Antidermatophytic activity of Solanum indicum L. from North East India.

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ABSTRACT

Chloroform, Methanol and Water extract of Solanum indicum Linn., a widely used food and medicinal plant of Assam, India was investigated for its in vitro antifungal activity against five different dermatophytic fungi viz., Trichophyton mentagrophytes, Trichophyton rubrum, Trichophyton tonsurans, Microsporum gypseum and Microsporum fulvum. Agar well diffusion method was used to determine the inhibition zone of the different extracts of the plant. The minimum inhibitory concentration of the extracts was determined by broth dilution method. When compared, the Chloroform extract of the plant was found to be highly active against all the tested fungi followed by the methanol extract. The water extract was found completely inactive against T. mentagrophytes and T. rubrum while it showed very low activity against M. gypseum, T. tonsurans and M. fulvum at the tested concentration. The results suggest that the leaves of S. indicum may possess some non polar antifungal compounds that can be used for treating dermatophytosis.

Key Words: Agar well diffusion, Broth dilution, dermatophytosis.

INTRODUCTION

In humans, infections pertaining to the skin and mucosal surface constitute a serious problem specially in the tropical and subtropical developing regions of the world (1). Skin infection due to dermatophytes has become a significant health problem in countries like India and may reach epidemic proportions in areas with a high rate of humidity, over population and poor hygienic conditions (2). Chronic dermatophytosis is a refractory condition, which runs a course of more than one year with episodes of exacerbations and remissions (3). Factors responsible for chronicity are the site of infection, poor penetration of the drug and drug resistance. Additionally associated conditions like atopic diathesis, disorders of keratinization, diabetes mellitus, Cushing’s syndrome, immunosuppression following cancer, organ transplants and HIV infection have further lead to increased predisposition to fungal infections (4). Most antifungal creams used in India are imported and very expensive, and are reported to be associated with several toxic side effects. Therefore, the development of an antifungal agent from local raw material is still necessary (5-6).

Northeast Indian states are the store house of medicinal plants which are naturally grown and available in the forests. The region is known for its high ethnic and biological diversity and is often referred to as optical hotspot (7-8). Plants used in folk medicine in Northeast India represent several species reported as a result of more than 150 ethnobotanical case studies. The state of Assam alone has about 2000-3000 species of medicinal plants useful in indigenous medicine (9). The use of medicinal and aromatic plants in the use of skin diseases caused by mycotic infections is an old age old process, mainly by the various ethnic tribes of Assam. Most tribal communities of this region still largely depend on their traditional system of medicine, inherited from their ancestors. Further owing to their scattered and far flung settlements, and problems arising due to transportation and communication, traditional medicine has remained as the most affordable and easily accessible source of treatment mainly for the tribal ethnic communities (10).

Solanum indicum Linn. belonging to the family Solanaceae is a spiny shrub, found wild along roadsides, forest margins and forest floors throughout North East India (11). Leaves of the plant are eaten as vegetables. Externally, the juice from the leaves is used for skin problems. In the Ayurveda, Yunami medicinal system the fruit is used in pruitus, leucoderma, bronchitis, asthma, fever, vomiting, loss of appetite and diseases of the eye. The smoke from burning seeds is said to relieve toothache. The plant is also useful in bronchitis, calculus affections, chronic sebile affections, colic, dysuria, cardiac weakness and urinary diseases. Half-ripe fruits are employed in the preparation of curries, chutneys and preserves. Root is carminative and expectorant, useful in coughs and catarhal affections, dysuria and colic. Root is pounded and used for the treatment of nasal ulcers. The root is also used to remove foulness of the mouth, beneficial in cardiac troubles, useful in leucoderma, fever, asthma, pain bronchitis, vomiting, pruritus etc (12-13). Keeping in view of its broad medicinal use, we in this study carried out the in vitro antidermatophytic activity of the chloroform, methanol and water extract of the leaves of S. indicum growing in Assam, India.

