Phytochemical screening and antifungal activities of three medicinal plants from Arunachal Pradesh, India

Yumge Yomgam, Rohita Sharma, Om Prakash Arya and Yumbi Yomgam

Abstract

Medicinal plants have been used as sources of traditional medicine in virtually all tribal cultures. Solanum torvum, Clerodendrum colebrookiannum, and Spinlanthes acmella: the three ethnomedical plants used in various ailments by tribal communities of Arunachal Pradesh were evaluated for their phytochemicals and antifungal activities against Aspergillus niger. Phytochemical analysis of selected plants revealed the presence of alkaloids, flavonoids, tannins, sterols, terpenoids, saponins, resins, carbohydrate in various plant parts. Ethyl acetate, Chloroform and Aqueous extracts of various parts of these plants were tested against Aspergillus niger by Agar well diffusion method. The obtained results showed that aqueous extract of roots of Clerodendrum colebrookiannum and ethyl acetate extract of leaves of Solanum torvum were the most active; while aqueous extract of Solanum torvum leaves did not show any activity against Aspergillus niger. Presence of various phytochemicals which are associated for various activities like, antimicrobial, antioxidant, anti-inflammatory, analgesic, anticancer, antiarrheal etc. supports the use of these plants in treatment of various ailments.

Keywords: Medicinal plant, Phytochemical, Antifungal activity, Arunachal Pradesh.

1. Introduction

The state of Arunachal Pradesh which covers a major portion of Eastern Himalaya is the largest state of north-eastern India with a geographical area of 83,743 sq. km. spreading over 19 districts with a population of 1.255 million [1, 2]. Arunachal Pradesh is home to about 26 major tribes and sub-tribes. Each district has its own composition of tribes with distinctive dialects, custom, traditional beliefs and cultural diversity. In Arunachal Pradesh there is 5,000 species of angiosperms, of which 500 species has been reported so far. These plants have been used by locals to cure various ailments and considered as potential source of economy to the state [3, 4]. Medicinal plants have been used as sources of traditional medicine in virtually all tribal cultures. Each tribe has its own traditional belief and histories of treating ailments through preparing herbal extracts from the surrounding and incantation of local hymns [5]. Over the centuries the local population of Arunachal Pradesh have gain an abundant knowledge on the utility of various plant species and forest commodity [6].

The tribal communities are mostly forest dweller who are engaged in agriculture, fishing, hunting, animal husbandry etc and increases the chance of skin infection due to various fungal and bacterial infections. Skin disease accounts for 34% of all occupational ailments [6, 7]. Over the past decade the incidence of fungal infection has risen substantially, which are higher in case of immunocompromised patients. Inappropriate and extensive use of immunosuppressant and antifungal drugs has increases the frequency of opportunistic fungal infection. Aspergillus spp., Candida albicans, Malassezia spp. and Fusarium Trichosporon are some of the most common opportunistic fungi causing life threatening invasive disease [8, 9]. A wide range of disorders including allergy, colonization and invasive disease is caused by Aspergillus species in immunocompetent and immunocompromised individual. It is estimated that the mortality due to fungal infection is around 1,350,000 patients per year across the world [10]. Antimicrobial activity is the ability of a substance to inhibit growth or kill microorganism. Medicinal plants have antimicrobial activity, as they produce wide range of bioactive molecules which kills or inhibit the growth of microorganism such as bacteria, fungi, or protozoa [11, 12]. A vast majority of plants have not been adequately evaluated, even though thousands of plant varieties from different parts of India have been tested for antimicrobial activities [13].
Three ethnomedicinal plants viz *Spilanthes acmella*, *Clerodendrum colebrookianum* and *Solanum torvum* (Fig.1) from Arunachal Pradesh, traditionally used in various ailments (Table 1) were undertaken to investigate the screening of secondary metabolites and antifungal activities against *Aspergillus niger*.

