ejpmr, 2020,7(9), 629-631

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

PHYTOCHEMICAL AND ANTIMICROBIAL ACTIVITY EVALUATION STUDIES OF BERBERIS ARISTATA

Roopa B.[1], Yogeesh M.*[1], Shwetha S. P.[1], Jayanth R.[1] and Netravathi R.[1]

[1]Department of PG studies and Research in Botany, Tumkur University, Tumkur-572106.

***Corresponding Author: Yogeesh M.**

Department of PG studies and Research in Botany, Tumkur University, Tumkur-572106.

Article Received on 20/07/2020 **Article Revised on 11/08/2020** **Article Accepted on 01/09/2020**

ABSTRACT

Berberis aristata (Berberidaceae) is an important medicinal plant & found in the different region of the world. It has significant medicinal value in the traditional Indian & European system of medicine. The aim of the present investigation was undertaken to find out phytochemical & antimicrobial activity of aqueous and alcoholic extract of *Berberis aristata*. In this study includes determination of phytochemical analysis (qualitative and quantitative), antimicrobial study & estimation of total steroid content. Preliminary phytochemical analysis showed by presence of carbohydrates, glycosides, flavonoid, tannin & steroid. Total steroid content was found to be 2.67%. Antimicrobial activity showed good activity against gram positive and negative bacteria but in case of fungi shoed positive result against *Penicillium brocae* and sensitive to *Aspergillus terreus*.

KEYWORDS: Antimicrobial activity, *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 Himalayan region and other parts of the world. It is traditionally used in India & Nepal to accelerate process wound healing, jaundice. It is use in the management of infect wounds has also been described in Ayurvedic classical texts (Sushrutha Samhita 1963). *Berberis aristata* commonly known as "Daruhaldhi & chitra". Is spinous herb shrub native to northern Himalaya region. The plant is a widely distributed from Himalayas to Srilanka, Bhutan & region. It grows at the height of 2000-3000m especially in kumaon and chammba region of himachal Pradesh. It is also found in nilgris hills in south india.

The world health organization (WHO) has also recommended the evaluation of the effectiveness for various plant treatments of disease conditions where we lack safe of modern drugs. The decoction of the root is used as a wash for infected wounds and ulcers and said to help healing and promote cicatrisation (Khory and Kartak, 1985). Despite the medicinal importance of the plant species especially root extract, reports on its antibacterial and antifungal activities are still limited (Dutta & Panse 1962). The antimicrobial activites of hydro alcoholic (50% extract) of four Berberis species, including that *Berberis aristata*.

In these present study, we developed solvent extract for phytochemicals by qualitative, quantitative and antimicrobial activity.

MATERIALS AND METHODS

Collection of Plant

Collection of plant material and identification: *Berberis aristata* plant material (root) was collected from the Belagum at Karnataka India.

Preparation of the plant extract

Both aqueous and alcoholic extracts were prepared as described by with slight modifications as adopted in previous studies. The root *Berberis aristata* were collected from Belgum district respectively in 9th February 2020. The root sample of *Berberis aristata* plant were mechanically 5g of powdered and subjected to extraction using methanol and ethanol (de castro and ayuso 1988). The crude extract were collected in air tight plastic and glass containers and stored in cool condition.

The Qualitative Analysis

The extracts obtained by solvent extraction were subjected to various qualitative tests detect the presence of plant constituents Carbohydrates, Alkaloids, Flavonoids, protein, Tannin and steroids etc. (Bhardwaj D, Nutan Kaushik,2012 and Tiwari *et al.*, 2011).

Test for Alkaloids

Add 2 ml of sample in clean test tube and add 3drops of picric reagent and observed for formation of light yellow precipitate.

Test for Flavonoids
Add 2 ml of sample in a test tube and added few drops of concentrated NH_3 (ammonia) solution and kept observation for yellow colour.

Test for Tannin
Add 2ml of sample in to dried test tube and add 3ml of distilled water and add few drops of 0.1% ferric chloride and observed for dark precipitate.

Test for Steroids
Add 1ml of sample to a test tube and add 2ml of glacial acetic acid and also add 2ml of con H_2SO4 and observed for colour change from violet to blue green.

Test for carbohydrates
Two ml sample were taken in test tube than add 10ml water, 2 drops of 20% ethanolic α-napthol and 2 ml of con H_2SO_4 and kept for observation to get reddish violet ring at the junction.

Test for Protein
1ml of sample in a test tube and 1ml of con H_2so_4Change in colour precipitate from white to yellow on boiling.

THE QUANTITATIVE ANALYSIS
Total Steroids content estimation
Determination of total steroids content was based on quantitative method. 5g powdered sample was dissolved in 50ml distilled water + HCL .filter using what man filter paper transfer the filtrate into separating funnel to that add equal volume ethyl acetate were added to it and mix well and allow to spear the 2 layer discard the aqueous layer. Collect the solvent extraction in a beaker the extract was dried at 100^0c for 5 min in a steam/ water bath. They were then heated with con amyl alcohol to extract the steroid. The mixture become turbid and cool, reweighed what man filter paper were used to filter the mixture kept the filter paper at 60^0c in oven for 5-10 min cool using desiccator for half an hour. (Yokosuka et al., 2000).

Test for Flavonoids
Weigh 5gm of plant powdered sample into a conical flask. Add 50ml distilled water. Add 2ml of Hcl solu & boil for 30 minute in a water bath & allow it cooling. The mixture is filtered through filter paper. Add 10ml of the ethyl acetate and shake vigorously for 2 minute, let the layers separate, drain out the lower aqueous layers, and filter the solvent extract into a pre-weigh filter paper. Confirm the complete filtration and remove the filter paper carefully with the help of a needle. Place the filter paper for drying in oven at 60^0C for at least 30 minute. After drying cool it, place in desiccator for constant temperature and weigh immediately.

