Mycopath (2003) **1**(1): 99-104

Effect of host species on antimicrobial activity of the ethanolic extracts of *Cuscuta reflexa* Roxb.

Nabeela Anjum and Zaheer-ud-Din Khan*

*Botany Department, Government Islamia College for Women, Cooper Road, Lahore (nabeela662000@yahoo.com) and *Botany Department, Govt. College University, Lahore, Pakistan*

Abstract

The present investigation deals with antimicrobial activity of ethanolic extracts of *Cuscuta reflexa* parasitizing on three different angiospermic hosts.namly *Populus euro- Americana* ,*Zizphus hysudrica* and *Clerodendron inerme* .The extracts were reported to exhibit significant antimicrobial activity against bacteria like *Pseudomonas aeruginosa* , *Escherichia coli, Bacillus subtilis* and *Bacillus licheniformis*, and fungi namely *Aspergillus niger* and *Trichoderma reesei.* The results when compared with the inhibition caused by standard reference discs of antibiotics for example Amikacine, Ciprofloxacin and Griseofulvin showed significant differences among three hosts with respect to antimicrobial activity.

Introduction

Cuscuta reflexa Roxb (Amarbel,Akasbel) an angiospermic haustorial advanced, obligate, hemiparasite belonging to family Cuscutaceae has been cited to have a broader host range due to its occurance on a number of host plants such as, *Populus euroamericana, Zizyphus hysudrica Clerodendron inerme Beta vulgaris,Lycopersicum esculentum* etc throughout the plains of Pakistan (Bhan *et al.,* 1973; Bhattacharya, 1976; Malik *et al*., 1980). Several physiological studies on the host parasite relationships have suggested that it may act as stronger sink for secondary host metabolites. Thus *Cuscut*a species provide an under utilized approach to study the effects of its hosts secondary metabolites (Arentsen and Arentsen, 1976; Cheema *et al.,* 1976; Raghava-Reddy *et al*., 1981; Forstreuter, 1984; Czygan *et al.*, 1988; Ishra, 1992; Jeschke, 1995). It was suggested that haustoria first produced in response to microbial parasitizims and were subsequently modified for water and nutrient uptake (Nicokrent, 2002). It also acts as allelopathic weed used for controlling water hyacinth weed) (Pandey *et al.*, 1993). Its therapeutic properties such as anticancer, antidiabetic; antiviral; and antiinflamatory are well documented (Shenqing *et al*., 1997). Cuscutin, Amarbelin, Antiviral protein, Wax of esters of higher aliphatic alcohols with saturated fatty acids, Carboxymethylcellulase and eight phenolic compounds have already been extracted from *Cuscuta reflexa* plant (Bhatanger, 1950; Awasti, 1981; Sharma *et al*., 1986; Gilani *et al*., 1972; Chatterjee *et al*., 1997; Loffler, 1997). Previously various workers have reported the antimicrobial activity on animals and plants (Deborah, 2001; Victor, 2001; Michael, 2002; Robert, 2001). Only few researchers have described the details of antimicrobial activity of *Cuscuta reflexa* (Khan *et al*., 2002). The effect of ethanolic extract of *Cuscuta reflexa* was studied on frogs perfused heart, dog's blood pressure, isolated rabbit duodenum, isolated guinea pig ileum and isolated guinea tracheal chain (Singh and Garg, 1973). Furthermore, no attempt so far has been made to study the antimicrobial activity of the parastic plant with respect to different hosts Keeping in view the strong allelopathic potential of *C. reflexa*, this preliminary investigation has been carried out to evaluate the antimicrobial activity of ethanolic extracts of the parasitic plant. Another anovation was to conclude the potency of the extracts within host species parasitized by *C. reflexa* There is greet need to study host parasite relationship in natural field communities for the biological control of parasites. Therefore, this pilot study has been done to describe the site of action of active compounds of *Cuscuta* stem extract (CSE) from three different hosts on various test organisms as a first step study.

