

IN-VITRO ANTIBACTERIAL STUDY ON LEAF EXTRACTS OF *ABUTILON INDICUM* LINN.

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ABSTRACT

The antimicrobial activity of petroleum ether, Chloroform and Methanol extract obtained from *Abutilon indicum* was tested against Gram-negative bacteria like *Escherichia coli* ATCC 69314, *Klebsiella pneumoniae* NCIM 2719, *Pseudomonas aeruginosa* NCIM 2200 and *Agrobacterium tumefaciens* NCIM 2943 and two strains of Gram-positive bacteria *Staphylococcus aureus* NCIM 2080 and *Bacillus subtilis* MTCC 441, using agar well diffusion method. In this study, the highest antibacterial potentials were observed against *Streptococcus aereus* (18.72±0.01mm), *Pseudomoas aeruginosa* (17.49±0.14mm). *Klebsiella pneumoniae* (17.15±0.03) and less activity found against *Escherichia coli* (9.75±0.13) Our finding suggest that the petroleum ether and methanol extract of *abutilon indicum* has potent antibacterial activity against the pathogenic strains of *pseudomonas aeruginosa*, *streptococcus aeures* and *Klebsiella pneumoniae*.

KEYWORDS

Antibacterial activity, *Pseudomoas aeruginosa*, *streptococcus aeures*.

INTRODUCTION

The use of plants to treat various diseases in India is dates back to the time of Rig-Veda (3500 to 1800 BC). The plants containing active compounds are important. The beneficial medicinal effects of the Plant materials typically result from the combinations of secondary products present in the plant. In plants, these compounds are mostly secondary metabolites such as alkaloids, steroids, tannins and phenol compounds, which are synthesized and deposited in specific parts or in all parts of the plant. The secondary metabolites with adequate antibacterial potency will be used for the treatment of the bacterial infections (1). The studies of medicinal plants not end just with the knowledge of their therapeutic uses. Various aspects of medicinal plants have to be studied in depth for their optimum utilization. Plants with possible antimicrobial activity should be tested against an appropriate microbial model to confirm the activity and to ascertain the parameters associated with it

The plant *Abutilon indicum* belongs to family Malvaceae, has been referred with different names as monkeybush, kangi, Indian mallow. This plant is native of Southeast Asia, now widespread as a tropical weed (3). Occurring in disturbed sites at low elevations near the ocean" (4). This plant is an erect velvety-pubescent subshrub with suborbicular-ovate cordate coarsely crenate-serrate long-petiolate alternate leaves; pubescence of soft stellate pale hairs; flowers are solitary in axils, on long pedicels (4-7 cm), usually longer than the petioles; petals orange-yellow, imbricate, deltoid-obovate, 1 cm long or slightly more, staminal-tube hirsute with stellate hairs; fruit are circular, of 11-20 radiating carpels, hirsute, brown when dry; each carpel flattened, somewhat boatshaped, apiculate by the short persistent style-remnant, about 8 mm long; seeds are reniform and stellate-pubescent" (3).

Abutilon indicum has herbal applications as a urinary antiseptic, as an antiasthmatic, as a demulcent and as a laxative. The root together with the

MATERIALS AND METHODS

Bacterial strains:

The standard bacterial strains were obtained from the Department of Microbiology, P.G. Center, Kuvempu University, Tolahunase, Davangere, Karnataka, India. Four strains of Gram-negative bacteria *Escherichia coli* ATCC 69314, *Klebsiella pneumoniae* NCIM 2719, *Pseudomonas aeruginosa* NCIM 2200 and *Agrobacterium tumefaciens* NCIM 2943 and two strains of Gram-positive bacteria *Staphylococcus aureus* NCIM 2080 and *Bacillus subtilis* MTCC 441 were used. The organisms were maintained on nutrient agar slants at 4°C and subcultured in to nutrient broth by a picking off technique for 24 hours before use (Aneja, 2003).

Preparation of nutrient agar media:

Nutrient agar (Hi Media, India) was used as the bacteriological medium. In the preparation of media, peptone-10g, beef extract-3g, sodium chloride-5g and agar-15g were dissolved in double distilled water and volume made up to 1000ml. the pH was adjusted to 7.2 to 7.5 by adding 0.1N NaOH or 0.1 N Hcl. To this nutrient broth, 15g of agar was added and kept on the boiling water bath to the agar. Later the medium was dispensed to conical flask and plugged with non-absorbent wool and sterilized in the autoclave for 15-30 min at 121°C.

Plant materials:

The leaves of *A. indicum* were initially rinsed with distilled water and dried in the laboratory

at $37\pm 1^{\circ}\text{C}$ for 24h. Preparation and preservation of plant extracts were obtained from air-dried plant materials according to the standard methods (Bhattacharjee *et al.*, 2006).

Extraction of plant constituents:

The dried leaves were powdered and soaked in the methanol for about 10-15 days then this cold extract is subjected to distillation at low temperature under reduced pressure in rotary flash evaporator and concentrated on water bath to get the crude extract. Then powdered leaves were subjected to soxhalation and are exhaustively extracted with petroleum ether, chloroform and methanol for 48 hours. The solvent was distilled off at lower temperature under reduced pressure in rotovapour and concentrated on water bath to get the crude extract.