![Fig 1: Ethnomedicinal plants under study (a) Clerodendrum colebrookianum, (b) Spilanthes acmella (c) Solanum torvum from Arunachal Pradesh](image)

**Table 1:** The ethnobotanical information of selected medicinal plants species

<table>
<thead>
<tr>
<th>Species Name</th>
<th>Family</th>
<th>Local name</th>
<th>Plant parts used</th>
<th>Traditional Uses</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Clerodendrum colebrookianum</em></td>
<td>Lamiaceae</td>
<td>Oen-tapen (Galo), ongin (Nyishi)</td>
<td>Tender leaves,</td>
<td>Blood pressure [13]; stomach trouble, malarial and bronchitis treatment [14]</td>
</tr>
<tr>
<td><em>Solanum torvum</em></td>
<td>Solanaceae</td>
<td>Baka (Galo), Byako (Adi), Byakta (Nyishi), Sathi Byako (Apatani)</td>
<td>Fruits, seed and roots</td>
<td>Infection in gums and toothache [15]; stomachache, high blood pressure [15] fever, cough, toothache [16]; malaria and stomach pain [17]</td>
</tr>
<tr>
<td><em>Spilanthes acmella</em></td>
<td>Asteraceae</td>
<td>Marsha(Galo), Marsang (Adi)</td>
<td>Leaves and young twigs</td>
<td>Stop bleeding, skin infections and gastric, fish poison [12]</td>
</tr>
</tbody>
</table>

* Words in parenthesis in the column 3 is the name of ethnic communities of Arunachal Pradesh

2. Materials & Methods

2.1. Plant collection and identification

The leaves, stem, bark and roots of *Spilanthes acmella*, *Clerodendrum colebrookianum* and *Solanum torvum* were collected from Itanagar and Diomukh area of Arunachal Pradesh, India. The plant was identified at BSI, SFRI and State Medicinal Plant Board (SMPB) Arunachal Pradesh.

2.2 Sample preparation

The plant material was sorted and cleaned in running water and the plant parts were air-dried in the shade at room temperature till constant weight was attained. The dried parts of plants were chopped into tiny pieces and then grinded with mortar pestle into powder form, sieved and packaged into air tight containers for further experimental work.

2.3 Plant Extracts Preparation

The extraction process was carried out with three different solvents viz. ethyl acetate, chloroform, and water. Plant samples were extracted with various solvents using Rotary shaker and Soxhlet extraction method as shown in Table 2. For extraction of plant material in water, powdered plant parts were kept in a rotary shaker for 3 days; while with other solvents extraction was performed using Soxhlet extraction method. All the extracts were first filtered through Whatman no. 1 filter paper to remove the debris. All the extracts were evaporated using rotary vacuum evaporator and percentage yield was thus calculated. The dried plant extracts were then solubilised in 2 ml of solvents and 2ml of 2% DMSO in normal saline was added to it and kept in centrifuge tubes. The centrifuge tubes levelled, sealed and kept at 4°C in the refrigerator for further screening of phytochemical and antifungal activity of these plant extract against *Aspergillus niger*.

<table>
<thead>
<tr>
<th>Species name</th>
<th>Plant parts</th>
<th>Solvent</th>
<th>Extraction method</th>
<th>Initial amount</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Clerodendrum colebrookianum</em></td>
<td>Leaves</td>
<td>Ethyl acetate</td>
<td>Soxhlet and rotary evaporator</td>
<td>4g</td>
</tr>
<tr>
<td><em>Clerodendrum colebrookianum</em></td>
<td>Leaves</td>
<td>Chloroform</td>
<td>Soxhlet and rotary evaporator</td>
<td>5g</td>
</tr>
<tr>
<td><em>Clerodendrum colebrookianum</em></td>
<td>Roots</td>
<td>Water</td>
<td>Rotary shaker and rotary evaporator</td>
<td>5g</td>
</tr>
<tr>
<td><em>Solanum torvum</em></td>
<td>Leaves</td>
<td>Ethyl acetate</td>
<td>Soxhlet and rotary evaporator</td>
<td>4g</td>
</tr>
<tr>
<td><em>Solanum torvum</em></td>
<td>Leaves</td>
<td>Water</td>
<td>Rotary shaker and rotary evaporator</td>
<td>5g</td>
</tr>
<tr>
<td><em>Solanum torvum</em></td>
<td>Stem</td>
<td>Ethyl acetate + Water</td>
<td>Rotary shaker and rotary evaporator</td>
<td>4g</td>
</tr>
<tr>
<td><em>Spilanthes acmella</em></td>
<td>Whole plants</td>
<td>Ethyl acetate</td>
<td>Soxhlet and rotary evaporator</td>
<td>3g</td>
</tr>
<tr>
<td><em>Spilanthes acmella</em></td>
<td>Whole plants</td>
<td>Water</td>
<td>Rotary shaker and rotary evaporator</td>
<td>3g</td>
</tr>
</tbody>
</table>

