

## Preliminary Phytochemical Screening and Antibacterial Activity of *Bauhinia racemosa* Leaves

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Phytochemical screening of *Bauhinia Racemosa* leaves reveals the presence of carbohydrate, alkaloids and tannin. Aqueous, ethanol, Chloroform, acetone, petroleum ether extract of leaves of *bauhinia racemosa* (*caesalpiniaceae*) were prepared and its antibacterial activity were studied by disc diffusion method against certain pathogenic bacteria such as *Staphylococcus aureus*, *Salmonella typhimurium*, *proteus vulgaris*, *pseudomonas aeruginose*, *B. megaterium*. The ethanol and acetone extracts were having good antibacterial activity as compared to other extract.

**Key words:** *Bauhinia racemosa*, Antibacterial activity, Aqueous extract, Ethanol extract, Chloroform extract, Acetone extract, Petroleum ether extract.

The use of plant compound for pharmaceutical purposes has gradually increased in India and throughout the world. About 80% of individuals from developed countries use traditional medicine, which involves compounds derived from medicinal plant<sup>1</sup>. The plant known as *Bauhinia racemosa* has been used traditionally for its various therapeutic properties like<sup>2,3</sup> hepatoprotective, astringent, dysentery, diarrhea, diuretic, tonic, anti inflammatory, anti neoplastic, antiulcer, antioxidant etc. Enteric or diarrhoeal infection are major public health problem in developing countries and contribute to the death of 3.3-6.0 million children annually. Enteric bacteria comprised of *salmonella sp.* *shigella sp.* *proteus*

*sp. klebsiella sp.* *E. coli*, *pseudomonas sp.* *vibrio cholera* and *staphylococcus aures* which are major agent of sporadic and epidemic diarrhea both in children and adult<sup>4-5</sup>. Literature survey revealed that almost no work is reported on the antibacterial property of *Bauhinia rascemosa*. There fore present work was carried out to evaluate the phytochemical and antibacterial properties of leaves of *Bauhinia rascemosa*.

### MATERIALANDMETHODS

The fresh leaves of *Bauhinia rascemosa* was collected from Mahadeo dara, District Beed. The fresh leaves were dried under shade, powdered and pass through 40 mesh sieve and stored in closed bottle for further use. The powder were extracted with different solvent ranging from non polar to polar solvent and using soxhlet extractor.

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The powdered leaves of *B. racemosa* were used for extraction purpose. Powdered leaves were refluxed with solvent for 3 hours. The solvent used for extract were water, ethanol, chloroform, acetone and petroleum ether. After each extraction, extract were filtered through cotton to remove other impurities. The extract were concentrated by vacuum distillation to reduce volume. Then concentrated extract were transferred to beaker and remaining solvent was evaporated on water bath with help of hair drier. Then they were cooled and placed in a dessicator to remove excessive moisture. The dried extract were placed in airtight container and used for further studies such as phytochemical screening and antimicrobial studies<sup>6</sup>.

#### **Phytochemical screening of plant material**

##### **Test for carbohydrate and glycosides**

A small quantity of the extract was dissolved separately in 4ml of distilled water and filtered. The filtrate was subjected to molisch test to detect presence of carbohydrate.

##### **Molisch test**

Filtrate was treated with 2-3 drops of 1% alcoholic  $\alpha$ - Naphthol solution and 2ml of conc.sulphuric acid was added along the sides of test tube. Appearance of brown ring at the junction of two liquids shows presence of carbohydrate. Another portion of the extract was hydrolysed with hydrochloric acid for few hours on water bath and the hydrolysate was subjected to legal's and Borntrager's test to detect the presence of different glycosides.

##### **Legal's test**

Hydrolysate was treated with chloroform and then chloroform layer was separated. To this equal quantities of dilute ammonia solution was added. Ammonia layer acquire pink colour, showing presence of glycosides.

##### **Test of alkaloids**

A small portion of the solvent free extract was stirred separately with few drops of dilute hydrochloric acid and filtered. The filtrate was tested with various reagents for presence of alkaloids.( Table.1)

##### **Test for phytosterols**

The extract was refluxed with solution of alcoholic potassium hydroxide till complete saponification had taken place. The ether layer was evaporated and residue was tested for presence of phytosterols.

##### **Test of saponins**

The extract was diluted with 20ml of distilled water and it was agitated in graduated cylinder for 15minutes. The formation of 1cm.layer shows presence of saponins.

##### **Test for phenolic compound and tannins**

Small quantities of extract were taken separately in water and test for presence of phenolic compound and tannins which is carried out with following reagent.

1. Dilute ferric chloride solution (5%)-violet colour.
2. 1% solution of gelatin containing 10% sodium chloride-white precipitate.
3. 10% lead acetate solution – white precipitate.

##### **Test for flavanoids<sup>7</sup>**

The flavanoid present in the extract was tested using sodium hydroxide solution it gives blue to violet colour (anthocyanins), yellow colour (flavones), yellow to orange (flavonones).

##### **Test for protein and amino acids**

Small quantities of the extracts were dissolved in few ml of water and treated with following reagent. The colour appears indicates presence of protein and amino acids.

1. Millions reagent – Appearance of red colour.
2. Ninhydrin reagent – Appearance of purple colour.

##### **Antibacterial activity using disc diffusion method**

The modified disc diffusion<sup>8</sup> method was employed to determine antibacterial activity of aqueous, ethanol, chloroform, acetone, petroleum ether extract of leaves of *Bauhinia racemosa*. Turbidity of inoculums was matched with Mc farland turbidity standard. Inoculums were spread over the nutrient agar plate using a sterile cotton swab in order to get uniform microbial growth. Then the prepared antibacterial disc were placed over the lawn and pressed slightly along with positive and negative controls. Disc were used as positive controls while disc soaked in sterile distilled water of various organic solvent and dried were placed on lawns as negative control. The plate were incubated for 18 hours at 37°C. The antibacterial activity was determined and diameters of inhibition zone were measured. Experiment was carried out in triplicate and average of diameter of zone of inhibition was recorded.

