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ANTIMICROBIAL ACTIVITY OF THE CRUDE EXTRACT OF *CUSCUTA REFLEXA* ROXB.

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Abstract

The present work was carried out to study the comparative antimicrobial activity of pure solvents, standard antibiotic discs *viz.* Amikacine, Ciprofloxacin, Griseofulvin and solvent extracts of *Cuscuta reflexa* against Gram (-) bacteria *viz. Pseudomonas aeruginosa, Escherichia coli*, Gram (+) bacteria *viz. Bacillus subtilis, Bacilus licheniformis* and fungi *viz. Aspergillus niger, Trichoderma ressei.* On the whole all the crude solvent extracts were found to be more resistant against test organisms. Chloroform and petroleum ether extracts appeared to be the most effective antifungal and antibacterial agents.

Introduction

The cosmopolitan genus *Cuscuta* of the family Cuscutaceae has 170 species distributed throughout the world, out of which 14 grow in Pakistan. *Cuscuta reflexa* Roxb. is an aggressive parasitic weed occurring on a number of host plants such as *Clerodendron inerme, Nicotiana tabacum, Vicia faba, Brassica juncea* and *Ricinus communis* etc. (Malik *et al.*, 1980; Kroll & Schustar, 1982; Ihl *et al.*, 1984; Ishra & Sanwal, 1992; Jeschke & Hilpert, 1997). Its therapeutic and economic benefits *i.e.* antibilious vulnerary, narcotic, hypnotic, aphrodisiac, anti-haemorrhoidal, diuretic, anti-prusitic, demulcent, expectorant antiviral, anticancer, antitumour, antioxidant, modulating autonomic and autocoid activities, mycotrophy *etc.* are well documented (Awasti, 1982; Bown, 1995; Chevallier, 1996; Khalid & Iqbal, 1996; Shenqing & Shu, 1999; Zhuanl *et al.*, 1999; Dahanukar *et al.*, 2000; Yaday *et al.*, 2000; Arshad & Rao, 2002; Khan *et al.*, 2002). Cuscutin, amarbelin, antiviral protein, wax of esters of higher aliphatic alcohols with saturated fatty acids, carboxymethylcellulase, soluble phenolic compounds, flavanoids, pactin methylesterase, starch phosphorylaserylase (Awasti, 1981; Sharma *et al.*, 1986; Gilani & Aftab, 1992; Sirivastava *et al.*, 1994; Garcia *et al.*, 1995; Chatterjee *et al.*, 1997; Loffler *et al.*, 1997). The present report describes the antimicrobial potential of solvent extracts of *Cuscuta reflexa* against test organisms.

Materials and Methods

Cuscuta reflexa Roxb. parasitzing on *Clerodendrone inerme* was collected from the Campus of Govt. College University, Lahore.

Test organisms: Pure cultures of Gram positive bacteria (*Bacillus subtilis, Bacillus licheniformis*) and Fungi (*Asperigillus niger* and *Trichoderma reesei*) were obtained form the culture collections of the Biotechnology Laboratory, Government College University, Lahore, while that of Gram negative bacterium *i.e. Pseudomonas aeruginosa* have obtained from the culture collection of Department of Pathology, King Edward Medical College, Lahore and *Escherchia coli* form the culture collection of Department of Pharmacy, University of the Punjab, Lahore.

Standard drug discs: The following standard drug discs were purchased from the local Pharmacy shops:

i. Amikacine (30 µg), ii. Ciprofloxacin (10 µg), iii. Griseofulvin (100 units).

The antibiotic Amikacine was used against *P. aeruginosa*, while Ciprofloxacin for *E. coli*, *B. subtilis* and *B. licheniformis* (bacteria) and Griseofulvin for fungi like *A. niger* and *T. reesei*.

Solvent extraction: Four hundred grams fresh plant material was soaked in ethanol for one week. The ethanol extract was filtered and evaporated on rotary evaporator to yield the residue (95 g) M1. The crude ethanol extract was defatted with Petroleum ether (5 g) M2 and the defatted extract was successively fractioned with different solvent systems on the polarity basis. The solvents were Chloroform (M3), Chloroform (PH=3, M4), Chloroform (PH=9, M5), Ethylacetate (M6) and Butanol (M7). The resulting aqueous portions were separated. The obtained yields were 5 g (M2), 2.1 g (M3), 1.80 g (M4), 1.03 g (M5), 0.85 g (M6) and 0.30 g (M7) respectively. Seven solvent extracts were obtained by solvent extraction method (Khan *et al.*, 2002).

