INVESTIGATION OF THE ANTIMICROBIAL ACTIVITY OF CRUDE ALKALOIDS EXTRACT OF *Dicentra scandens* (D. DON) WALP. TUBEROUS ROOT

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ABSTRACT

*Dicentra scandens* (D.Don) Walp. (*Fumariaceae*) has been used as folk remedy for a variety of ailments by certain tribal communities living in the Central and Eastern Himalayan region. The aim of this study was to evaluate the antidermatophytic and antibacterial activity of *D. scandens* tuberous root crude alkaloids extract. Crude alkaloids extract was assayed against seven fungi namely, *Trichophyton mentagrophytes*, *Microsporum gypseum*, *Microsporum fulvum*, *Trichophyton rubrum*, *Trichophyton tonsurans*, *Trichosporon beigeslii*, *Candida albicans* and four bacteria namely, *Bacillus mycoides*, *Escherichia coli*, *Bacillus subtilis* and *Enterobacter cloacae* by the agar well diffusion method. Crude alkaloids extract exhibited antimicrobial activity against all tested pathogens, further confirmed its fungicidal effect in the present study. Inhibition of growth was observed in all the tested pathogens although inhibition percent varied from species to species. A maximum of 53.13% inhibition was observed against *T. mentagrophytes*. The present study indicated that *D. scandens* possess potential broad spectrum antimicrobial activity which could be explored for pharmaceutical applications.

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1 Introduction

Medicinal plants have been in use for centuries as remedies for human diseases. It has been estimated that 14 - 28% of higher plant species are used medicinally and that 74% of pharmacologically active plant derived components were discovered after following upon ethno medicinal use of the plants as reported by Ncube et al., 2008. Acceptance of traditional medicine as an alternative form of health care and the development of microbial resistance to the available antibiotics has led researchers to investigate the antimicrobial activity of medicinal plants (Bisignano et al., 1996; Lis-Balchin and Deans, 1996; Moaz and Neeman, 1998; Hammer et al., 1999). Medicinal plant based antimicrobials represent a vast untapped source of pharmaceuticals and further exploration of plant antimicrobials need to occur for treatment of infectious diseases both in plants and humans while simultaneously for mitigating many of the side effects that are often associated with synthetic antimicrobials. According to Hostettmann (1999), only a small portion has been investigated both phytochemically and pharmacologically out of the several medicinal plant species around the globe. This shows that medicinal plants represent a vast untapped source of medicines having an enormous therapeutic potential not only effective in the treatment of the diseases alone, but also play a vital role in mitigating many side effects that are often associated with synthetic antimicrobial drugs.

*D. scandens* is a perennial herb exhibiting a dual habit that is climber when supports are present or creep on the ground, has been used for years as folk medicine against wounds, helminthic gastrointestinal disorder such as dysentery, diarrhea, fever, hypertension, toothache as reported by various researchers (Manandhar, 1993; Pfoze and Chiezou, 2006). However, till date no report is available on skin related problems. Therefore the aim of the present study was to explore the antidermatophytic and antibacterial potential of crude alkaloids extract of *D. scandens* tuberous root for the treatment of skin related problems.

2. Materials and methods

2.1 Collection of plant material

*D. scandens* was collected in the month of September, 2008 from Senapati district of Manipur. A voucher specimen of the plant was deposited in the herbarium of the Department of Botany, North Eastern Hill University, Shillong (collection No. 160 and accession No. NEHU 11 877).

2.2 Preparation of crude alkaloids extract

Collected tuberous roots were washed with distilled water and shade dried for two weeks. Dried root were powdered by using mechanical grinder. About 200 g of shed dried ground sample was soaked in water and ethanol (1:4) for 48 h. Filtrate collected using Whatman No.1 filter paper was concentrated under reduced pressure in rotary vacuum evaporator (RV10 Control, IKA). The dried extract was extracted into weakly acidic solvent which was further extracted in dichloromethane. Aqueous phase was basified and extracted with dichloromethane to get crude alkaloids (yield of 5%), which was further confirmed for the presence of alkaloids using different alkaloids reagents. The resultant brownish sticky crude alkaloid extract was stored in freeze at -20°C till further analyses or use.

2.3 Preparation of test extract

10% (w/v) of crude alkaloids extract was prepared by dissolving initially in small amount of dimethyl sulphoxide (DMSO) and then final volume was adjusted with double distilled water. Prepared solutions were sterilized by filtering through Millipore (0.2μm).

2.4 Antimicrobial assay

Test microorganisms used in the present investigation include both bacteria and fungi. Four different species of bacteria namely, *B. mycoides* (MTCC645), *B. subtilis* (MTCC736), *E. coli* (MTCC40) and *E. cloacae* (MTCC509) and seven fungi including two species of yeast viz. *T. rubrum* (MTCC8477), *T. mentagrophytes* (MTCC8476), *T. tonsurans* (MTCC8475), *M. fulvum* (MTCC847), *M. gypseum* (MTCC8469), *C. albicans* (MTCC854) and *T. beigelii*. Microbial strains preserved in nutrient agar (NA) at 4°C were revivified in nutrient agar (NA) and potato dextrose agar (PDA) for bacterial and fungal, and incubated at 37±1°C and 28±2°C for 18 – 24 h, respectively and were used as inoculums for antibacterial and antifungal studies. Agar well diffusion method was employed for qualitative antimicrobial screening according to Collins et al., (1989) by measuring zone of inhibition (ZI) in millimeters in diameter, which is further calculated in percentage and given in Table1. The experiment was performed in triplicate. Percentage of inhibition was calculated according to Vyas et al. (2006) as Percentage of inhibition = inhibition zone (mm)/Control (mm) x 100, where control is the diameter of agar plate used in which the growth of fungi or bacteria occurs. To study the fungicidal or fungistatic property of the extract, plugs from the zone of inhibition were taken out and re-incubated into fresh media that were then examined for their growth after 96 h of incubation according to Karuba et al. (2001)

