



## *In-vitro* Antibacterial Efficacy of Solvent Extracts of Leaves of *Bauhinia racemosa* Lam. (*Caesalpinaceae*) Against Enteric Bacterial Pathogens

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### ABSTRACT

Phytochemical screening of the plant leaves reveals the presence of carbohydrates, alkaloids, flavonoids, steroids, and tannins. Petroleum ether extract, chloroform extract, ethyl acetate extract and methanol extracts of leaves of *Bauhinia racemosa* Linn. were prepared and antibacterial activity were studied by disc diffusion method against certain enteric bacterial pathogens such as *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Salmonella typhi*, *Staphylococcus epidermidis* and *Proteus vulgaris*. The Methanol extracts had wide range of antibacterial activity against enteric bacterial pathogens than the petroleum ether extract, where as ethyl acetate extract were slightly higher antibacterial activity than chloroform extract. Antibacterial activity of various extract of leaves of *Bauhinia racemosa* was carried in attempt to develop a new pharmaceutical drug from natural origin for prevention of enteric infection.

**Keywords:** Antibacterial activity, *Bauhinia racemosa*, enteric bacterial pathogens.

### INTRODUCTION

The use of plant compounds for pharmaceutical purposes has gradually increased in India. About 80% of individuals from developed countries use traditional medicine, which involves compounds derived from medicinal plants.<sup>[1]</sup> Therefore, such plants should be investigated to better understand their properties, safety and efficiency. The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments.<sup>[2]</sup> Hence, studies involving the use of plants as therapeutic agents should be emphasized, especially those related to the control of antibiotic resistant microbes.<sup>[3]</sup> The plant *Bauhinia racemosa* (L). belongs to the *Caesalpinaceae* family. It occurs frequently in India, Ceylon, China, and Timor. The stem bark of the plant is an astringent and is used in the treatment of headache, fever, skin diseases, tumors, blood diseases, dysentery, and diarrhea.<sup>[4]</sup> The fresh flower buds of the plant showed antiulcer activity.<sup>[5-6]</sup> Enteric or diarrhoeal infections are major public health problems in developing countries and contribute to the death

of 3.3 to 6.0 million children annually. Enteric bacteria comprised of *Salmonella* sp., *Shigella* sp., *Proteus* sp., *Klebsiella* sp., *E. coli*, *Pseudomonas* sp., *Vibrio cholerae* and *Staphylococcus aureus* which are major etiologic agents of sporadic and epidemic diarrhea both in children and adults.<sup>[7-8]</sup> Therefore the review revealed that the leaves of *Bauhinia racemosa* were used in various metabolic disorders, but far their antibacterial properties were not demonstrated. Hence attempt was made to find out the antibacterial properties of leaves of *Bauhinia racemosa* against enteric bacterial pathogens.

### MATERIALS AND METHODS

#### Plant materials

Fresh plant of *Bauhinia racemosa* (L). were collected from local region of Ahmednagar District in India. The leaves were identified by Mr. P. S. N. Rao, Joint Director, Botanical survey of India (BSI), Koregaon road, Pune.

#### Preparation of extracts

1.5 kg of the plant material in each batch was exhaustively extracted by soxhlet extraction method using Petroleum ether, Chloroform, Ethyl acetate and Methanol. The solvent used in each batch was recovered under pressure until dry extracts were obtained and then labeled and stored separately at 4°C in amber colored airtight bottles.

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**Phytochemical Screening of Plant materials**

The presence of saponins, tannins, carbohydrates, alkaloids, flavonoids glycosides, steroids, proteins and alkaloids, were detected by simple qualitative methods.<sup>[9]</sup>

**Bacterial cultures**

The standard pathogenic bacterial cultures were procured from IMTECH, Chandigarh, India and used in the present study (Table 1). The bacterial cultures were rejuvenated in Mueller- Hinton broth (Hi-media laboratories, Mumbai, India) at 37°C for 18h and then stocked at 4°C in Mueller-Hinton Agar. The inoculum size of the bacterial culture was standardized according to the National committee for Clinical Laboratory Standards (NCCLS, 2002) guideline. The pathogenic bacterial culture was inoculated into sterile Nutrient broth and incubated at 37°C for 3h until the culture attained a turbidity of 0.5 McFarland units. The final inoculum size was standardized to 10<sup>5</sup> CFU/mL with the help of SPC and Nephlo-turbidometer.

