Antifungal metabolites of *Bulbophyllum nilegiriensis* Wt. from western ghats of karnataka, India

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**ABSTRACT**

*Bulbophyllum nilegiriensis* Wt., an orchid of ethno medical significance, from Western Ghats of Karnataka was studied for antifungal and phytochemical properties. The phytochemical constituent analysis showed different phytoconstituents in different extracts. Petroleum ether, alcohol and chloroform extract showed alkaloid, steroid and terpinoids. Flavonoids were present in chloroform and alcohol extracts. Tannins were observed in petroleum ether and chloroform extract. Tannins and Flavonoids were present in chloroform extract and absent in petroleum ether extracts. In vitro antifungal efficacy of all the four extracts was tested by well in agar method. Both yeasts and filamentous fungi were inhibited with zone of inhibition upto 26 mm. Candida albicans was significantly inhibited in all four tested extracts followed by Candida krusei and Fusarium oxysporum. Saccharomyces cerevisiae was unaffected by all the extracts. A zone of 20mm was observed for Cryptococcus neoformans only in water extracts. The purity of the chloroform extract was assessed by Column Chromatography, HPLC and UV absorption studies with a single peak and absorption of 257nm.

**KEYWORDS**

Orchid; Anti fungal; *Bulbophyllum nilegiriensis* Wt well in agar; Western Ghats,

**INTRODUCTION**

India possesses rich floristic wealth and diversified genetic recourses of medicinal plants. Orchidaceae members constitute the vital herbal medicines of India and China. Orchidaceae is known for its beautiful flowers. *Bulbophyllum nilegiriensis* Wt. (Orchidaceae) is a fleshy native Indian epiphyte reputed to cure stomach ache in traditional Indian medicine. It is a robust epiphytic or lithophytic herb with slender, creeping rhizome. Pseudobulb is conical-ovoid, yellowish green, fleshy with terminal leaf. (Rao, 1998).

The genus *Bulbophyllum*, belonging to the Orchidaceae consists of about 1000 species found in Asia, America and Africa. It contains mainly phenanthrenes and bibenzyls. *B. odoratissimum* (J.E. Smith) Lind is widely distributed in China, Nepal, Bhutan, India, Burma, Thailand, Laos and Vietnam it is used in folk medicine to treat tuberculosis, chronic inflammation and investigation on the compounds from *B. odoratissimum* have revealed the presence of phenanthrene, lignin, flavonoids, bibenzyls, phenolic glycosides, aldehydes and acids (Chen et al, 2007). *Bulbophyllum nilegiriensis* Wt is distributed in
Shimoga, South Canara and Kodagu districts of Karnataka and also in northern districts of Tamilnadu and Kerala.

The present study concentrated on the antifungal metabolites of *Bulbophyllum nilegiriensis* Wt, and its pharmacological constituents as little work has been done on these parameters. Plant products are also a source of very potent and powerful drugs that have stood the test of times and modern chemistry has not been able to replace most of them. Plant based drugs constitute a major share of all the officially recognized systems of health in India, China viz., Ayurveda, yoga, unani, siddha, homeopathy and naturopathy, except allopathy[4]. In many countries including India and China, thousands of tribal communities still use folk here medicinal plants for the cure of various diseases. It has been confirmed by WHO, that traditional medicines, based largely on different species of plants and animals serve the health needs of large number of people; especially for millions of people in the vast rural areas of developing countries[2]. Serving to the peoples all over the world, Phytochemicals of medicinal plants serve a lead compounds is drug discovery and design. Medicinal plants are rich source of novel drugs that forms the ingredients is traditional systems of medicine, modern medicines, pharmaceutical intermediates, bioactive principles and lead compounds in synthetic drugs[8]. The orchids are one of the largest groups of Angiosperms belonging to the family Orchidaceae. They occur in diverse habitat conditions of our country. There are about 20,000 species grouped about 800 genera distributed all over the world except polar region and dry deserts. India is one of the richest orchid habitats with about 2500 species in 167 genera represented in six sub families, 17 tribes and 30 sub tribes. Various phytogeographical situation accompanied by the variation in elevation, temperature, rain fall and humidity have contributed to the rich diversity of orchids in India. The orchids have been described as the ‘Royal family’ and contain their own specialized characters such as intriguing flowers, exciting colours, varied shapes and great diversity of growth habitats would certainly agree that they are rather special plants[6].

**MATERIALS AND METHODS**

**Preparation and extraction of plant material**

The whole plant, except roots, was collected from forest trees of Shimoga and South Canara districts of Karnataka in March–April 2008. The plant was identified by at the Department of Botany, Sahyadri Science College, Shimoga by referring literature. A fresh specimen weighing 750 g was surface sterilized in 70% ethanol with two drops of surfactant, tween 20 for 1 minute and washed with sterile distilled water for 2-3 times. The air dried specimen was then cut into 5 mm pieces aseptically and shade dried aseptically for 30 days in laboratory. The dried plant material was powdered with help of blender.

