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ANTIMICROBIAL POTENTIAL OF *CUSCUTA REFLEXA* ROXB. SUCCESSIVE EXTRACTS GROWN ON *NERIUM OLEANDER* L. AS PARASITIC PLANT AGAINST SELECTED TEST MICROBES

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Cuscuta reflexa Roxb. is a parasitic plant having many medicinal properties and used as traditional medicine in Ayurveda for the treatment of various disorders. In the present research work, researchers have investigated antimicrobial ability of *C. reflexa* grown on *Nerium oleander* host plant successive extracts against selected human pathogenic bacteria. *Escherichia coli* (MTCC 730), *Proteus vulgaris* (MTCC 1771), *Staphylococcus aureus* (MTCC 7443), *Bacillus subtilis* (MTCC121) and *Pseudomonas aeruginosa* (MTCC 4673) were used as the test pathogens. Hot extraction technique was used to prepare the plant extracts. Antimicrobial activity was tested using disc diffusion method. Chloroform extract of *Cuscuta reflexa* grown on *Nerium oleander* host plant showed maximum activity against *B. subtilis* (20 mg/disc; IZ= 27mm, AI= 1.35), (10mg/disc; IZ= 25mm, AI= 1.3) which is more than the standard disc tetracycline. Chloroform extract also showed appreciable activity against *P. vulgaris* and *P. aeruginosa*. Ethyl acetate extract also showed maximum activity against *P. aeruginosa* (20mg/disc; IZ= 16mm, AI= 1.14) and *E. coli* (5 mg/disc; IZ= 22mm, AI= 1.1). Chloroform extract and ethyl acetate extract of *C. reflexa* grown on *Nerium oleander* host plant is capable to inhibit the growth of *E. coli*, *B. subtilis* and *P. aeruginosa* effectively. Therefore, these extracts can also be used for the isolation of pure compounds/ volatile compounds with potentials to be used as therapeutics.

Key words: Antimicrobial Activity, *Cuscuta reflexa*, parasitic plant and Medicinal Plants.

Introduction

The amarbel, *Cuscuta reflexa* Roxb. (Convolvulaceae), is a parasitic plant having different medicinal properties and known as a miracle plant in the ethnomedicine[1]. *Cuscuta reflexa* Roxb.grows in the parasitic manner over the host plant and sucks nutrients from the host plant for its growth and development[2]. Ancient literature also reveals the medicinal potentials of *Cuscuta* species. In present investigations author studied research on medicinal properties of *C. reflexa* growing on different host plant.

Cuscuta reflexa Roxb.is a plant with medicinal value and have very important place in Ayurveda. Seeds of *Cuscuta* are used in cold infection as carminative and depurative in stomach ache. Stems of *C.*

reflexa are used in constipation, fatigue, bilious disorder and liver problems. *Cuscuta* seeds are used as blood purifier along with the *Sarsaparilla*[3].

C. reflexa is useful in treatment of androgen induced alopecia[4]. It also gives anti-inflammatory and anti-cancer activity[5]. The aqueous and alcoholic extract of *C. reflexa* has diuretic activity[6]. The crude water extract of the *C. reflexa* also shows the anti HIV activity[7].

Cuscuta reflexa has antioxidant activity due to the presence of phenolics compounds in its chemical constitution that have free radical scavenging activity[8]. Many chemical compounds have been isolated from the *C. reflexa* such as Cuscutin, Amarbellin, Stigmasterol, Sitosterol and many more having therapeutic potentials[9]. *C. reflexa* is a source of various bioactives and still new compounds can be isolated from *C. reflexa* with biopotentials.

Cuscuta reflexa also possess hepatoprotectic activity. It is reported that hydroalcoholic extract of *C. reflexa* showed hepatoprotectic activity in albino rats against paracetamol induced hepatic damage[10].

Many antibiotics of different types are being used for human therapy as well as for animals and aquaculture, resulted in development of resistance against multiple drugs in pathogenic microorganisms. Multiple drug resistance strains (MDRS) are developed may be due to the accumulation of various genes within a single cell each coding resistance against different drugs[11]. Therefore, there is an urgent need for the exploration of new sources having therapeutic potentials to deal with these day by day developing microorganisms[12]. Chemical compounds isolated from *Cuscuta reflexa* showed therapeutic potential against various disorders[13]. Thus, present research work deals with antimicrobial ability of *Cuscuta reflexa* grown on *Nerium oleander* host plant. Successive extracts of *C. reflexa* were screened for antimicrobial potentials against selected human pathogenic bacterial strains.

