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Antibacterial and antifungal activity of aerial parts of *Rubus racemsosus*

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ABSTRACT

Five different crude extracts: petroleum ether, ethyl acetate, chloroform, methanol and aqueous extracts of Rubus racemosus have been studied for in vitro antibacterial activity and antifungal activities by disc diffusion method. The minimum inhibitory concentration was also determined by agar streak dilution method. All the extracts showed significant and moderate activity against various gram positive and gram negative bacteria and two fungal species when compared with standard.

Keywords: *Rubus racemosus*, Antibacterial activity, Antifungal activity.

INTRODUCTION

Rubus racemosus is a deciduous shrub belongs to the family *Rosaceae*[1]. Traditionally, it is used as astringent, abortifacient, muscle relaxant, Anticonvulsant and free radical scavenging agents[2]. Family Rosaceae is known as folk medicine for treatment to nervous disorders[3]. Decoction of the root is useful for relaxed bowel and dysentery[4]. The chief active constituents are flavanoids, phenolic, glycosides and tannins. Literature review revealed no documentation on antibacterial and antifungal activity of various extracts of *Rubus racemosus*. The present study is therefore an attempt to assess the efficacy of this plant in different concentrations against gram positive, gram negative and fungal species.

MATERIAL AND METHODS

Plant material and extraction

The aerial parts of *Rubus racemosus* were collected from Nilgiri Hills in the month of August in the year 2006. The plant was authenticated by Dr.S.Rajan, Field Botanist, Survey of Medicinal Plants & Collection Unit, (Central Council for Research in Homoeopathy), Department of AYUSH. The aerial parts were shade dried for seven days and then powdered. Powdered material was extracted in soxhlet extractor successively with petroleum ether, ethyl acetate,

chloroform, methanol and water. The residues were collected by evaporation of solvent under reduced pressure by rotary evaporator.

Determination of minimum inhibitory concentration

Agar streak dilution method[5]

MIC of the extract was determined by agar streak dilution method. A stock solution of the extract (100 µg mL^{-1}) in respective solvent was prepared and incorporated in specified quantity of molten sterile agar (nutrient agar for anti-bacterial activity and sabourand dextrose agar medium for anti-fungal activity). A specified quantity of the medium (40 – 50^0C) containing the extract was poured into a petridish to give a depth of 3-4 mm and allowed to solidify. Suspension of the microorganism were prepared to contain approximately 10^5 cfu mL^{-1} (colony forming unit per milliliter) and applied to plates with serially diluted extracts in respective solvent and incubated at 37^0C for 24 hr and 48 hr for bacteria and fungi, respectively. The MIC was considered to be the lowest concentration of the test substance exhibiting no visible growth of bacteria of fungi on the plate.

Antibacterial Activity[6]

The antibacterial activities of different extracts were studied by Disc Diffusion method against the following organisms.

- *Staphylococcus aureus (gram positive)*
- *Staphylococcus epidermides (gram positive)*
- *Bacillus cereus (gram positive)*
- *Micrococcus luteus (gram positive)*
- *Klebsiella pneumoniae (gram negative)*
- *Pseudomonas aeruginosa (gram negative)*
- *Escherichia coli (gram negative)*

Extracts were used in the concentrations of 25, 50 and 100 mcg/ml, using their respective solvents comparing with Ciproflaxacin (5 mcg/disc) as standard. The disc diffusion method was employed for the screening of antibacterial activity.

Disc Diffusion Method[7]

A suspension of *Micrococcus luteus* was added to sterile soyabean casein digest agar media at 45^0C. The mixture was transferred to sterile petri dishes and allowed to solidify. Sterile discs of 5mm in diameter (made from whatmann filter paper previously sterilized by U.V.lamp) dipped in solutions of the different extracts; standard and a blank were placed on the surface of agar plates.

The plates were allowed to stand for 1 hour at room temperature as a period of preincubation diffusion to minimize the effects of variation in time between the applications of the different solutions. Then the plates were incubated at 37^0C for 18 hours and observed for antibacterial activity. The diameters of the zones of inhibition was observed and measured. The average area of zone of inhibition was calculated and compared with that of the standards.

A similar procedure was carried out for the study of antibacterial activity of other extracts against *Staphylococcus aureus, Staphylococcus epidermides, Bacillus cereus, Kl.pneumoniae, Pseudomonas aeruginosa and E.coli.*

Antifungal activity

The antifungal activities of the extracts were studied by disc diffusion method against the following organisms.

- *Aspergillus niger*
- *Aspergillus fumigates*

Extracts were used in the concentrations of 25, 50 and 100 mcg/ml using their respective solvents. The standard used was ketaconazole (50 mcg/disc) against both the organisms. The disc diffustion method was employed for the screening of anti fungal activity.