250 g powder was subjected to soxhlet extraction using Petroleum Ether, Chloroform, Ethyl Alcohol and Water as solvent, the crude extract was obtained by removing the solvent using flash rotary evaporator, similarly water extracts were prepared by hot water extraction. The dried crude extracts were stored in dry air tight glass vials.

**Preliminary phytochemicals screening**

Phytochemical tests were carried out for the solvent extracts. Petroleum ether, chloro form. Ethanol and aqueous extracts were subjected to routine qualitative chemical analysis to identify the nature of phytochemical constituents. Standard procedures were followed to identify the described by sofowara (1993), Harborne (1973) and Brindha *et al.*, (1982)[3].

1. **Test for Terpenoids** 5 ml of the extract was mixed with 2 ml of chloroform and concentrated sulphuric acid to form a layer. A reddish brown coloration in the interface showed the presence of terpenoids.

2. **Test for flavonoids** 5 ml of the diluted ammoniasolution a portion of the aqueous extract was added, followed by addition of concentrated sulphuric acid. Appearance of yellow coloration indicates the presence of flavonoids.

3. **Test for Reducing Sugars** 2 ml of test solution was added with a 2 ml Fehling’s reagent. A (or) B. and 2 ml of water formation of reddish orange colour indicates the presence of reducing sugar.

4. **Test for Phenols** 2 ml of test solution in alcohol was added with one drop of neutral ferric chloride 5% solution. Formation of intense blue colour indicates the presence of phenols.
**Full Paper**

5. **Test for Catechins** 2 ml of test solution in alcohol was added with Ehrich reagent and a few drops of concentrated HCl formation of pink colour indicate the presence of catechins.

6. **Test for saponins** 2 ml of test solution was added with H2O and shacked formation of foamy eather indicates the presence of saponins.

7. **Test for Tannins** ml of test solution was added with H2O and concentrated sulphuric acid. Formation of while precipitate indicates the presence of tannins.

8. **Test for Anthroquinone** 2 ml of test solution was added with magnesium acetate. Formation of pink colour indicates the presence of Anthroquinones.

9. **Test for Quinine** 1 ml of extract, 1 ml of concentrated sulphuric acid was added and was allowed to and for some time to develop colour. Development of red colour shows the presence of quinine.

10. **Test for Coumarin** 1 ml of extract, 1 ml of 10% NaOH was added and was allowed to stand for some time development of yellow colour shows the presence of coumarin.

11. **Test for Glycosides** 1 ml of the extract, 1 ml of alpha napthol was added to which chloroform was added along the sides and it was looked for the development of colour and the result was recorded. Development of Violet colour indicates the presence of glycosides.

12. **Test for Carbohydrate** Aqueous or alcoholic solution of substance was added with 10% aqueous solution of alpha Naphthol shaken and added concentrates sulphuric acid along the side of the tube. Violet ring at the Junction of two liquids shows presence of Carbohydrates.

13. **Test for Sugar** 0.5 ml of the Filtrate. 0.5 ml Benedict’s reagent was added. The mixture was heated on boiling water both for 2 minutes. A characterises of red coloured precipitate shows presence of sugar.

**In-vitro Antifungal Assay**

**Sample preparation**

10% of extracts was prepared by dissolving all four extracts separately in DMSO, which was used for the antibacterial activity.

**Antifungal Activity**

The extracts were tested for the antifungal activity. The fungal strains employed in the biological assays were

**Test Organisms**


**Determination of antifungal activity**

Agar well diffusion method was followed but nutrient medium used was Sabouraud Dextrose Agar (SDA) and Potato Dextrose Agar (PDA). The Agar plates were swabbed (Sterile Cotton Swabs) with 72 h old broth culture of the respective fungi. A sterile cork borer was used to place four wells, each measuring 8 mm in diameter, in each of the plates, about 0.1 ml each of 50, 25, 12.5 and 6.25 mg/ml of concentrated test samples with DMSO was added into the wells using sterilized dropping micro pipettes and allowed for diffusion at room temperature for 2h. The plates were incubated at 28°C for 48-72 h. Diameter of the inhibition zones was recorded. The experiment was repeated thrice and the average values were calculated for antifungal activity. Similarly standard antibiotics antifungal assay was done.

