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Antimicrobial and phytotoxic properties of *Conyza bonariensis*

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ABSTRACT

The present study was conducted to evaluate *Conyza bonariensis* for its antimicrobial and phytotoxic potentials. The crude methanolic extract and its subsequent solvent fractions were tested for its antibacterial, antifungal and phytotoxic effects. Regarding the antimicrobial effect, tested samples were effective against *Escherichia coli, Pseudomonas aureginosa, Klebsella* and the rest bacteria showed resistance against all the tested samples. The chloroform and ethyl acetate fractions showed maximum activity with zone of inhibition 14 and 13 mm, while the *n*-hexane fraction was not effective at lower dose. The standard drug (streptomycin) was far most effective than the tested extracts having zone of inhibition 35 mm. The maximum fungicidal effect against *Cladosporium cucumerinum* was demonstrated by ethyl acetate followed by chloroform and crude extract with percent inhibitory activity of 38, 32 and 29 respectively. The ethyl acetate fraction was also most effective against *Candida albicans* with percent activity of 35 followed by chloroform and crude methanolic extract with percent inhibitory effect of 28 and 25. The maximum phytotoxic effect was produced with chloroform fraction followed by ethyl acetate with LD_{50} values 16.6 and 17.78. On the basis of the present research work, *Conyza bonariensis* is recommended for its antimicrobial and phytotoxic effect.

Keywords: *Conyza bonariensis,* antibacterial, antifungal, phytotoxic.

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INTRODUCTION

From the very beginning of human existence, man has familiarized himself with plants and used them in a variety of ways throughout the ages. In search of food and to cope successfully with human suffering, primitive man began to distinguish those plants suitable for nutritional purpose from others with definitive pharmacological action. This relationship has grown between plants and man, and many plants came to be used as drugs. The growth of knowledge to cure disease continues at an accelerating pace, and number of new plant-derived drugs increase likewise. Herbal medicine is currently experiencing a revival in Western society, along with other complementary therapies such as traditional Chinese Medicines, Osteopathy and Homeopathy (Shinwari and Gilani, 2003).

The genus Conyza composed of 50 species which are found on the tropical Himalaya from Nepal to Sikhim, extending to Assam, Khasia hills, Chittagong and Burma. The species *C. bonariensis* (Asteraceae) is distributed in many parts of the Punjab (Pakistan) province along the edges of roads, gardens and maize crops lands. It is used in the treatment of constipation and diarrhea (Bukhari et al., 2013). The genus Conyza is found to be very rich in terpenoids such as celarodanes (Zdero et al., 1990), sesquiterpenes (Bohlmann and Wagner, 1982) and diterpenes (Ahmad et al., 1992; Mata et al., 1997).

Spasmolytic and anti-inflammatory activities are shown by some of the species containing these secondary metabolites (De-las et al. 1998). In the current research work, we tested the crude methanolic extract and its various solvent fractions *C. bonariensis* for its antimicrobial and phytotoxic effects.

MATERIALS AND METHODS

Plant collection

Plant was collected from Azad Kashmir, in July 2011; the collected plant was identified at the Department of Botany, Malakand University, Chakdara, Pakistan.

Extraction and fractionation

The collected plant was washed with tap water and shade dried, pulverized and powder plant materials was obtained. These materials were macerated to get crude methanolic extract (Barkatullah and Muhammad, 2011; Khan et al., 2011; Raziq et al., 2011). The crude methanolic extract was further fractioned with various solvents on the basis of polarity (*n*-hexane, chloroform, ethyl acetate, *n*-butanol and aqueous fractions). The crude methanolic as well as the subsequent solvent fractions were screened for antibacterial, antifungal and phototoxic.

Antibacterial assay

The antimicrobial effect was screened out following agar well diffusion method. The experimental bacterial cultures were first grown on nutrient broth and incubated for 24 h prior to experiments. The nutrient agar was melted, then cooled to 40°C, poured to sterilized Petri dishes and allowed to solidify. Wells were then bored in agar using 6 mm diameter with the help of sterile metal cork borer keeping a distance of 24 mm between two consecutive well. 4 to 8 h old bacterial culture was spread on the surface of nutrient agar in petri dishes with the help of sterilized cotton swab. These processes were repeated thrice turning the plate 60°C between each streaking. About l00 µl of 3 mg/ml of respective extract dissolved in dimethyl sulfoxide (DMSO) were then added to the wells. Other wells were supplemented with DMSO and 10 µg ciprofloxacin. The zones of inhibition were then measured after 24 h incubation period. All the experiments were conducted in triplicate (Rahman et al., 2011a; Rahman et al., 2011b).

