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Research Article

IN VITRO ANTIMICROBIAL ACTIVITY AND PRELIMINARY PHYTOCHEMICAL ANALYSIS OF BERBERIS ARISTATA

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ABSTRACT

Berberis aristata (Berberidaceae) is an important medicinal plant and found in the different region of the world. It has significant medicinal value in the traditional Indian and Chinese system of medicine. The aim of the present investigation was undertaken to find out the phytochemical presence and Antimicrobial activity of aqueous and alcholic extract of Berberis aristata. Present study includes determination of phytochemical analysis, antimicrobial study and estimation of total flavonoid content. Preliminary phytochemical analysis showed the presence of carbohydrate, glycoside, alkaloid and flavonoid. Total flavonoid content was found to be 0.98%. Antimicrobial activity shows good positive result with gram positive bacteria. According to observed result it can be said that the concentration of 50µg/ml of plant extracts is the maximum inhibitory concentration.

KEYWORDS: Antimicrobial, Berberis aristata, aqueous and alcoholic, phytochemical.

INTRODUCTION

Berberis aristata belongs to the family Berberidaceae, is an important medicinal plant. It is also called as ‘daruharidra’, found in the Himalayas and other parts of the world. A very valuable ayurvedic preparation ‘Rashut’ is prepared by this plant and are used for the curing of diseases such as, jaundice ophthalmic and skin diseases [1-3]. The plant is an important medicine for the treatment of oxidative stress, remittent fevers, and is used as a cooling laxative to children and as a tonic medicine for liver and heart. The plant contains berberine, oxyanthine, epiberberine, palmatine, dehydrocaroline, jatrorhizine and columbamine,[4] karachine,[5] Four alkaloids, pakistanine, 1-Omethylpakistanine, pseudopalmatine chloride and pseudoberberine chloride[4,6]. It has hypotensive, immuno-stimulating, antiinflammatory, antinfectious, antiprotozoal, activities. It has also antifungal, tuberculostatic antihelminthic, properties. The Bacteria related, parasitic intestinal infections diarrhea, and ocular infections are the most well-known clinical uses of berberine. It has been reported that berberine exhibits local anesthetic, enzyme inhibitory and antipyretic activities [7-10]. Berberis extracts and decoctions have showed significant antimicrobial activity against a variety of organisms including bacteria, viruses, fungi, protozoans, helminths, and Chlamydia. Currently, the predominant clinical uses of berberine include bacterial diarrhea, intestinal parasite infections, and ocular trachoma infections. The most active ingredient of the plant is berberine, a quaternary isoquinoline alkaloid and the content of berberine is used for monitoring the quality of the plant. It is typically found in the roots, rhizomes and stem bark.
MATERIALS AND METHODS

Collection of Plant material and identification: Berberis aristata plant material (root) was collected from the panchmari at Madhya Pradesh India. Identification of plant material was done in the Scan Research Laboratory Indirupuri Bhopal and placed in herbarium and was well documented.

Preparation of the Plant extract

Both aqueous and alcoholic extracts were prepared as described by [11] with slight modifications as adopted in previous studies [12].

Aqueous extracts

Fresh roots of B. aristata (20 g) were thoroughly washed in sterile distilled water; surface sterilized in 70% ethanol (v/v) for 30 sec, and then washed three times in sterile distilled water. The sterilized materials were grounded with a sterile pestle and mortar in sterile distilled water (200 ml). The homogenized tissue was centrifuged at 7,000 rpm for 15 min, supernatant was filter-sterilized and used as the aqueous extract.

Alcoholic extracts

To prepare alcoholic extracts, fresh roots (20g) were homogenized in 95% ethanol (30 ml) and centrifuged as outlined above. The supernatant was put in a hot water bath at 60 °C to evaporate the organic solution. The extract was re-dissolved in 95% ethanol to get the preferred concentrations (100 mg/ml). The extracts were filter-sterilized before use.

The Qualitative Phytochemical Tests

(Khandelwal, 2005, Kokate, 1994 and Tiwari et al., 2011). The extracts obtained by solvent extraction were subjected to various qualitative tests detect the presence of plant constituents Carbohydrates, Alkaloids, Saponins, Tannins and Triterpenoids etc.

Test for Alkaloids

1ml of the extract, 1ml of Dragendorff’s reagent (Potassium bismuth iodide solution) was added an orange-red precipitate was formed which indicates the presence of alkaloids.

Test for Glycosides

1gm of powered drug was extracted with 10ml of 70% ethanol for 2 minutes, filtered and to the filtrate 10ml of water and 0.5ml of strong solution of lead acetate was added and then again filtered. The filtrate was shaken with 5ml of chloroform. The chloroform layer was separated in a porcelain dish the solvent is removed by gentle evaporation. The cooled residue was dissolved in 3ml of glacial acetic acid containing 2 drops of 5% ferric chloride solution. This solution was carefully transferred to the surface of 2ml of concentrated sulphuric acid. A reddish brown layer is formed at the junction of two liquids and the upper layer slowly becomes bluish green, darkening with standing.

