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Antimicrobial properties of extracts and compounds isolated from *Berberis jaeschkeana*

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Abstract

The present study was undertaken to evaluate the anti microbial properties of *Berberis jaeschkeana* Schneid Var. *jaeschkeana* for the first time. The screening of *B. jaeschkeana* for its phytochemical constituents showed the presence of alkaloids, glycosides, flavonoids, steroids, saponins reducing sugars and terpenoids. Crude ethanolic extract and different fractions showed good anti-microbial properties. Five compounds isolated for the first time from this plant also showed good anti-microbial properties. Columbamine was found to have excellent anti-microbial properties among all the compounds.

Introduction

Medicinal plants are very important for the healthy lives of most of the people across the world. The medicinal importance of these plants is due to the physiological actions of several classes of biologically active compounds. These classes of compounds include alkaloids, flavonoids, tannins and phenolic compounds. (Hill, 1952).

Berberis is an ever green shrub with yellow stem and simple leaves. The genus *Berberis* have about 400-450 species found in Asia, Mediterranean region and America. In Pakistan *Berberis* is represented by 20 species which are mostly found in the mountainous parts (Jafri, 1975). The most important chemical constituents of *Berberis* are isoquinoline alkaloids. *Berberis* species have established cardiovascular, gastro-intestinal and wound healing activities (Mohsen and Hossein, 2008). The most important *Berberis* alkaloid is berberine which has shown many pharmacological activities like anti-inflammatory, antimicrobial, anti-tumor, anti-diabetic, anti-hepatitis and antidiarrhea (Rashmi et al., 2008).

Material and Methods

Plant collection

Berberis jaeschkeana Schneid var. *jaeschkeana* was collected from Azad Kashmir Pakistan during July 2009 and was identified by Prof. Tanveer Akhtar (Chairperson, Botany Department, University of Azad Jammu and Kashmir). A voucher specimen number 9615-B was deposited in the herbarium of Botany Department University of Peshawar.

Extraction and fractionation

B. jaeschkeana Schneid var. *jaeschkeana* root along with bark (6 Kg) was grinded to powder with a grinder. The powdered plant material was soaked in commercial grade ethanol for ten days. The dilute extract was concentrated with rotary evaporator to yield a gummy residue. (412 g) The crude residue was fractionated following acidification and basification method for alkaloids. The acidic fraction, obtained by treating crude extract with 5% HCl, was termed as fraction A (127 g). After the removal of fraction A, the filtrate was extracted with dichloro-methane to yield fraction B (28

g) and basic fraction was obtained with EtOAc after basification with ammonia and it was termed as fraction C (93 g). While the left over aqueous fraction after the separation of fractions A, B and C was termed as fraction D.

Phytochemical screening

Phytochemical tests were carried out for crude ethanolic extract, fraction A, B and C to identify the chemical constituents (Edeoga et al., 2005; Uddin et al., 2012).

Alkaloids

0.2 g of crude ethanolic extract and each fraction were warmed with 2% H_2SO_4 for 2 min. After filtration of the reaction mixture a few drops of Dragendroff's reagent were added to each filtrated fraction. Orange red precipitate indicates the presence of alkaloids.

Glycosides

Ethanolic extract and each fraction was first acidified with dilute HCl and then neutralized with NaOH solution. Now a few drops of Fehling's solution A and B were added to each mixture. Formation of red precipitate indicates the presence of glycosides.

Flavonoids

Five milliliters of dilute ammonia solution was added to the aqueous filtered solution of each fraction followed by the addition of conc. H_2SO_4. The appearance of yellow color indicated the presence of flavonoids. The yellow color disappeared after some time.

Steroids

Two mL of acetic anhydride was added to 0.5 g ethanolic extract of each fraction followed by adding 2 mL H_2S0_4. The color changed from violet to blue or green indicated the presence of steroids.

Saponins

About 2 g of the powdered sample was boiled in 20 mL of distilled water in a water bath and filtered. 10 mL of the filtrate was mixed with 5 mL of distilled water and shaken vigorously. The appearance of frothing indicated the presence of saponins.