Material and Methods

Collection of plant sample

Plant samples were collected from the various parts of Jaipur, Rajasthan, India. *Cuscuta reflexa* grown on *Nerium oleander* host plant was collected from C-scheme and near Jaipur Engineering College and Research Centre, Tonk Road, Near Choki dhani, Jaipur, Rajasthan, India.

Plant sample of *Cuscuta reflexa* was identified and submitted in Ethno-medicinal Herbarium, Centre with potentials of Excellence funded by Department of Science and Technology, JECRC University, Jaipur, India. Further, voucher specimens of *Cuscuta reflexa* was deposited at herbarium of University of Rajasthan, Jaipur, India and was verified by senior taxonomist of department and provided with accession no. RUBL211577. Collected plant material was screened for foreign material, shed dried and grinded to powdered form.

Microbial strain

Five species of human pathogenic bacterial strains were obtained from Institute of Microbial Technology (IMTECH), Chandigarh, India. IMTECH is an Indian Repository for preservation of authentic microbial cultures with their identification number as Microbial type culture Collection and Gene Bank (MTTC). Bacterial strains were *Escherichia coli* (MTCC 730), *Proteus vulgaris* (MTCC 1771), *Staphylococcus aureus* (MTCC 7443), *Bacillus subtilis* (MTCC121) and *Pseudomonas aeruginosa* (MTCC 4673).

Extraction of plant sample

Dried plant material was powdered (750g) and subjected to extraction to prepare crude plant extracts. Hot extraction technique was used to prepare successive plant extracts with petroleum ether, toluene, chloroform, ethyl acetate and methanol at various temperatures with continue stirring for

maximum yield. Solvents used for the extraction was according to the polarity of solvents for the complete extraction of phytoconstituents from plant material. Then, each homogenate was filtered and concentrated by vacuum distillation method *in vitro* concentrated plant extract was further used to screen antimicrobial ability of plant sample[14].

Paper disc of crude plant extracts was prepared using Whattman No.1 paper of 6mm diameter. Disc prepared was of three different weights-

A_1= 5mg of test extract/disc

A_2= 10mg of test extract/disc

A_3= 20mg of test extract/disc

Prepared discs were air dried at room temperature to removal of any residual solvent that might interfere with the results of antimicrobial ability of plant extract. Tetracycline disc was used as reference drug (standard disc) separately.

Disc diffusion method was adopted to screen the antimicrobial activity of plant extract because of the exactitude and precision of the method[15]. Nutrient agar medium (NAM) was used for microbial culture. Spread plate method was used for bacterial culture using sterile swabs. Prepared discs were placed on the inoculated culture plates. Zone of inhibition was calculated around the placed discs after incubation for 24hr at 37^0 C.

The zone of inhibition of each result was recorded and activity index was calculated with the zone of standard reference drug. (AI= inhibition zone of test sample/ inhibition zone of standard). Five replicates of each test extract were examined and the mean values and standard deviation was calculated and reported.

Results and Discussion

Parasitic plant *Cuscuta reflexa* is screened for its antimicrobial activity grown on *Nerium oleander* host plant. All five extracts of plant sample; petroleum ether, toluene, chloroform, ethyl acetate and methanol was tested for the identification of antimicrobial activity against selected human pathogenic bacteria *Escherichia coli* (MTCC 730), *Proteus vulgaris* (MTCC 1771), *Staphylococcus aureus* (MTCC 7443), *Bacillus subtilis* (MTCC121) and *Pseudomonas aeruginosa* (MTCC 4673) which are responsible for the development of various life threatening diseases.

Table 1 shows the inhibition zone and activity index of all five successive extracts against selected test pathogens. Figure 1 shows the graphical representations of inhibition zones of plant extracts (in mm) against test microbes. *Cuscuta reflexa* is a parasitic plant; it draws nutrients from host plant for its survival so it also has some properties of its host plant in its chemical constitution along with its own properties. *C. reflexa* were studied for antimicrobial potential before, but evaluation of antimicrobial activity of *Cuscuta reflexa* grown on *Nerium oleander* host plant was not performed so far against selected test microbes.

Chloroform extract of *Cuscuta reflexa* grown on *Nerium oleander* host plant showed maximum activity against *B. subtilis* (20 mg/disc; IZ= 27mm, AI= 1.35), (10mg/disc; IZ= 25mm, AI= 1.3) which is more than the standard disc tetracycline. Chloroform extract also showed appreciable activity against *P. vulgaris* and *P. aeruginosa*.