Test for Flavonoids

Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoid

Test of Saponins

Small quantity of alcoholic and aqueous extract was taken separately and 20ml of distilled water was added and shaken in graduated cylinder for 15 minutes length wise. A 1cm layer of foam indicates the presence of saponins. No saponins were indicated in extract.

Test of Fats and Fixed oils

1ml of the extract few drops of 0.5N alcoholic Potassium hydroxide along with a drops of phenolphthalein was added. The mixture was then heated on a water bath for 1-2 hours. The formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats. But there was no such formation of soap which indicates the absence of fats.

Test for Triterpenoids

Two or three granules of tin metal were dissolved in 2 ml of thionyl chloride solution. Then 1ml of the extract was poured into test tube and warmed, pink color was produced which indicates the presence of triterpenoids. No such colour is produced which shows the absence of triterpenoids.

Test for Carbohydrates

2ml of the extract, 1ml of α- naphthol solution and sulphuric acid was added. Purple color is formed at the junction of the two liquids which reveals the presence of carbohydrates.

Test for Protein

1 ml of 40 % sodium hydroxide solutions and 2 drops of 1 % copper sulphate solution was mixed in test tube till a blue color is produced, and then 1ml of the extract was added. If Pinkish color is formed shows presence of proteins but no such colour is formed indicating absence of proteins.

Total flavonoids content estimation

Determination of total flavonoids content was based on aluminium chloride method. 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 25-150g/ml were prepared in methanol.
Screening for antimicrobial activity

Disk diffusion method was used for the determination of antimicrobial activity. Clinical isolates of Gram-positive and Gram-negative bacteria were collected from the Scan Research laboratory Indirpuri Bhopal for anti microbial activity assay. The clinical bacterial species used were *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhimurium*. Discs containing aqueous and alcoholic extracts at concentrations of 50, 25 and 12.5 μg/ml were used for susceptibility testing. Ciprofloxacin was used as standard antibiotics.

Statistical Analysis

Data were statistically analyzed by using either one way or two way analysis of variance (ANOVA)

RESULTS

Preliminary phytochemical analysis of *Berberis aristata* showed the presence of carbohydrate, glycoside, alkaloid, flavonoid while protein, saponin, triterpenoids and fats and fixed oils were absent. Total flavonoid content was determined and found to be 0.98%. In the present analysis calibration curve of standard berberine was found to be linear \( y = 0.004x + 0.001, \ R^2 = 0.98 \), which was presented in the Figure. The different three concentrations (12.5μg/ml, 25μg/ml and 50μg/ml) of *Berberis aristata* plant extracts were show antimicrobial effect against the gram positive and gram negative bacteria. According to this observed result it can be said that the concentration of 50μg/ml of plant extracts is the maximum inhibitory concentration.

Presence of Phytochemicals

Different methods were followed to find out qualitatively the presence of phytochemical constituents in the extract. The extract of the plant possessed alkaloids, glycosides,flavonoids and carbohydrates while sasponins, fats and fixed oils, triterpenoids and proteins were absent.

<table>
<thead>
<tr>
<th>Chemical tests</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+Ve</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+Ve</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+Ve</td>
</tr>
<tr>
<td>Saponins</td>
<td>-Ve</td>
</tr>
<tr>
<td>Fats and Fixed Oils</td>
<td>-Ve</td>
</tr>
<tr>
<td>Triterpenoids</td>
<td>-Ve</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+Ve</td>
</tr>
<tr>
<td>Proteins</td>
<td>-Ve</td>
</tr>
</tbody>
</table>

+Ve and –Ve signs report the relative presence and absence of constituents in *Berberis aristata*

Total flavonoids content estimation (TFC)

Total flavonoids content was calculated as quercetin equivalent (mg/g) using the equation based on the calibration curve: \( Y=0.004 \ X+0.001, \ R^2=0.996 \), where X is the absorbance and Y is the quercetin equivalent (QE). The flavonoids in the presence of aluminum chloride have an deep yellow fluorescence which observed under UV spectrophotometer at 510 nm. The calibration curve with quercetin at different concentrations 25mg/L, 50mg/L, 75mg/L, 100mg/L, 125mg/L and 150mg/L is shown in figure.
Table 2: Estimation Total flavonoids content (TFC)

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Conc. (μg/ml)</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>25</td>
<td>0.119</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>0.195</td>
</tr>
<tr>
<td>3</td>
<td>75</td>
<td>0.297</td>
</tr>
<tr>
<td>4</td>
<td>100</td>
<td>0.387</td>
</tr>
<tr>
<td>5</td>
<td>125</td>
<td>0.517</td>
</tr>
<tr>
<td>6</td>
<td>150</td>
<td>0.626</td>
</tr>
</tbody>
</table>