Antifungal bioassay

Antifungal activity of plant extract and sub fractions was determined by the Agar tube dilution Method. The crude extract was dissolved in DMSO (24 mg/ml). Sterile Sabouraud's dextrose agar medium (5 ml) was placed in a test tube and inoculated with the sample solution (400 µg/ml) kept in slanting position at room temperature overnight. The fungal culture was then inoculated on the slant. The samples were incubated for 7 days at 29°C and growth inhibition was observed and percentage growth inhibition was calculated with reference to the negative control by applying the formula:

$$\% \text{ inhibition} = \frac{\text{Growth in sample tube (mm)}}{\text{Growth in control tube (mm)}} \times 100$$

Some antifungal drugs like miconazole and amphotericin B were used as standard drugs, while miconazole, Amphotericin B and DMSO were used as positive and negative controls (Muanza et al., 1994).

Phytotoxic activity

Phytotoxic activity of plant extract and subsequent solvents fractions were determined by using the recommended protocol of *Lemna minor* (Muhammad and Saeed, 2011; Rahman et al., 2011b). The medium was prepared by mixing various constituents in 1000 ml distilled water and the pH was adjusted (5.5 to 6.5) by adding KOH solution. The medium was then autoclaved at 121°C for 15 min. The extracts dissolved in ethanol (20 mg/ml) served as stock solution. Nine sterilized flasks, three for each concentration, were inoculated with 1000, 100 and 10 µl of the stock solution (1000, 100 and 10 µg/ml, respectively). The solvent was allowed to evaporate overnight under sterile conditions. To each flask, medium (20 ml) and plants each containing a rosette of three fronds of *L. minor*, was added. All flasks were plugged with cotton and kept in the growth cabinet for 7 days. The number of fronds per flask were counted and recorded on day seven and their growth regulation in percentage was calculated by the following formula:

$$\% \text{ Growth Inhibition} = \frac{\text{100 - No. of fronds in test flask}}{\text{No. of fronds in negative control}} \times 100$$

The result was calculated with reference to the positive and negative control. Paraquat was used as a standard drug, while paraquat and volatile solvent were used as positive and negative controls.

RESULTS

Antibacterial activity

The antibacterial effects of the crude methanolic as well as the subsequent fractions of *Conyza bonariensis* against gram positive and gram negative bacteria is presented in Table 1. All the tested samples were applied against *E. coli, P. aureginosa, Klebsella, Shigella, Proteus, S. aureus* and *Bacillus* at three different concentrations (6, 12 and 18 mg/disc). The extracts were effective against *E. coli*, *Klebsella* and *P. aureginosa* and the remaining bacteria show 100% resistance against all the tested samples. The chloroform and ethyl acetate fraction showed maximum activity as shown in Table 1, while the n-hexane fraction showed little bacterial activity. The standard drug (streptomycin) was far most effective than the tested extracts, while crude methanolic extract shows significant anti-bacterial activity against respective microorganisms.

Antifungal activity

Except butanol and aqueous fractions of the plant, all the tested samples were effective against *C. albicans*, *Claudosporium cucumerinum* and *A. niger*. The maximum

Table 1. Antibacterial profile of various solvent fractions of *Conyza bonariensis*.

Bacterial strains	Conc. (mg/disc)	Inhibition zone (mm)						
		Meth.	Hex.	Chl.	Ethy.	But.	Aqu.	Std. Drug
	06	6	-	8	9	-	-	35
E.coli	12	9	2	11	10	-	-	35
	18	12	3	14	13	-	-	35
	06	-	-	-	-	-	-	-
P.aureginosa	12	4	2	5	8	-	-	--
	18	6	4	7	10	-	-	-
	06	-	-	5	4	-	-	-
Klebsella,	12	5	-	7	5	-	-	-
	18	7	-	9	8	-	-	-
	06	--	-	-	-	-	-	-
Shigella	12	-	-	-	-	-	-	-
	18	-	-	-	-	-	-	-
	06	-	-	-	-	-	-	-
Bacillus	12	-	-	-	-	-	-	-
	18	-	-	-	-	-	-	-

Meth = Methanol, Hex. = hexane, Chl. = Chloroform, Ethy. = Ethyl acetate, But. = Butanol, Aqu. = Aqueous, Std. = Standard.

