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 more 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 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 lead 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 specie belongs to genus conyza, family asteraceae. The plant is used for rheumatism, anti diarrhoeal and as antihaeorrhoidal [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 materials was obtained. These powder plant materials were subjected to maceration to get crude methanolic extract according to well establish 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 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 activities.

Antibacterial Assay: Nutrient agar media plates were seeded with 18 to 24 h cultures of microbial inoculums (a standardized inoculums 1-2 107 CFU ml-1 0.5 McFarland Standard). 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 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/ml). 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:

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

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

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*

<table>
<thead>
<tr>
<th>Fungal Strain</th>
<th>Meth</th>
<th>Hex</th>
<th>Chl</th>
<th>Ethy</th>
<th>But</th>
<th>Aqu</th>
<th>Standard Drug</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. albicans</em></td>
<td>25±0.81</td>
<td>40±0.33</td>
<td>45±0.81</td>
<td>33±0.91</td>
<td>-</td>
<td>-</td>
<td>Miconazole</td>
</tr>
<tr>
<td><em>A. niger</em></td>
<td>10±0.91</td>
<td>25±0.88</td>
<td>30±0.56</td>
<td>25±0.56</td>
<td>-</td>
<td>-</td>
<td>Miconazole</td>
</tr>
<tr>
<td><em>M. canis</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Amphotericin B</td>
</tr>
<tr>
<td><em>F. solani</em></td>
<td>-</td>
<td>-</td>
<td>10±0.45</td>
<td>25±0.65</td>
<td>-</td>
<td>10±0.67</td>
<td>Miconazole</td>
</tr>
<tr>
<td><em>C. glabratu</em></td>
<td>-</td>
<td>-</td>
<td>10±0.29</td>
<td>15±0.94</td>
<td>-</td>
<td>-</td>
<td>Miconazole</td>
</tr>
</tbody>
</table>

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*

<table>
<thead>
<tr>
<th>Samples</th>
<th>10 ppm</th>
<th>100 ppm</th>
<th>1000 ppm</th>
<th>LD50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanolic</td>
<td>10±0.77</td>
<td>15±0.78</td>
<td>55±0.78</td>
<td>530±0.34</td>
</tr>
<tr>
<td>Hexane</td>
<td>20±0.81</td>
<td>40±0.81</td>
<td>75±0.91</td>
<td>165±0.65</td>
</tr>
<tr>
<td>Chloroform</td>
<td>35±0.85</td>
<td>75±0.89</td>
<td>90±0.99</td>
<td>15.6±0.76</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>30±0.67</td>
<td>75±0.67</td>
<td>85±0.56</td>
<td>17.78±0.98</td>
</tr>
<tr>
<td>Butanol</td>
<td>15±0.00</td>
<td>30±0.98</td>
<td>35±0.76</td>
<td>435±0.89</td>
</tr>
<tr>
<td>Aqueous</td>
<td>5±0.56</td>
<td>10±0.49</td>
<td>45±0.09</td>
<td>795±0.81</td>
</tr>
</tbody>
</table>

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

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 phytotoxic effect of the crude 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. glabara*

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. 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 [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.

REFERENCES


