

## Short Communication

# Antimicrobial activity of the ethanolic extract of *Bryonopsis laciniosa* leaf, stem, fruit and seed

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**Antimicrobial activity of ethanolic extract of the leaf, stem, seed and fruit of an Indian medicinal plant, *Bryonopsis laciniosa*, used traditionally as potent medication in healing several ailments such as adenopathy, ague, asthma, bronchitis, cholera, colic, consumption, convulsion, cough, fertility and phthisis, was tested against different pathogenic microorganisms by agar well diffusion method. Leaf and stem extracts of *B. Laciniosa* exhibited antimicrobial activity against different Gram positive and Gram negative bacteria. The extents of the growth inhibition of bacteria were measured for each extract and *Staphylococcus aureus*, *Micrococcus luteus* and *Bacillus cerues* exhibited significant growth inhibition zone. Minimum inhibitory concentrations (MIC) exhibited by stem extract against the tested organisms ranged between 0.156 and 5 mg/ml; and for leaf extracts it varied between 0.625 and 10 mg/ml. Antimicrobial activities of the crude plant extracts were comparable to those of the standard antibiotics. This study concluded that *B. Laciniosa* used as a traditional medicinal plant has antimicrobial activity against pathogenic microorganisms.**

**Key words:** Medicinal plant, *Bryonopsis laciniosa*, ethanol extract, antimicrobial activity.

## INTRODUCTION

Finding healing powers in plants is an ancient idea. People on all continents have long applied poultices and imbibed infusions of hundreds, if not thousands, of indigenous plants, dating back to prehistory (Cowan, 1999). Over three-quarters of the world population relies mainly on plants and their extracts for health care. The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment (Joy et al., 1998). Biologically active compounds present in the medicinal plants have always been of great interest to scientists working in this field. In recent years this interest to evaluate plants possessing antibacterial activity for various diseases is growing (Clark and Hufford, 1993). For instance in developed countries, 25% of the medical drugs are based on plants and their derivatives (Principe, 1991). Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased (Cohen, 1992).

In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions (Ahmad et al., 1998). Therefore there is a constant need to establish and develop antimicrobial drugs from natural origin that are much safe, reliable and less expensive.

*Bryonopsis laciniosa* is a shrub known as "Shivlingi" in India and it is used commonly as an aperient medicine and tonic. *B. laciniosa* belongs to the Cucurbitaceae family and traditional healers use the leaves and the seeds of this plant for treatment of fevers. It is also taken in impotency and used as a tonic. Whole plant is used to treat adenopathy, ague, asthma, bronchitis, carbuncles, cholera, colic, consumption, convulsions, cough, delirium, fertility, headache, megalosplenly, paralysis, phthisis, snake bite. The chloroform extract of *B. laciniosa* has exhibited significant anti-inflammatory activity (Gupta et al., 2003). Analgesic and antipyretic activity of methanol extract of *B. laciniosa* also has been shown in standard animal models (Sivakumar et al., 2004).

Evidently there are no scientific studies about this plant on activities against pathogenic microorganism. Therefore, the objective of this study was to investigate the

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antimicrobial activity of this plant. We here report a comparative study on the antimicrobial properties of various parts of plant.

## MATERIALS AND METHODS

### Collection of plant material

The fresh parts of plant were collected from Katraj Dairy medicinal plant garden, Pune, India during the month of August. The collection was under specialist supervision and it was finally authenticated by Botanical Survey Institute of India (BSI). A voucher specimen (DPNDP1) is deposited at the Herbarium of Botanical Survey of India. Each specimen was washed under running tap water, labeled, weighed and annotated with the date of collection. Then each specimen was dried at 37°C for 48 h, powdered and stored in air tight container.

### Preparation of extracts

Exactly 10 g of each powdered sample (leaf, stem, seed and fruit) was soaked in 50 ml of 80% ethanol for 3-5 days and filtered through Whatman No.1 filter paper (Mohan, 2004). Further extraction of the residue was repeated 3 times until a clear colorless supernatant extraction liquid was obtained. The combined filtrates were concentrated half of original volume by rotary evaporator at 70°C and completely evaporated in water bath maintained in 70°C till the dried extracts were obtained. Subsequently a known weight of each air-dried extract was dissolved in known volume of 5% DMSO to give the desired concentration of each extract. Then extracts obtained were placed in sterile screw capped bottles and stored at 4°C.

### Microorganisms used

Microorganisms were identified and obtained from National Chemical Laboratory (NCL), Pune, India. The bacteria studied were three strains of gram-negative bacteria, *Escherichia coli* (DH5 $\alpha$ ), *Salmonella typhimurium* (NCIM 2501), and *Pseudomonas aeruginosa* (NCIM 2200) and three strains of gram-positive bacteria, *Bacillus cereus* (NCIM 2155), *Staphylococcus aureus* (NCIM 2079) and *Micrococcus luteus* (NCIM 2871). They were stored in slants in McCartney bottles containing nutrient agar for further use. The bacterial suspensions were cultured in enriched nutrient broth for 12 h and 0.1 ml of  $1.5 \times 10^8$  cfu/ml fresh inoculums (0.5 McFarland Standard) were utilized for antimicrobial activity test.