Disc Diffusion Method[7]

A suspension of *Aspergillus niger* was added to Sabouraud Dextrose Agar Media at 45^0C. The mixture was transferred to sterile petridishes and allowed to solidify. Sterile discs 5mm in diameter (made from whatmann filter paper previously sterilized in U.V.lamp) dipped in solutions of the different extracts, standard and a blank were placed on the surface of agar plates. The plates were allowed to stand for 1 hour at room temperature as a period of preincubation diffusion to minimize the effects of variation in time between the applications of the different solutions. Then the plates were incubated at 37^0C for 18 hours and observed for antifungal activity. The diameter of the zones of inhibition was measured for the plates in which the zone of inhibition was observed. The average area of zone of inhibition was calculated and compared with that of the standards. A similar procedure was carried out for the study of antifungal activity of other extracts against *Aspergillus fumigates.*

RESULTS AND DISCUSSION

Antibacterial Activity

Methanol, Chloroform, Petroleum ether and Aqueous extracts exhibited significant activity at the concentration of 100µg/ml whereas it showed moderate activity at the concentration of 25µg/ml and 50µg/ml against *Staphylococcus aureus*. The Results were shown in Table 1. Methanol, Chloroform, Petroleum ether and Aqueous extracts exhibited significant activity at the concentration of 100µg/ml whereas it showed moderate activity at the concentration of 25µg/ml and 50µg/ml against *Staphylococcus epidermidis.* The Results were shown in Table 2. Methanol, Chloroform, Petroleum ether and Aqueous extracts exhibited significant activity at the concentration of 100µg/ml whereas it showed moderate activity at the concentration of 25µg/ml and 50µg/ml against *Bacillus cereus*. The Results were shown in Table 3. Methanol, Chloroform, Petroleum ether and Aqueous extracts exhibited significant activity at the concentration of 100µg/ml whereas it showed moderate activity at the concentration of 25µg/ml and 50µg/ml against *Micrococcus luteus*. The Results were shown in Table 4. Methanol, Chloroform, Petroleum ether and Aqueous extracts exhibited significant activity at the concentration of 100µg/ml whereas it showed moderate activity at the concentration of 25µg/ml and 50µg/ml against *Klebsiella pneumoniae*. The Results were shown in Table 5. Methanol, Chloroform, Petroleum ether and Aqueous extracts exhibited significant activity at the concentration of 100µg/ml whereas it showed moderate activity at the concentration of 25µg/ml and 50µg/ml against *Pseudomonas aeruginosa*. The Results were shown in Table 6. Methanol, Chloroform, Petroleum ether and Aqueous extracts exhibited significant activity at the concentration of 100µg/ml whereas it showed moderate activity at the concentration of 25µg/ml and 50µg/ml against *Escherichia coli*. The Results were shown in 7.

Antifungal Activity

Methanol, Chloroform, Petroleum ether and Aqueous extracts exhibited significant activity at the concentration of 100µg/ml whereas it showed moderate activity at the concentration of 25µg/ml and 50µg/ml against *Aspergillus Niger* and *Aspergillus fumigatus*. The Results were shown in Tables 8 and 9. The minimum inhibitory concentration values of all the extracts were determined.

Table:1 In Vitro evaluation of Anti-microbial activity of different extracts of Rubus Racemosus on Staphylococcus aureus

Extract	Micro-Organism	Zone of Inhibition (mm)					MIC Values mcg/ml
		Std.	Control	25 (mcg/ml)	50 (mcg/ml)	100 (mcg/ml)	
Methanol	Staphylococcus aureus	30	0	16	19	25	15
Chloroform		30	0	17	24	27	14
Petroleum ether		30	0	18	20	24	13
Aqueous		29	0	17	19	22	14

Table:2 In vitro evaluation of Anti-microbial activity of different extracts of Rubus racemosus on Staphylococcus epidermidis

Extract	Micro-Organism	Zone of Inhibition (mm)					MIC Values (mcg/ml)
		Std.	Control	25 (mcg/ml)	50 (mcg/ml)	100 (mcg/ml)	
Methanol	Staphylococcus epidermidis	31	0	18	21	24	17
Chloroform		30	0	17	19	21	16
Petroleum ether		30	0	16	18	20	15
Aqueous		30	0	15	17	20	14

Table:3 In vitro evaluation of Anti-microbial activity of different extracts of Rubus racemosus on Bacillus cereus