**Table 1: Bacterial cultures used in study (IMTECH, Chandigarh, India)**

| Bacterial Pathogens               | MTCC Number |
|-----------------------------------|-------------|
| <i>Proteus vulgaris</i>           | 426         |
| <i>Staphylococcus epidermidis</i> | 435         |
| <i>Staphylococcus aureus</i>      | 96          |
| <i>Escherichia coli</i>           | 739         |
| <i>Pseudomonas aeruginosa</i>     | 424         |
| <i>Klebsiella pneumoniae</i>      | 109         |
| <i>Salmonella typhi</i>           | 733         |
| <i>Enterobacter aerogenes</i>     | 111         |
| <i>Salmonella typhimurium</i>     | 98          |

**Preparation of disc for antibacterial activities**

The Petroleum ether, Chloroform, Ethyl acetate and Methanol extracts were prepared in their respective solvents and the sterile blotting paper disc (10 mm) were soaked in the diluted extract in such concentration that the amount of solution absorbed by each disc was 1mg, 2mg, 3mg, 4mg, 5mg of each extracts of *Bauhinia racemosa*. The prepared disc were dried in controlled temperature to remove excess of solvent and used in study.

**Antibacterial activity using disc diffusion method**

The modified paper disc diffusion<sup>[10]</sup> was employed to determine the antibacterial activity of The Petroleum ether, Chloroform, Ethyl acetate and Methanol extracts of leaves of *Bauhinia racemosa*. Turbidity of inoculums was matched with McFarland turbidity standard. Inoculums were spread over the Nutrient agar plate using a sterile cotton swab in order to get a uniform microbial growth. Then the prepared antibacterial disc were placed over the lawn and pressed slightly along with positive and negative controls. Ampicillin 10 mcg/disc (Hi-Media, Mumbai) were used as positive control while disc soaked in sterile distilled water or various organic solvents and dried were placed on lawns as negative control. The plates were incubated for 18h at 37°C. The antibacterial activity was evaluated and diameters of

**Table 2: Phytochemical analysis of *Bauhinia racemosa* leaves.**

| S. No | Phytochemical Constitutes | Petroleum ether extract | Chloroform extract | Ethyl acetate extract | Methanol extract |
|-------|---------------------------|-------------------------|--------------------|-----------------------|------------------|
| 1     | Alkaloid                  | +                       | +                  | +                     | +                |
| 2     | Flavonoids                | -                       | -                  | +                     | +                |
| 3     | Carbohydrates             | +                       | +                  | -                     | -                |
| 4     | Glycosides                | -                       | -                  | -                     | -                |
| 5     | Saponins                  | -                       | -                  | -                     | +                |
| 6     | Proteins                  | +                       | -                  | -                     | -                |
| 7     | Steroids                  | -                       | -                  | +                     | -                |
| 8     | Tannins                   | +                       | -                  | -                     | -                |

Where: + = the presence of constitute, - = the absence of constitutes

inhibition zones were measured. Experiment was carried out in triplicate and the averages diameter of zone of inhibition was recorded. The antibacterial activity was classified as strong (>20mm), moderate (16-19mm) and mild (12-15mm) and less than 12mm was taken as inactive.

**RESULTS AND DISCUSSION**

The photochemical investigation (Table 2) of the various solvent extract of leaves of *Bauhinia racemosa* showed the Petroleum ether extract to only alkaloids and steroids which, occurring in higher concentration. The chloroform extract and ethyl acetate extract content similar photochemical constitutes, alkaloids and steroids in higher concentration, Carbohydrates, Flavonoids, glycosides, saponins. Methanol extract contented Alkaloids, Steroids, Flavonoids, Saponins and Tannins, but did not contented any proteins.

According to antibacterial profile (Table 3), maximum inhibitory effect of the Petroleum ether extract observed only on *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Salmonella typhi* and moderate antibacterial against *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Salmonella typhimurium* but mild inhibitory effect on *Proteus vulgaris*. Chloroform extract showed strong antibacterial effect against *Staphylococcus epidermidis* and *Staphylococcus aureus* and moderate antibacterial against *Proteus vulgaris*, *Escherichia coli*, *Enterobacter aerogenes*, *Salmonella typhi* and *Salmonella typhimurium* but mild effect on *Pseudomonas aeruginosa*. Ethyl acetate extract showed maximum inhibitory effect on *Staphylococcus epidermidis*, *Staphylococcus aureus* *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes* and *Salmonella typhimurium*, but moderate effect on *Proteus vulgaris* and *Salmonella typhi*. Methanol extract showed maximum inhibitory effect on *Staphylococcus aureus*, *Proteus vulgaris*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Salmonella typhimurium*, but moderate inhibitory effect on *Escherichia coli*, *Enterobacter aerogenes*.