Test for carbohydrates
Take 100mg of sample in a boiling tube. Hydrolyze by keeping it in a boiling water bath for 3 hours with 5ml of 2.5N Hcl & cool. Neutralize it with soild sodium carbonate untill the effervescence. Make up to 100ml & centrifuge. Collect the supernatant and take 0.5 & 1ml aliquots for the analysis. Prepare the standards by taking 0, 0.2, 0.4, 0.6, 0.8 & 1ml of the working standard.'o' serves as blank. Make up the volume to 1ml in all the tubes inculding the sample tubes by adding distilled water. Then add 1ml of dinitro salicylic acid reagent. Heat for 8 minutes in a boiling water bath. Cool rapidly and add 5ml of distilled water. Read the absorbance at 540nm. Draw a standard graph by taking concentration of the standard on the X- axis and absorbance on the Y-axis.

- From the graph calculate the amount of carbohydrate present in the sample.

TEST FOR PROTEINS
a) Extraction of protein from Sample
Extraction is usally carried out with buffers used for the enzyme assay. Weigh 500mg of the sample and grind wellusing pestle and mortar by adding 5-10ml of the buffer. estimation Centrifuge and use the supernatant for protein estimation.

(b) Estimation of protein
standard into a series of test tubes. Pipette out 0.1ml and 0.2ml of the sample extract in two other test tubes. Make up the entire test tubes to 1ml by adding distilled water. A tube with 1ml of water serves as the blank. Add 5ml of reagent C to each tube including the blank and mixed well and allow to stand for 10 min. Add 0.5ml of reagent D and mix well and incubate at room temp, in the dark for 30 minute. Blue color is developed. Read the absorbance at 660nm. A standard graph was drawn and the amount of protein in the sample was calculated.

Test for Tannins
Take 2ml of sample to 1litere conical flask. Add 25ml of indigo solution & 750ml of distilled water. Titrate against 0.1N $KMNO_4$ & until blue colour solution to green colour, add again few drop $KMNO_4$ & until solution become golden yellow. Prepare blank by in same manner using distilled water 25ml indigo solution + 750ml distilled water + titrate against $KMNO_4$ end point (blue to green) (green to golden yellow).

Antimicrobial activity
Antimicrobial and antifungal studies were carried out by using nutrient agar and czpeck dox agar. The diffusion method was used for the determination of antimicrobial activity. The gram positive and gram negative bacteria (*Staphylococcus aureus* and *Escherichia coli*) were used for anti- microbial activity assay. The sample were extracted from Soxhlet, the extract were added to the plates and observed for zone of inhibition.

RESULTS AND DISCUSSION
The plant roots were collected from Belagahvi. The roots were extracted from solvent (Ethanol, and methanol solvent) and extract were used for Preliminary phytochemical analysis (qualitative) of *Berberis aristata*

showed the presence of carbohydrates, flavonoid, protein, tannin, steroids and absence of alkaloids (Table-1). The total steroid content was determined and found to be 2.67%. in the analysis of quantitative estimation secondary metabolites. *Berberis aristata* plant extracts were show antimicrobial effect against the gram positive and gram negative bacteria and also showed antifungal activity of the extract. The results were observed and documented in table-3.

Basanta Lamichhane et al., (2014) were highlighted the phytochemical constituents in qualitative screening. They found 2.45% crude alkaloid content in quantitative analysisOther phytochemicals like steroids, flavonoids, coumarin and terpenoids are also present in trace amount. Experimental observation declared that the plant extract were sensitive against *C Albicans*, *S typhii*, *P aeruginosa* and *E coli*, while it didn't show any activity against *K pneumonia*, *S aureus*. Our results are good agreements with Basanta Lamichhane et al., (2014).

Zakir Hussain Maliket al., (2017) were discussed Qualitative phytochemical analysis of the methanolic extracts of *B.aristata* was done to screen the presence of phytochemical constituents. In his study highlighted with antimicrobial activity of the extracts against *E.coli*, *S.aureus*, etc.

Seema saxena et al., (2014) worked on antimicrobial activity of six human pathogenic bacteria by using leaves extracted from *Berberis aristata*. Gahlaut and Chhillar (2013) in their study on *B. aristata* leaves reported MIC of 5 mg/ml against *E.coli* towards aqueous extract. This significantly supports present findings on the effectiveness of plant extracts against *E. coli.*

Table 1: Shows Presence/ Absence of Phytochemicals.

Secondary metabolites	Results
Alkaloids	-ve
Flavonoids	+ve
Protein	+ve
Tannin	+ve
Steroids	+ve
Carbohydrates	+ve

Table 2: Quantitative Phytochemicals of *Berberis aristata.*

Sample	Flavonoids	Protein	Tannin	Steroids	Carbohydrates
Root extract	1.992%	0.6 mg/ml	2.1mg	2.67%	930mg/ml

Table 3: Antimicrobial activity of *Berberis aristata.*

Solvent used for Root sample	*Staphylococcus aureus* (ZI in cm)	*E-coil* (ZI in cm)	*Pencillium brocae* (ZI in cm)	*Aspergillus terreus* (ZI in cm)
Ethanol	0.8	0.65	0.8	S*
Methanol	0.9	0.7	0.2	S*

Where, S*= Sensitive and ZI= Zone of inhibition

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