

Antimicrobial and Phytotoxic Profile of *Conyza sumatrensis*

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Abstract: The crude extract and its various solvent fractions of *Conyza sumatrensis* were tested for their antibacterial, antifungal and phytotoxic activities. The tested samples were only effective against *E. coli* and *P. aureginosa* and the remaining bacteria demonstrated 100 % resistance against all the tested samples. The chloroform and ethyl acetate fraction showed maximum activity with zone of inhibition 16 and 12 mm while, the *n*-hexane fraction was not effective at lower dose while at higher dose it showed activity against *E. coli* and *P. aureginosa* with zone of inhibition 10 and 11 mm respectively. The standard drug (streptomycin) was far most effective than the tested extract having zone of inhibition 35 mm. The maximum fungicidal effect against *C. albicans* was produced by chloroform followed by *n*-hexane and ethyl acetate fractions with percent inhibitory activity 45, 40 and 33 respectively. The chloroform fraction was also most effective against *A. niger* with percent activity 30 followed by *n*-hexane and ethyl acetate with percent inhibitory effect 25 of each. The maximum phytotoxic effect was produced by chloroform fraction followed by ethyl acetate with LD₅₀ values 15.6 and 17.78. The current study supports the use of this plant in various microbial infections.

Key words: *Conyza sumatrensis* antibacterial • Antifungal and phytotoxic

INTRODUCTION

The genus *Conyza* belongs to family Asteraceae which comprises of 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 led to the isolation of secondary metabolites, some of which have been reported to exhibit biological activities including anti-inflammatory, [2-4]. *Conyza canadensis* is one of the species belongs to genus *Conyza*, family Asteraceae. The plant is used for rheumatism, anti-diarrhoeal and as anti-haemorrhoidal [5-7]. Traditionally *Conyza sumatrensis* is used in the treatment of facial pimples and stomach disorder. It also serves as a good source of food for the fowls. Crude ethanolic extract of this plant has antimicrobial activity [8-10].

MATERIALS AND METHODS

Extract Preparation: *Conyza sumatrensis* was collected (in July 2009), dried, pulverized and 5 kg dried powder plant material was obtained. These powder plant materials were subjected to maceration to get crude methanolic extract according to well established reported protocols [11-13]. After filtration and concentration under vacuum at 40°C, 600 g crude methanolic extract was obtained. The crude methanolic extract was further fractionated 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 activities.

Antibacterial Assay: Nutrient agar media plates were seeded with 18 to 24 h cultures of microbial inoculums (a standardized inoculum 1-2 10⁷ CFU ml⁻¹ 0.5 McFarland Standard). Whatman No. 1 filter paper discs (6 mm in

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diameter) were placed with the help of a sterile forceps on the media and then plant extracts 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 [14-18].

Antifungal Bioassay: The antifungal activity was determined by the Agar tube dilution Method. 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 by applying the formula:

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

Miconazole and amphotericin B were used as standard drugs, while miconazole, Amphotericin B and DMSO were used as positive and negative controls [19].

Phytotoxic Activity: Phytotoxic activity was determined by using the recommended protocol of *Lemna minor* [20-23]. 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 at 121°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:

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

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 AND DISCUSSION

Antibacterial Activity: The antibacterial effects of the crude methanolic as well as the subsequent fractions of *Conyza sumatrensis* 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*, *Steph. aureus* and *Bacillus* at three different concentrations (6, 12 and 18 mg/disc). The samples were only effective against *E.coli* and *P.aureginosa* and the remaining bacteria show 100 % resistance against all the tested samples. The chloroform and ethyl acetate fraction showed maximum activity with zone of inhibition 16 and 12 mm while, the *n*-hexane fraction was not effective at lower dose while at higher dosed it showed activity against *E.coli* and *P.aureginosa* with zone of inhibition 10 and 11 mm. The standard drug (streptomycin) was far most effective than the tested extracts having zone of inhibition 35 mm as cleared from table and figures.

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

Bacterial Strains	Conc. (mg/disc)	Zone of Inhibition (mm)							Std. Drug
		Meth	Hex	Chl	Ethy	But	Aqu		
<i>E. coli</i>	06	-	-	10 ±1.22	9±1.45	-	-	35±0.11	
	12	5±0.89	8±1.34	11±0.88	10±0.88	-	-	35±0.23	
	18	7±0.73	10±0.46	16±2.11	12±0.99	-	-	35±0.13	
<i>P. aureginosa</i>	06	-	-	-	-	-	-	-	
	12	3±0.35	8±0.81	-	-	-	-	-	
	18	6±0.27	11±0.89	-	-	-	-	-	

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

Table 2: Antifungal assay of crude extract and fractions of *Conyza sumatrensis*

Fungal Strain	% Inhibition						Standard Drug
	Meth	Hex	Chl	Ethy	But	Aqu	
<i>C. albicans</i>	25±0.81	40±0.33	45±0.81	33±0.91	-	-	Miconazole
<i>A. niger</i>	10±0.91	25±0.88	30±0.56	25±0.56	-	-	Miconazole
<i>M. canis</i>	-	-	-	-	-	-	Amphotericine B
<i>F. solani</i>	-	-	10±0.45	25±0.65	-	10±0.67	Miconazole
<i>C. glabarata</i>	-	-	10±0.29	15±0.94	-	-	Miconazole

