Comparative evaluation of antimicrobial potential of different extracts of *Cuscuta reflexa* growing on *Acacia arabica* and *Zizyphus jujuba*

Chhabra Summit* Thakral Jatin*, Kamboj Pradeep, Paliwal Yogesh

* Department of Pharmacy, Manav College of Pharmacy, Hisar
* Corresponding Author: chhabrasumit28@gmail.com

Abstract

C.reflexa Roxb. (Cuscutaceae or Convolvulaceae) is a golden yellow leafless plant commonly known as amarbel, akashbel, swaranlata, devils guts, hair weed and love-wine. The methanol extracts of *C. reflexa* is implicated as an antimicrobial. Plant extracts of *C.reflexa* growing on different sources (*Acacia arabica* and *Zizyphus jujube*) were prepared using aqueous and various organic solvents viz. benzene, acetone, ethanol and methanol. Agar well diffusion technique was used to assess the antimicrobial potential of plant from different sources against gram positive bacteria (*Staphylococcus aureus* & *Staphylococcus epidermidis*), gram-negative bacteria (*Escherichia coli* & *Pseudomonas aeruginosa*) and fungus (*Aspergillus niger*). The diameter of zone of inhibition was taken as an indicator of antimicrobial effect. The present study showed a strong inhibitory effect of ethanol and methanol extracts of *C.reflexa* (*jujuba* and *arabica*) on most of the gram positive and gram negative bacteria. The aqueous extract of *C.reflexa* (*arabica*) failed to show any antimicrobial activity while *C.reflexa* (*jujuba*) showed very little effect. Thus *C.reflexa* growing on *Zizyphus jujuba* could be considered as a potential source of natural antimicrobials.

Keywords: C. reflexa, antimicrobial activity, zone of inhibition.

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***Author for Correspondence:** chhabrasumit28@gmail.com

INTRODUCTION

Over the last few decades, a great interest has developed in searching for antimicrobial drugs from natural plant products. This interest primarily arises from the belief that drugs derived from plants are safe and dependable compared with synthetic drugs that may have adverse effects on host besides their high cost. Natural antimicrobials come from a wide array of sources including plants, animals and microorganisms[1]. Researchers have so far discovered approximately over 10,000 biologically active compounds of microbial origin[2]. Recently, many bacterial pathogens are becoming resistant to existing antibiotics due to their indiscriminate use in the treatment of infectious diseases[3,4]. Therefore, there is an exigency to discover new and efficient antimicrobials from other sources such as plants[5,6].

Cuscuta reflexa has profound use in Ayurveda and folklore medicine. *C.reflexa* Roxb. (Cuscutaceae or Convolvulaceae) is a golden yellow leafless plant with zig-zag stem, mostly found in all the region[7,8]. It has no underground roots and grows commonly on *Zizyphus jujuba, Clerodendron inerme, Acacia arabica* and other shrubs and trees as a parasitic twiner[9]. Being parasitic plant it earn sustenance from host plant[10]. White crystalline compound cuscutalin and yellow crystalline solid cuscutin ($C_{15}H_{12}O_9$) have been isolated from the hot alcohol extract of *C. reflexa* stem[11]. In the present study, an attempt was made to screen comparative potential of different extracts of *C.reflexa* obtained from two sources *Acacia arabica* and *Zizyphus jujuba* for its antimicrobial action against Gram-positive and Gram-negative bacteria and fungi.

MATERIAL AND METHOD

Cuscuta reflexa was collected from two different host plants i.e. *Acacia arabica* and *Zizyphus jujuba* from wild areas of Hisar and Jind in the month of November and were authenticated by Dr. H.B. Singh Director, Department of Raw Material Herbarium & Museum, National Institute of Sciences Communication and Information Resources,

New Delhi under reference no: NISCAIR/RHMD/Consult/-2008-09/1136/168. The stems of plant (both sources) were dried and then grinded.

