Antimicrobial and Phytochemical Screening of *Rubus fruticosus*

Rajeswari T[1*], Venil C.K[1], Sathya R[1], Sunitha K[2] and Umavisalakshi S[2]

[1]Department of Microbiology, [2]Department of Industrial Biotechnology
Karpagam University, Coimbatore - 641021, Tamil Nadu, India
Email: rajeswarithamarai@rediffmail.com

Summary

Since plants are used as therapeutic agents, the present study was conducted to evaluate the phytochemical profile of *Rubus fruticosus* seed by using petroleum ether, chloroform, ethyl acetate, ethanol and water extract. The antimicrobial activities were tested against bacteria: *Staphylococcus aureus, Bacillus subtilis* and fungi: *Aspergillus niger, Penicillium* sp by agar disc diffusion method. Among the various extract, aqueous extract showed the highest antibacterial activity. Presence of phytochemicals such as alkaloids, steroids, flavanoids, saponins, tannins, terpenoids and carbohydrates were observed.

Keywords: *Rubus fruticosus*; antimicrobial activity; phytochemical screening; aqueous extract

Introduction

Infectious diseases are the leading cause of death worldwide. The clinical efficiency of many existing antibiotics is being threatened by emergence of multidrug resistant pathogens[1]. *Rubus* species have been traditionally used for therapeutic purposes. For instance, extracts of leaves and roots of this genus have been used for the treatment of diabetes mellitus, rheumatism, sore throat, hemorrhoid, diarrhea and similar enteric disorders[2 - 6]. *Rubus fruticosus* commonly called as naval seed in Tamil, belongs to the family Rosaceae. It is widely used in traditional medicine for arresting diarrhea, settling an upset, nervous stomach and even soothing a stomach-flu. The blackberry is known to contain polyphenol antioxidants, naturally occurring chemicals that can up regulate certain beneficial metabolic processes in mammals. The astringent blackberry root is sometimes used in herbal medicine as a treatment for diarrhea and dysentery[7].

The development of antibacterial resistance is multifactorial, including the specific nature of the relationship of the bacteria to antibiotic, how the antibacterial is used, host characteristics and environmental factors. To overcome the problem of antibiotic resistance, medicinal plants have been extensively studied as alternative treatments for diseases

The aim of the present study was to determine the antimicrobial activity and phytochemical screening of various extracts of *Rubus fruticosus* seeds which is having traditional claim for several diseases.

Materials and Methods

Plant Material

The seed materials used in this study were collected from various locations in Tamil Nadu, India. The plant was identified with the herbarium of Botanical Survey of India, Southern circle, Coimbatore, India. The seed material were washed under running tap water, shade dried and then homogenized to fine powder and stored in airtight container.

Preparation of extracts

About 20 g of powdered plant material of *Rubus fruticosus* was taken. The sample was mixed with 100ml of petroleum ether. It is then subjected to occasional shaking for 24 hours. After 24 hours, the sample mixed with petroleum ether has been filtered through Whatmann No.1 filter paper .The extracts thus obtained is concentrated by evaporation at room temperature. Similarly Benzene, Chloroform, Ethyl acetate, Ethanol and Water extracts were prepared using 100 ml of solvents. Yields of each extracts were 8.3% for petroleum ether, 9.2% for Benzene, 17.6% for chloroform, 25.2% for Ethylacetate, 47.2% for Ethanol, 60% for water.

Antimicrobial susceptibility testing agar disc diffusion method[8]

Antimicrobial activity of the plant extracts was tested using the disc diffusion method. Sterile Muller Hinton agar plates were inoculated with the broth cultures of *Staphylococcus aureus, Bacillus subtilis*, *Aspergillus niger* and *Penicillium* sp. using sterile cotton swabs. The filter paper disc of 6mm diameter were prepared and sterilized. 10mg/ml, 5mg/ml, 2.5 mg/ml concentrations of the plant extracts were added to each disc of holding capacity 15 micro liters. The sterile impregnated disc with plant extract were placed on the agar surface with flamed forceps and gently pressed down to ensure complete contact of the disc with the agar surface. Then the plates were incubated at inverted position. After incubation the size (diameter) of the inhibition zones were measured.

Phytochemical screening [9]

The collected extracts were subjected to preliminary phytochemical testing for the detection of major chemical groups. The details of the tests are as follows:

Mayer's reagent test for Alkaloids**:** To 1ml of the extract 2ml of Mayer' reagent was added. Dull white precipitate indicates the presence of alkaloids.

Salkowski's test for Steroids and sterols: The extract is dissolved in 1ml of chloroform and equal volume of concentrate H2SO4 was added through the sides of the test tube. The upper layer turns red and the lower sulphuric acid layer turns yellow with green fluorescence shows the presence of sterols and steroids.

Flavonoids: To 1ml of the extract, 1ml of Ferric chloride is added. The formation of brown color confirms the presence of flavonoids.

