Paper title:
An ethnomedicinal study on medicinal plant of Mayong circle, Assam (India) used in leucorrhoea and assessment of antifungal activity of *Solanum torvum* on *Candida albicans*

Paper subtitle: Plants for curing leucorrhoea and assessment of antifungal activity of *Solanum torvum*

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families which are being used as herbal remedies for leucorrhoea. Among these plants *Solanum torvum* was selected for the screening of antifungal effects due to its popularity and the plant extract showed clear inhibitory activity against *Candida albicans* (causative factor of pathological leucorrhoea) in a concentration of 200 mg/ml in our laboratory condition. The results demonstrate that *Solanum torvum* possess antifungal property against *Candida albicans* and suggest that it may have therapeutic potentials in leucorrhoea and other similar ailments of fungal origin.

Keywords: Antifungal activity; *Candida albican*; Ethnobotanical; Herbal medicine; Mayong; *Solanum torvum*.

I. INTRODUCTION

Herbal medicine is the oldest form of healthcare known to mankind. India has rich medicinal plants of more than 7500 species. Of these, 4635 species are used commercially on a fairly large scale. Over 50% of all modern clinical drugs are of natural product origin and thus these products play important roles in drug development in the pharmaceutical sciences. The compounds found in the plants that are not
required for normal functioning of the body, but have a beneficial effect on health or play an active role in amelioration of diseases. Nowadays, the demand for more and more drugs from plant sources is continuously increasing. It is therefore essential to evaluate plants of medicinal value systematically for various ailments that are used in traditional medicine.

Leucorrhoea is a common term for the women passing through reproductive age. It refers to a whitish secretion from vagina which acts as a moisturizer and a protective coating over the vaginal wall. However, if vagina is infected by microorganisms the normal vaginal floras become more sticky, odorous and the whitish secretion become yellowish or greenish depending on the microbes that cause the infection. These symptoms collectively termed as pathological leucorrhoea. These usually present with other symptoms like vaginal or vulval pruritus, dyspariunia or pelvic pain. *Candida albicans*, an yeast like fungal strain is generally responsible for this pathological leucorrhoea. Moreover, sometimes *Gardnerella vaginilis* and *Tricomonas vaginilis*, a bacterial and a protozoal strain respectively are also infective.

*Candida* belongs to the phylum Ascomycota, class Saccharomycetes, order Saccharomycetales and family Saccharomycetaceae. *Candida albicans*, one of the major causative factors of pathological leucorrhoea, stay in the women but not always in infective mode. Due to different causes like illness, wearing of dirty undergarments, using of too much antibiotics could cause overgrowth of *Candida albicans* which cause infection and transformed to leucorrhoea. They generally live in the mouth, throat, intestine and genitourinary tract of most humans and is usually considered to be normal part of bowel flora (the organisms that coexists with us in our lower digestive tract known as fungi). *Candida* coexists in our bodies with many bacterial species in a competitive balance. In healthy condition, the immune system keeps Candida proliferation under control, but if immune response is weakened, Candida growth can precede unhindered. Other major culprits of Candida overgrowth are antibiotics and sulfa drugs that kill the good floras, which normally keep the Candida under control. A cost of illness study has indicated that the annual cost of treating hospitalized patients suffering from Candidiasis is to the tune of $US 250 million [1].

So far literature is concern, lots of herbal species have been traditionally used for curing leucorrhoea in different parts of the world. In North-eastern Region of India also, numerous ethno-medicinal studies have been done on the pharmaceutical use of different indigenous plant species [2, 3, 4, 5, 6]. The area taken for the present study was Mayong, which is regarded as treasure of ethnomedicinal plants and also very often as the land of magic powers specially the evil ones. This area was famous for its necromancy. Due to rich diversity of ethno-medicinal herbs and extraordinary traditional knowledge, witchdoctors who spent more than half of their life practicing to cure of people from different diseases in this remote area. But instead of their very rich traditionality, no study was made in this region to investigate such important issue in the light of modern science. Hence, there is an urgent need to screen medicinal plants for their promising biological activity. The present study was based on those medicinal plants which are being still in use by the people to cure leucorrhoea which is very prevalent in the women of this economically as well as educationally weak region of North-East India. Among the reported plants from the present survey, *Solanum torvum* was selected and the methanol and petroleum ether extracts of the plant were tested for antibiotic/antibacterial activities in laboratory condition.

