



## Phytochemical screening study and antibacterial evaluation of *Symplocos racemosa* Roxb

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### Abstract

Preliminary phytochemical screening of petroleum ether and alcohol extract of *Symplocos Racemosa* Roxb was carried out which indicates that alcoholic extract contain carbohydrate, glycoside, saponin and terpenoid & alkaloid and ether extract indicated presence of glycoside, phytosterol and steroid. An antibacterial evaluation of the petroleum ether and ethanolic extract was carried out and it is found that ethanolic extract possess a good antibacterial action.

**Key Words:** Phytochemical Screening, *Symplocos Racemosa* Roxb, Antibacterial evaluation

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### Introduction

*Symplocos Racemosa* Roxb (*Symplocaceae*) is distributed throughout North East India, up to 2,500 ft., from the terai of Kumaon to Assam and Pegu, Chota Nagpur, Burma. It is a small evergreen tree with stem up to 6 m. height and 15 cm diameter.

Bark is useful in bowel complaints such as diarrhea, dysentery, in dropsy, eye disease, liver complaints, fevers, ulcers; scorpion-string etc. bark is often employed in the preparation of plasters and is supposed to promote maturation or resolution of stagnant tumors.[1-3] In cases of menorrhagia due to relaxation of the uterine tissue, given two to three times a day for three to four days. Alcoholic extract and watery extract of "lodh" as astringent for looseness of the bowels. A decoction of the bark or wood is used as gargle for giving firmness to spongy and bleeding gums and relaxed uvula. [4-6] It is one of the constituent of a plaster or lap used to promote maturation of boils and other malignant growths. Knowledge of chemical constituent of the plant is not only essential for the discovery of therapeutic agents but also for economical source of the alkaloids carbohydrates etc. [7-9]

## Materials and Methods

### Experimental

#### Extraction

The bark of *Symplocos racemosa* was dried under shade with occasional shifting and then made into a coarse powder with a mechanical grinder and stored in airtight container for further use. The dried powder material of bark (was first extracted with petroleum ether (60-80<sup>0</sup>C) and the solvent was removed by rotary evaporator. After the extraction with petroleum ether the same plant material was dried and again extracted with ethanol (95%v/v) and dried using rotary flash evaporator.

#### Preliminary Phytochemical Screening

The petroleum ether extract and ethanol extract were subjected to chemical tests to identify chemical constituent of the plant. [10-16]

#### Test for Sugars

Small quantity of extract was dissolved in 4 ml of distilled water and filtered and the filtrate was subjected to Molisch's test and Iodine Test.

#### Test for Glycosides

A few mg of residue was dissolved 4 ml of distilled water and filtrated and the filtrate was subjected to Legal Test and Borntrager's test.

#### Test for Flavonoids

Shinoda test

The extracts were dissolved in alcohol. One piece of magnesium followed by concentrated hydrochloric acid was added drop wise and heated. Appearance of magenta color demonstrated the presence of flavonoids.

#### Test for Sterols

Salkowaski test

10 mg of extract was dissolved in 2 ml of chloroform and 2ml of concentrated sulphuric acid was added from the side of the test tube. Test tube was shaken for few minutes. The development of red color in chloroform layer indicated the presence of sterols.

#### Liebermann – Burchard Test

1 ml of concentrated sulphuric acid was added to 10 mg of extract in 1ml of chloroform. A reddish – blue color exhibited by chloroform layer and green fluorescence by the acid layer suggests the presence of sterols.

#### Test for Alkaloids

Few mg of extract was taken in 5 ml of 1.5% v/v hydrochloric acid and filtered. These filtrates were then used for testing alkaloids.

**Dragendorff's test**

Dragendorff's reagent was added in 2ml of filtrate. Formation of orange-brown precipitate indicated the presence of alkaloids.

**Mayer's reagent**

To a 1ml of test filtrate in a watch glass, a few drops of mayers reagent were added. If the formation of cream colored precipitate it shows the presence of alkaloids.

**Test for Tannins**

The test extract was taken in water, warmed and filtered. 5 ml of filtrate was allowed to react with 1ml of 5% ferric chloride solution. If dark green or deep blue color is obtained, tannin is present.

**Test for Saponins**

## a) Foam test

1ml solution of extract was diluted with distilled water to 20 ml and shaken in a graduated cylinder for 15 minutes. Development of stable foam suggests the presence of saponins.

b) 1ml extract was treated with 1% lead acetate solution. Formation of white precipitates indicates the presence of saponins.

**Test for Terpenoids***Knollar's test*

5 mg of extract is treated with 2ml of 0.1% anhydrous stannic chloride in pure thionyl chloride. A deep purple color that changes to red indicates the presence of terpenoids.

**Test for Protein and Amino Acid**

Small quantity of the extract was dissolved in few ml of water and filtered. Filtrate was subjected to **Millons** test and Biuret test

**Test for Resins**

Few mg of extract was treated with caustic soda a red color was developed if resins are present.