Saponins: To 1ml of the extract, alcoholic vanillin solution and a few drops of concentrated H_2SO_4 were added. A deep violet color confirms the presence of saponins.

Keller-Killiani test Glycosides: Dissolve the extract in acetic acid containing traces of Ferric chlorides and transfer to a test tube containing sulphuric acid. At the junction, formation of a reddish brown color, which gradually becomes blue, confirms the presence of glycosides.
Tannins: To 1ml of extract add a few ml of 5% ferric chloride solution, a dark blue or bluish black color shows the presence of tannins.
Terpenoids: Add Tin metal pieces and thionyl chloride to ethanol extract. Pink color indicates the presence of terpenoids.
Carbohydrates: Add 5ml of Benedicts solution to plant extract and boil in water bath. A red yellow or green precipitate indicates presence of reducing sugars.

Results and Discussion

The antimicrobial activity of *Rubus fruticosus* of aqueous and ethanolic extract against the microorganism against different concentration of 0.5mg/ml, 2.5 mg/ml, 5.0 mg/ml, 7.5 mg/ml and 10 mg/ml concentration was assessed quantitatively by the presence or absence of inhibition zone.

The antimicrobial activity of *Rubus fruticosus* of petroleum ether, benzene, chloroform, ethyl acetate, ethanol and aqueous extract against *Staphylococcus aureus* and *Bacillus subtilis* were tabulated in Table-1. The aqueous extract showed the highest activity than other extract.

The aqueous extract of *Rubus fruticosus* against *S. aureus* exhibits maximum zone of inhibition about 8 mm and 14 mm and ethanolic extract exhibit about 6 mm, 8 mm and 12 mm zone of inhibition when compared with ethanolic and aqueous extract. The same dilutions were subjected to *B. subtilis,* the maximum zone exhibited only in aqueous extract about 19mm, 20mm and 24 mm and the control drug penicillin shows lowest zone of inhibition.

The extract of *Rubus fruticosus* were subjected against *Aspergillus niger*, the maximum zone of inhibition was obtained only in the aqueus extract about 12mm, 19mm and 24mm and control drug nistatin exhibited lower zone of inhibition about 11mm, 15mm and 20mm. The same dilutions were subjected to *Penicillium* and the maximum zone of inhibition was found in the aquous extract of about 12mm, 20mm and 23mm and control drug nistatin exhibited lowest zone of inhition of about 11mm, 15mm and 20mm.

Further the plant extract was subjected to preliminary phytochemical screening for the presence or absence of different chemical groups (Table - 2). From the results, it reveals that all the extracts of *Rubus fruticosus* showed the positive result for the presence of alkanoids, flavanoids, glycosides and carbohydrates.

The present study was aimed to focus the antimicrobial activity of *Rubus fruticosus*. The inhibitory effect of the extract justified the medicinal use of *Rubus fruticosus* and further study is required to find out the active component of medicinal value.

Table-1: Antibacterial activity of *Rubus fruticosus* on *S.aureus*, *B.substilis*.

S.No.	Name of the organism	Concentration (mg/ml)	Zone of inhibition						
			Rubus fruticosus						
			Petroleum ether	Benzene	Chloroform	Ethylacetate	Ethanol	water	Control* Penicillin /Nystatin
1.	*Staphylococcus aureus*	2.5	-	-	6	-	6	-	4
		5.0	-	-	7	8	8	8	6
		7.5	-	-	7	9	10	12	11
		10.0	-	-	8	10	12	14	12
2.	*Bacillus subtilis*	2.5	-	12	13	-	12	19	4
		5.0	-	14	16	8	19	20	6
		7.5	-	15	18	12	22	21	11
		10.0	-	16	19	14	23	24	12
3.	*Aspergillus niger*	2.5	-	8	5	8	10	12	11
		5.0	-	14	8	16	20	19	15
		7.5	-	15	12	18	21	22	17
		10.0	-	16	14	19	23	24	20
4.	*Penicillium* sp.	2.5	-	6	13	13	13	12	11
		5.0	-	8	16	19	16	20	15
		7.5	-	10	18	22	18	22	17
		10.0	-	14	19	23	19	24	20

* Penicillin for bacteria; Nystatin for fungi

TABLE 2:-PHYTOCHEMICAL SCREENING RESUTS

EXTRACTS/TESTS	Petroleum Ether	BENZENE	CLOROFORM	Ethyl Acetate	ETHANOL	WATER
ALKALOIDS	+	+	+	+	+	+
STEROID& STEROLS	–	–	–	–	–	–
FLAVAROIDS	+	+	+	+	+	+
SAPONINS	–	–	–	–	–	–
GLYLOSIDES	+	+	+	+	+	+
TANNINS	–	–	–	–	–	–
TERPENOID	–	–	–	–	–	–
CARBOHYDRATE	+	+	+	+	+	+

+ Presence - Absence

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