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### RESEARCH ARTICLE

#### PHARMACOLOGICAL STUDY OF AERIAL PARTS OF TWO MALVACEOUS PLANTS.

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#### Manuscript Info

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Hibiscus micranthus, Abutilon bidentatum, Aerial parts, Ethanolic extract, Antimicrobial activity.

#### Abstract

The present study deals with the pharmacological studies of ethanolic extract of aerial parts of two Malvaceous plants : Hibiscus micranthus Linn. and Abutilon bidentatum Hochst. The ethanolic extracts of aerial parts of plants were examined for antibacterial and antifungal studies by using Disc diffusion method. *Escherichia coli*, *Klebsiella aerogenes*, *Proteus vulgaris* and *Pseudomonas aeruginosa* as Gram -ve and *Staphylococcus aureus* as Gram +ve bacteria were selected for antibacterial efficacy and *Aspergillus flavus*, *Aspergillus niger*, *Fusarium moniliforme* and *Rhizoctonia bataticola* fungi were selected for antifungal efficacy. In case of antibacterial efficacy, the ethanolic extract of H. micranthus did not exhibit any activity against selected bacteria but A. bidentatum exhibited trace activity against *S. aureus* and in case of antifungal efficacy, extract of both the plants inhibited growth of almost all the test fungi. Out of selected fungi, the growth of *F. moniliforme* inhibited the most by ethanolic extract (aerial parts) of both the plants.

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#### Introduction:-

Medicinal plants are the nature's gift to human being to make disease free healthy life. The medicinal importance of plant(s), attracted the attention of chemists to study the natural products. Plant products are being increasingly tried upon and utilized for various purposes in the field of pharmaceuticals. It is believed that the extracts and products of plants being natural, are harmless to human beings and their environment. The present work was, therefore, undertaken with the antimicrobial studies of two plants belongs to family **Malveceae**. The genus Hibiscus comprises about 46 species, 20 of which are found in India and some of these are found to be medicinally very important (Chopra et al., 1971; I.C.M.R., 1987; Yoganarsimhan et al., 1979). H. micranthus is a herb growing in the hotter parts of India. It is used as a febrifuge (Kirtikar et al., 1975). In India certain parts of Gujarat, the fruits and flowers of this plant are used as hypoglycemic agent (Kakrani et al., 2005). The plant has been scientifically validated for its antipyretic, anti-inflammatory, hematological effects (Al-yahya et al., 1987). A survey of literature revealed that no work on the antimicrobial studies is reported so far. The genus Abutilon comprises mainly of shrubs and small trees. About 10 species are found in India, some of which are found to be medicinally useful. A. indicum have several applications in medicinal world (Chopra et al., 1971; Kirtikar et al., 1975; Sinha et al., 1985; Sharma et al., 1989). A. bidentatum is a shrub growing throughout the hotter parts of India. A perusal of literature revealed that this plant remained unexplored by the pharmacologists but their sister species have been found to possess promising activity of one type or another. The ethanolic extracts of aerial parts of both the plants were examined for selected test bacteria and test fungi.

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## Material and Methods:-

### Plant Materials:-

The plant material (aerial parts) of *H. micranthus* Linn. and *A. bidentatum* Hochst. were collected from the campus university of Rajasthan, Jaipur and were identified for authenticity in the department of botany, University of Rajasthan, Jaipur (Herbarium sheet No. RUBL 754 and 824 respectively).

### Extraction:-

Powdered aerial parts of both the plants were extracted on a steam bath for 8 X 3 hrs. with ethanol separately. Later, each of these extract was filtered, the residue re-extracted (2 X) for complete exhaustion, the extracts were pooled individually and concentrated under reduced pressure and stored in dark colored bottle at 4°C in a refrigerator.

### Sources of Test Organisms:-

**Bacteria:-** Pure culture of all test organisms, namely *Escherichia coli*, *Klebsiella aerogenes*, *Proteus vulgaris* and *Pseudomonas aeruginosa* as Gram -ve and *Staphylococcus aureus* as Gram +ve bacteria, the human pathogens were obtained through the courtesy of SMS Medical College, Jaipur, which were maintained on Nutrient Broth Medium.

**Fungi:-** The pure cultures of test fungi, namely *Aspergillus flavus*, *Aspergillus niger*, *Fusarium moniliforme* and *Rhizoctonia bataticola* were obtained from the Seed Pathology Laboratory, Department of Botany, University of Rajasthan, Jaipur, which were maintained on Potato Dextrose Agar (PDA) medium.

