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Antimicrobial and Phytotoxic Study of *Conyza canadensis*

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Abstract: The crude methanolic extract and its various solvent fractions of *Conyza canadensis* were evaluated for antimicrobial and phytotoxic profile. The tested samples were only effective against *E.coli*, *P. aureginosa, S. aureus* and the remaining bacteria showed 100 % resistance. The methanolic extract, chloroform and ethyl acetate fraction demonstrated maximum activity with zone of inhibition 14, 12 and13 mm respectively while, the *n*-hexane fraction was devoid of antibacterial effect at lower dose and exhibited low activity at higher dose against *E. coli, P. aureginos and S. aureus* with zone of inhibition 10, 11 and 9 mm respectively. The standard drug (streptomycin) was far most effective than the tested extracts having zone of inhibition 35 mm. The maximum fungicidal effect against *C. albicans* was produced by ethyl acetate followed by chloroform and methanol with percent inhibitory activity 45, 40 and 35 respectively. The ethyl acetate and chloroform fraction was also most effective against *A. niger* with percent activity 40 and 35 followed by methanolic extract and *n*-hexane with percent inhibitory effect 30 and 25. The maximum phytotoxic effect was produced by chloroform fraction followed by ethyl acetate that is 80 and 77% activity.

Key words: *C*onyza *canadensis* antibacterial · Antifungal and phytotoxic activities

INDRODUCTION

The genus *Conyza* belong*s* a well known family that is Asteraceae which almost round about fifty species, which are mainly found in tropical and subtropical regions. Some species of this genus are traditionally used for a variety of pharmacological applications including treatment of smallpox, chickenpox, sore throat, ringworm and other skin related diseases, toothache and to stop bleeding from injuries [1]. Studies on some species have lead to the isolation of secondary metabolites, some of which have been reported to exhibit biological activities including anti-inflammatory, [2-3]. *Conyza canadensis* is one of the specie belongs to genus conyza, family asteraceae. The plant is used for rheumatism, antidiarrhoeal and as antihaemorrhoidal [4, 5]. Traditionally *C*onyza *sumatrensis* is used in the treatment of facial pimples and stomach disorder. Crude ethanolic extract of this plant has antimicrobial activity [6-8]. *Conyza Canadensis* also have antiviral activity. In continuation of our research work on Pakistani medicinal plants [9-16] here we are reporting phytochemical and biological profile of *Conyza canadensis*.

MATERIALS AND METHODS

Extract Preparation: *Conyza canadensis* was collected, dried, pulverized and 5 kg dried powder plant materials was obtained. These powder plant materials were subjected to maceration to get crude methanolic extract according to well establish reported protocols [17-20]. After filtration and concentration under vacuum at 40°C, 600 g crude methanolic extract was obtained. 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 phytotoxic activity.

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Antibacterial Assay: Antibacterial assay was carried out by disc diffusion method, Nutrient agar media plates were seeded with 18 to 24 h cultures of microbial inoculums. Whatman No. 1 filter paper discs (6 mm in diameter) were placed with the help of a sterile forceps on the media and then plant extract and fractions in concentrations of 6, 12 and 18 mg / disc were applied on the discs. Antibiotics streptomycin as positive control and DMSO as negative control were also applied on the discs. Inoculated plates were then incubated at 37°C for 18 to 24 h. After 24 h, zones of inhibition were recorded in mm around the discs in each plate. Streptomycin was used as standard drug [21-24].

Antifungal Bioassay: The antifungal activity was determined by the Agar tube dilution Method according to standard protocol [25-28]. The crude extract was dissolved in DMSO (24 mg/1ml). Sterile Sabouraud's dextrose agar medium (5ml) 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. Miconazole and amphotericin B were used as standard drugs, while DMSO was used negative controls.

Phytotoxic Activity: Phytotoxic activity was determined by using the recommended protocol of *Lemna minor* [29]. The medium was prepared by mixing various constituents in 1000 ml distilled water and the pH was adjusted (5.5-6.5) by adding KOH solution. The medium was then autoclaved at121°C for 15 minutes. The extracts dissolved in ethanol (20 mg/ml) served as stock solution. Nine sterilized flasks, three for each concentration, were inoculated with 1000 µl, 100 µl and 10 µl of the stock solution for 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 *Lemna minor* L., 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:

$$\%\text{Inhibition}=10-\frac{\text{Number of frounds in test sample}}{\text{Number of frounds in control}}\text{X}100$$

The result was calculated with reference to the positive and negative control. Paraquat was used as a standard drug.