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

Table 3: Phytotoxic assay of *Conyza sumatrensis*

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

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

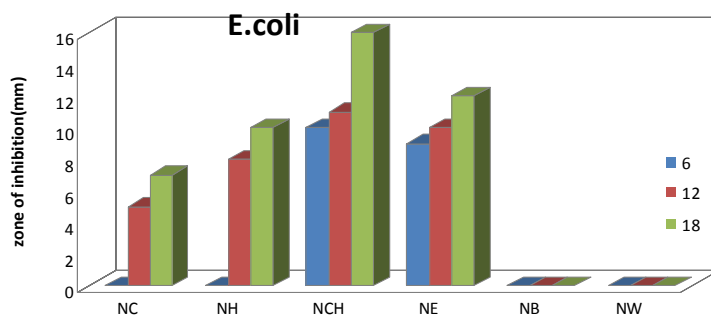


Fig. 1: Antibacterial activity of crude extract and fractions against *E. coli*

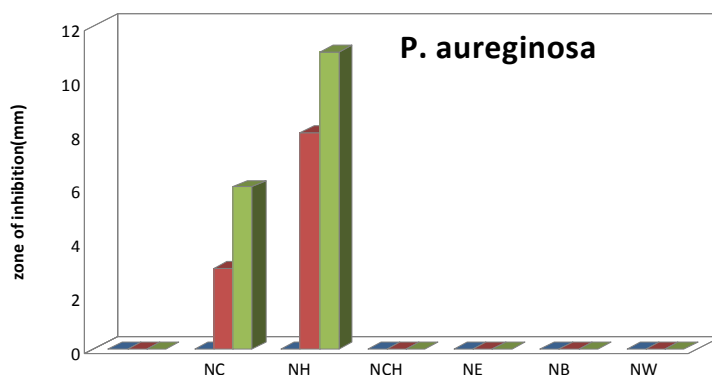


Fig. 2: Antibacterial activity of crude extract and fractions against *P. aureginosa*

Antifungal Activity: Except butanol and aqueous fractions of the plant all the tested samples were effective against *C. albicans* and *A. niger*. The maximum fungicidal effect against *C. albicans* was produced by chloroform followed by n-hexane and ethyl acetate fraction with percent inhibitory activity 45, 40 and 33 respectively. The chloroform fraction was also most effective against *A. niger* with percent activity 30 followed by n-hexane

and ethyl acetate with percent inhibitory effect 25 of each. The crude methanolic extract showed low (25 and 10 %) activity against *C. albicans* and *A. niger* as shown in Table (2) and Figures (3-5).

Phototoxic Activity: The phototoxic effect of the crud methanolic and solvent fractions of *Conyza sumatrensis* is presented in Table 3. The tested samples were applied