* ~ 279 ~
2.4 Phytochemical analysis

Chemical tests for the screening of different phytoconstituents in *Spilanthes acmella*, *Clerodendrum colebrockianum* and *Solanum torvum* were performed following the standard methods [18-20]

**Test for alkaloids**

1 ml of extract mixed with some drops of dilute HCl and then filtered out. From this 1 ml of extract is taken and 1 ml of Meyers reagent was added. Creamy white precipitation indicates the presence of alkaloids.

a) **Test for saponins**

In 1 ml of extract, 2 ml of distilled water added and shake continuously for 10 minutes. Formation of foam on the mixer surface after for 20 minutes confirms the existence of saponins.

b) **Test for flavonoid**

i. **Alkaline reagent test**

Few drops of NaOH solution blended with 2 ml of extract solution. Development of intense yellow colour confirms the presence of flavonoid.

ii. **Lead acetate test**

2 ml of extract put together with few drops of 10% lead acetate, presence of yellow colour confines the existence of flavonoids

c) **Test for sterols**

i. **Sulphuric acid test**

Extract from plant mixed with sulphuric acid along with ethanol gives rise to blue green colouration suggests the presence of sterol

d) **Test for tannins**

1 ml of extract combined 2 ml of 5% ferric chloride. Dark brownish green colour shows the presence of both phenols as well as tannins.

e) **Test for Terpenoids**

1 ml of plant extract and 2 ml of chloroform blended thoroughly, and then 3 ml of sulphuric acid added to it leads to formation of layer. Reddish brown colouration indicates the presence of terpenoids.

f) **Test for carbohydrate**

2 mg of extract added to 1 ml of distilled water. The mixture is then filtered to remove the debris.

i. **Benedict’s test**

0.5 ml of filtrate from the above mixture was combined with 0.5 ml of Benedict’s reagent. The solution was then heated in water bath for 2 minutes. Characteristic precipitate formation determines the occurrences of carbohydrate.

g) **Test for resins**

i. **Acetone water test**

Small amount of water and 1 ml of acetone was added to extract. The mixer is shaken well. Appearance of turbidity in the solution indicates the presence of resins in the extract.

2.5 Test microorganism and maintenance

In the present investigation, *Aspergillus niger* (MTCC 501) was collected from Lovely Professional University (LPU) laboratory, Punjab.

2.6 Antifungal activity

i. **Fungal culture**

In the present investigation, *A. niger* was cultured in potato dextrose agar by spread plate method in three plates and streak plate method in other two plates and then incubated at 28°C for 7 days. On the second day, inoculums appeared as creamy coloured colonies in the petri dishes. On the seventh day mature blackish dark green numerous spore colonies as dots was observed. Sub culturing was done by both spread plate and streak plate method once culture reached confluence. Inoculation and harvesting was prepared as described by Yazdani et al. [21] with few modifications. On the 7th day conidia from matured colonies was harvested by flooding (0.5% v/v) of 5 ml tween 20 aseptically inside the laminar flow hood. Then the solution is filtered through Whatman No.1 filter paper. To check the viability and verify the absence of contamination two plates were inoculated from the above prepared solution and incubated for seven days. On seventh day mature dark spore conidia without any contamination was obtained. The suspended conidia spores kept at 4°C in the refrigerator for further use.

ii. **Media & plate preparation**

Every glassware and plastic ware along with nutrient agar was sterilized in autoclave and hot air oven. The media used for antifungal activity were Potato dextrose agars. 20 ml of pre autoclaved Potato dextrose agar (MHA) was poured into pre sterilized Petri plates. These Petri plates were led to solidify at room temperature.

iii. **Agar well diffusion method**

The agar well diffusion was chosen as it is globally accepted testing method for microbial susceptibility [23]. Antifungal activities of all the plant extract were tested using agar well diffusion method according to Varaprasad et al. [24] with slight modification. After solidifying of the media, 50 µl of conidia (*A. niger*) suspended in 0.5% v/v tween 20 was taken in a 100 µl pipette then spread thoroughly using a glass rod. Then the wells were punched using autoclaved cork borer. Plant extract were placed in the well carefully using a micro pipette. The plates were then sealed with parafilm and subjected to incubation in upright position at 28°C for initiation of fungal growth. To ensure the sterility all the work was carried out in laminar flow hood. After incubation, the diameter of zone of clearance of inhibition produced around each well were measured in cm and compared with standard antifungal drugs.