II. EXPERIMENTAL

A. Study Area

The site of study taken for the present investigation was Mayong, is a part of Morigaon district in Assam (India). It is 58 km away from Guwahati city. This region lies between 26°12’ North to 26°15’ North latitude and 92°2’ East and 92°5’ East longitude. Mayong, though is a very remote and socio-economically backward area, has been able to draw special impetus in the traditional scenario of India, harbouring very rich diversity of ethno-medicinal plants and their use to cure various ailments by the native
people. The Mayong area has a unique characteristic with its diverse tribes and communities and with their own socio-cultural heritage. The indigenous people in this area are the Tiwas or the Lalungs who, in fact, form the socio-cultural base of this area. The Mayong circle, which constitute the present study area comprised of 179 revenue villages with a total population on 2,78,367 [7].

B. Survey Methodology

Plants for the present study were collected in the field, in markets, and at the homes of traditional healers. An extensive and intensive field study was undertaken using the methodology of Jain and Rao (1969) covering the whole area of Mayong administrative circle from January 2010 to December 2010 with an initial start of the investigation in the year 2009 for intrusion into the trust of the people of the tribes [8]. The informants (healers and market vendors) in this area were repeatedly interviewed during this time, using structured questionnaires. The questionnaires did not include any reference as to disease concepts, plant parts or preparation. In contrast, the participants were asked simple questions along the lines "What is this plant used for, which part, which quantity, how is it prepared, are any other plants added to the mixture." All questions were asked in the same order [9]. The villages were selected on the basis of the following consideration: (i) areas where the tribes constitute more than 50% of the population and are distributed in a contiguous belt; (ii) areas where medical facilities are scanty or rudimentary; (iii) areas where people are economically weak and (iv) areas where people approach first the village medicine man for treatment of most of the ailments. Meticulous efforts were made in this direction so as to gather as much as information on each and every plant species. From each village 10 respondents were selected at random to make the final sample size. The general procedures for collection of data regarding use of medicinal plants for control of fertility and for treatment of reproductive anomalies were followed. During field works, the informants were requested to accompany to the field and to show the plants they use in their treatment. During the study, prior information consent (PIC) of community has been taken from knowledge providers. Interaction sessions were held at Rajamayong, Burhamayong, Pobitora and the other remote areas of this region where local peoples were also invited to tap the information of medicinal plants commonly used by these traditional healers. Medicinal properties of plants were learned through informal interviews also. A number of group discussions were also conducted during the period of investigation. The information collected from the people were ascertained by consulting with different literatures and faculty members and scientists of the Department of Botany, Gauhati University. The herbarium specimens were prepared and numbered and were preserved by the authors for future reference [10].

C. Collection of plants and preparation of extracts

Fresh fruits of Solanum torvum were collected from the study area (Mayong circle) and washed them properly with tap water and shed-dried and made fine powder using a mechanical grinder. Then the 500g of fruit powder was soaked in 1 L of methanol and 1 L of petroleum ether separately. After 96 hr the solvents were filtered using filter paper (Whatman). The filtrate mixtures were then allowed to evaporation under separate vacuum evaporator to get the semisolid extracts. The final extracts were then stored in 4°C.

D. Culturing of Candida albicans

A pure culture of Candida albicans was purchased from Microbial Type Culture Center (MTCC) of Chandigarh, India (MTCC No. 3455). The freeze dried culture was revived in a Nutrient Broth (Merck Specialities Pvt. Ltd., Mumbai). Then a sub culture of Candida albicans was made using nutrient agar plate. From these sub culture a slant or nutrient agar was made and stored in 4°C for future use.

