



RESEARCH ARTICLE

MICROBIOLOGY

EVALUATION OF ANTIBACTERIAL ACTIVITY OF CUSCUTA REFLEXA AND ABUTILON INDICUM.**AYESHA MATEEN^{*1}, P.V.K.SURESH¹, PARWEZ AHMED².**¹Central Research Institute of Unani Medicine, Opp, E.S.I. Hospital, Erragadda, Hyderabad – 500038, India.²Regional Research Institute of Unani Medicine, University of Kashmir, Srinagar- 190006, India.**AYESHA MATEEN**Central Research Institute of Unani Medicine, Opp, E.S.I. Hospital, Erragadda,
Hyderabad – 500038, India**ABSTRACT**

There is a currently worldwide upsurge in the use of herbal preparations and active ingredients of medicinal plant in health care. This is particularly true in the rural areas of Asian countries where herbal medicines are the only choice for treating human ailments. Present study reveals the difference in the antimicrobial activity pattern extracts of *Cuscuta reflexa* and *Abutilon indicum* in ethanol, chloroform, ethyl acetate and aqueous solvents against the pathogenic organisms *E.coli* (ATCC-25922), *Staphylococcus aureus* (ATCC-25923), *Pseudomonas aeruginosa* (ATCC-27853), *Proteus vulgaris*(ATCC 6380), *Salmonella paratyphi*(ATCC-9150), *Shigella sonnei*(ATCC-25931), *Salmonella typhimurium*(ATCC-25241), and *Klebsiella pneumonia*(ATCC-27736). Among all the extracts Ethanol and Chloroform showed greater activity with a zone of inhibition ranging from 6mm to 17mm. Ethanol extract of *Cuscuta reflexa* exhibited better antibacterial activity with IC₅₀ value range 16.93-55mg/ml when compared with ethanol extract *Abutilon indicum*. The results of crude extracts were compared with standard Antibiotic *Chloramphenicol*(30 mcg).



KEY WORDS

Abutilon indicum, *Cuscuta reflexa*, Antibacterial, Solvent extracts, MTT.

INTRODUCTION

Antibiotics are one of our most important weapons in fighting bacterial infections and have greatly benefited the health-related quality of human life since their introduction. However, over the past few decades these health benefits are under threat as many commonly used antibiotics have become less and less effective against certain illnesses not only because many of them produce toxic reactions but also due to emergence of drug resistant bacteria. It is essential to investigate newer drugs with lesser resistance. Systematic studies among various pharmacological compounds have revealed that any drug may have the possibility of possessing diverse functions and thus may have useful activity in completely different spheres of medicine.

Drugs derived from natural sources play a significant role in the prevention and treatment of human diseases. In many developing countries, traditional medicine is one of the primary health care systems^{1, 2}. Herbs are widely exploited in the traditional medicine and their curative potentials are well documented³. About 61% of new drugs developed between 1981 and 2002 were based on natural products and they have been very successful especially in the areas of infectious disease and cancer⁴. Recent trends, however, show that the discovery rate of active novel chemical entities is declining⁵. Therefore, there is a need to bio prospect new sources and if possible from less explored regions and habitats to maximize the discovery of novel bioactive metabolites.

Abutilon indicum (*Malvaceae*) is extensively grown in Bangladesh, India, Pakistan, Srilanka⁶. The plant is considered as astringent, antibacterial, anthelmintic, carminative and diuretic. It is used locally for colds, high fever, mumps, tuberculosis, bronchitis, diabetes, carbuncle, haemorrhoids, hernia, diarrhoea and various types of worm infections⁵. Previous

phytochemical investigation of the plant revealed the presence of chemical constituents namely luteolin, chrysoeriol, apigenin 7-O-beta rhamnopyranosyl, quercetin, triacontanoic acid, ursenol, methylstigmasterol, glucopyronoside etc⁷. Bioactivity guided isolation of *Abutilon indicum* yielded eugenol (4-allyl-2-methoxyphenol), which was found to possess significant analgesic activity⁸ in acetic acid induced writhing test. *Cuscuta reflexa* (*convulvulaceae*), commonly known as Akashbela in Hindi. The plant is distributed throughout India, Ceylon and Malaya. *Cuscuta reflexa* has been investigated for antispasmodic, haemodynamic, bradycardia¹⁰, antisteroidogenic¹¹, antihypertensive, muscle relaxant, cardiotoxic¹², psychopharmacological¹³ and antiviral and anticonvulsant¹⁴ activities. Many chemical constituents have been isolated from *Cuscuta reflexa* such as, Cuscutin, amarbelin, myricetin, quercetin, coumarin and oleanolic acid¹⁵. As a part of our continuing study on chemical and biological characterization of different plants, attempt was made this time to investigate the antimicrobial activity of *Abutilon indicum* and *Cuscuta reflexa* against different Gram-positive and Gram-negative bacteria²⁰.