**Antibacterial Activity**

In the present research work, the antibacterial activity spectrum of petroleum ether and ethanolic extract of *Symplocos racemosa* was analyzed.[17-20] Three Gram positive bacteria, *Staphylococcus aureus* (MTCC 737), *Enterococcus faecalis* (MTCC 439), *Bacillus cereus* (MTCC 430) and three Gram negative bacteria *Klebsiella pneumoniae* (MTCC 109), *Pseudomonas aeruginosa* (MTCC 2642), *Escherichia coli* (MTCC 1687) were used. Inoculum size was adjusted to 1 to 2 × 10<sup>7</sup> CFU (Colony Forming Units)/ml by serial dilution with sterilized nutrient broth media. Nutrient agar (pH 7.2-7.4) was used for routine susceptibility testing of nonfastidious bacteria. Stock solution of 10000µg/ml was prepared in 20 % v/v water in DMSO. Using the stock solution, 7000µg/ml, 5000µg/ml, 3000µg/ml and 1000µg/ml solutions were prepared from which 100 µl solution was taken for assay. Ciprofloxacin was used as a standard. 20 % v/v WFI in DMSO was used as a control. Antibacterial assay was carried out by agar well diffusion method. After 16 to 18 hours of incubation, each plate is examined.

## Result and Discussion

Preliminary phytochemical screening showed that of *symplocos racemosa* contain higher amount of Carbohydrate, Glycoside, Saponin and Terpenoid Glycoside, Phytosterol and steroid. (Table 1)

**Table 1. Preliminary phytochemical screening of the two extracts of *symplocos racemosa***

Phytochemical test	Type of extract	
	Petroleum ether (60-80)	Ethanol (95% v/v)
Carbohydrate	--	++
Glycoside	++	++
Alkaloid	--	++
Phytosterol and steroid	++	--
Flavonoid	--	--
Protein and amino acid	--	--
Tannin	--	--
Saponin	--	++
Gum of mucilage	--	--
Terpenoid	--	++

++ = Present; -- = Absent

**Table 2. Zone of inhibition of ethanolic extract of *Symplocos racemosa***

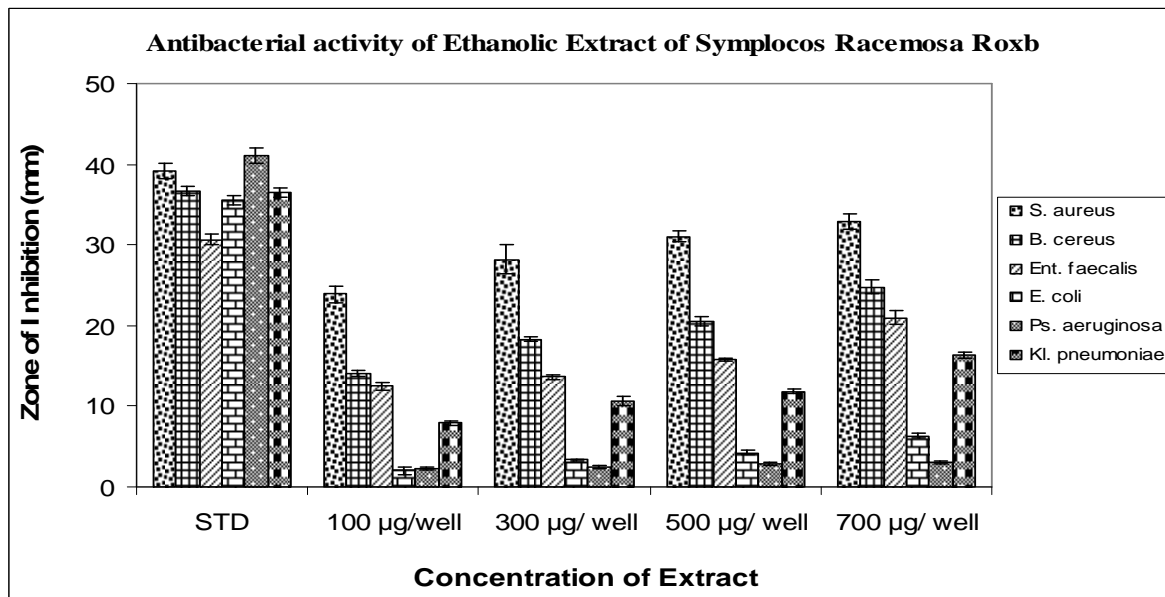
	<i>S. aureus</i>	<i>B. cereus</i>	<i>Ent. faecalis</i>	<i>E. coli</i>	<i>Ps. aeruginosa</i>	<i>Kl. pneumoniae</i>
<b>STD</b>	39.10 ± 0.95	36.67 ± 0.61	30.67 ± 0.61	35.60 ± 0.53	41.07 ± 1.01	36.53 ± 0.61
<b>100 µg/well</b>	23.93 ± 1.03	14.13 ± 0.41	12.47 ± 0.42	2.00 ± 0.40	2.20 ± 0.20	7.93 ± 0.31
<b>300 µg/ well</b>	28.23 ± 1.86	18.33 ± 0.31	13.60 ± 0.35	3.33 ± 0.15	2.47 ± 0.12	10.60 ± 0.60
<b>500 µg/ well</b>	31.07 ± 0.72	20.53 ± 0.61	15.80 ± 0.20	4.27 ± 0.31	2.80 ± 0.20	11.87 ± 0.31
<b>700 µg/ well</b>	32.90 ± 0.95	24.80 ± 0.80	21.00 ± 0.87	6.30 ± 0.26	3.00 ± 0.20	16.33 ± 0.31