Well plate method:

The bioassay for bacterial strains was subjected to well plate method. On the nutrient agar medium culture were spread and well were made by borer of 5mm size. To the well different volumes of (30 μl , 40 μl and 50 μl) of leaf extract were placed and plates were incubated for 24hrs at 37° . The diameter of zone of inhibition in mm recorded after incubation. The experiment was performed in triplicates and average diameter of the colony obtained.

RESULTS AND DISCUSSION

The well plate method for antibacterial activity showed significant reduction in bacterial growth in terms of zone of inhibition around the well. Among bacterial forms tested *P. aeruginosa*, *S. aureus* and *K. pneumoniae* were found more sensitive to the crude extract and *E. coli* shows less sensitive to the crude extract. The zone of inhibition was increased with increase of concentration of extract in well.

Table 1: Antibacterial activity of petroleum ether extracts of *A.indicum*

Bacterial strains	30 μg	40 μg	50 μg	Ciprofloxacin (50 μg)
<i>S. aureus</i> NCIM 2080	13.21 \pm 0.25	15.30 \pm 0.10	16.83 \pm 0.12	17.95 \pm 0.37
<i>P. aeruginosa</i> NCIM 2200	9.83 \pm 0.17	11.72 \pm 0.22	14.91 \pm 0.48	19.01 \pm 0.25
<i>K. pneumoniae</i> NCIM 2719	9.48 \pm 0.32	11.13 \pm 0.28	12.57 \pm 0.30	18.79 \pm 0.86
<i>B. subtilis</i> MTCC 441	8.02 \pm 0.50	9.84 \pm 0.25	12.05 \pm 0.39	20.31 \pm 0.33
<i>A. tumefaciens</i> NCIM 2943	7.09 \pm 0.21	6.35 \pm 0.23	9.40 \pm 0.26	16.80 \pm 0.78
<i>E. coli</i> ATCC 69314	6.49 \pm 0.32	5.97 \pm 0.22	7.75 \pm 0.30	14.49 \pm 0.32

In the case of *P. aeruginosa*, *S. aureus* and *K. pneumoniae* the zone of inhibition of Petroleum ether extract is (16.83 \pm 0.12mm, 14.91 \pm 0.48mm and 12.57 \pm 0.30mm) respectively, for

chloroform extract (13.54±0.12mm, 12.64±0.08mm and 10.73±0.09 mm) respectively and Methanol extract (18.72±0.01mm, 17.49±0.14 mm and 17.15±0.03 mm) respectively. All the three solvent system showed very less zone of inhibition against *A. tumefaciens* and *E.coli*. The results revealed that, the zone of inhibition was varying between 19.12±0.1mm to 14.13±0.03mm. These data indicated that petroleum ether and methanol crude extract of plant exhibited strong antibacterial activity compared to chloroform crude extract.

Table 2: Antibacterial activity of chloroform extracts of *A. indicum*

Bacterial strains	30 µg	40 µg	50 µg	Ciprofloxacin (50µg)
<i>S. aureus</i> NCIM 2080	10.05±0.14	12.06±0.22	13.54±0.12	15.21±0.13
<i>P. aeruginosa</i> NCIM 2200	8.04±0.10	11.86±0.08	12.64±0.08	17.61±0.07
<i>K. pneumoniae</i> NCIM 2719	9.21±0.13	11.01±0.17	10.73±0.09	16.86±0.08
<i>B. subtilis</i> MTCC 441	7.00±0.15	7.87±0.15	11.75±0.12	18.81±0.03
<i>A. tumefaciens</i> NCIM 2943	5.01±0.17	7.65±0.10	7.08±0.15	15.68±0.07
<i>E. coli</i> ATCC 69314	4.98±0.21	4.82±0.12	6.05±0.12	14.879±0.03

Table 3: Antibacterial activity of Methanol extracts of *A. indicum*

Bacterial strains	30 µg	40 µg	50 µg	Ciprofloxacin (50µg)
<i>S. aureus</i> NCIM 2080	15.84±0.05	16.17±0.11	18.72±0.01	19.12±0.01
<i>P. aeruginosa</i> NCIM 2200	14.89±0.10	14.95±0.12	17.49±0.14	19.54±0.12
<i>K. pneumoniae</i> NCIM 2719	14.12±0.02	15.76±0.02	17.15±0.03	18.97±0.08
<i>B. subtilis</i> MTCC 441	12.29±0.12	13.75±0.12	16.73±0.08	18.87±0.03
<i>A. tumefaciens</i> NCIM 2943	8.19±0.01	9.65±0.03	11.76±0.2	15.87±0.07
<i>E. coli</i> ATCC 69314	6.83±0.12	6.13±0.09	9.75±0.13	14.13±0.03

In the present study the antibacterial activity of *A. indicum* may be attributed to individual or synergistic effect of phytoconstituents present in it. The petroleum ether and methanol extracts of leaf plant exhibited significant wide spectrum of antibacterial activity against both Gram's positive and Gram's negative bacteria.

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