Reducing sugars

Each sample was shaken with distilled water first and then filtered. To each filtrate a few drops of Fehling's solution A and B were added and boiled for few minutes. The appearance of an orange red precipitate confirmed the presence of reducing sugars.

Terpenoids

About 0.2 g of the each sample was mixed with 2 mL of chloroform first and then 3 mL of concentrated H_2SO_4 was added to each mixture. The formation of a reddish brown coloration at the interface indicated the presence of terpenoids.

Microbial culture preparation

Five strains of bacteria namely *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Bacillus subtilis* and *Staphylococcus epidermidis* were used for the anti-microbial activity. The bacterial strains were collected from stock culture of Phytopharmaceutical and Neutraceutical research laboratories (PNRL), Institute of Chemical Sciences, University of Peshawar Pakistan. These organisms were placed in Muller-Hinton agar in the refrigerator at 4°C prior to subculture.

Anti-microbial activity of the various extracts and pure compounds against selected bacterial species

Modified agar-well diffusion method was used to test the anti-bacterial properties of the crude extract, fractions and pure compounds. Muller-Hinton agar was used as medium. The cultures were taken in triplicates at incubation temperature of 37°C for 24 to 72 hours. The broth culture (0.6 mL) of the test organism was placed in a sterile Petri-dish and 20 mL of the sterile molten MHA was added. Holes were bored in the medium using 0.2 mL of the extract, fractions and pure compounds. Streptomycin was used as standard anti-microbial agent at a concentration of 2 mg/mL. Inoculation was done for 1 hour. Incubation was done at 37°C for 24 hours and the diameters of the zone of inhibition of microbial growth were measured in mm.

Results and Discussion

The crude ethanolic extract of *B. jaeschkeana* was evaluated for its antimicrobial potential. Upon positive results the crude extract was fractionated into acidic and basic fractions according to the fractionation scheme for alkaloids. Then each fraction was evaluated for its antibacterial properties which also showed positive results (Table I; Table II). Five known compounds namely berberine (Lenka et al., 2007), columbamine (Tian et al., 2004), syringic acid (Sheng-Ming et al., 2006), berberine chloroform (Radek et al., 2003) and jatrorrhizine (Trinh, et al., 2006) were isolated from different fractions of *B. jaeschkeana* (Table III). The structures were confirmed by com-paring 1D and 2D NMR data of all the five compounds with the literature.

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Table I

Results of preliminary screening

Class of compound	Crude extract (ethanol)	*B. jaeschkeana-A*	*B. jaeschkeana-B*	*B. jaeschkeana-C*
Alkaloids	Present	Present	–	Present
Glycosides	Present	–	Present	Present
Flavonoids	Present	–	Present	Present
Steriods	Present	–	Present	–
Saponins	Present	–	–	Present
Reducing sugars	Present	–	Present	–
Terpenoids	Present	–	Present	–

Table II

Antimicrobial results for crude and fractions

Sample	*Staphylococcus aureus*	*Escherichia coli*	*Staphylococcus epidermidis*	*Klebsiella pneumoniae*	*Bacillus subtilis*	Streptomycin
Crude ethanol extract	14	12	16	14	16	30
RBJ- A	16	18	18	16	18	28
RBJ- B	14	14	16	14	16	30
RBJ- C	16	18	16	14	18	28

RBJ-A = Acidic fraction; RBJ-B = Dichloromethane fraction; RBJ-C = Basic fraction of *B. jaeschkeana*

Table III

Antimicrobial results for pure compounds

Sample	*Staphylococcus aureus*	*Escherichia coli*	*Staphylococcus epidermidis*	*Klebsiella pneumoniae*	*Bacillus subtilis*	Streptomycin
Berberine	14	16	18	18	20	26
Columbamine	20	18	14	22	20	28
Syringic acid	20	18	16	20	18	28
Berbreine chloroform	16	20	18	14	18	28
Jatrorrhizine	16	20	18	12	20	26

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