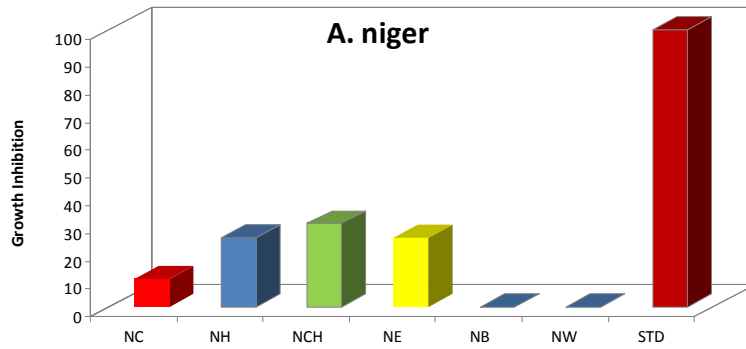


Fig. 3: Antifungal activities of crude extract and fraction against *A. niger*

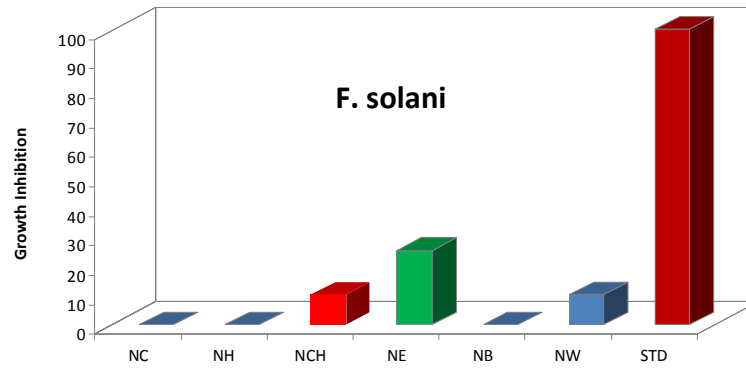


Fig. 4: Antifungal activities of crude extract and fraction against *F. solani*

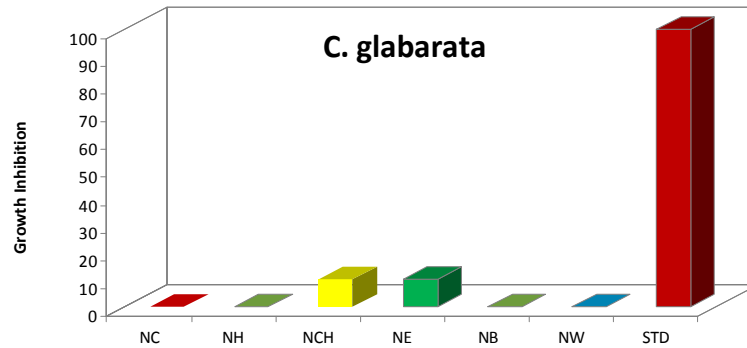


Fig. 5: Antifungal activities of crude extract and fraction against *C. glabarata*

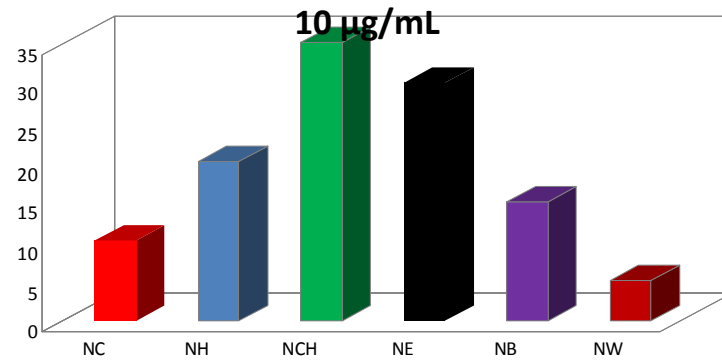


Fig. 6: Phytotoxic activities of *C. sumatrensis* at 10 µg/mL concentration

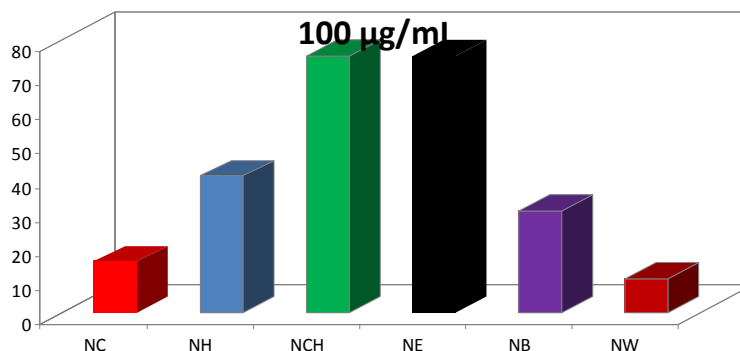


Fig. 7: Phytotoxic activity of *C. sumatrensis* at 100 µg/mL concentration

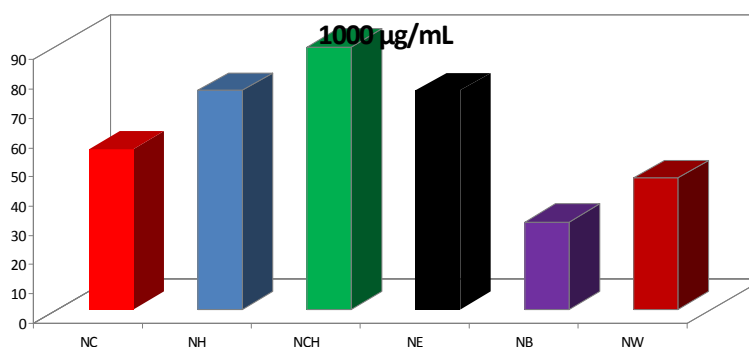


Fig. 8: Phytotoxic activity of *C. sumatrensis* at 1000 µg/mL concentration

NC = Crude Extract
 NH = *n*-Hexane Fraction
 NCH = Chloroform Fraction
 NE = Ethyl Acetate Fraction
 NB = Butanol Fraction
 NW = Aqueous Fraction

in three different concentrations i.e. 10, 100 and 1000 ppm. The maximum phytotoxic effect was produced chloroform fraction followed by ethyl acetate with LD₅₀ values 15.6 and 17.78. The lowest effect was observed against aqueous and methanolic extract as shown in Table (3) and Figures (6-8).

According to one of the survey conducted by 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 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 [24-26]. 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 [27, 28]. In the present study our tested samples were effective against two human pathogen i.e. *E. coli* and

P. aureginosa. *E. coli* is mostly responsible for the urinary tract infection and GIT disorders [29], while the later is mostly causes respiratory tract infections, ear and wound infection, urinary tract infections, dermatitis, soft tissue infections, bacteremia, bone and joint infections. Systemic infections are common particularly in patients with severe burns, cancer and immunosuppressed patients (AIDS) [30]. 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 maxed 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 the *Conyza sumatrensis* can be used as antibacterial and antifungal specially 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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