RESULTS AND DISCUSSION

Antibacterial Effect: The antibacterial effect of the crude methanolic extract as well as the subsequent fractions of *Conyza canadensis* against gram positive and gram negative bacteria is presented in Table 1. All the tested samples were applied against *E. coli, P. aureginosa, Klebsella, S. aureus and Bacillus* at three different concentrations (6, 12 and 18 mg/disc). The samples to be tested were only effective against *E. coli*, *P. aureginosa and S. aureus* and the remaining bacteria show almost 100 % resistance against all the tested samples. The crude methanolic extract and ethyl acetate fraction showed maximum activity with zone of inhibition 14 and13 mm while, the *n*-hexane fraction was not effective at lower dose while at higher dose it showed activity against *E. coli, P. aureginosa and S. aureus* with zone of inhibition 10, 11 and 9 mm respectively. The standard drug (streptomycin) was far most effective than the tested extracts having zone of inhibition 35 mm.

Antifungal Effect: Except butanol and aqueous fractions of the plant all the tested samples were effective against *C. albican, A. niger* and up to some extent *C. glabarata*. The maximum fungicidal effect against *C. albicans* was produced by ethyl acetate followed by chloroform and methanolic extract with percent inhibitory activity 45, 40 and 35 respectively. The ethyl acetate fraction was also most effective against *A. niger* with percent activity 40 followed by chloroform and methanolic extract and with percent inhibitory effect 35 and 30. The ethyl acetate fraction showed activity against *C. glabarata* with percent inhibitory activity 25 as shown in Table 2.

Phytotoxic Profile: The phytotoxic effect of the crud methanolic and solvent fractions of *Conyza sumatrensis* is presented in Table 3. The tested samples were applied in three different concentrations i.e. 10, 100 and 1000 ppm. The maximum phytotoxic effect was produced by chloroform fraction followed by ethyl acetate that is 80 and 77% the lowest effect was observed against aqueous and methanolic extract.

According to WHO, it is estimated that round about 43 % of total deaths in growing countries occurred due to the contagious diseases. The search for new useful

Table 1: Antibacterial profile of various solvent fractions of *Conyza canadensis*

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

Meth= methanolic, Hex= n-hexane, Chl = chloroform, But = butanol and Aqu = aqueous.

Table 2: Antifungal assay of *Conyza canadensis*

	% Inhibition						
Fungal Strain	Meth	Hex	Chl	Ethy	But	Aqu	Standard Drug
C. albicans	35±0.81	30±0.33	40±0.81	45±0.91	-	-	Miconazole
A. niger	30±0.91	25±0.88	35±0.56	40±0.56	-		Miconazole
M. canis	-	-	-	-	-	-	Amphotericine B
F. solani	-	-	10±0.45	25±0.65	-	10±0.67	Miconazole
C. glabarata	20±0.65	20±0.88	15±0.29	25±0.94	-	-	Miconazole

Meth= methanolic, Hex= n-hexane, Chl = chloroform, But = butanol and Aqu = aqueous. Data are presented as mean ± SEM (n= 3)

Table 3: Phytotoxic assay of *Conyza canadensis*

Samples	10 ppm	100 ppm	1000 ppm	LD_{50}
Methanolic	20±0.77	45±0.78	70±0.78	530±0.34
Hexane	20±0.81	40±0.81	65±0.91	165±0.65
Chloroform	35±0.85	73±0.89	80±0.99	15.6±0.76
Ethyl acetate	30±0.67	70±0.67	77±0.56	17.78±0.98
Butanol	15±0.00	30±0.98	30±0.76	435±0.89
Aqueous	5±0.56	10±0.49	25±0.09	795±0.81

Data are presented as mean ± SEM (n= 3)

antimicrobial drugs is needed due to the microbial resistance and occurrence of opportunistic infections that is most of the antimicrobial agents are now not use or their use is limited because of most resistance and due to this resistance a lot of secondary problem are generated that have further complication [30-33]. The drug resistant bacteria have further complicated treatment of infectious diseases in immuno-compromised and cancer patients. Ethno botanical 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 [33, 34]. In the present study our tested samples were effective against some bacteria, fungi and also have good phytotoxic activity.

It is concluded the *Conyza canadensis* 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.

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