**RESULTS**

Preliminary phytochemical analysis of various solvent extracts such as ethanol and aqueous extracts are recorded. Petroleum ether extract contain alkaloid and steroid. Chloroform extract contain alkaloid, steroid, flavonoid and triterpinoid. Alkaloid, steroid, tannin and triterpinoid were present in ethanol extract. Where as aqueous extract contain only alkaloids. Except chloroform extract flavonoid was absent in rest of extracts.

These solvent extracts are subjected to antifungal assay, the inhibition zone is tabulated (TABLE 1), the value ranging from 10-26mm. Aqueous extract inhibits all fungal organism tested except *Saccharomyces*
cerevisiae, which showed resistance towards all four extract tested. Cryptococcus neoformens was inhibited only by aqueous extract, other three extracts showed no inhibition zone. Petroleum ether extract and chloroform extract showed similar inhibition efficacy (11-24mm), except Saccharomyces cerevisiae and Cryptococcus neoformens. Ethanol extract has showed high degree of inhibition except Saccharomyces cerevisiae, Cryptococcus neoformens and Aspergillus pumilis. Inhibition zone ranging from 14-26mm. Candida species were inhibited by all four extracts to maximum extent 23-26mm. The ethanol extract showed 26mm inhibition towards Candida albicans. The filamentous fungi also showed considerable degree of inhibition 11-21mm. Curvularia, Trichophyton cutanium, Aspergillus niger were inhibited by all four extract tested. Aspergillus pumilis did not show inhibition towards ethanol extract, Fusarium oxysporium was inhibited by all four extract tested, chloroform extract showed maximum inhibition of 21mm. The standard antifungal antibiotics 10mg/ml concentration moderately inhibited the test isolates. When compared with all the four solvent extracts (TABLE 2).

<table>
<thead>
<tr>
<th>Sl.No.</th>
<th>Test Organisms</th>
<th>Zone of Inhibition in mm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pet.Ether</td>
</tr>
<tr>
<td></td>
<td>Saccharomyces cerevisiae</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>Curvularia Sp</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Trichophyton cutanium</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Aspergillus niger</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Aspergillus pumilus</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Fusarium oxysporium</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Candida lipolytica</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Candida albicans</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>Candida krusei</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Cryptococcus neoformens</td>
<td>--</td>
</tr>
</tbody>
</table>

TABLE 1 : Antifungal activity of Bulbophyllum nilegiriensis Wt.

<table>
<thead>
<tr>
<th>Test organism</th>
<th>Griseofulvin</th>
<th>Fluconazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.krusei</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>C.albicans</td>
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<td>0</td>
</tr>
<tr>
<td>S.cerevisiae</td>
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<td>14</td>
</tr>
<tr>
<td>C.lipolytica</td>
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<td>25</td>
</tr>
<tr>
<td>C.neoformens</td>
<td>0</td>
<td>28</td>
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<tr>
<td>A.niger</td>
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<td>0</td>
</tr>
<tr>
<td>A.pumilis</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>Fusarium</td>
<td>15</td>
<td>12</td>
</tr>
<tr>
<td>Curvularia</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>T.cutaneum</td>
<td>23</td>
<td>0</td>
</tr>
</tbody>
</table>

TABLE 2 : Antifungal Activity of Standard Antibiotics

DISCUSSION

Kalaiarasan A et al (2012) enumerated antimicrobial activity of Bulbophyllum kaitense and performed antibacterial and antifungal assay. The present investigation showed the promising results of Bulbophyllum neilghirensis Wt. antifungal property. The extract showed inhibitory effect on human pathogenic fungi like Candida albicans, Trichophyton cutanium (11-26mm). These extracts are also effective against phytopathogenic fungi like Curvularia Sp and Fusarium oxysporium (14-21mm). Similar study was conducted on Acanthephippium bicolor by Shanmugavalli et al (2009)77. Since plant grow in abundance and they are widely distributed. This plant can be used in organic fungicide preparation. The present work emphasized on antifungal potential of Bulbophyllum neilgherrense Wt in solvent extracts, where the pseudobulbs were collected in summer and winter and assessed for their antifungal potential. Antifungal activity of pseudobulb remained same, even though collected in different seasons, confirmed the same results.

Kala S and Senthilkumar.S(2010)8 studied antibacterial activity of Acanthephippium bicolor, Lindley,
tested 35 bacteria and found out that, seasonal change does not influence on the antimicrobial property of plant.  

Priya K and Krishnaveni C (2005) tested different solvent extracts of *Bulbophyllum neilgherrense* against bacterial isolates, where it had shown moderate activity, while the present investigation clearly showed presence of best antifungal principles, from same species of orchid. Since the inhibited fungi includes human pathogenic yeasts, dermatophytes and plant pathogens, the extraction of active fraction from both ethanol and aqueous medium may provide a novel antifungal agent from this orchid. This can be exploited in scientific medicine.

**REFERENCES**