Materials and Methods

Sample Plant Collection

Samples of the parasitic plant *Cuscuta reflexa* were collected from Government Islamia College Coper Road, Lahore and Government College University (GCU). Sampling of *C. reflexa* was done from three different hosts namely *Populus euroamericana, Zizyphus hysudrica* and *Clerodendron inerme*. The former two hosts trees were growing in Government Islamia College

Cooper Road while the later was present in the premises of GCU (Plate 1,2).

Retreival of Cultrures of Test Organisms

Pure cultures of gram positive bacteria (*Bacillus subtilis, Bacillus licheniformis*) and Fungi (*Aspergilli\us niger* and *Trichoderma reesei*) were obtained from 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 Edwared medical College, Lahore and *Ecscherchia coli* from 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 (10μg)
iii). Griesofulvin (100 units)

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

Preparation of Extraction

Four hundred grams of each type of plant material was soaked in ethanol (2000ml) for one week. Three ethanolic extracts (Extract A, Extract B and Extract C) were filtered and evaporated on rotary evaporator to yield the rsesidues.

Preparation of Antimicrobial Activity

The three ethanolic extracts of powdered plant material of *Cuscuta reflexa* Roxb. were studied for any antimicrobial activity by the disk diffusion Method (Bauer *et al.*, 1962).

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 ethanolic 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 centre of the petridishes. The purpose of the experiment was to compare the antimicrobial activity of the standard antibiotic and antifungal discs and solvents with that of the crude ethanolic extracts of *C. reflexa*.

All the steps involved in the preparartion 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, solvents, and around the standard dsic were measured with the help of ruler and recorded cording to Software Costat (version 3.03) to evaluate the significant difference between various means of zone of inhibition of the crude ethanolic extracts, pure solvent and standard discs.

Results

The antimicrobial activity was recorded as zone of inhibition in mm for all the materials used as follows.

Antimicrobial Activity of Pure Solvent Ethanol

It exhibited very small zone of inhibition against *P. aeruginsa, E. coli, B. subtilis, B. licheniformis, A. niger* and *T. reesei* i.e., 3.33mm, 0.80mm, 1.66mm, 1.60mm, 2.87mm and 3mm respectively.

Antimicrobial Activity of Standard Antibiotic and Antifungal Discs

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

Antimicrobial Activity of Crude Ethanolic Extracts of *C. reflexa* Parasitysing on three Different Hosts

Zizyphus hysudrica

Significantly high antimicrobial activity of extract B was observed in *P. aeruginosa, B. subtilis, B. licheniformis* (21.00 mm, 19.33 mm and 19.33 mm) followed by *E. coli, T. reesei* and *A. niger* (13.00 mm, 11.33 mm and 7.33 mm) respectively.

Clerodendron inerme

Significant inhibition zones of extract C were recorded against *A. niger, B. licheniformis, P. aeruginosa, B. subtilis* (21.66 mm, 19.30 mm, 18.00 mm and 16.33 mm) while non-significant against *T. reesei* and *E. coli* (11.66 mm and 10.00 mm) respectively.

Populus euroamericana

Extract A also had worth noticing results against the test organisms. An appreciable antimicrobial activity was found against *P. aeruginosa* and *B. subtilis* (17.20 mm, 14.00 mm). However, insignificant activity was recorded in the case of *B.licheniformis, T. reesei, E. coli* and *A. niger*. The values for antimicrobial activity ranged from 13.33 to 9.86 mm.

Plate 1: *Cuscuta reflexa* parasitizing *Clerodendron inerme*

Plate 2: Plant body of *Cuscuta reflexa* Roxb.