3. Results

3.1. Extract yield

The yield of different parts of the plants under study is given below in the table (Table 3). Ethyl acetate gives a higher yield for the leaves of *Clerodendrum colebrockianum* and *Solanum torvum* amongst other solvents. Water have different yield for different plants which also varies according to their plant parts. Whole plant of *Spilanthes acmella* have highest yield (22.4%) followed by Stem of *Solanum torvum* (17%) and whole plant of *Spilanthes acmella* (14.7%) amongst all the plant parts.
were absent from the carbohydrate and alkaloids, however flavonoids and sterols the presence of tannin, resins, saponins, terpenoids, vital role in defence system of plants and also use in blood, diuretic and chloeretic functions. Experiments on terpenoids play a nutritive value. Terpenoids play a characteristics are found in saponins. Flavonoids are bestowed membrane. Antioxidant, anticancer and antimutagenic healing of wounds, antitumor and inflamed mucous terpenoids all other tested secondary metabolites was present in the Clerodendrum colebrookianum. Spilanthes oleracea reported the absence of sterols, saponins and flavonoids, whereas alkaloids, tannins, resins, carbohydrate and terpenoids were present. The presence or absence of particular phytochemical also depends on the nature/abilities of the solvent used.

3.2. Phytochemical analysis
Phytochemical analysis of selected plants revealed presence of various phytochemicals Table 4. Solanum torvum showed the presence of tannin, resins saponins, terpenoids, carbohydrate and alkaloids, however flavonoids and sterols were absent from the Solanum torvum extracts. Except terpenoids all other tested secondary metabolites was present in the Clerodendrum colebrookianum. Spilanthes oleracea reported the absence of sterols, saponins and flavonoids, whereas alkaloids, tannins, resins, carbohydrate and terpenoids were present. The presence or absence of particular phytochemical also depends on the nature/abilities of the solvent used.

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Plant Parts</th>
<th>Solvent</th>
<th>Mode of extraction</th>
<th>Final amount</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clerodendrum colebrookianum</td>
<td>Leaves</td>
<td>Ethyl acetate</td>
<td>Soxhlet and rotary evaporator</td>
<td>0.55g</td>
<td>13.75%</td>
</tr>
<tr>
<td>Clerodendrum colebrookianum</td>
<td>Leaves</td>
<td>Chloroform</td>
<td>Soxhlet and rotary evaporator</td>
<td>0.47g</td>
<td>9.4%</td>
</tr>
<tr>
<td>Clerodendrum colebrookianum</td>
<td>Roots</td>
<td>Water</td>
<td>Rotary shaker and rotary evaporator</td>
<td>0.60g</td>
<td>12%</td>
</tr>
<tr>
<td>Solanum torvum</td>
<td>Leaves</td>
<td>Ethyl acetate</td>
<td>Soxhlet and rotary evaporator</td>
<td>0.55g</td>
<td>13.75%</td>
</tr>
<tr>
<td>Solanum torvum</td>
<td>Leaves</td>
<td>Water</td>
<td>Rotary shaker and rotary evaporator</td>
<td>0.50g</td>
<td>10%</td>
</tr>
<tr>
<td>Solanum torvum</td>
<td>Stem</td>
<td>Ethyl acetate + Water</td>
<td>Rotary shaker and rotary evaporator</td>
<td>0.68g</td>
<td>17%</td>
</tr>
<tr>
<td>Spilanthes acmella</td>
<td>Whole plants</td>
<td>Ethyl acetate</td>
<td>Soxhlet and rotary evaporator</td>
<td>0.44g</td>
<td>14.7%</td>
</tr>
<tr>
<td>Spilanthes acmella</td>
<td>Whole plants</td>
<td>Water</td>
<td>Rotary shaker and rotary evaporator</td>
<td>0.67g</td>
<td>22.4%</td>
</tr>
</tbody>
</table>