Experimental design: Above mentioned crude solvent extracts were studied for antimicrobial activity by the disk diffusion Method (Bauer *et al.*, 1966). Three series of experiments were conducted. Initially pure solvent was used and tested for its antimicrobial activity against above mentioned bacteria and fungi. In the second series of experiments, different crude solvent extracts were used. In the third series of the experiments, commercially available standard antibiotic discs (Amikacine and Ciprofloxacine) and antifungal discs (Griseofulvin) were placed on the top of the medium in the center of the Petridishes. The purpose of the experiment was to compare the antimicrobial activity of the standard antibiotic and antifungal discs and solvents with those of the crude solvent extracts of *C. reflexa*. All the steps involved in the preparation of the inoculum and Petridishes were performed in aseptic conditions. The experiments were run in triplicates.

The Petridishes with inoculated bacteria and fungi were then placed in different incubators having different temperature for growth. After the time intervals of 24 hours for bacteria and 72 hours for fungi, a zone of inhibition around the crude extract or solvent or standard disc was measured with the help of ruler and recorded according to Software Costat (version 3.03) to evaluate the significant difference between various means of zone of inhibition of the crude solvent extracts, pure solvent and standard discs.

Statistical analysis: Mean values and standard errors were calculated and the Duncan's multiple range test was also carried out by using Costat (version 3.03) to find out significant differences.

Results

Inhibition zones of test organisms for solvent extracts, standard reference discs and solvent were significantly different at $P<0.05$. All extracts exhibited appreciable antimicrobial activity as compared to standard reference discs and pure solvents.

1. Ethanol extract (M1): The maximum inhibition zones were observed against *P. aeruginosa, B. subtilus, B. licheniformis, A. niger* (25.56, 20.50 & 17.33 mm), while minimum inhibition zones against *E. coli, B. subtilis* and *T. reesei* (2.50, 4.00 & 3.42 mm) respectively (Table 1). The antimicrobial activity was highly significant against *P. aeruginosa* and *B. licheniformis* as compared to antibiotic discs (Fig. 1).

2. Petroleum ether extract (M2): The maximum inhabitation zones were recorded against *P. aeruginosa, A. niger* and *T. reesei* (16.00, 32.66 & 18.33 mm), minimum against *E. coli* and *B. subtilis* (1.33 & 20.50 mm) respectively (Table 1). The antimicrobial activity against *P. aeruginosa, A. niger, T. reesei* was highly significant as compared to the antibiotic discs (Fig. 2).

3. Chloroform extract (M3): The maximum inhibition zone was observed against *P. aeruginosa* (27.67 mm) moderate zone against *E. coli, T. reesei* (16.00 & 15.00 mm) and minimum against *B. subtilis, B. licheniformis* and *A. niger* (4.20, 7.33 & 2.50 mm) respectively (Table 1). The antimicrobial activity against *P. aeruginosa* was highly significant as compared to the antibiotic discs (Fig. 3).

4. Chloroform extract (M4): The maximum inhibition zone was observed against *P. aeruginosa, E. coli, B. subtilis, B. licheniformis* and *T. ressei* (30.33, 18.46, 20.50, 27.66 & 25.55 mm), while minimum zone against *A. niger* (3.4 mm) respectively (Table 1). The antimicrobial activity against *P. aeruginosa, E. coli, B. subtilis, B. licheniformis* and *T. ressei* was highly significant as compared to the antibiotic discs (Fig. 3).

5. Chloroform extract (M5): The maximum inhibition zone was observed against *B. subtilis* (26.56 mm), moderate zone against *P. aeruginosa, E. coli, B. licheniformis, T. ressei* (16.33, 10.66, 14.33 & 13.33 mm) and minimum zone against *A. niger* (3.4 mm) respectively (Table 1). The antimicrobial activity was highly significant against *P. aeruginosa* and *B. subtilis* as compared to the antibiotic discs (Fig. 3).

6. Ethyl acetate extract (M6): The maximum inhibition zone was recorded against *E. coli, A. niger* (20.00 & 33.66 mm), moderate against *B. licheniformis* (4.33 mm) and minimum against *P. aeruginosa, B. subtilis* and *T. reesei* (5.00, 3.00 & 4.20 mm) respectively (Table 1). The antimicrobial activity against *E. coli* and *A. niger* was highly significant as compared to the antibiotic discs (Fig. 3).