MATERIALS AND METHODS

PLANT MATERIAL

Flowers of *Abutilon indicum* (*Malvaceae*) were collected from local area (Hyderabad, Andhra Pradesh, India). Plant material of *Cuscuta reflexa* (*convulvulaceae*) were also procured from local area (Hyderabad, Andhra Pradesh, India) and were also collected from Herbal garden CRIUM, Hyderabad. These were authenticated by a Botanist at CRIUM, Hyderabad (A.P, India).



BACTERIAL CULTURES

Bacterial strains used in this study were *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853), *Proteus vulgaris* (ATCC-6380), *Salmonella typhimurium* (ATCC-25241), *Salmonella paratyphi* (ATCC-9150), *Shigella sonnei* (ATCC-25931), *Klebsiella pneumonia* (ATCC-27736) obtained from Himedia, Mumbai, India.

EXTRACTION

The collected plant material were washed and air-dried for 48 h at the room temperature, chopped and grounded into small pieces and then soaked with different types of solvent at room temperature. The extract was filtered and then entire extract was concentrated to dryness using rotary evaporator under reduced pressure.

EVALUATION OF ANTI-BACTERIAL ACTIVITY

Anti-Bacterial activity of the extract was determined by Agar diffusion assay¹⁶. Bacterial strains were first grown in *Mueller Hinton broth* (MHB) under shaking condition for 24 h at 37°C and after incubation period, 0.1ml of the test inoculums was spread evenly with a sterile glass spreader on *Mueller Hinton Agar* (MHA) plates. The seeded plates were allowed to dry in the incubator at 37°C, wells were made using sterile 6mm cork borer in the inoculated MHA plate. The wells were filled with 100µl of the extracts (re-suspended in respective solvents). The concentration of stock extracts were 100 mg/ml. The inoculated plates were incubated at 37°C for 24 h. The plates were observed for the presence of inhibition of bacterial growth that was indicated by a clear zone around the wells. The size of the zones of inhibition was measured and the antibacterial activity was expressed in terms of average diameter of the zone of inhibition in millimeters. The results were compared with the standard antibiotics. The photograph was taken in U.V-Visible documentation system.

MTT ASSAY

Inoculum of the microorganisms were prepared from the 24 h *Mueller-Hinton broth* (Himedia) cultures and suspensions were adjusted to 10⁵ CFU/ml. Percentage of bacterial growth inhibition of the extracts were determined based on a micro-well dilution method¹⁷. The 96-well sterile plates were prepared by dispensing 100 µl of the inoculated broth plus a 20 µl aliquot of the plant extract in each well and 20 µl of broth as a negative control, and then made up to 200 µl using sterile broth. *Chloramphenicol* (Himedia, Mumbai) were included as positive control. Plates were covered and incubated for 2 h at 37°C. Bacterial growth was determined after addition of 10µl of MTT [(3-(4-5-Dimethyl-2-yl)-2,5-diphenyl tetrazolium bromide, A.R.; *Thiazoyl blue*, A.R.) 5mg/ml] and incubate for half an hour till the blue color developed in the negative control. At the end, the absorbance was determined by ELISA reader.

RESULTS AND DISCUSSION

The present study has been designed to evaluate antibacterial activity of crude ethanolic extracts of *Abutilon indicum* (Flower) and *Cuscuta reflexa* plants. The results of MTT Assay show that the crude ethanolic extract of *Abutilon indicum* and *Cuscuta reflexa* exhibit good percentage of inhibition. *E.coli* and *Shigella sonnei* shown more sensitivity with 95.9% and 96.3% of inhibition. *Staphylococcus aureus*, *Salmonella typhi*, *Salmonella paratyphi*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Proteus vulgaris* comparably exhibit less sensitivity with 86.8%, 90.9%, 86.08, 87.9, 87.9 and 81.9% (Table-I) of inhibition at concentration of 100mg/ml, with IC₅₀ value range 51.92 to 61.05mg/ml (Table-II) by using Ethanol extract of *Abutilon indicum* (Flower). *Klebsiella pneumonia* shown higher sensitivity among all the bacterial strains used with IC₅₀ value 16.93mg/ml, *Staphylococcus aureus*, *Shigella sonnei*, *Pseudomonas aeruginosa*, *E.coli* and *Proteus vulgaris* also exhibit better sensitivity with IC₅₀ values 21.55, 19.20, 23.94, 24.60 and 20.03 mg/ml (Table-III)

by using crude Ethanol extract of *Cuscuta reflexa*.