E. Determination of the effects

The antifungal activity of both Petroleum Ether and Methanolic extract of
Solanum torvum plant against Candida albicans was determined by using Microdilution Broth Assay [11, 12]. Varying concentration of the extracts 12.5, 25, 50, 100, 150, 200, 250 and 300 mg/ml were prepared and 1 ml of each concentration was added to each 9 ml of broth containing 0.1 ml of standardized test organism of Candida albicans. The tubes were then incubated at 37°C for 24 hr. Negative controls were equally set up by using saline water and test organisms only, while Ketoconazole (a synthetic antifungal drug) served as positive control agents (30 mg/ml). After incubation period is over, the minimum inhibitory concentration (MIC) was determined at the concentration in which the fungal growth was inhibited which was clearly visible at necked eyed observation [13].

III. RESULTS AND DISCUSSION

A. Ethnomedicinal plants used for female reproductive ailments in Mayong

In the present study 21 numbers of plant species from 15 different families were documented and identified as herbal remedies for Leucorrhoea in Mayong. Mostly used plant species were from the family Fabaceae, Euphorbiaceae, Moraceae, Poeceae, Solanaceae and Malvaceae. Other families are less important because they contributed only one plant species each to the pharmacopoeia.

The majority of herbal preparations for leucorrhoea were prepared from bark (19.04%), root (14.28%), leaf (14.28%), flower (9.52%), fruit (9.52%) and whole areal parts (9.52%) while other plant parts were used much less frequently. In almost 85% of the cases fresh plant material was used to prepare remedies, while in others dried powder was used. Over 70% of the remedies were applied orally while the remaining was applied topically. Many remedies were prepared as mixture of multiple ingredients by mixing with goat milk and honey. A complete overview of all plants encountered is given in Table 1.

B. Antifungal activity of the extract of Solanum torvum

The results of the Microdilution Broth Assay clearly showed that the plant extract had antifungal activity against Candida albicans (Table 2). Both methanol as well as petroleum ether extracts of the plant exhibited the results similar with the results as observed in the positive control agent (Ketoconazole). Interestingly, no inhibitory effects were observed in the tube containing saline water with test organism (negative control). The methanolic extract showed no inhibition up to the concentration of 100 mg/ml, while in 150 mg/ml concentration showed a very little inhibition (indicated by ‘+’). A clear visible inhibition of fungus was seen in the concentration of 200 mg/ml which was considered as MIC (indicated by ‘+++’). On the other hand, petroleum ether extract showed no inhibitory effects up to 50 mg/ml concentration, while in 100 mg/ml concentration showed very little inhibition. Similarly 150 mg/ml extract dose showed a higher response than 50 mg/ml concentration and finally a clearer visible inhibition i.e., MIC was observed in the concentration of 200 mg/ml (Table 2).

Among the people of Mayong there has lots of information about medicinal plants they used locally. The natives of this locality have been using these plants as a remedy of different diseases instead of modern allopathic treatments. This land of black magic needs our support to prove the believes of its natives scientifically, so that their traditional knowledge over plants and herbs can be used for betterment of the mankind. In the present study 21 numbers of plants and herbs are documented used as herbal remedies for Leucorrhoea by the people of Mayong region. Recently, in a similar kind of survey on ethnomedicinal plants, Bussmann and Glenn (2010) reported a total of 105 plant species belonging to 62 families are documented and identified as herbal remedies for reproductive problems in Northern Peru [9].

There are several reasons that people use plants for medication. This includes improvement of health after herbal treatment, low cost of the drugs, non availability of synthetic drugs particularly in the rural areas as Mayong, or where available were either fake or expired [14]. Very small information could be
found to prove the effectiveness of the plants with antifungal activity being used in Mayong. Ramya and his associates (2008) have reported antifungal activity of Terminalia arjuna in laboratory condition which is used as ethnomedicine for curing diseases including different reproductive anomalies [15]. Cassia sp. an anti leucorrhoeal plant shows good antifungal effect against Candida [16], while Emblica officinalis shows good MIC value against different fungus [17]. A group of scientists successfully tested the antifungal property of Allium stivum, Punica granatum, Cassia torra, Solanum spp. and Syzygium spp. being used as ethnomedicine for leucorrhoea [18]. The extracts of these plants also showed a good MIC value against different pathogenic fungus.