7. Butanol extract (M7): The maximum inhibition zone was recorded against *B. licheniformis* and *T. reesei* (32.50 & 19.33 mm), while moderate against *P. aeruginosa, E. coli* and *B. subtilis* (12.00, 16.33 & 11.33 mm) and minimum zone against *A. niger* (6.33 mm) respectively (Table 1). The antimicrobial activity against *B. subtilis* and *T. reesei* was highly significant as compared to the antibiotic discs (Fig. 3).

Antimicrobial activity of solvents: Almost all the solvents exhibited very small zone of inhibition against *P. aeruginosa, E. coli, B. subtilis, B. licheniformis, A. niger* and *T. reesei.*

Table 1. Zone of inhibition produced by the seven solvent extracts of *Cuscuta reflexa* against bacteria and fungi.

Test organism	Zone of inhibition (in mm ± S.E.) for solvent extracts						
	M1	M2	M3	M4	M5	M6	M7
Bacteria							
Pseudomonas aeruginosa	25.56a± 2.10	16.00a±0.02	27.67a±8.16	13.33b±2.37	16.33b±3.67	5.00b±1.81	12.00a±1.01
Escherichia coli	2.00b ± 0.61	1.33b-1:0.17	16.00b±1.45	18.46a±1.45	10.66c±1.15	25.00a±2.89	16.33a±3.67
Bacillus subtilis	4.00b±0.49	2.50b±1.44	4.20c±1.81	20.50a±4.77	26.56a+6.01	3.00b±0.08	11.33a±0.68
Bacilus lichenformis	20.50a±4.77	11.00b.1-2.00	7.33c±5.96	27.66a±0.06	14.33b±2.96	14.33a±2.96	32.50a±0.33
Fungi							
Aspergillus niger	17.33b±1.69	32.66a±0.33	2.50b±1.73	3.41b±2.15	3.40b±3.29	33.66a±7.50	6.33b± 1.88
Trichoderma reesei	3.41b±2.15	18.33a±0.88	15.00±1.73	25.55a±0.84	13.33±2.68	4.20b±1.81	19.33a±0.69

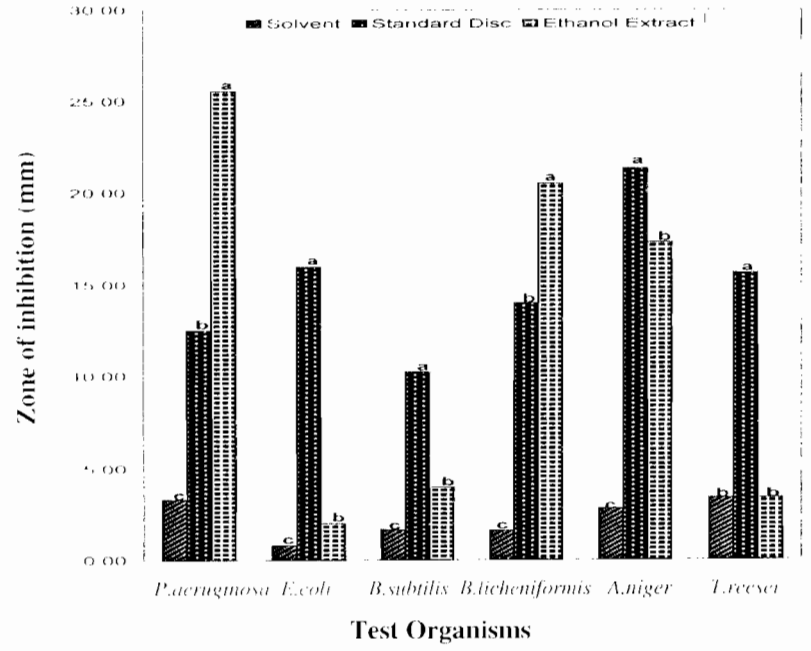

Fig. 1. Mean zones of inhibition for ethanol extract, with respect to solvent and standard disc.