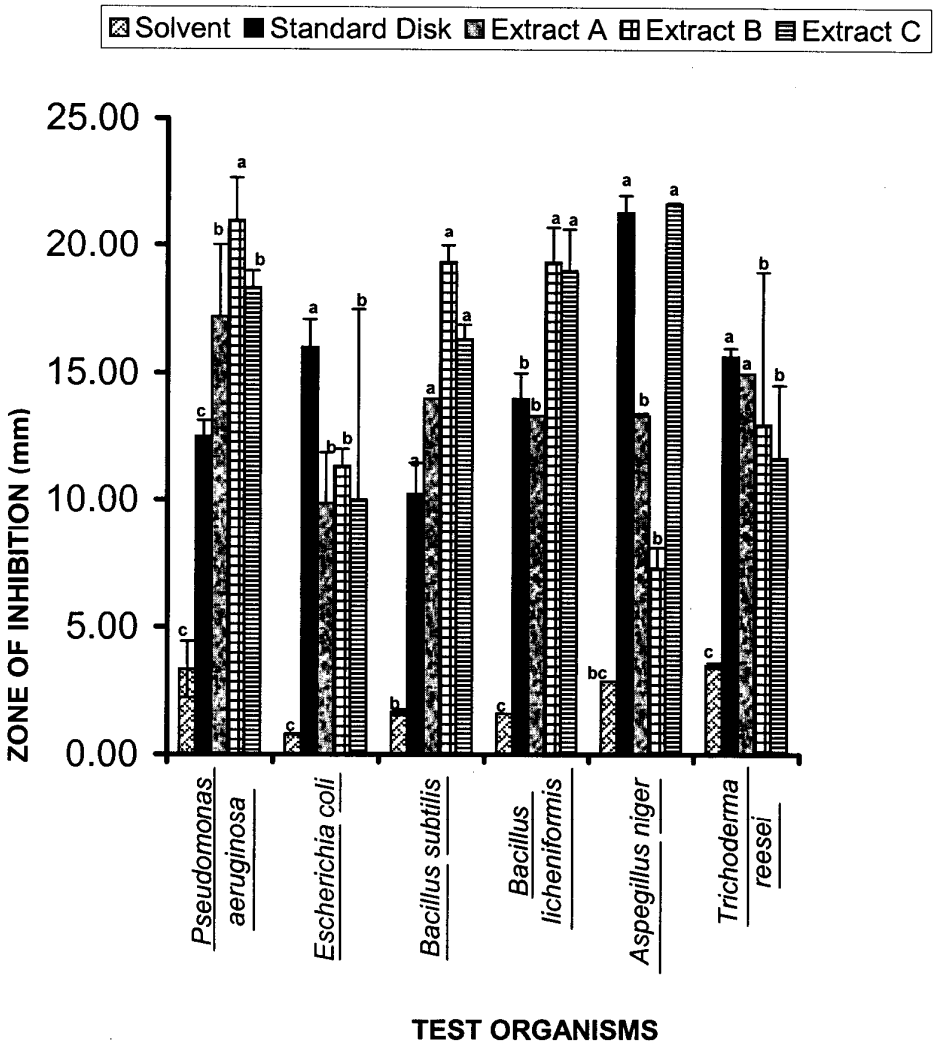

Fig. Distribution of mean zone of inhibition for three ethanolic extract with respect to solvent and standard disc. (The bars represent standard error, while lower case letters represent Duncan's multiple range test).

*A. **Populus euro-americana***
*B. **Zizyphus hysudrica***
*C. **Clerodendron inerme***

C- *Clerodendron inerme*: The third ethanolic extract of plant material also exhibited significant zone of inhibition against *P.areuginosa,B.subtilus B. licheniformis* and *A. niger* i.e., 18.33mm, 16.33mm, 19.30mm and 121.66mm while non-significant zone against *E .coli* and *T. reesei* i.e., 10.00mm and 11.66mm respectively.

Comparative Effect of three Ethanolic Extracts on Test Organisms

Extract B showed excellent antimicrobial activity against bacteria, *P. aeruginosa, E. coli, B. subtilis* and *B. licheniformis* i.e., 56%, 23.333%, 62%, 46% respectively as compared to extract A and extract C which showed appreciable antimicrobial activity i.e., 31.66% and 33.66% against fungi, *T. reesei* and *A. niger* respectively.

