion method, the fruit extract of Berberis vulgaris has shown its efficacy by inhibiting the growth of Pseudomonas aeroginosa only and it did not exhibit any antibacterial activity against any other pathogen. Although this activity was weak and only13 mm size zones were recorded in each experiment. It might be due to the presence of some factors, which hindered the absorption of extract into the medium. [15] who described that there are some factors including culture medium composition, microorganisms tested and solubility of the extract in the culture medium can change results and there is no relation between diffusion power and antimicrobial activity. The result was supported by Gundidza in 1987, who proved that most plant extracts have low diffusion properties and that is why results in diffusion assays may be different from the results of dilution assays.

In the dilution method, very different results were obtained as compared to the results of the diffusion method. In this method, the extract of Berberis vulgaris inhibited almost all microorganisms but also showed growth promotion along with inhibition (Table 3.6) since all the components of the crude extract were in contact with the test microbes and it might have less growth promoting compounds and thus more inhibitory to all pathogens [6]. The presence of Antioxidant activity

in this fruit extract enhanced the antimicrobial activity [22].

Conclusion: In the new era this type of drug research will open the field for scientists to develop safe drugs from such types of plants, exist in the country and industry to serve not only the nation rather the humanity.

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