### Antimicrobial screening

0.1 ml of each fresh bacterial suspension standardized to 0.5 McFarland Standards was mixed with 20 ml of enriched nutrient agar in Petri dishes. Once the agar was solidified, they were punched using a sterile cork borer. Then wells (4 ml in diameter) were filled with 20 microliters of different plant extracts or antibiotic. Ampicillin (100 mg/ml) and DMSO (5%) were used as positive and negative control, respectively. The plates were incubated at 37°C for 24 h and the inhibition zones were compared with that of the standard antibiotic ampicillin. Each experiment was repeated three times.

### Modified dilution method for determination of MIC (de Paiva et al., 2003)

Those extracts that showed antimicrobial activity were tested to

determine the Minimal Inhibitory Concentration (MIC) for each bacterial sample by dilution method. The microdilution method was performed in 96-well microtiter plates. Bacterial samples were diluted in enriched nutrient broth at a density adjusted to 0.5 McFarland turbidity. The final inoculum was  $1.5 \times 10^8$  cfu/ml of bacterial colony. The wells were filled with 100  $\mu$ l of sterile distilled water and 100  $\mu$ l of the plant extracts or ampicillin (as positive control) were added to the wells by serial two fold dilution from 40 and 100 mg/ml suspension of plant extract Stock solution and ampicillin, respectively. Then each well was inoculated with 100  $\mu$ l of 0.5 McFarland standard bacterial suspensions. The plates were covered in plastic bags and incubated at 37°C for 24 h and MIC was evaluated as compared with controls by visual reading. The MIC was defined as the lowest concentration of the extract inhibiting the visible growth of each microorganism.

## RESULT AND DISCUSSION

Table 1 shows the effect of ethanol extract of different parts of *B. laciniosa* through well diffusion method. There were fine responses of the organisms to the leaf and stem extracts compared with standard antibiotics, while organisms did not show any susceptibilities to fruit and seed extracts. *S. aureus*, *M. luteus*, *B. cereus* and *P. aeruginosa* were susceptible to leaf and stem extract at all concentrations except *P. aeruginosa* for 10 mg/ml. *E. coli* and *S. typhimurium* were resistant to all extracts. Judging by the diameter of the zone of inhibition *B. cereus* and *S. aureus* were identified as the most susceptible organisms to the stem and leaf extracts of *B. lacinioca*. In general antibacterial activity increases with increase in concentration of extract as evident by the zone of inhibition.

The MIC values of different extracts are represented in Table 2. The MIC values ranged between 0.156 and 5 mg/ml for stem extract and varied between 0.625 and 10 mg/ml for leaf extract against tested microorganisms. Therefore the minimum inhibitory concentration identified 0.156 mg/ml for stem extract and 0.625 mg/ml for leaf extract. The standard ampicillin had MIC values varying between 0.048 and 3.125 mg/ml. Comparing the MIC results, it is evident that tested bacteria are more sensitive to stem extract than leaf extract.

The increase of antibiotic resistance of microorganisms to conventional drugs has necessitated the search for new, efficient and cost effective ways for the control of infectious diseases. The results of different studies provide evidence that some medicinal plants might indeed be potential sources of new antibacterial agents (Kone et al., 2004). The use of medicinal plants is part of the Indian tradition. Many local regions all over India have a great variety of vegetation used by the local population to treat and prevent diseases. From this study we can concluded that, this medicinal plant has a wide range of antibacterial activity and supports the traditional use of these plants as medicines. This study demonstrated that herbal medicine can be as effective as modern medicine to combat pathogenic microorganisms. Using different purification methods the activity of antimicrobial com-

**Table 1.** Antimicrobial activity of various ethanolic extracts of *Bryonopsis laciniosa*.

Test organism	Zone of inhibition (mm)												Ampicilin (mg/ml)	
	Leaf extract (mg/ml)			Stem extract (mg/ml)			Fruit extract (mg/ml)			Seed extract (mg/ml)				
	10	20	30	10	20	30	10	20	30	10	20	30		
<i>E. coli</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	15
<i>P. aeruginosa</i>	-	5	6	-	6	7	-	-	-	-	-	-	-	17
<i>S. typhimurium</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	21
<i>S. aureus</i>	8	10	13	8	11	14	-	-	-	-	-	-	-	30
<i>M. luteus</i>	6	9	12	6	7	11	-	-	-	-	-	-	-	20
<i>B. cereus</i>	9	11	14	7	10	13	-	-	-	-	-	-	-	22

**Table 2.** MIC values of active ethanolic extracts of *Bryonopsis laciniosa* on test bacteria.

Test organism	Leaf extract (mg/ml)	Stem extract (mg/ml)	Ampicilin (mg/ml)
<i>S. aureus</i>	0.625	0.156	0.048
<i>M. luteus</i>	0.625	0.156	0.097
<i>P. aeruginosa</i>	10	5	3.125
<i>B. cereus</i>	1.25	1.25	0.097

pounds can be improved for further pharmaceutical uses.

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