### Culture of Test Microbes:-

For the cultivation of bacteria, Nutrient Broth Medium (NBM) was prepared using 8% Nutrient Broth (Difco) in distilled water and agar-agar and sterilized at 15 lbs for 25-30 min. The agar test plates were prepared by pouring - 15 ml of NBM into the petri-dishes (10 mm) under aseptic conditions. The peptone saline solution was prepared (by mixing 3.56 g  $\text{KH}_2\text{PO}_4$  + 7.23 g  $\text{NaH}_2\text{PO}_4$  + 4.30 g NaCl + 1.00 g peptone in 1000 ml distilled water, followed by autoclaving) and the bacterial cultures were maintained on this medium by regular sub-culturing and incubation at 37°C for 24 hrs.

However, for the cultivation of fungi, Potato Dextrose Agar (PDA) medium was prepared by mixing 1000 ml potato infusion prepared from 200 g potatoes, 20 g agar and 20 g glucose, followed by autoclaving; the test fungi were incubated at 27°C for 48 hrs and the cultures were maintained on the same medium by regular sub-culturing.

To prepare the test plates, in both bacteria and fungi, 10-15 ml of the respective medium was poured into the petri-dishes and used for screening.

For assessing the bactericidal efficacy, a fresh suspension of the test bacteria was prepared in saline solution from a freshly grown agar slant, while for fungicidal efficacy, a uniform spread of the test fungi was made using sterile swab.

### Bactericidal and fungicidal Assay:-

For both bactericidal and fungicidal assays Disc diffusion method (Gould et al.,1952) was adopted, because of reproductivity and precision. The different test organisms were preceded separately using a sterile swab over previously sterilized culture medium plates and the zone of inhibition were measured around sterilized dried discs of Whatman No. 1 paper (6 mm in diameter), which were containing 500 µg and 1000 µg of the test extracts and control amikacin (10 µg/ml) or mycostatin (100 units /ml) as reference drugs separately. These plates were initially placed at low temperature for 1 h, so as to allow the maximum diffusion of the compound from the discs into the agar plate and later, incubated at 37°C for 24 h in case of bacteria and 48 h for fungi, after which the zones of inhibition could be easily observed.

**Table 1:-** Bactericidal activity of selected plants.

Plant species	Dose µg/disc	E.coli		<i>K.aerogenes</i>		<i>P.vulgaris</i>		<i>P. arruginosa</i>		<i>S. aureus</i>	
		IZ*	AI*	IZ	AI	IZ	AI	IZ	AI	IZ	AI
H. micranthus	1000	-	-	-	-	-	-	-	-	±	-
	500	-	-	-	-	-	-	-	-	±	-
A. bidentatum	1000	-	-	-	-	-	-	-	-	±	-
	500	-	-	-	-	-	-	-	-	-	-
IZ : inhibition zone (in mm) including the diameter of disc (6mm)											
AI : activity index=( inhibition zone of sample/inhibition zone of standard)											
Standard : Amikacin = 10 µg/ml.											
( ± ) Trace activity: ( - ) No activity											

**Table 2:-** Fungicidal activity of selected plants.

Plant species	Dose µg/disc	<i>A. flavus</i>		<i>A. niger</i>		<i>F. moniliforme</i>		<i>R. bataticola</i>	
		IZ*	AI*	IZ	AI	IZ	AI	IZ	AI
H. micranthus	1000	11.00	0.50	11.00	0.48	13.00	0.57	10.00	0.41
	500	9.00	0.41	7.00	0.30	10.00	0.43	7.00	0.32
A. bidentatum	1000	11.00	0.50	12.00	0.52	17.00	0.74	-	-
	500	6.00	0.27	8.00	0.35	14.00	0.61	-	-
IZ: inhibition zone (in mm) including the diameter of disc (6mm)						AI :			
activity index=( inhibition zone of sample/inhibition zone of standard									
Standard : Mycostatin = 100 units/disc .									

**Result and Discussion:-**

In case of bactericidal activity ethanolic extract of aerial parts of both the plants failed to demonstrate any significant activity against any test bacteria except trace activity against *S.aureus*. *H. micranthus* showed activity at both concentrations ( 1000 µg/disc and 500 µg/disc) while *A. bidentatum* exhibited activity only at 1000 µg/disc. Table 1. In case of fungicidal efficacy , both the extracts showed maximum activity against *F. moniliforme* . In comparison, *A. bidentatum* is more active ( IZ=17 mm at 1000 µg/disc , IZ=14 mm at 500 µg/disc ) than *H. micranthus* ( IZ=13 mm at 1000 µg/disc , IZ=10 mm at 500 µg/disc ) against this test fungi. On the other hand , ethanolic extract of aerial parts of *H. micranthus* demonstrated antifungal activity against *R. bataticola* ( IZ= 10 mm at 1000 µg/disc , IZ=7 mm at 500 µg/disc ) while *A. bidentatum* failed to exhibit any activity against this test fungi. Table 2.

**Conclusion:-**

The present investigation revealed that both the Malvaceous plants are potentially against fungi and can be used as safe and harmless antifungal agents.

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