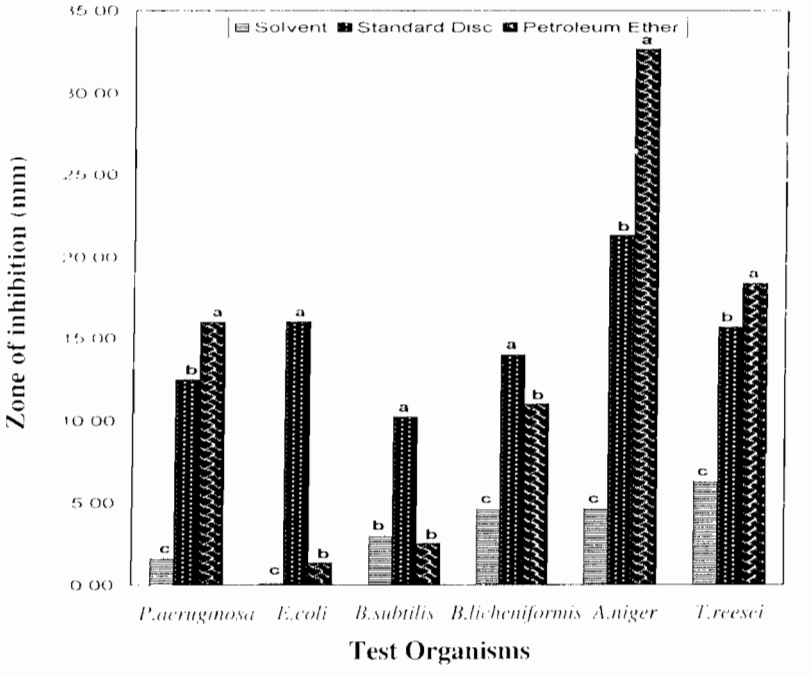

Fig. 2. Mean zones of inhibition for petroleumether extract, with respect to solvent and standard disc.

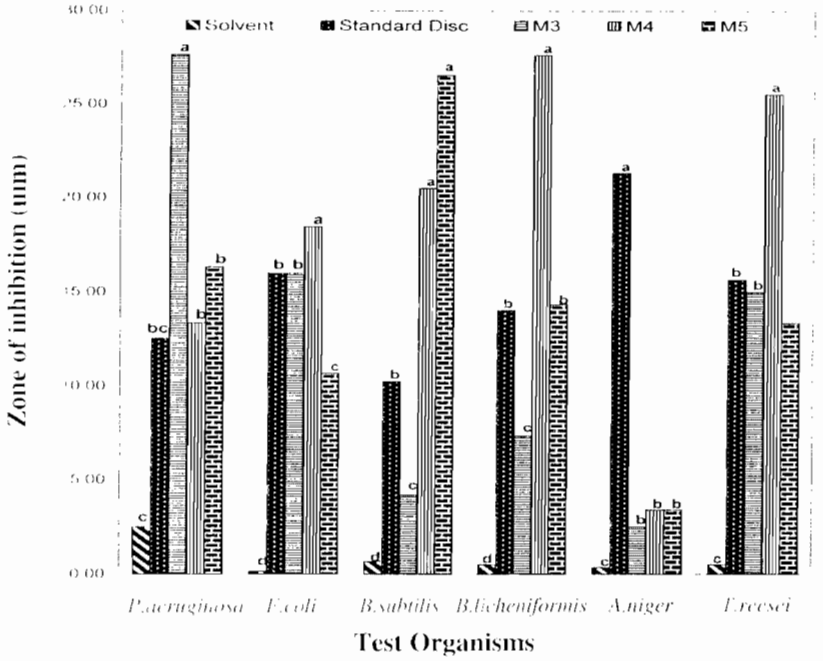

Fig. 3. Mean zones of inhibition for chloroform extract, with respect to solvent and standard disc.