Disscussion

Cuscuta reflexa is one of the medicinal plants that is commonly used by the local practitioners for various human ailments. but only one or two attempts have been made to study its antimicrobial activity against some microbes. In present study the crude extracts of the plant material obtained in polar and non-polar solvents were tested against three types of the test organisms. This was entirely a new attempt that was made to study the antimicrobial activity of *Cuscuta* plant from three different hosts. The antibacterial and antifungal activity thus studied was compared with the standard antibiotic (Amikacine and Ciprofloxacine) and antifungal (Griseofulvin) discs. The three ethanolic extracts showed higher antibacterial and antifungal activities as compared to the standard discs .Flavonoids, alkaloids and phenolic compounds have been already extracted from *Cuscuta reflexa* plant by Loffler *et al.* (1997) and Da-Nian Qin *et al.* (2000). These compounds have antibacterial and antifungal activities. Therefore the antibacterial and antifungal activity recorded in the present study could be attributed to these compounds. These compounds are polar compounds and such compounds are usually extracted well in polar solvent such as ethanole found to be highly antimicrobial against most of the microbes used Extract A Extract C were found to be highly antifungal against *T. reesei* and *A. niger*. While Extract B was found to be highly antibacterial This was further supported by Duncan's multiple range test. It was found out that the antimicrobial activity was highly significant against almost all the microorganisms used as compared to the standard discs. The important point to mention here is that the significance difference in antifungal and antibacterial activity with respect to different hosts might be due to the difference in the ssecondary metabolites of the host. Because *Cuscuta reflexa* is considered as stronger sink of secondary metabolites of the host.

Acknowledgement

The author sincerely thanks Prof. Dr. Amin–ul-Haq Khan, Chairman, Department of Botany, G.C.U.; Prof. Dr. Rukhsana Bajwa, Department of Botany, University of the Punjab, Lahore, for encouragement and moral support.

References

Arentsen OJ, Arentsen SS, 1976. Economic importance of *Cuscuta* for sugarbeet stands [Chile]. *Simiente*. **46**: 30-32.

Awasthi .LP, 1981. The purification and nature of an antiviral protein from *Cuscuta reflexa plants. Arch. Virol.*, **703***:* 2215-23.

Bhan AK, Kaul MLH, 1973. Host diversity and chromosomal polymorphism in *Cuscuta reflexa* Roxb. *Sci. Cult*., **39**: 403-404.

Bhatnagar S, Chopra SRN, Prashad B, 1950. The Wealth of India. New Delhi.

Bhattacharya MK, 1976. Growth requirements of *Cucuta reflexa* (growing on *Duranta plumeiri*). *Biol. Plant*, **18**: 415-420.

Chatterje U, Chauhan HOS, Sanwal GG, 1997. Physico-chemical and function characterization of a high molecular weight carboxymethylcellulase from *Cuscuta reflexa. Ind. J. Biochem. Biophys*., **34**: 354-364.

Cheema SS, Kapur SP, Kapur SK, 1976. *Cuscuta reflexa* - a new host for *Aphis craccivora* [Citrus]. *Current Science*, **45**: 35-35.

Chopra RN, Badhwar RL, Ghosh S, 1940. Poisnous Plants of India., pp.712-713.

Czygan FC, Wessinger B, Warmuth K, 1988. *Cuscuta* and its property to take up and to accumulate alkaloids of the host plant, *Cuscut*a und ihre Fahigkeit zur Aufnahme und Speicherung Von Alkaloiden der Wirtspflanzen. Biochem. Physiol. Pflanz. **183:** 495-501.

Da-Nian QIN, Bai-rong SHE, Yun-Chu SHE, Jian Hong Wang, 2000. Effects of flavonoids from Semen Cuscutae on the reproductive system in male rats. *Asian J. Andro*l, 99-102.

Deborah A, Kimbrell, Bruce Beutler, 2001. *Nature Reviews Genetics*, **2**: 256–267.

Forstreuter W, Weber HC, 1984. Parasitism of *Cuscuta* on *Euphorbi*a hosts [*Cuscuta reflexa* and *Cuscuta odorata* on *Euphorbi* Tirucalli, euphorbia milii and Euphorbia pulcherrima]. Zum Parasitismus von

Cuscuta auf Euphorbia-Wirten. *Beitr. Biol. Pflanzen*, **59**: 31-54.

