

ANTIMICROBIAL ACTIVITY AND PRILIMINARY PHYTOCHEMICAL SCREENING OF ABUTILON INDICUM

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ABSTRACT

The aim of the present study was to investigate the antibacterial properties and phytochemical evaluation of of *Abutilon indicum*. The organic solvent (Ethanol, Methanol, Hexane) and water extracts from the whole plant of *Abutilon indicum* (Malvaceae) were tested against, *Salmonella typhimurium*, *Proteus vulagaris*, *Shigella dysenteriae* and a fungal pathogen *Candida albicans* by Agar disc diffusion method. The results showed prominent antimicrobial activity against the tested microbial pathogens. Of all those, Ethanol extract was found to give a strong antimicrobial effect when compared to the other extracts (Methanol, Hexane and water). Phytochemicals like tannins, flavonoids, alkaloids, steroids and terpenoids are found. The Anthraquinones were found to be absent in plant material observed.

KEY WORDS

Abutilon indicum, Agar Disc Diffusion Method, Phytochemicals, Antimicrobial Properties

INTRODUCTION

Abutilon indicum (Linn.) Sweet (Malvaceae) is a shrub distributed throughout India and other topical regions of the world. The various parts of the plant (leaves, roots, seeds and seed and seed oil) are widely used in variety of ailments in traditional system of medicine such as Ayurveda and Siddha. The roots are useful in treating uterine hemorrhagic discharges. Leaves are useful in treatment toothache, lumbago, piles and all kinds of inflammation. The aim of present research is, to determine the preliminary phytochemical constituents, antimicrobial activity of various extracts of the leaves and stems of *Abutilon indicum*.

Traditional medicines derived from medicinal plants are used by about 60% of the world's population. Though there are various approaches

to control diseases and their secondary complications, herbal formulations are preferred due to lesser side effects and low cost. The use of and search for drugs and dietary supplements derived from plants has been increased in recent years. Botanists, Ethno pharmacologists, microbiologists, and chemists are combing the earth for phytochemicals and drugs which could be developed for treatment of highly infectious diseases in a natural way.. While 30 to 50% of current pharmaceuticals are derived from plants, only few of them are used as antimicrobials. Traditional healers have long used plants to prevent or cure infectious conditions. Plants are rich in a wide variety of secondary metabolites, such as Terpenoids, Tannins, Alkaloids, Flavonoids, saponins and Anthraquinones which

have been found in vitro to have antimicrobial properties.

Abutilon indicum belonging to family Malvaceae, commonly called as Country mallow (English), Kanghi (Hindi), Atibala (Sanskrit). *Abutilon indicum* is a perennial shrub, softly tomentose and upto 3 m in height. The leaves are evergreen, Base-cordate, stipulate, fili form, ovate, acuminate, toothed, rarely subtrilobate and 1.9-2.5 cm long. Petiole 1.5-1.70cm long, cylindrical, yellowish in colour, stellate and hairy. The flowers are yellow in color, peduncle jointed above the middle. The petioles are 3.8-7.5 cm long; stipules 9mm long; pedicels often 2.5-5mm long, axillary solitary, jointed very near to top and the seeds are 3-5mm, kidney shaped, reniform, tubercled or minutely stellate hairy, black or dark brown.^[1-3] This work attempts to find out the anti microbial properties of *Abutilon indicum*, against select list of microbes and extraction, isolation and characterization of compounds that give these properties to these plants.



Figure:1. *Abutilon indicum* with fruits and flower

MATERIALS AND METHODS

Abutilon indicum plants were collected from various places in and around the areas of Kurnool. Whole Plants of both the species were collected from mature plants and identified by comparing with herbarium specimens. The plants were air- dried and powdered. The dry powder was extracted by refluxed in 100 mL methanol for 24 h, using a Soxhlet apparatus (Khan *et al.*, 1988). The extract was filtered using Whatman

filter paper, No. 1. The filtrate was then evaporated using rotatory evaporator and dried at 55°C. Ethanol, methanol, hexane and distilled water extracts are obtained and all the extracts are preserved. Dried extract was stored at 20°C in labeled, sterile capped bottles. Stock cultures of microbes are maintained at a temperature of 4 degrees centigrade, active cultures are prepared by growing in tubes of Muller-Hinton (MHB) / Potato dextrose agar (PDA) for bacteria and Sabouraud dextrose broth (SDB) for fungi.

Microorganisms:

The bacterial colonies were isolated from hospital samples at Kurnool, their pure cultures were maintained in nutrient agar and stored at 4°C. Three gram negative bacterial species were grown, namely *Salmonella typhimurium*, *Proteus vulgaris*, *Shigella dysenteria* and the fungus *Candida albicans*.

Antimicrobial assay:

Sensitivity tests were performed by disc diffusion with standard antibiotics, following Kirby-Bauer method (Bauer *et al.*, 1966). The assessment of antimicrobial activity was done based on measurements of the diameter of inhibition zones (NCCLS, 1998). Of the four extracts, ethanolic extract has given interesting results and the aqueous extract showed no response.

phytochemical screening:

Phytochemical testing is done for the promising extract of all the four types of extracts as it has shown the interesting activity.