MATERIALS AND METHODS

Plant Material and Extract Preparation

Leaves of S. indicum were collected from Kamrup district, Assam in the month of February 2010, authenticated by a Taxonomist from Gauhati University and herbarium was prepared and deposited as voucher specimen (No. IASST/MEP/H No.50) in the Medicinal Plant and Biochemistry Laboratory of IASST for future reference.

Freshly collected plant materials was dried under shade and 100 gm of the fine dried powdered materials were then extracted separately with 1000 ml of chloroform (Merck) and methanol separately in an extractor at room temperature. The extracts were concentrated in a rotary evaporator (Buchi R124, German) at <40°C. The yield of the Chloroform and Methanol extract was 7.50 gm and 4.25 gm respectively. For the aqueous extract, 100 gm of powdered plant materials were heated in 1000 ml water for one hour in a water bath at 35°C, filtered and finally lyophilized to dryness; it yielded 6.0 gm of extract. The plant extracts were dissolved in dimethyl sulfoxide (DMSO) to reach a concentration of 10 mg/ml. All the extracts were kept in refrigerator at 4°C for future use.

Procurement of Fungi and inoculum preparation

Fungal strains were procured from Institute of Microbial Technology (IMTECH), Chandigarh- 160036 (India). The organisms tested were Tricho-
phyton rubrum (MTCC 8477), Trichophyton mentagrophytes (MTCC 8476), Trichophyton tonsurans (MTCC 8475) Microsporum gypseum (MTCC 8469) and Microsporum fulvum (MTCC 8478). The procured samples were sub cultured and maintained in Sabouraud Dextrose Agar (HIMEDIA) slants at 4 °C. For the experiment, freshly cultured slants were used for preparing spore suspension in 0.9% saline water. The fungal spore suspension was adjusted to give a final concentration of 10^6 cfu/ml.

Antifungal activity

The in vitro antifungal screening was performed using agar well diffusion method (14). In brief, 200 µl of fungal suspension were uniformly spread over solidified Sabouraud Dextrose Agar (SDA) plates with the help of a sterilized spreader. Wells of 6 mm diameter were made in the centre of these agar plates by a sterile cork borer. The wells were filled with 200µl of the respective test extracts (Concentration 10mg/ml) and allowed to diffuse at room temperature for an hour. Next the plates were incubated at 28 ± 2°C for 72-96 hours to 2 weeks depending on the growth rate of the test pathogen. The antifungal activities of the extracts were determined by measuring the diameter of the inhibition zone around the well that was filled with the extracts. Clotrimazole (0.5 mg/ml) and DMSO (2%) were used as positive and negative controls respectively.

Determination of the Minimum Inhibitory Concentration (MIC) of all the extract was carried out by the visual broth macro dilution method (15) using Sabouraud Dextrose broth medium. The concentration of the extract which exhibited no visible growth of the fungus was considered as the MIC. The Minimum Fungicidal Concentration (MFC) was determined using the method of Rotimi et al. (16). The tube of extracts which showed no visible growth after 21 days of incubation when sub cultured on to extract free Sabouraud Dextrose Agar plated using as inoculum size of 0.5 ml is considered to be the MFC.

RESULTS AND DISCUSSION

Table 1 represents the several ethno-medicinal uses of various parts of S. indicum by the people of Assam, India. The results of the in vitro antifungal property of chloroform, methanol and aqueous extracts of S. indicum against Trichophyton and Microsporum species is presented in Fig 1. Both the chloroform and methanol extract of S. indicum was highly active against all the tested fungi where chloroform extract was found superior in activity than methanol. The highest inhibition zone for chloroform and methanol extract was produced against T. rubrum. The water extract of the plant was completely inactive against T. rubrum and T. mentagrophytes while weakly active against Microsporum gypseum, Trichophyton tonsurans, and Microsporum fulvum at the tested concentration. Clotrimazole (Positive control) was found to be active against all the tested fungi at 0.5 mg/ml while dimethyle sulfoxide DMSO (Negative control) was inactive at 2% concentration (Table 2). Minimum inhibitory concentrations and Minimum fungicidal concentration of the extracts against all the tested dermatophytes is given in Table 3.