Ethyl acetate extract also showed maximum activity against *P. aeruginosa* (20mg/disc; IZ= 16mm, AI= 1.14) and *E. coli* (5 mg/disc; IZ= 22mm, AI= 1.1) (Figure 2). Toluene extract and methanol extract both showed appreciable activity against microbes but petroleum ether extract doesn't showed very good antimicrobial activity and proved to be non compatible to be used as antimicrobial agent. Methanolic extract of *C. reflexa* also showed promising results

Solvent Type			EC	BS	SA	PV	PA
Standard (Tetracycline)	A	IZ	20	18	20	31	11
	B	IZ	22	20	23	38	14
	C	IZ	28	23	24	44	15
Petroleum Ether	A	IZ	7± 0.11	8±0.19	-	-	-
		AI	0.3	0.44	-	-	-
	B	IZ	7± 0.25	8±0.78	-	-	7±0.78
		AI	0.31	0.4	-	-	0.5
	C	IZ	9±0.54	10±0.88	-	-	6±1.2
		AI	0.32	0.43	-	-	0.4
Toluene	A	IZ	19±0.75	-	10±1.7	8±0.81	-
		AI	0.9	-	0.5	0.25	-
	B	IZ	11±0.54	9±0.79	11±1.72	9±0.67	7±0.35
		AI	0.5	0.45	0.47	0.23	0.63
	C	IZ	9±1.56	10±0.79	10±1.35	14±0.81	9±0.69
		AI	0.32	0.43	0.41	0.31	0.6
Chloroform	A	IZ	14±1.84	-	19±0.98	14±0.45	-
		AI	0.7	-	0.95	0.45	-
	B	IZ	15±0.79	25±0.82	21±1.26	22±1.71	22±0.74
		AI	0.68	1.3	0.91	0.57	1.5
	C	IZ	20±0.95	27±1.24	23±0.86	26±0.78	20±1.65
		AI	0.71	1.35	0.95	0.59	1.3
Ethyl Acetate	A	IZ	22±0.76	17±1.28	20±0.93	14±0.56	17±1.67
		AI	1.1	0.94	1	0.45	1.5
	B	IZ	19±1.78	20±0.22	17±0.34	16±0.27	16±0.63
		AI	0.86	1	0.73	0.42	1.14
	C	IZ	25±0.74	22±0.58	18±0.71	17±.91	19±0.42
		AI	0.89	0.95	0.75	0.38	1.2
Methanol	A	IZ	14±0.72	14±0.64	15±1.45	13±0.21	9±0.59
		AI	0.7	0.77	0.75	0.41	0.81
	B	IZ	16±1.67	13±0.29	14±1.23	14±0.56	13±0.34
		AI	0.72	0.65	0.60	0.36	0.92
	C	IZ	17±0.34	14±1.25	13±0.76	20±0.38	14±0.93
		AI	0.60	0.60	0.54	0.45	0.93

Table 1. Antibacterial activity of petroleum ether, toluene, chloroform, ethyl acetate and methanol extracts of *C. reflexa* with *Nerium oleander* host plant

I.Z. = Inhibition zone showed by extract against microorganism in mm; AI = Activity index of extract; EC= *Escherichia coli* (MTCC 730), PV= *Proteus vulgaris* (MTCC 1771), SA= *Staphylococcus aureus* (MTCC 7443), BS= *Bacillus subtilis* (MTCC121) and PA= *Pseudomonas aeruginosa* (MTCC 4673); - = no inhibition zone; A = 5 mg/disc; B = 10 mg/disc; C = 20 mg/disc.