Table I
Antibacterial activity of Different Plant Extracts on various Bacterial strains.
DIAMETER OF ZONE OF INHIBITION (mm)

Plant Extracts	Staphylococcus aureus	Pseudomonas aeruginosa	Salmonella typhi	Salmonella paratyphi	Proteus vulgaris	Klebsiella pneumoniae	E.coli	Shigella sonnei
1. Abutilon indicum (Eth)	17	11	08	11	12	12	13	09
2. Abutilon indicum(Aq)	ND	ND	ND	ND	ND	ND	ND	ND
3. Abutilon indicum(Chl)	N	06	N	N	09	15	N	12
4. Abutilon indicum (Ethact)	ND	ND	ND	ND	ND	ND	ND	ND
5. Cuscuta reflexa (Eth)	14	10	10	14	N	13	N	08
6. Cuscuta reflexa (Aq)	N	N	N	N	17	N	N	N
7. Cuscuta reflexa (Chl)	N	N	11	08	14	14	N	N
8. Cuscuta reflexa (Eth act)	N	N	N	N	N	N	N	N
9. Chloramphenicol (25mcg)	21	08	09	09	08	19	08	08

Note: Aq- Aqueous; Eth- Ethanol; Chl-Chloroform; Ethact- Ethylacetate; ND – Not done; N-No activity.

Table II
Represents percentage of inhibition and IC-50 Values of Ethanol extract of Abutilon indicum.

Plant species	Organism	PERCENTAGE OF INHIBITION (%)				
		20mg/ml	40mg/ml	80mg/ml	100mg/ml	IC 50 Value
Abutilon indicum	Staphylococcus aureus	21.5	6.8	49.9	86.8	57.00
	Salmonella typhi	11.2	31.8	63.8	90.9	55.00
	Salmonella paratyphi	28.2	40.4	73.7	86.08	58.08
	E.coli	33.07	45.3	94.2	95.9	52.13
Abutilon indicum	Shigella sonnei	26.5	22.8	56.9	96.3	51.92
	Pseudomonas aeruginosa	15.5	5.6	42.7	87.9	56.88
	Klebsiella pneumoniae	7.5	2.2	42.3	87.9	56.88
	Proteus vulgaris	44.8	39.7	59.7	81.9	22.2 - 61.05

Table III
Represents percentage of inhibition and IC-50 Values of Ethanol extract of
***Cuscuta reflexa*.**
 PERCENTAGE OF INHIBITION (%)

<i>Plant species</i>	<i>Organism</i>	25mg/ml	50mg/ml	100mg/ml	IC ₅₀ Value
	<i>Staphylococcus aureus</i>	58	87.4	90.7	21.55 -55.12
	<i>Salmonella typhi</i>	39.3	65.4	95.5	52.35
	<i>Salmonella paratyphi</i>	37.3	55.9	87.1	57.40
<i>Cuscuta reflexa</i>	<i>E.coli</i>	50.8	63.9	97	51.54
	<i>Shigella sonnei</i>	65.1	73.6	73.6	19.20-33.96
	<i>Pseudomonas aeruginosa</i>	52.2	70.5	91.5	23.94-54.64
	<i>Klebsiella pneumonia</i>	73.8	84.4	90.9	16.93-55.00
	<i>Proteus vulgaris</i>	62.4	79.7	79.9	20.03-31.36

Different organic extracts (Aqueous, Ethanol, Chloroform and Ethylacetate) of *Abutilon indicum* and *Cuscuta reflexa* has been evaluated using agar well diffusion assay. Among all the extracts Ethanol show better activity compared with other extracts, *Staphylococcus aureus* found to be more sensitive toward ethanolic extract of *Abutilon indicum* with a zone of inhibition diameter 17mm, least antibacterial activity was shown by *Shigella sonnei* with a zone of inhibition diameter 9mm. *Cuscuta reflexa* aqueous extract was only effective on *Proteus vulgaris* with a zone of inhibition diameter 17mm, poor sensitivity was shown by *Pseudomonas aeruginosa* with a zone of inhibition diameter 6mm. *Klebsiella pneumoniae* shown good sensitivity for Chloroform extract of *Abutilon indicum* with a zone of inhibition diameter 15mm, *Staphylococcus aureus* and *Salmonella paratyphi* were shown sensitivity towards Ethanolic extract of *Cuscuta reflexa* with zone of inhibition 14mm. *Proteus vulgaris* and *Klebsiella pneumoniae* were sensitive for Chloroform extract of *Cuscuta reflexa* with a zone of inhibition diameter 14mm. Ethylacetate

extract of *Cuscuta reflexa* showed nil antibacterial activity. The results of crude organic extracts were compared with standard antibiotic *Chloramphenicol* (30 mcg).

The phytochemical compounds present in medicinal plants are known to play an important role of bioactivity in medicinal plants. Flavonoids exhibit anti-inflammatory, antibacterial, antiallergic and analgesic activity. The presence of the saponin compound in these plants supports the usefulness in the managing inflammation. Thus these plants not only possess antibacterial property but can improve overall health status of the patient.

CONCLUSION

The plant is said to be a source of many bioactive principles acting against some human ailments. The present study provides scientific authentication of *A. Indicum* and *C. reflexa* for their potential activities against infectious microbes.



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