**RESULT AND DISCUSSION**

The Ayurvedic system of medicine includes number of plant and minerals, which should be investigated to determine the hidden potential by using the modern methodology. The plant *Bauhinia racemosa* commonly called as 'Ari',

is an indigenous herb which was chosen for this study<sup>9</sup>. The plant belongs to family *caesalpinceae*. The plenty availability of information on this plant facilitates the study on it<sup>10</sup>. Since ages various parts of this plant are being used for their medicinal use. The attempts were made to study phytochemical constituents and antimicrobial studied.

**Table 1.** Reagent used to test alkaloid

S. No.	Reagent	Colour of Precipitate
1.	Mayers reagents	Cream precipitate
2.	Dragendroff's reagents	Orange brown precipitate
3.	Hager's reagents	Yellow precipitate
4.	Wagner's reagents	Reddish brown precipitate

**Table 2.** Ash analysis of *Bauhinia Racemosa* leaves.

S. No	Type of ash	Percentage total ash
1.	Total ash	6.22%
2.	Acid insoluble ash	4.32%
3.	Water soluble ash	5.01%

**Table 3.** Extractive value of various extract of leaves of *Bauhinia Racemosa*

S. No	Name of extract	Percentage
1	Aqueous extract	6.85%
2	Ethanol extract	14.71%
3	Chloroform extract	1.72%
4	Acetone extract	3.14%
5	Petroleum ether extract	6.58%

**Table 4.** Phytochemical constituents of *Bauhinia Racemosa* leaves

S. No	Chemical constituents	Aqueous extract	Ethanol extract	Chloroform extract	Acetone extract	Petroleum ether extract
1	Carbohydrate	-	+	+	-	+
2	Glycoside	+	+	-	-	-
3	Alkaloid	-	-	+	-	+
4	Phytosterols	+	-	-	+	-
5	Saponins	+	-	-	-	-
6	Phenol	+	-	-	+	+
7	Tannin	+	+	-	+	+
8	Flavonoids	+	+	-	-	-
9	Protein & Amino acid	-	-	-	-	+

**Table 5.** Antibacterial activity of *Bauhinia Racemosa* leaves in different solvent

S. No	Name of organism	Aqueous extract	Ethanol extract	Chloroform extract	Acetone extract	Petroleum ether extract
1	<i>Staphylococcus aureus</i>	-	5	-	-	-
2	<i>Salomonella typhimurium</i>	-	5	-	5	-
3	<i>Proteus vulgaris</i>	2	4	-	3	-
4	<i>Pseudomonas aeruginosa</i>	-	2	-	7	-
5	<i>B.negativerium</i>	-	3	-	6	-

Phytochemical investigation of various solvent extracts leaves of *Bauhinia racemosa* shows presence of phenols, tannin, protein and amino acid in aqueous extract and petroleum ether. The leaves of *Bauhinia racemosa* shows total ash as 6.22 %, acid insoluble ash 4.32 %, while water soluble ash is 4.01 % shown in table.2. The extractive value of different solvent i.e aqueous , ethanol, chloroform, acetone and petroleum ether is as 6.85 %, 14.71 %, 1.72 %, 3.14 & 6.58 % respectively shown in table 3. The different phytochemicals present is shown in table. 4.

According to antibacterial profile table.5, maximum inhibitory effect was observed by petroleum ether and ethanol extract as compared other three extract. The result of the antibacterial assay shows promising evidences for the antibacterial effect of leaves of *Bauhinia racemosa*. From above evidences it is clear that plant extract have great potential as antibacterial compound. Therefore that can be used in treatment of enteric infection.

#### REFERENCES

- 1 Chopra R. N, Nayar S. N, Chopra I. C, Glossary of Indian medicinal plant(Including the supplement). Council of scientific and industrial Research, New Delhi, 1986.
- 2 The Wealth of India, A Dictionary of Indian materials and Industrial products, Published by publications and information directorate, CSIR Hill side road, New Delhi-110012; 1998; **2**(b): 53-55.
- 3 Raj K. Jagg Md Shalini Kapoor. *Indian drugs*, 1999; **36**(11): 668-678.
- 4 World Health Organisation, 5<sup>th</sup> Programme Report, Programme for control of diarrheal diseases, Geneva. *WHO Bulletin*, 1985; **63**:557-772.
- 5 Ballal M. screening of medicinal Plant used in rural folk medicin for treatment of diarrhea, Internet; <http://www.pharmoinfo.net>, 2005.
- 6 Kokate C. K, Practical pharmacognosy, published by M. K. Jain for Vallabh prakashan, Ap-53A, Pitam pura, Delhi-110088, 4<sup>th</sup> edition: 2004; 107.
- 7 Trease and Evans. pharmacognosy, W.C Evans, International student edition ISBN 0702026182, 15<sup>th</sup> edition, 2004, 247.
- 8 NCCLS (National committee for clinical laboratory standards) Performance standards for antimicrobial susceptibility testing. 8<sup>th</sup> Informational suppliment. M100 512 National committee for clinical laboratory standards, Villanova, Pa 2002.
- 9 Michael J. pelezar, JR, ECS chan, Noel R. Krieg. The text book of Microbiology, published by Tata McGraw-Hill, 5<sup>th</sup> edition, 2003, 106.
- 10 Ananthanarayan R and panikar's. Text book of Microbiology, published by orient Longman private Ltd., 160, Anna salai, Chennai - 600002, 7<sup>th</sup> edition, 2005, 37.