MICROORGANISMS

Strains, including fungi and bacteria were obtained from the Microbial Type Culture Collection, Institute of Microbial Technology, Chandigarh. Two gram-positive strains, *Staphylococcus aureus* and *Staphylococcus epidermidis*, one gram-negative strains, *Escherichia coli* and one fungus; *Aspergilus niger* were used in the present investigation as test organisms.

CULTURE MEDIA AND CHEMICALS

The culture media, Nutrient Agar (NA), Nutrient Broth (NB) and Czapek Yeast Extract Agar (CYA) were purchased from Hi- Media. All other chemicals, including organic solvents used for the extraction of the plant metabolites, were of analytical grade.

PREPARATION OF PLANT EXTRACTS

130 gm of coarsely ground plant material from both the sources were defatted with petroleum ether (60–80°C) and then the solvent free marcs were extracted with benzene, acetone, methanol and 90% ethanol, using soxhlet. The marcs were extracted with distilled water by maceration. The solvents were removed by *in-vacuo* in rotary evaporator. The dried extracts were stored in a desiccator till further comparative studies.

ANTIMICROBIAL ACTIVITY

Antimicrobial activity of all the extracts was determined, using the agar well diffusion assay method[12]. Approximately 20ml of molten and cooled media (NA and CYA) were poured in sterilized petri dishes. The plates were left overnight at room temperature to check for any contamination . The bacterial test organisms (*S. aureus, S. epidermidis* and *E. coli*) were grown in nutrient broth for 24 h. A 100µl nutrient broth culture of each bacterial organism was used to prepare bacterial lawns. For *A. niger*, a spore suspension of the fungus was prepared in 2 ml sterilized distilled water in a test tube and 100µl of spore suspension was spread on CYA plates. Five agar wells (four on the periphery and one in the center) of 5mm diameter were prepared with the help of a sterilized stainless steel cork borer. The wells were loaded with 100µl of various extracts (for both plants). The central well in each plate were used as a control and loaded with 100µl of solvent or sterilized distilled water. Various antibiotics and fungicides (Table 2) were used as standard control. The plates containing the bacteria and extracts were incubated at 37°C (for *E. coli* and *S. epidermidis*) and at 30°C (for *S. aureus and A. niger*). All the tests were repeated in triplicates.

The antimicrobial activity was assessed on the basis of diameter of zone of inhibition, which was measured at cross-angles after 24 h of incubation (Table 1). Percent inhibition of bacterial/fungal microorganisms was calculated after subtracting the value of control (solvents) from the value of extracts.

STATISTICAL ANALYSIS

All the results were expressed as mean ±S.E.M. Data was analyzed using one way analysis of variance test (ANOVA), p values <0.05 were considered as statistically significant (13).

RESULTS AND DISCUSSION

All the plant extracts tested, exhibited a broad spectrum of antimicrobial activity against the tested microorganisms (Table 1). The aqueous extracts of *Cuscuta reflexa* (*Zizyphus arabica*) did not show any antimicrobial activity, but a significant ($p=0.05$) effect was seen in case of *C.reflexa* (*Zizyphus jujuba*) against *A. niger*. In addition, results show that most of the extracts were significantly effective ($p=0.05$) against the fungus *A. niger*. Moreover, the ethanol and methanol extracts of *C.reflexa* showed significant results ($p=0.05$) against all tested microorganisms with the inhibition zone ranging from 8.2 to 26.8 mm and 13.3 to 25.6mm, respectively, this antimicrobial effect were acceptable with respect to the standard antibiotics (Table 2). The activity of methanol and ethanol extracts is attributed due Dulcitol which is reported in plant as a major component in addition to luteolin and quercitin . Benzene extracts of *Cuscuta reflexa (Zizyphus jujuba*) do not exhibit significant antimicrobial activity against the test microorganisms. The acetone extracts were effective ($p=0.05$) against most test microorganisms. *E. coli* was quite resistant to all the extracts. However, *S. aureus*, the common wound pathogen, was sensitive to most of the extracts. The results support the use of this plant in folklore medicine for treatment of infectious diseases especially for *C.reflexa* (*Zizyphus jujuba*).