1. Braemer's test for Tannins
2. Liebermann-burchardt test for Steroids
3. Liebermann-burchardt test and Salkowski test for Terpinoids
4. Dragendroff's reagent test for Alkaloids
5. Shinoda test for Flavanoids
6. KOH test FOR Anthraquinones
7. Keller-Kilianii test for Cardiac glycosides
8. Frothing test for saponins

Antimicrobial disc diffusion assay:

Antibacterial and antifungal activities of the four plant extracts were investigated by the disc diffusion method^[4]. The MHA plates, containing an inoculum size of 106 colony-forming units (CFU)/mL of bacteria or 2x10⁵ CFU/mL yeast cells on SDA were spread on the solid plates with a glass rod. Then discs (4.0-mm diam.) impregnated with 20 µL of each extract at a concentration of 100.0mg/mL were placed on the inoculated plates. Similarly, each plate carried a blank disk by adding solvent control alone in the centre, and antibiotic discs (6.0-mm diam.) of (20 µg/ml, Streptomycin sulphate for bacteria) and Nystatin (20 µg/ml, for fungal) were also used as a positive control. All of the plates were incubated at 37°C for 18 hours for bacteria and at 28°C for 48 hours for fungi. The zones of growth inhibition around the discs were measured after 18 hours of incubation at 37°C for bacteria and 48 hours for fungi at 28°C, respectively. The sensitivity of the microorganism species to the plant extracts was determined by measuring the sizes of inhibitory zones on the agar surface around the discs.

RESULTS AND DISCUSSION

The aqueous and hexane extracts of plant has shown negligible antimicrobial activity on tested pathogens, whereas the Methanol extract of plant has shown maximum inhibition on *Salmonella typhimurium* (10.03 ± 1.1) and minimum on *Shigella dysenteriae* (9 ± 0.9) and it has no effect on *Proteus vulgaris*. Ethanol extract of plant has shown maximum inhibition on *Shigella dysenteriae* (12 ± 0.7) and minimum on *Salmonella typhimurium* (11 ± 0.9) and it has no effect on *Candida albicans*. Of all the extracts ethanolic extracts have shown maximum

inhibition, so it is used for phytochemical screening of secondary metabolites.

The results of the phytochemical screening to test the presence of tannin, anthraquinone, alkaloid, saponin, phlobatannin, flavonoid, cardiac glycosides, volatile oils, terpenoids and steroids in the extracts from various parts of *Abutilon indicum* are shown in Table I. The preliminary phytochemical screening study revealed that the leaf of *Abutilon indicum* contains moderate amounts of tannins and steroids, small amounts of cardiac glycosides, alkaloids, flavonoid, terpenoid & saponins. The roots of *Abutilon indicum* contain small amounts of flavonoid, tannins and steroids. The flowers contain moderate amount of flavonoids and small amounts of saponins & steroids. The stem contains small amounts of saponins, alkaloids and terpenoids. Anthraquinones were found to be absent in the entire plant.

Treating Gram-negative bacterial infections can be difficult because of several unique features of these bacteria. For example, the unique nature of their cell wall makes them resistant to several classes of antibiotics. Infections have typically been treated with broad-spectrum antibiotics, such as beta-lactams followed by carbapenems. However, even these drugs have become ineffective against some bacteria, leaving researchers to go for natural resources, which are medicinal plants. New drugs to combat Gram-negative bacterial infections are needed. In addition, researchers are unravelling the molecular mechanisms of drug resistance in Gram-negative bacteria to identify novel strategies to combat these pathogens. This paper helps in formulating natural principles to combat drug resistance of certain gram negative bacteria.

Table.I. Antimicrobial activity of *Abutilon indicum*.

Solvent extracts	μL	Zone of inhibition in mm			
		<i>Salmonella typhimurium</i>	<i>Shigella dysenteriae</i>	<i>Proteus vulgaris</i>	<i>Candida albicans</i>
Aqueous	20	-N-	-N-	-N-	-N-
Methanol	20	10.3 $\pm$ 1.1	9 $\pm$ 0.9	-N-	7.1
Ethanol	20	11 $\pm$ 0.9	12 $\pm$ 0.7	12 $\pm$ 0.4	-N-
Hexane	20	-N-	-N-	-N-	-N-
Streptomycin sulphate	20	29 $\pm$ 0.7	25 $\pm$ 0.8	21 $\pm$ 0.5	-
Nystatin	20	-	-	-	17 $\pm$ 1.8

-N- --No activity

Table II. Phytochemical Screening of Secondary Metabolites from *Abutilon indicum* Ethanolic extract

S.NO	Secondary metabolites	Name of the test	Leaf	Stem	Flower	Root
1.	Tannins	Braemer's test	2+	--	--	1+
2.	Flavonoids	Shinoda test	1+	--	2+	1+
3.	Anthraquinone	KOH test	--	--	--	--
4.	Saponins	Frothing test	1+	1+	1+	--
5.	Cardiac glycosides	Keller-Kiliani test	1+	--	--	--
6.	Alkaloid	Dragendorff test	1+	1+	--	--
7.	Steroids	Lieberman Burchardt test	1+	--	1+	1+
8.	Terpenoids	Steroids test	2+	--	--	--
		LiebermannBurchardt test	1+	1+	--	--
		Salkowski test	1+	1+	--	--

'2+' Moderate, '1+' Small amounts, '--' absent

CONCLUSION

In conclusion, all the extracts investigated possessed activity against at least one strain of bacteria and/or fungi. Further studies aimed at the isolation and identification of active substances from the Ethanol extracts of *Abutilon indicum* could also evolve compounds with effective natural medicinal values for the cure of microbial disorders. The plant is said to be a source of many bioactive compounds acting against some human diseases. The present study

provides proof of *A. Indicum* for its fight against infectious microbes.

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