In developing countries, phytomedicines are a major component of traditional system of healing, which have been an integral part of their history and culture. Besides widespread use of botanicals as medicinal products in developing countries, such products are becoming part of the integrative healthcare system of industrialized nations, known as complementary and alternative system of medicines. The existing costly therapy is not affordable well for the millions of inhabitants particularly of the developing world. Plants and their preparations are the cheap and easily available resource to them. Plants are great source of thousands of new useful phytochemical of great diversity, which have inhibitory effects on all types of microorganisms in vitro (6).

In the present study three solvents viz. Chloroform, methanol and water were used for the extraction process. The antifungal activity was significantly recorded in chloroform and methanol extracts. This might be due to the better solubility of the active components in the organic solvents (27). Further the antifungal activity was higher in chloroform than methanol extracts which indicates that the most active components against the dermatophytes tested, found in the leaves of S. indicum are more likely to be non polar. Based on the inhibition zones produced the activity of the extracts can be stated as Chloroform extract > Methanol extract > Water extract. In the present investigation the MIC of S. indicum was found ranging between 2.5-10 mg/ml for chloroform and methanol extracts and for the water extracts it was found greater than 10mg/ml against all the tested dermatophytes.

Antimicrobial activities of other species of Solanum have also been studied (28-31). The ethanolic and aqueous extracts of berries of Solanum nigrum showed high to very high antifungal activity against Aspergillus fumigatus, Aspergillus flavus, Aspergillus terreus and Aspergillus glaucus while moderate to low antifungal activity against a number of dermatophytic fungi (32). However the methanolic leaf extract of the same plant showed weak antifungal activity against Aspergillus flavus and A. niger. (33). The ethanolic seed extract of Solanum surattense revealed higher degree of antifungal potency against human pathogens like C. albicans, C. tropicalis, Aspergillus niger, Aspergillus fumigates and Aspergillus flavus eight fungal species (34). Other

Table 1: Various ethno-medicinal use of S. indicum in Assam, India.

<table>
<thead>
<tr>
<th>Parts of S. indicum</th>
<th>Mode of use against various ailments</th>
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<tr>
<td>Leaves</td>
<td>Leaf juice is taken with fresh ginger to stop vomiting. Fresh leaves paste along with sugar is used externally to relieve itching (17).</td>
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<tr>
<td>Fruits</td>
<td>Fried fruit is taken with meal for removal of intestinal worms, also considered medicinal for skin diseases (18, 19). Paste of fruit is also directly applied on the vagina for treatment of leucorrhoea (20). Decoction of dried fruits is used to cure high blood pressure (21), also used to cure Jaundice (22). Fruit is boiled with mustard oil and tied with a piece of cloth just below ear or cheek to cure toothache (23).</td>
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<tr>
<td>Roots</td>
<td>Roots are used to cure toothache, Asthma and in cough (24). Root paste mixed with little amount of Piper nigrum L. seed powder is prescribed three times a day in irregular menstruation. Root paste along with several other plants is used to cure Melina (25).</td>
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studies showed the antibacterial activity of five different plants belonging to Solanaceae viz., Solanum nigrum L., Solanum torvum Sw., Solanum trifoliatum L., Solanum surattense Burm. and Solanum melongena L. (35). However to the best of our knowledge, reports on the antidermatophytic activity of S. indicum Linn. from North East India is still lacking. The plant studied in this present investigation is largely available in North East India, widely used both as a food and medicinal plant. The long standing traditional use of this plant suggests that there is no undesirable effect to human. In this investigation potential antidermatophytic activity of the leaf extracts of the plant against human ringworm pathogens was observed. Further investigation of the plant may yield bioactive compounds with potential antifungal activity.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

REFERENCES


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