Extract	Micro-Organism	Zone of Inhibition (mm)					MIC Values (mcg/ml)
		Std.	Control	25 (mcg/ml)	50 (mcg/ml)	100 (mcg/ml)	
Methanol	Bacillus cereus	31	0	16	19	26	15
Chloroform		30	0	17	20	27	14
Petroleum ether		30	0	16	19	21	15
Aqueous		30	0	14	18	22	13

Table:4 In vitro evaluation of Anti-microbial activity of different extracts of Rubus racemosus on Micrococcus luteus

Extract	Micro-Organism	Zone of Inhibition (mm)					MIC Values (mcg/ml)
		Std.	Control	25 (mcg/ml)	50 (mcg/ml)	100 (mcg/ml)	
Methanol	Micrococcus luteus	30	0	17	22	25	16
Chloroform		31	0	15	21	24	14
Petroleum ether		31	0	16	20	23	15
Aqueous		30	0	17	21	24	16

Table:5 In vitro evaluation of Anti-microbial activity of different extracts of Rubus racemosus on Klebsiella pneumoniae

Extract	Micro-Organism	Zone of Inhibition (mm)					MIC Values (mcg/ml)
		Std.	Control	25 (mcg/ml)	50 (mcg/ml)	100 (mcg/ml)	
Methanol	Klebsiella pneumoniae	30	0	17	19	22	14
Chloroform		29	0	16	20	23	15
Petroleum ether		28	0	14	17	22	13
Aqueous		28	0	14	16	21	12

Table:6 In vitro evaluation of Anti-microbial activity of different extracts of Rubus racemosus on Pseudomonas aeruginosa

Extract	Micro-Organism	Zone of Inhibition (mm)					MIC Values (mcg/ml)
		Std.	Control	25 (mcg/ml)	50 (mcg/ml)	100 (mcg/ml)	
Methanol	Pseudomonas aeruginosa	31	0	18	20	22	16
Chloroform		30	0	18	20	23	15
Petroleum ether		32	0	15	18	21	13
Aqueous		32	0	14	19	23	12

Table:7 In vitro evaluation of Anti-microbial activity of different extracts of Rubus racemosus on Escherichia coli

Extract	Micro-Organism	Zone of Inhibition (mm)					MIC Values (mcg/ml)
		Std.	Control	25 (mcg/ml)	50 (mcg/ml)	100 (mcg/ml)	
Methanol	Escherichia coli	30	0	15	19	23	14
Chloroform		32	0	18	22	26	17
Petroleum ether		31	0	16	21	25	15

Aqueous		30	0	15	19	24	14

Table:8 In Vitro evaluation of Anti-fungal activity of different extracts of Rubus Racemosus on Aspergillus niger

Extract	Micro-Organism	Zone of Inhibition (mm)					MIC Values (mcg/ml)
		Std	Control	25 (mcg/ml)	50 (mcg/ml)	100 (mcg/ml)	
Methanol	Aspergillus niger	31	0	15	19	22	14
Chloroform		30	0	14	18	23	13
Petroleum ether		30	0	15	19	22	14
Aqueous		30	0	13	17	21	12

Table:9 In Vitro evaluation of Anti-fungal activity of different extracts of Rubus Racemosus on Aspergillus fumigatus

Extract	Micro-Organism	Zone of Inhibition (mm)					MIC Values mcg/ml
		Std	Control	25 (mcg/ml)	50 (mcg/ml)	100 (mcg/ml)	
Methanol	Aspergillus fumigatus	31	0	17	20	23	16
Chloroform		30	0	16	21	24	15
Petroleum ether		31	0	17	22	25	14
Aqueous		32	0	15	18	21	13

Petroleum ether extract exhibited significant activity against all the tested organisms at the concentration of 100µg/ml whereas it showed moderate activity at the concentration of 25µg/ml and 50µg/ml when compared with standard drug. Methanolic extract exhibited significant activity against all the tested organisms at the concentration of 100µg/ml whereas it showed moderate activity at the concentration of 25µg/ml and 50µg/ml when compared with standard drug. Chloroform extract exhibited significant activity against all the tested organisms at the concentration of 100µg/ml whereas it showed moderate activity at the concentration of 25µg/ml and 50µg/ml when compared with standard drug. Aqueous extract exhibited significant activity against all the tested organisms at the concentration of 100µg/ml whereas it showed moderate activity at the concentration of 25µg/ml and 50µg/ml when compared with standard drug.

The Minimum Inhibitory concentration values of all the extracts were determined. From the above studies it reveals that all the extracts were active against tested bacterial and fungal organisms.

CONCLUSION

Petroleum ether, Ethyl acetate, Chloroform, Methanol and Aqueous extracts of Rubus racemosus have showed significant and moderate activity against anti-bacterial and anti-fungal organisms.

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