3.3. Antifungal activity
The inhibitory effect of various plant extract on mycelium growth of Aspergillus niger varies as shown in Table 5. The highest inhibitory zone (2.475 cm) was shown by Clerodendrum colebrookianum in water. Solanum torvum leaf extract in ethyl acetate and Spilanthes acmella in ethyl acetate exerted an inhibition of 1.50 cm and 1.575 cm respectively. Clerodendrum colebrookianum leaf extract in chloroform also showed significant antifungal activity of 1 cm i.e. diameter of zone of inhibition. Others showed medium activities against the mycelium growth that is between 0.6 cm to 0.75 cm. Solanum torvum extracted in water did not show any antifungal activity against Aspergillus niger. Three controls were used for this study: - Itraconazole, voriconazole and amphotericin B. Itraconazole showed the highest inhibitory activity of 2.38 cm against the Aspergillus niger. A diameter of 2.3 cm was given by voriconazole around the wells, while amphotericin B showed 1.70 cm of inhibition zone. The Diameter of zone of inhibition by different plant extract exhibited by different plant extract also depends on solvents.

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Spilanthes acmella</th>
<th>Solanum torvum</th>
<th>Clerodendrum colebrookianum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvents</td>
<td>Phytochemicals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Flavanoid</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sterols</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Tannin</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Tarpenoid</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Resins</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

3.4. Discussion
Alkaloids are associated with properties such as antibacterial, analgesic and antipsamodic. Tannins are found to be astringent in nature, antimicrobial, anticancer, hasten the healing of wounds, antitumor and inflamed mucous membrane. Antioxidant, anticancer and antinfectious genetic characteristics are found in saponins. Flavonoids are bestowed with activities such as antioxidant, cytotstatic, antiallergic, analgesic, anti-inflammatory, antiinflammatory and antimicrobial. Whereas, experiments on phenols showed various activities like antioxidant, reduce blood pressure, decrease viscosity of blood, diuretic and chloeretic functions. Experiments on sterols confirm anti-inflammatory and analgesic activities. Carbohydrate accounts for nutritive value. Terpenoids play a vital role in defence system of plants and also use in manufacturing sustainable pesticide and abiotic stress protection [25, 26]. Previously, researcher have reported the presence of alkaloids, flavonoids, saponins, steroids, tannins, terpenoids, glycosides and phenolic compounds in the leaves and seeds of Solanum torvum [27, 28]. Researchers have reported presence of alkaloids, carbohydrates, tannins, steroids, carotenoids, glycosides, flavonoids, terpenoids, phlobatannins, fats and fixed oils, sesquiterpenes and amino acids in different extracts of Spilanthes acmella plant parts [29, 30 31]. Experiments have reported the presence of glycosides, terpenoids, tannins, saponins, steroids, phenols, flavonoids, alkaloids, carotenoids, carbohydrate and proteins in Clerodendrum colebrookianum [26, 32]. This study was the first of its kind from Arunachal Pradesh for these three plants, results may be vary from earlier researchers’ who worked on
the same plants, because of different habitat of these plants and use of different solvents.

5. Conclusion
The present study showed that different plant parts show significant antifungal activities against *Aspergillus niger*. The different plant parts also revealed presence of different phytochemicals which are associated for various activities like, antifungal, antioxidant, anti-inflammatory, analgesic, anitcancer, antidiarrheal etc. supports the use of these plants in treatment of various ailments by ethnic communities of Arunachal Pradesh. Further studies should be performed for the isolation and characterization of bioactive compounds so that selected plant species may be use as potent drugs.

6. Acknowledgements
Authors are thankful to Head of the Department, School of Biotechnology & Biosciences, Lovely Professional University, Punjab for providing the support and facilities. Authors are also thankful to the local communities of the Doimukh and Itanagar for their support and help in collection of plant material.

7. References
26. Thangi J, Ashwini HA, Singh KG. Qualitative and quantitative phytochemical analysis of ethnomedicinal folklore plant- *Clerodendrum colebrookianum*. Journal of