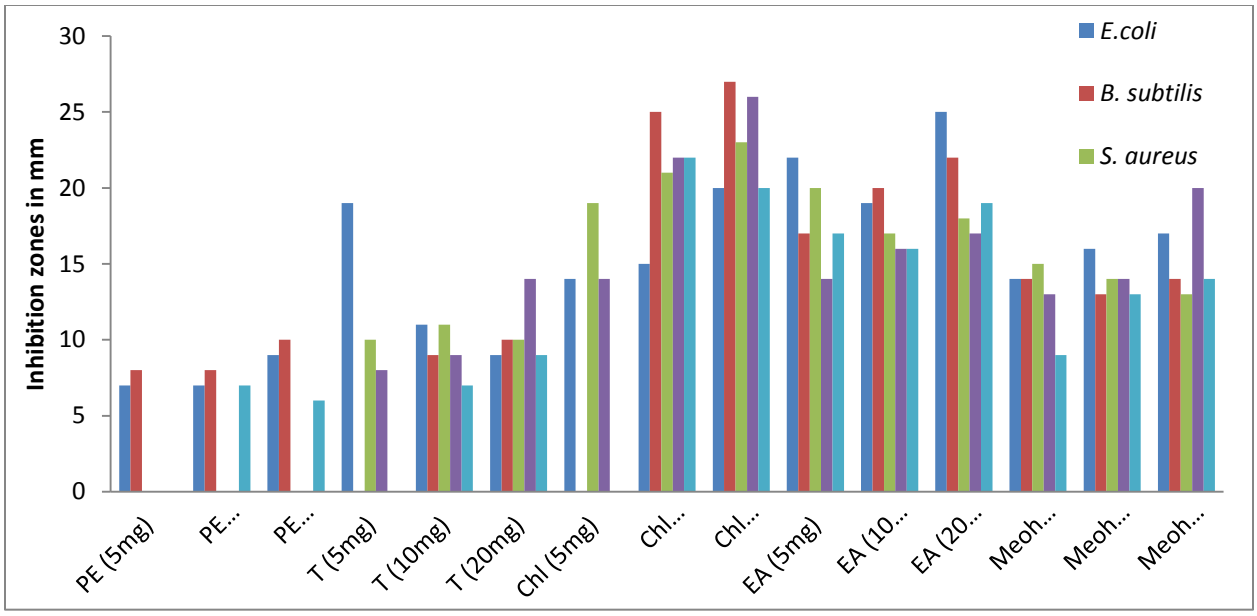

Figure 1 – Graphical representations of Antimicrobial activity of *Cuscuta reflexa* with *Nerium oleander* host plant all five successive extracts. PE= Petroleum ether; T= Toluene; Chl= Chloroform; EA= Ethyl acetate; Meoh= Methanol.

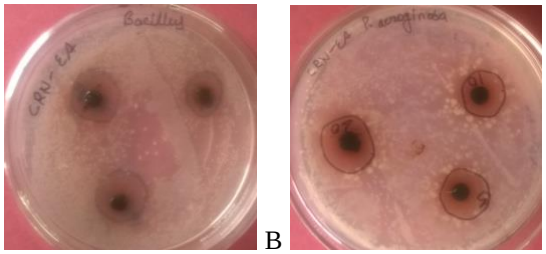

Figure No. 2– Antimicrobial activity of *Cuscuta reflexa* grown on *Nerium oleander* host plant Ethyl acetate extract against A= *Bacillus subtilis* (MTCC121), B= *Pseudomonas aeruginosa* (MTCC 4673)

against all the selected test pathogens (Figure 3).

It is noteworthy that extract of *C. reflexa* possess antimicrobial efficacy more than standard in selected test pathogens proved its prominent role in antibiotics. Further, these microbes are responsible for the development of various life threatening diseases and hence detail studies on *C. reflexa* can be performed for isolation of novel bio actives as leading antibiotics.

C. reflexa ethanol, chloroform, ethyl acetate and aqueous solvents against pathogenic organisms *E. coli, S. aureus, P. aeruginosa, P. vulgaris, S. paratyphi, Shigella sonnei, S. typhimurium* and *K. pneumonia*, ethanol and chloroform extract showed greater activity with a zone of inhibition ranging from 6mm to 17 mm[16]. Further, antimicrobial activity were also studied using ethanolic extract of *C. reflexa* on selected pathogens like *S. aureus, S. epidermidis, E. Coli, Micrococcus luteus, P. aeruginosa.* The zone of inhibition was calculated and results revealed that maximum activity was screened and maximum efficacy against *P. aeruginosa* and *E. coli* was found in rainy season samples where as spring season showed maximum activity against *S. aureus*[17]. Even antifungal efficacy was also screened for ethanol, methanol and acetone extract of stem of *C. reflexa* against *Fusarium sps, Aspergillus sp* and *Penicillum sps.*[18]. The antimicrobial activities were assessed against *Bacillus subtilis, Pasteurella multocida* and *Staphylococcus aureus* bacteria strains and *Aspergillus niger* and *Aspergillus flavus* fungal strains[19]. Therefore, present research work deals with

antimicrobial efficacy of *C. reflexa* grown on *Nerium oleander* host. This study was done for the first time with selected host and possessed good efficacy results. Ethyl acetate extract also showed maximum activity against *P. aeruginosa* which is appreciable and more than standard drug. These studies will further, used for bioactivity guided fractionation which will lead to isolation of pure compounds with antimicrobial efficacy.

Conclusion

C. reflexa grown on *Nerium oleander* host plant was studied for the screening of its antimicrobial activity against selected test pathogens. Chloroform extract and ethyl acetate extract of plant sample is capable to inhibit the growth of *E. coli*, *B. subtilis* and *P. aeruginosa* effectively. Therefore, these extracts can also be used for the isolation of pure compounds/ volatile compounds with potentials to be used as therapeutics.

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