Table 2. Antifungal assay of crude extract and fractions of *Conyza bonariensis.*

Fungal strain	% Inhibition						Standard Drug
	Meth.	Hex.	Chl.	Ethy.	But.	Aqu.	
C. albicans	25	-	28	35	-	-	Miconazole
A. niger	20	-	15	25	-		Miconazole
M. canis	-	-	-	-	-	-	Amphotericine B
F. solani	15	-	10	25	-	10	Miconazole
C. glabarata	-	-	10	15	-	-	Miconazole
C. cucumerinum	29		32	38			Miconazole

Meth = Methanol, Hex. = hexane, Chl. = Chloroform, Ethy. = Ethyl acetate, But. = Butanol, Aqu. = Aqueous, Std. = Standard.

maximum fungicidal effect against *C. cucumerinum* was produced by ethyl acetate, chloroform followed by crude methanolic extract with percent inhibitory activities of 38, 32 and 29, respectively. The ethyl acetate fraction was also effective against *C. albicans* with percent activity of 35, followed by chloroform and crude methanolic extract with percent inhibitory effect 28 and 25 (Table 2).

Phytotoxic activity

The phytotoxic effect of the crud methanolic and solvent fractions of *C. bonariensis* is presented in Table 3. The tested samples were applied in three different concentrations: 10, 100 and 1000 ppm. The maximum phytotoxic effect was produced by chloroform fraction followed by ethyl acetate with LD_{50} values 15.6 and 17.78. The lowest effect was observed against aqueous and methanolic extract.

DISCUSSION

According to one of the survey conducted by Word Health Organisation, it is estimated that about 43% of total deaths in growing countries occurred due to contagious diseases. The search for new, safe and effective antimicrobial drugs is needed due to the

Table 3. Phytotoxic assay of *Conyza bonariensis.*

Samples	10 ppm	100 ppm	1000 ppm	LD_{50}
Methanolic	10 ± 0.77	15 ± 0.78	55 ± 0.78	530 ± 0.34
Hexane	20 ± 0.81	40 ± 0.81	75 ± 0.91	165 ± 0.65
Chloroform	35 ± 0.85	75 ± 0.89	90 ± 0.99	15.6 ± 0.76
Ethyl acetate	30 ± 0.67	75 ± 0.67	85 ± 0.56	17.78 ± 0.98
Butanol	15 ± 0.00	30 ± 0.98	35 ± 0.76	435 ± 0.89
Aqueous	5 ± 0.56	10 ± 0.49	45 ± 0.09	795 ± 0.81

microbial resistance and occurrence of opportunistic infections (Rojas et al., 1992; Srinivasan et al., 2001; Van Vuuren, 2008). The drug resistant bacteria have further complicated treatment of infectious diseases in immuno-compromised and cancer patients. Ethnobotanical data have proved to be helpful in search for new antimicrobial agents and many antibiotics were isolated from natural sources (microbes or medicinal plants) so many antibiotic have been isolated from natural sources (Martini et al., 2004; Sohn et al., 2004). In the present study, our tested samples were effective against two human pathogens, that is, *E. coli* and *P. aureginosa. E. coli* is mostly responsible for the urinary tract infection and GIT disorders (Welch et al., 1981), while the latter mostly causes respiratory tract infections, ear and wound infection, urinary tract infections, dermatitis, soft tissue infections, bacteremia, and bone and joint infections. Systemic infections are common particularly in patients with severe burns, cancer and immunosuppressed patients (AIDS) (Liu, 1974). The chloroform and ethyl acetate fraction should be further screened in the hope of finding new, safe and effective antimicrobial compounds. The same fractions are also effective fungicidal, therefore it is very interesting that the theses fractions are helpful in controlling mixed infection of the fungi and bacteria. It is seen that the chloroform and ethyl acetate fractions were also phytotoxic in a dose dependent manner. The search for the new and safe weedicidal agent is the need of the agriculture as a lot of the food crops and ornamental plants are destroyed or their growth is inhibited by weeds. It is concluded that the *C. bonariensis* can be used as antibacterial and antifungal especially the chloroform and ethyl acetate fraction of the plant can be subjected for the isolation of new and safe compounds. It very clear from the results, that these fractions are effective in all the performed *in-vitro* pharmacological studies. As a crude drug, these both fractions are recommended as antibacterial, antifungal and phytotoxic.

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