Results of Antimicrobial Study

The extract showed wide antibacterial activity against Gram-positive bacteria and gram-negative bacteria; best being given by extracts at a concentration of 50μg/ml (Table 3). Alcoholic and aqueous extract showed antimicrobial activity against four tested bacteria. Positive controls formed considerably sized inhibition zones against tested bacteria. *Barberis aristata* showed highest zone of inhibition for *Bacillus subtilis* followed by *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhimurium*. According to this observed result it can be said that the concentration of 50μg/ml of plant extracts is the maximum inhibitory concentration. The antibacterial activity of the extract against clinical isolates was comparable to that of standard strains (Table 3). It is interesting to note down that the Gram-negative *Bacteria* reported here as prone to the extracts of *B. aristata* are important human pathogens responsible for causing diarrhea and dysentery.
**DISCUSSION**

Physicochemical standards are usually used for determining the character, purity and strength of the drug source. These characters are also used to confirm the actual nature of the crude drug. Hence it plays an important role in preventing the possible steps of adulteration. The photochemical analysis conducted on *Berberis aristata* extracts revealed the presence of alkaloid, carbohydrate, glycoside, flavonoid and other phytoconstituents. The phytochemical analysis revealed the presence of different phytoconstituents including triterpenoidal, phenolic and flavonoidal compounds. The medicinal properties of plants are due to the presence of different classes of secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, sterols, etc [13]. In the existing literature, berberine has been reported to be produced by various Berberis Spp. Including *B. nepalensis*, *B. asiatica*, *B. vulgaris* and *B. lycium*. [14], *B. stolonifera* [15]. The most active constituent of *Berberis aristata* is berberine, a quaternary isoquinoline alkaloid and the content of berberine is used as biomarker of the plant. Flavonoids have a membrane permeability effect and are considered as potential antioxidants and have caring action against allergies, inflammation, platelet aggregation, microbes, ulcers and hepatoxins, [16 – 19]. Phenols and phenolic compounds are greatly used in skin infections, wound healing, inflammation; antioxidant, immune enhancers, anti clotting and hormone modulators [17].

Ethno botanically, *Berberis aristata* has been used against a broad range of ailments [20]. Uneven activities of different plant parts (root, stem) of this plant have been reported against different bacterial and fungal human pathogens in different solvents [21 – 22]. A majority of the described antimicrobial effects of *Berberis aristata* have been accredited to their secondary metabolites, particularly alkaloid compounds [11]. In this study, the extract of *Berberis aristata* are found to be most active in inhibiting the growth of pathogens viz., *B. subtilis*, *S. aureus*, *E. coli* and *S. typhimurium*. The root extract of *Berberis aristata* were found to be mainly active in inhibiting the growth of gram positive bacteria and gram negative bacteria. The antimicrobial activity of *Berberis aristata* extract against tested bacteria may be due to the presence of secondary metabolites mainly berberine, an isoquinoline alkaloid with a bright yellow colour. Published reviews have described a majority of antimicrobial effects of the extracts of *Berberis* species have been accredited to their secondary metabolites, especially alkaloids. The present study clearly reveals the antimicrobial nature of *Berberis aristata* and recommended that this plant could be exploited in the management of diseases caused by microbes in plants and animals.

**CONCLUSION**

On the basis of result obtained it can be concluded that *Berberis aristata* root possess phytochemical constituents and have antimicrobial activity. We have been able to demonstrate that the root of *Berberis aristata* contains different compounds that have health benefits. The amount of alkaloids is high, because the plant is reported to have berberine, a major plant alkaloid. The plant extract also showed antibacterial activity against major pathogens.

**REFERENCES**


**Table 3: Inhibition zones (mm) of aqueous and alcoholic extract of Berberis aristata against Gram-positive and Gram-negative bacteria species**

<table>
<thead>
<tr>
<th>Bacterial test</th>
<th>Alcoholic extract (µg/ml)</th>
<th>Aqueous extract (µg/ml)</th>
<th>Ciprofloxacin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50</td>
<td>25</td>
<td>12.5</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>26.8±2.6</td>
<td>21.1±2.7</td>
<td>14.0±2.1</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>27.0±0.4</td>
<td>15.0±2.4</td>
<td>10.1±0.1</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>13.5±1.4</td>
<td>9.4±0.3</td>
<td>9.4±0.4</td>
</tr>
<tr>
<td><em>Salmonella typhimurium</em></td>
<td>10.0±2.7</td>
<td>10.0±2.5</td>
<td>8.1±1.4</td>
</tr>
</tbody>
</table>

Diameter of zone of inhibition is a mean of triplicates ± SE (mm). Differences were assessed statistically using one way ANOVA.


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