In our study, the result of the antimicrobial activity of Solanum torvum extract is particularly important considering this opportunistic organism (Candida albicans), which can be inhibited more efficiently by using this plant in a scientific manner. Considered the antifungal property of the extract it is clear that the plant contains some substances that are true antifungal agent. Isolation of the active constituents of this plant could make the field of pharmacology fruitful.

Our study documented 21 anti-leucorrhoeal ethnomedicinal plants used by the natives of the Mayong region of Northeastern part of India. Among these plants Solanum torvum was selected for the screening of antifungal effects due to its popularity among traditional healers of Mayong. The plant extract showed clear inhibitory effects in a concentration of 200 mg/ml in our laboratory condition. Plenty of research opportunities and necessities on these ethnomedicinal plants are still being remained a large gap at this area. One of the major problems is the lack of proper documentation on these treasures of traditional healing knowledge. As the people of Mayong are extremely talented, they just memorized the knowledge and transfer it to generation after generation. But now it needs a good scientific documentation. Proper scientific documentation and clinical analysis could save this kind of information and their pharmaceutical use for fighting with different health problems in a more efficient manner.

ACKNOWLEDGMENT

We gratefully acknowledge and thank to Prof. Gajen Sharma, Department of Botany, Gauhati University for his kind help in proper identification of the plants. We express our thanks and gratitude to Prof. Krishna Sharma, Department of Microbiology, Assam Agricultural University for his support in carrying out the bacteriological experiments and also to Microbial Type Culture Center, Chandigarh for supplying the bacterial strain. We also acknowledge all medical practitioners, traditional healers and the people of Mayong for their kind information, help and cooperation during the survey.

REFERENCES

Table 1. List of the medicinal plants used by local communities around the Mayong region of Northeast India for curing leucorrhoea showing different parts used, mode of preparation and administration.