3. Results and Discussion

Result of antimicrobial efficacy of the crude alkaloids extract against eleven human pathogens by using agar well diffusion method is summarized in table 1. The extract showed zones of inhibition ranging from 12.3-42.5 mm in diameter. The highest zone of inhibition was observed against *T. mentagrophytes*
(42.5 mm) (figure 1) while the least zone was against C. albicans (12.3 mm). The sensitivity pattern of the microorganisms to the extract was found to decrease among the fungal and yeast strains in the following order: T. mentagrophytes > M. gypseum > M. fulvum > T. rubrum > T. tonsurans > T. beigeslii > C. albicans. Upon re-incubation of fungi inoculums from the zones of inhibition observed no further growth suggesting that the mode of action of the crude methanolic extract is fungicidal. Activity against human pathogen such as C. albicans and T. beigeslii is of clinical importance as these are listed among the top opportunistic infections in the immune-compromised host such as Aids patients (Ministry of Public Health, Thailand, 2005).

Table 1. Antimicrobial potential of crude alkaloids extract of *D. scandens* tuberous root.

<table>
<thead>
<tr>
<th>Test microorganisms</th>
<th>MTCC</th>
<th>Inhibition zone (mm)</th>
<th>Percent inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dermatophytes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Trichophyton mentagrophytes</em></td>
<td>MTCC8476</td>
<td>42.5</td>
<td>53.13</td>
</tr>
<tr>
<td><em>Trichophyton rubrum</em></td>
<td>MTCC8477</td>
<td>29</td>
<td>36.25</td>
</tr>
<tr>
<td><em>Trichophyton tonsurans</em></td>
<td>MTCC8475</td>
<td>28.6</td>
<td>35.75</td>
</tr>
<tr>
<td><em>Microsporum gypseum</em></td>
<td>MTCC8469</td>
<td>35.2</td>
<td>44</td>
</tr>
<tr>
<td><em>Microsporum fulvum</em></td>
<td>MTCC847</td>
<td>33.6</td>
<td>42</td>
</tr>
<tr>
<td><strong>Yeast</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>MTCC854</td>
<td>12</td>
<td>15</td>
</tr>
<tr>
<td><em>Trichosporon beigeslii</em></td>
<td>-----------</td>
<td>18.6</td>
<td>23.25</td>
</tr>
<tr>
<td><strong>Bacteria</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Bacillus mycoides</em></td>
<td>MTCC645</td>
<td>19.2</td>
<td>24</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>MTCC736</td>
<td>20.2</td>
<td>25</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>MTCC 40</td>
<td>20.8</td>
<td>25.25</td>
</tr>
<tr>
<td><em>Enterobacter cloacae</em></td>
<td>MTCC509</td>
<td>21.2</td>
<td>26.5</td>
</tr>
</tbody>
</table>

Values are represented as mean of 3 replicates.

Figure 1. Antimicrobial activity of crude alkaloids extract of *D. scandens* tuberous root against: A - T. mentagrophytes and B - M. fulvum
Under certain conditions, dermatophytosis can be complicated by secondary bacterial infections. Therefore further investigation was carried out to check whether the same extract exerts in addition to its antifungal effects, a significant antibacterial activity against gram negative and gram positive bacteria. It was observed that the extract showed moderate activity on B. mycoides, E. coli, B. subtilis and E. cloaceae.

Screening of plant extract against antimicrobial activity has resulted in identification of a large number of plant secondary metabolites such as phenolics and polyphenols, alkaloids, terpenoids, etc. exhibiting such activity. The antimicrobial properties of alkaloids have been well established and Verpoorte, 1998 has reported about 300 alkaloids showing such activity. Berberine, a quaternary alkaloid for instance exerts not only a strong antifungal and antibacterial activities but is potentially effective against trypanosomes and plasmodia as per the report of Freiburghaus et al., 1996 and Omulokoli et al., 1997 respectively. Our data showed that the crude alkaloids extracts of D. scandens tuberous root was active against tested fungal and bacterial strains of human pathogens. Most importantly, this is the first report that D. scandens is antidermatophytic.

In conclusion, D. scandens may be added to the growing list of plants having antimicrobial activity. The results of the present investigation showed that the crude alkaloids extract is of a broad spectrum agent exhibiting a pronounced antidermatophytic and antibacterial activity in all the tested pathogens. Therefore a more comprehensive bioactivity guided fractionation and bioactive components or compounds isolation from D. scandens may be taken up and explore the potential source for treatment and/or management of opportunistic pathogens and other fungal infections of clinical importance and skin related problems.

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