



## Research Article

### ANTIMICROBIAL POTENTIAL OF *Abutilon indicum* EXTRACT

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#### ABSTRACT

*Abutilon indicum* has been known for its antibacterial potential. Leaf, stem and root extracts of *Abutilon* were made in three solvents viz, ethanol, acetone and chloroform using Soxhlet extraction unit. Each of the extract was tested by agar disc diffusion method for antibacterial potential against *Bacillus subtilis*, *Escherichia coli* and *Lactobacillus sp.* Results were expressed in terms of diameter of zone of inhibition. *In vivo* and *In vitro* grown plantlets were compared for results. Leaf extracts from all solvents recorded significant activity against all the test bacteria. Most of times, maximum inhibition zone appeared against *Bacillus subtilis*.

**Keywords:** *Abutilon*, antibacterial activity, zone of inhibition, disc diffusion method

#### INTRODUCTION

Plants have provided a source of inspiration for novel drug compounds as plant derived medicines have made significant contribution towards human health. Successive isolation of botanical compounds from plant material is largely dependent on type of solvent used for extraction. Since ancient times, various ailments have been treated by using plant extracts<sup>1</sup>. The overuse of antibiotics for curing diseases has led to emergence of multi drug resistant organisms which have raised the need for search of alternative source of antimicrobial agents.

*Abutilon* is distributed throughout the hotter parts of India and also found in hilly areas upto 1200 m. The flowers are used to increase semen in men<sup>2</sup>. Methanol extract of *A. indicum* had some antimicrobial properties<sup>3</sup>. From ancient times, this plant has been used as ayurvedic medicine with greater benefits<sup>4</sup>. A chemical compound,  $\beta$ -sitosterol, which has been identified as the active ingredient in many medicinal plants, is present in *A. indicum* and a petroleum ether extract provided larvicidal properties against the mosquito larvae *Culex quinquefasciatus*<sup>5</sup>. Results given by<sup>6</sup> revealed that methanolic extracts from *Abutilon indicum* were most susceptible to Gram positive bacteria *Escherichia coli* followed by *Pseudomonas aeruginosa*. Plantlets of *Abutilon* were grown *In vitro* and extract made in acetone, chloroform and ethanol was tested for antimicrobial activity against three bacterial species.

#### MATERIALS AND METHODS

##### Collection of Sample

*In vivo* plant material was collected randomly from its natural habitat in Sri Ganganagar and *In vitro* was collected from plantlets grown in Plant Tissue Culture laboratory, Department of Biotechnology at Seth G.L. Bihani S.D. (P.G.) College, Sri Ganganagar.

##### *In vitro* culture

The nodal parts were surface sterilized by preliminary cleaning with tap water, followed by treatment of 0.1% HgCl<sub>2</sub> (w/v) for 2-3 minutes. Finally the nodes were thoroughly rinsed with deionised water from 3-5 times and grown on MS media. Different concentrations of growth regulators were tried. The callus obtained was grown in Erlenmeyer's flasks of 250 ml capacity containing 50 ml medium having same composition as that of former but growth regulator concentration varied for shooting and rooting.

##### Preparation of Plant extract

Different plant materials namely, stem, root and leaf from *In vivo* and *In vitro* plants were taken and air dried followed by drying in oven for complete removal of moisture. The dried material was finely crushed to powder. 20 g of dried powder in 200 ml of solvent was subjected to Soxhlet extraction for a continuous period of 20-24 hours. 60% ethanol, 60% chloroform and 60% acetone each were used for extraction. After 24 h, the extracts were centrifuged at 5000 rpm for 10 min, the supernatant was collected, solvents were evaporated, and the dry extract was stored at 4°C in airtight bottles

##### Microbial cultures

Antimicrobial activity was tested against three bacterial species viz. *Bacillus subtilis* (ATCC 11774), *Escherichia coli* (ATCC 13763) obtained from IMTECH, Chandigarh and *Lactobacillus sp.* procured from stock cultures of Microbiology laboratory of the Biotechnology department of Seth G.L. Bihani S.D. (P.G.) College, Sri Ganganagar. The cultures were maintained on nutrient agar slants, stored at 4±1°C until further use and subcultured at weekly intervals. The culture was grown in Erlenmeyer's flasks of 250 ml capacity containing 50 ml medium having same composition as that of maintenance

medium except agar and incubated at 25°C for 24 h under stationary conditions to provide a suspension of 10 CFU/ml.

**Antimicrobial Assay**

Plant extract prepared in Soxhlet unit by using 60% ethanol, 60% chloroform and 60% acetone each was tested for antimicrobial activity against three bacterial species by agar disc diffusion method<sup>7,8</sup>. Zone of inhibition (in mm) was compared with similar extracts from *in vivo* grown *Abutilon*.

**RESULTS AND DISCUSSION**

**Control**

A sterilized Whatman’s filter paper disc saturated in each of the solvent was used as a control against each bacterial species. No zone of inhibition appeared in any case.

**Standard**

Imbibed filter paper discs dipped in standard solutions of each of Streptomycin and Tetracycline were placed as a positive control during each test. Tables (1-6) reveal that both these antibiotics had maximum zone of inhibition against *Bacillus subtilis* and minimum against *Lactobacillus sp.*

**Extract in 60% Ethanol**

Tables 1 and 4 indicate the diameters of inhibition zones of leaf, stem and root extract from *In vivo* and *In vitro* *Abutilon* tested against three bacterial species. Leaf extract from micropropagated plant showed maximum inhibition (18±2 mm) against *Bacillus subtilis*. Minimum diameter (10±1 mm) was from root extract of *In vivo* plant against *E.coli*.