<table>
<thead>
<tr>
<th>Scientific name</th>
<th>Local name</th>
<th>English name</th>
<th>Family</th>
<th>Parts used</th>
<th>Mode of preparation and administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Terminalia arjuna</td>
<td>Arjun</td>
<td>Arjuna</td>
<td>Combretaceae</td>
<td>Bark</td>
<td>25 ml water extract of bark mixed with 10 ml turmeric juice and drunk every morning in empty stomach for few days.</td>
</tr>
<tr>
<td>2. Sarca indica</td>
<td>Ashok</td>
<td>Ashoka</td>
<td>Fabaceae</td>
<td>Bark</td>
<td>Bark powder mixed with hot water and drunk twice daily for few days.</td>
</tr>
<tr>
<td>3. Emblica officinalis</td>
<td>Amlokhi</td>
<td>Indian Gooseberry</td>
<td>Euphorbiaceae</td>
<td>Seed</td>
<td>Fruit juice mixed with honey and drunk in morning in empty stomach for few days.</td>
</tr>
<tr>
<td>4. Carica papaya</td>
<td>Amita</td>
<td>Papaya</td>
<td>Caricaceae</td>
<td>Latex</td>
<td>The white latex directly use on ring worm for one month daily.</td>
</tr>
<tr>
<td>5. Bambusa balcooa Roxb.</td>
<td>Bhaluka bahn</td>
<td>Bamboo</td>
<td>Poaceae</td>
<td>Leaf</td>
<td>Leaf pasted with honey and drunk with water once daily for few months.</td>
</tr>
<tr>
<td>6. Acacia Arabica</td>
<td>Babul</td>
<td>Acacia tree</td>
<td>Fabaceae</td>
<td>Bark</td>
<td>Bark used in the form of decoction for a vaginal douche.</td>
</tr>
<tr>
<td>7. Ficus bangalences</td>
<td>Bargos</td>
<td>Banayan tree</td>
<td>Moraceae</td>
<td>Bark</td>
<td>Bark powder directly use on vagina.</td>
</tr>
<tr>
<td>8. Adhatoda vasica Nees</td>
<td>Bahoka</td>
<td>Malbarnut</td>
<td>Acanthaceae</td>
<td>Root</td>
<td>Root juice mixed with honey and drunk twice daily for few days.</td>
</tr>
<tr>
<td>9. Phyllanthas fraternus</td>
<td>Bhuiaml-okhi</td>
<td>Leaf flower</td>
<td>Euphorbiaceae</td>
<td>Areal part</td>
<td>Plant juice drunk once daily for few days.</td>
</tr>
<tr>
<td>11. Cassia tora</td>
<td>Dadidiga</td>
<td>Sickle pod</td>
<td>Caesalpiniaceae</td>
<td>Areal part</td>
<td>Fine paste of plant applies directly on vagina for few days.</td>
</tr>
<tr>
<td>12. Ficus hispida</td>
<td>Dimor</td>
<td>Creeping fig</td>
<td>Moraceae</td>
<td>Ripe fruit</td>
<td>Dried ripe fruit powder along with sugar taken once daily for few days.</td>
</tr>
<tr>
<td>13. Cynodon dactylon</td>
<td>Dubori</td>
<td>Dog grass</td>
<td>Poeceae</td>
<td>Whole plant</td>
<td>Fresh juice of whole plant along with honey drunk thrice daily for few days.</td>
</tr>
<tr>
<td>14. Ageratum conyzodes</td>
<td>Gendhalibhon</td>
<td>Goat weed</td>
<td>Asteraceae</td>
<td>Leaf</td>
<td>Leaf paste directly applies on vagina once in a day for one week.</td>
</tr>
<tr>
<td>15. Ferula foetida</td>
<td>Heeng</td>
<td>Asafoetida</td>
<td>Umbelliferae</td>
<td>Latex</td>
<td>Latex mixed with ghee, goat milk and honey and taken once daily for few days.</td>
</tr>
<tr>
<td>16. Solanum torvum</td>
<td>Hati vekuri</td>
<td>Turkey berry</td>
<td>Solanaceae</td>
<td>Raw fruit</td>
<td>Paste of fruit directly applies on the vagina for few days.</td>
</tr>
<tr>
<td>17. Hibiscus rosa sinensis</td>
<td>Jaba</td>
<td>Chinese hibiscus</td>
<td>Malvaceae</td>
<td>Flower</td>
<td>Paste of 3 - 4 fresh flowers along with goat milk and drunk once in a day for 15 days.</td>
</tr>
<tr>
<td>18. Syzygium cumini</td>
<td>Jamuk</td>
<td>Jambul</td>
<td>Myrtaceae</td>
<td>Root</td>
<td>10 ml of root juice along with milk and honey drunk after meal at night daily for one month.</td>
</tr>
<tr>
<td>19. Gossypium harboreum</td>
<td>Kopah</td>
<td>Cotton</td>
<td>Malvaceae</td>
<td>Root</td>
<td>Root juice along with honey drunk twice daily for 2-3 months.</td>
</tr>
<tr>
<td>20. Allium sativum</td>
<td>Nohoru</td>
<td>Garlic</td>
<td>Alliaceae</td>
<td>Bulb</td>
<td>One bulb covered by cotton cloth inserted on vagina for one hour daily for 18 - 20 days.</td>
</tr>
<tr>
<td>21. Solanum indicum</td>
<td>Tita vekuri</td>
<td>Indian Nightshade</td>
<td>Solaeanae</td>
<td>Raw fruit</td>
<td>Paste of fruit directly applies on the vagina for few days.</td>
</tr>
</tbody>
</table>
### Table 2. Minimal inhibitory concentrations and Antifungal activity of various extracts of *Solanum torvum* against *Candida albicans* (mg/ml)

<table>
<thead>
<tr>
<th>Plant extract (mg/ml)</th>
<th>12.5</th>
<th>25</th>
<th>50</th>
<th>100</th>
<th>150</th>
<th>200</th>
<th>250</th>
<th>300</th>
<th>Positive control (30mg/ml Ketoconazole)</th>
<th>Negative control (saline water)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanolic extract</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>NI</td>
</tr>
<tr>
<td>Petrolium ether extract</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>MI</td>
</tr>
</tbody>
</table>

NI, No inhibition; + very little inhibition; ++ little inhibition; +++ Clearly visible inhibition (considered as MIC); ++++ Complete inhibition.

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