Gilani AH, Aftab K, 1982. Pharmacological actions of *Cuscuta reflexa,I nt. J. Pharamacog*, **30**(11): 296-302.

Ishra S, Sanwal GG, 1992. Alterations in lipid Composition of seed oil from *Brassica juncea* upon infection by *Cuscuta reflexa*. *J. Agric. Food Chem*., **40:** 52-55.

Jeschke WD, Baumel P, Rath N, 1995. Partitioning of nutrients in the *Cuscuta reflexa-Lupinus albus* association. *Asp. Appl.boil.*, **42:** 71-79.

Kenneth Huttner, Richard L., Gallo 2001. Innate antimicrobial peptide protects the skin from invasive bacterial infection. *Nature*, **414**: 454–457.

Khan MTH, Lampronti I, Martello D, Bianchi N, Jabbar S, Chouldhuri MSK, Datta BK, Gambari R, 2002. Identification of Pyrogallol as an Antiproliferatice compound present in extracts from the medicinal plant *Emblica officinlis;* Effects on in. vtro cell growth of human tumor cell Lines. *Int. J. Oncol.* **20**: 187-192.

Loffler C, Czygan, FC, Proksch P, 1997. Phenolic constituents as Taxonomic markers in the genus *Cuscuta* Cuscutaceae). *Biochem. Syst. Ecol*., **25**(4): 287-303.

Malik CP, Komal A, Singh M, 1980. Histochemical Localization of enzymes in *Cuscuta reflexa* and its host *Clerodendron inerme*. Histochem. Dev. Struct. Anat. Angiosp. Symp. Ill. xx.

Michael Zasloff, 2002. Antimicrobial peptides of multicellular organisms. *Nature*, **415**: 389–395.

Nicokrent DL, 2002. Parasitic Plants of the World. Chapter 2, pp. 7-27 in *J.A.*

Pandy DK, Kauraw LP, Bhan VM, 1993. Inhibitory effect of Parthenium residue on growth of water hyacinth. *J. Chem. Ecol*., **19**: 2561-2561-2562.

Raghava-Reddy J, Redi AP, 1981. *Duranta repens* and its parasite, *Cuscuta reflexa*-new host rcords for Leveillula Taurica Indian *J.Mycol. Plant Pathol*., **11**: 317-317.

Rajput MTM, Tahir SS, 1988.. *Fl. Pak.* **189**: 1-24.

Sharma S, Khanna R.. Sanwal GG, 1985. Lipids of *Cuscuta reflexa* and Sahm, A., Czygan FC., Proksch P. ,1994 Resistance of tomato (*Lycopersicon esculentum*) to dodder *(Cuscuta reflexa*). *Acta Hortic*., **381**: 650-653.

Sharma S,. Khanna R, Sanwa G, 1986. Neutral and glycolipids of *Cuscuta reflexa* and of hosts upon parasitism by *Cuscuta. physiol. Veg.* , **24:** 443-451.

Shenqing FZ, Shu GS, 1999. Ciarette containing Chinese medicine for Stoping cough and asthama and preventing cancer. *Applied* pp. 99,100,103.

Singh GS, Garg KN, 1973. Some pharmacological studies on *Cuscuta reflexa* plant. *Ind. J. Pharmac*., **5**: 344-345.

Stojanovic D, Boric B, 1981. Study of mysoflora of parasitic phanerogams from the genera *Cuscut*a and *Orobanche* Biological control of weeds. *Zast. Bilja. Plant Prot*., **32***:* 135-145.

Tian,Yu Z, 1985 A forma specialis of *Collectotrichum gloeosporioides* on *Cuscuta spp. Acta Mycologica Sinica 4*, 234-249.

Victor Nizet, Takaaki Ohtake, Xavier Lauth, Janet Trowbridge, Jennifer Rudisill, Rubert A. Dorschner, Vasumati Pestonjamasp, Joseph Piraino,

Zhuanl FS, Gongka,. lu. S,1999. Antidiabetic capsule containing Chinese medicine. *Applied*, pp.*98*, 113, 999.