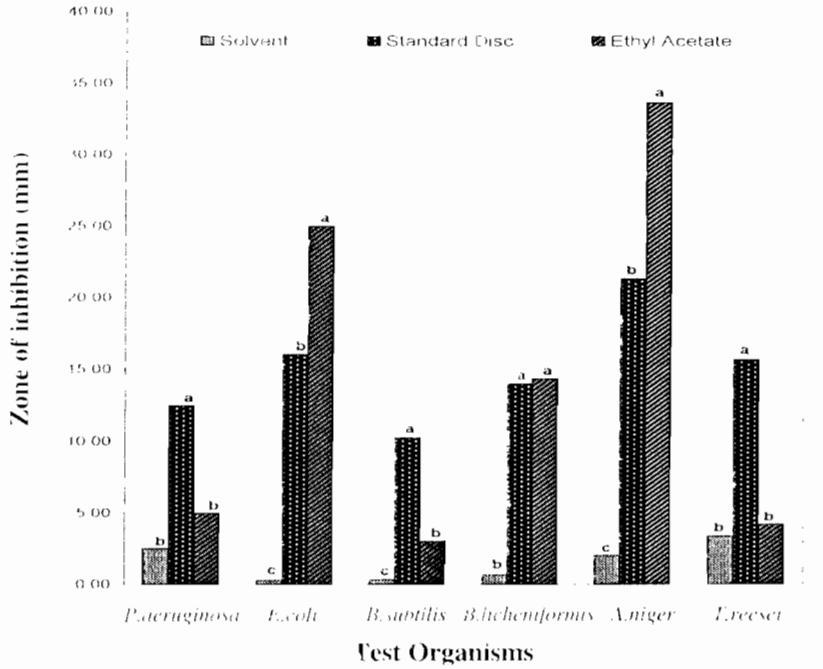

Fig. 4. Mean zones of inhibition for ethyl acetate extract, with respect to solvent and standard disc.

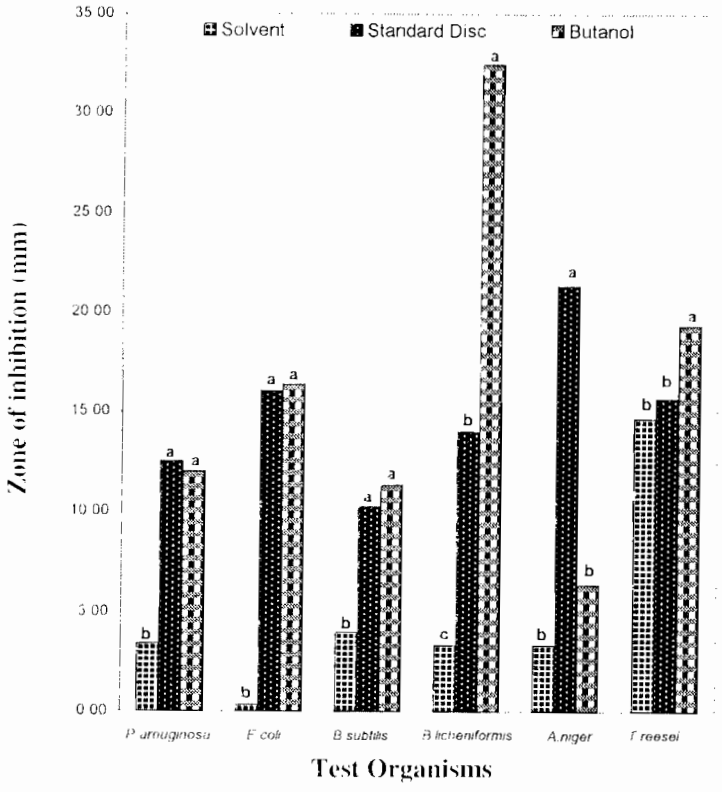

Fig. 5 Mean zones of inhibition for butanol extract, with respect to solvent and standard disc.

Discussion

In the present study crude extracts of the plant material obtained in polar and non polar solvents were tested against Gram positive, Gram negative bacteria and fungi. This was the first attempt to study the antimicrobial activity of *Cuscuta reflexa*. The extracts obtained in different solvents showed higher antimicrobial and antifungal activities as compared to standard discs. Flavonoid alkaloids and phenolic compounds have been extracted from *Cuscuta reflexa* plant by Peroz-Amudar *et al.* (1996), Loffler *et al.* (1997) and Garcia *et al.* (1995).

The antimocrobial and antifungual activities recorded in the present work could be attributed to these compounds. These polar compounds are usually extracted well in polar solvents such as ethanol, butanol and to some extent in chloroform having an intermediate polarity. This may be the reason that chloroform extract showed maximum antimicrobial activity against the bacteria used in the present investigation. Petroleum ether extract was found to be highly antifungal which may be due to the long chain fatty esters present in *Cuscuta*, as detected by Aukema & Stiger (1998). There is a general view that fatty compounds are usually antifungal. On the basis of above mentioned facts it can be suggested that *Cuscuta* can be used against bacteria and fungi as most potent antimicrobial agent.

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