**Table 3. Zone of inhibition of pet ether extract of *symplocos racemosa***

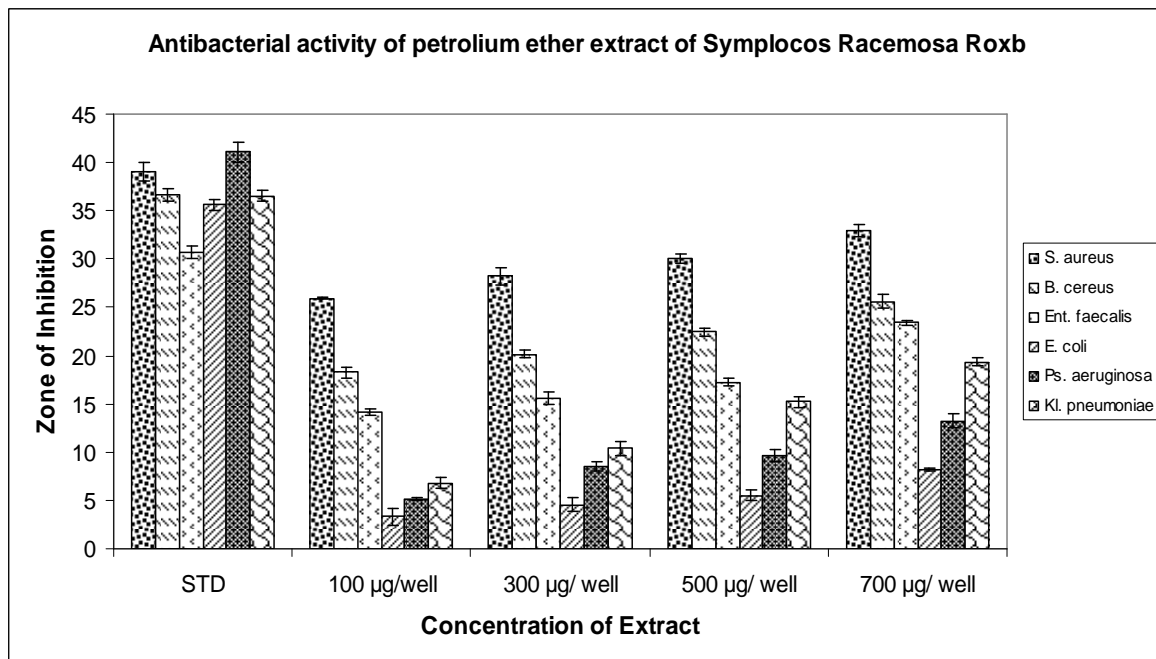
	<i>S. aureus</i>	<i>B. cereus</i>	<i>Ent. faecalis</i>	<i>E. coli</i>	<i>Ps. aeruginosa</i>	<i>Kl. pneumoniae</i>
<b>STD</b>	39.10 ± 0.95	36.67 ± 0.61	30.67 ± 0.61	35.60 ± 0.53	41.07 ± 1.01	36.53 ± 0.61
<b>100 µg/well</b>	25.93 ± 0.13	18.26 ± 0.56	14.12 ± 0.32	3.33 ± 0.85	5.13 ± 0.22	6.78 ± 0.55
<b>300 µg/ well</b>	26.55 ± 0.84	20.12 ± 0.39	15.56 ± 0.65	4.58 ± 0.69	8.50 ± 0.47	10.42 ± 0.70
<b>500 µg/ well</b>	30.07 ± 0.52	22.42 ± 0.45	17.25 ± 0.45	5.54 ± 0.63	9.67 ± 0.66	15.23 ± 0.59
<b>700 µg/ well</b>	32.90 ± 0.63	25.61 ± 0.76	23.40 ± 0.20	8.23 ± 0.12	13.23 ± 0.70	19.36 ± 0.47

The results of preliminary antibacterial evaluation showed that ethanolic extract of *Symplocos Racemosa* Roxb possess good antibacterial activity as compare to petroleum ether extract. However *Symplocos Racemosa* Roxb has poor antibacterial activity against gram negative micro organism like *P. aeruginosa* and *E. coli*.

**Figure 1. Graphical presentation of zone of inhibition against test microorganism**



**Figure 2. Graphical presentation of zone of inhibition against test microorganism**



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