**Extract in 60% Acetone**

The results of inhibition zones of leaf, stem and root extract from *In vivo* and *In vitro* *Abutilon* tested against three bacterial species have been summarized in tables 2 and 5. In case of natural growth, leaf extract had maximum zone diameter (14±2 mm) against *Bacillus subtilis* and root extract had minimum diameter (9±3 mm) against both *E.Coli* and *Lactobacillus*. During tissue culture, stem extract had maximum zone diameter (18±1 mm) against *Lactobacillus* and leaf extract had minimum diameter (11.5±3mm) against *E.Coli*.

**Table 1: Zone of Inhibition (in mm) of different extracts of *In vivo* grown *A. indicum* in 60% Ethanol.**

	<i>Bacillus subtilis</i>	<i>Lactobacillus bulgaricus</i>	<i>Escherichia coli</i>
60% Ethanol	-	-	-
Leaf Extract	17±2	14±2	13±1
Stem Extract	13±2	13±1	12±3
Root Extract	12±2	12±1	10±1
Streptomycin	44±2	35±1	37±1
Tetracycline	45±3	33±1	35±1

**Table 2: Zone of Inhibition (in mm) of different extracts of *In vivo* grown *A. indicum* in 60% Acetone.**

	<i>Bacillus subtilis</i>	<i>Lactobacillus bulgaricus</i>	<i>Escherichia coli</i>
60% Acetone	-	-	-
Leaf Extract	14±2	12.5±3	12±3
Stem Extract	11.2±2	11±1	10.5±3
Root Extract	10.5±1	9±3	9±3
Streptomycin	46±1	35±1	37±1
Tetracycline	46±2	33±1	35±1

**Table 3: Zone of Inhibition (in mm) of different extracts of *In vivo* grown *A. indicum* in 60% Chloroform.**

	<i>Bacillus subtilis</i>	<i>Lactobacillus bulgaricus</i>	<i>Escherichia coli</i>
60% Chloroform	-	-	-
Leaf Extract	20±2	14±3	13±3
Stem Extract	16±3	12±2	12±2
Root Extract	13±2	11±2	10±2
Streptomycin	38±2	29±1	31±1
Tetracycline	38±2	28±1	26±2

**Table 4: Zone of Inhibition (in mm) of different extracts of *In vitro* grown *A. indicum* in 60% Ethanol.**

	<i>Bacillus subtilis</i>	<i>Lactobacillus bulgaricus</i>	<i>Escherichia coli</i>
60% Ethanol	-	-	-
Leaf Extract	18±2	15±2	14±1
Stem Extract	15±2	13±2	12.5±3
Root Extract	11±2	14±1	12±1
Streptomycin	44±2	35±1	37±1
Tetracycline	45±3	33±1	35±1

**Table 5: Zone of Inhibition (in mm) of different extracts of *In vitro* grown *A. indicum* in 60% Acetone.**

	<i>Bacillus subtilis</i>	<i>Lactobacillus bulgaricus</i>	<i>Escherichia coli</i>
<b>60% Acetone</b>	-	-	-
<b>Leaf Extract</b>	15±2	18±1	14.5±3
<b>Stem Extract</b>	13±2	14±1	12±3
<b>Root Extract</b>	12.5±1	15.2±1	13.1±3
<b>Streptomycin</b>	46±1	35±1	37±1
<b>Tetracycline</b>	46±2	33±1	35±1

**Table 6: Zone of Inhibition (in mm) of different extracts of *In vitro* grown *A. indicum* in 60% Chloroform.**

	<i>Bacillus subtilis</i>	<i>Lactobacillus bulgaricus</i>	<i>Escherichia coli</i>
<b>60% Chloroform</b>	-	-	-
<b>Leaf Extract</b>	20.5±2	16±2	15±3
<b>Stem Extract</b>	18±2	13.5±2	14±2
<b>Root Extract</b>	14.4±2	12±2	12.8±2
<b>Streptomycin</b>	38±2	31±1	29±1
<b>Tetracycline</b>	38±2	26±1	28±2

### Extract in 60% Chloroform

Filter paper discs saturated with each of leaf, stem and root extracts were kept on petriplates with bacterial species to be tested. Leaf extract has maximum zones of inhibition against all three microbes in both *In vitro* and *In vivo* cultures. On the other hand, root extract has minimum diameter against *B. subtilis*, *E. coli* and *Lactobacillus* (Tables 3 and 6).

### CONCLUSION

Antimicrobial activity assayed by agar disk diffusion method revealed that during *In vivo* growth, extracts in chloroform had maximum inhibition zones against *B. subtilis*. Ethanolic extracts showed maximum zone diameter against *Lactobacillus sp.* and against *E. coli*, ethanol and chloroform extracts shared almost similar results. Leaf extracts from all solvents had highest antibacterial ability against all the tested species during *In vitro* growth as compared to stem and root extracts. Same trend was followed while *In vivo* growth. Compared to stem and root extracts, leaf extracted in all solvents had highest antibacterial ability against all the tested species. Mostly, highest inhibition zone diameters were visible against *Bacillus subtilis*. Leaves of *Abutilon* are immense source of phytochemicals and antibacterial properties. Although phytochemical content of roots is less compared to leaves and stem, but it shows comparable diameters of zones of inhibition indicating good antimicrobial activity.

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