CONCLUSION

It is evident from the present study that the extracts of *C.reflexa* (both sources) are active against the tested

Table 1. *In vitro* antimicrobial activity (zone of inhibition in mm) comparatively various extracts of *Cuscuta reflexa* collected from two different sources i.e. *Acacia arabica* and *Zizyphus jujuba*

Extract	Organism	Control	C.*reflexa* (Acacia Arabica)	C.*reflexa* (*Zizyphus jujuba*)
Ethanol	***Staphylococcus aureus***	12.0±0.1	14.3±0.3 (20)	26.8±0.4* (134)
	Staphylococcus epidermidis	12.0±0.1	–	18.8±0.2 (61)
	Escherchia coli	13.0±0.2	8.2±0.2 (–4)	11.8±0.2 (–10)
	Pseudomonas aeruginosa	11.3±0.3	25.1±0.2* (133)	13.8±0.2 (24)
	Aspergillus niger	8.6 ±0.1	13.5±0.1 (64)	21.8±0.1 (22)
Benzene	***Staphylococcus aureus***	7.2±0.2	12.5±0.1 (85)	11.0±0.2 (61)
	Staphylococcus epidermidis	4.5±0.1	15.5±0.2* (314)	12.1±0.2* (217)
	Escherchia coli	20.0±0.3	11.8±0.1 (–43)	–
	Pseudomonas aeruginosa	–	11.0±0.1	–
	Aspergillus niger	11.0±0.4	15.1±0.4 (41)	14.0±0.1 (30)
Acetone	***Staphylococcus aureus***	8.0±0.2	8.3±0.4 (4)	7.2 (–11)
	Staphylococcus epidermidis	8.3±0.1	12.5±0.2 (58)	10.3 (27)
	Escherchia coli	12.0±0.5	17.0±0.4 (45)	–
	Pseudomonas aeruginosa	–	–	–
	Aspergillus niger	13.0 ±0.6	15.2±0.2 (18)	15.5 (21)
Methanol	***Staphylococcus aureus***	12.0±0.3	14.4±0.2 (22)	13.3±0.2 (11)
	Staphylococcus epidermidis	9.8±0.4	15.5±0.3(64)	13.6±0.2 (43)
	Escherchia coli	11.6±0.3	10.4±0.4 (–11)	12.9±0.2 (12)
	Pseudomonas aeruginosa	12.1±0.2	13.6±0.4 (14)	15.6±0.2 (32)
	Aspergillus niger	11.8±0.1	*14.6*±0.2	25.6±0.2* (128)
Aqueous	***Staphylococcus aureus***	–	–	–
	Staphylococcus epidermidis	–	–	–
	Escherchia coli	–	–	–
	Pseudomonas aeruginosa	–	–	13.8
	Aspergillus niger	–	–	17.5

The values in parenthesis show percent increase or decrease (minus values) over the control excluding the diameter of agar wells.
– = No activity

Table 2. Antimicrobial activities of various antibiotics used as positive controls.

Antibiotics	Diameter of zone of inhibition (mm)			
	Escherchia coli	**Staphylococcus aureus**	**S.epidermidis**	***Aspergillus niger***
Streptomycin	15±0.1	16±0.2	12±0.7	N.D
Penicillin	17±0.4	16±0.3	14±0.2	N.D
Ampicilin	18±0.2	17±0.1	15±0.4	N.D
Tetracycline	21±0.1	21±0.2	17 ± 0.3	N.D
Fluconazole	N.D	N.D	N.D	15±0.2
Clotrimazol	N.D	N.D	N.D	17±0.3
Ketoconazole	N.D	N.D	N.D	12±0.2

All values represent ±S.E. of the mean (n=3), * *= significant (p< 0.05) compared to positive control.*

microorganisms. Based on results, *C. reflexa* (jujuba) had dominant antimicrobial activity in comparison to *C.reflexa* (arabica). Further work on isolation of various responsible plant active principles is required to ascertain the antimicrobial potential of *C.reflexa*.

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