The result of the antibacterial assay show promising evidence for the antibacterial effect of leaves of *Bauhinia racemosa*. From the above evidence, it is clear that plant extracts have great potential as antibacterial compounds against enteric pathogens and that they can be used in the treatment of enteric infectious. It is hoped that this study would lead to the establishment of some compounds that could be used to formulate new and more potent antimicrobial drugs of natural origin.

**ACKNOWLEDGEMENTS**

The authors are very much thankful to Honorable Shri Amit Dada Kolhe, Chairman, Sanjivani Rural Education society's for providing all the facilities and support for this research work.

**Table 3: Antibacterial activity of *Bauhinia racemosa* leaves extracts against enteric bacterial pathogens (Zone of inhibition of growth in mm, average of 3 readings)**

| Bacterial Pathogens   | The Petroleum ether extract |          |          |          |          | Chloroform extract |          |          |          |          | Ethyl acetate extract |          |          |          |          | Methanol extract |          |          |          |          | controls  |            |               |          | Ampicillin (10mcg) |
|-----------------------|-----------------------------|----------|----------|----------|----------|--------------------|----------|----------|----------|----------|-----------------------|----------|----------|----------|----------|------------------|----------|----------|----------|----------|-----------|------------|---------------|----------|--------------------|
|                       | 5mg/disc                    | 4mg/disc | 3mg/disc | 2mg/disc | 1mg/disc | 5mg/disc           | 4mg/disc | 3mg/disc | 2mg/disc | 1mg/disc | 5mg/disc              | 4mg/disc | 3mg/disc | 2mg/disc | 1mg/disc | 5mg/disc         | 4mg/disc | 3mg/disc | 2mg/disc | 1mg/disc | Pet ether | Chloroform | Ethyl acetate | Methanol |                    |
| <i>P. vulgaris</i>    | 15                          | 13       | 12       | -        | -        | 17                 | 15       | 12       | -        | -        | 19                    | 17       | 15       | 15       | 12       | 20               | 17       | 15       | 13       | 12       | -         | -          | -             | -        | 16                 |
| <i>S. epidermidis</i> | 23                          | 20       | 17       | 15       | 13       | 21                 | 19       | 17       | 16       | 14       | 24                    | 22       | 20       | 17       | 15       | 26               | 18       | 20       | 18       | 14       | -         | -          | -             | -        | 25                 |
| <i>S. aureus</i>      | 22                          | 21       | 18       | 17       | 16       | 24                 | 22       | 20       | 18       | 16       | 20                    | 19       | 17       | 15       | 14       | 22               | 19       | 18       | 16       | 13       | -         | -          | -             | -        | 24                 |
| <i>E.coli</i>         | 19                          | 17       | 15       | 12       | 12       | 17                 | 15       | 14       | 12       | -        | 22                    | 19       | 17       | 14       | 12       | 17               | 15       | 14       | 12       | -        | -         | -          | -             | -        | 11                 |
| <i>P. aeruginosa</i>  | 16                          | 15       | 14       | 13       | 12       | 15                 | 14       | 13       | -        | -        | 21                    | 17       | 15       | 14       | 13       | 20               | 17       | 16       | 15       | 13       | -         | -          | -             | -        | 16                 |
| <i>S. typhi</i>       | 21                          | 20       | 17       | 14       | 12       | 18                 | 16       | 15       | 14       | 13       | 18                    | 17       | 16       | 15       | 14       | 22               | 20       | 18       | 15       | 14       | -         | -          | -             | -        | 18                 |
| <i>E. aerogenes</i>   | 17                          | 15       | 15       | 13       | 12       | 18                 | 16       | 15       | 13       | 11       | 22                    | 19       | 17       | 14       | 13       | 17               | 15       | 13       | 13       | 12       | -         | -          | -             | -        | 14                 |
| <i>S. typhimurium</i> | 18                          | 17       | 15       | 13       | 11       | 18                 | 17       | 14       | 12       | -        | 20                    | 17       | 17       | 15       | 14       | 22               | 20       | 17       | 14       | 12       | -         | -          | -             | -        | 19                 |

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