Phytochemical and TLC studies on stem and leaves of the orchid *Dendrobium panduratum* subsp. *villosum* Gopalan & A. N. Henry

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The objective of the present study is to report the phytochemistry of the orchid, *Dendrobium panduratum* subsp. *villosum* Gopalan & A. N. Henry. Stem and leaves were dried and powdered using the homogenizer. Powdered materials were extracted with distilled water, benzene, ethanol, acetone and petroleum ether separately. Preliminary phytochemical screening of secondary metabolites was carried out by using Brindha et al. method. Phytochemical studies confirmed the presence of steroids, triterpenoids, alkaloids, tannins, phenols and flavonoids. Physico-chemical and fluorescence analysis were carried out using various organic and inorganic solvents. The colour of the extracts was observed both under ordinary and UV light. TLC studies were carried out using Toluene: Ethyl acetate and Chloroform: Methanol as mobile phase for the presence of phenolics and steroids. These studies provide information in respect of their identification, chemical constituents and physicochemical characters which may be used for standardization of herbal drugs of the present era and enrichment of Ayurvedic pharmacopoeia.

Keywords: Phytochemistry, *Dendrobium panduratum* subsp. *villosum*, Pharmacognosy, Fluorescence, Pharmacopoeia, Orchidaceae

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Introduction

Plants have been used for the treatment of disease all over the world before the introduction of modern clinical drugs. Natural phytochemicals are known to contain substance that can be used for therapeutic purposes or as precursor for the synthesis of novel useful drugs. Use of plant as a source of medicine has been inherited and is an important component of the health care system. In India nearly 20 thousands species of plants are used for various diseases by traditional healers. In recent years, the scientific evaluation constructed an overlay for the discovery of number of life saving drugs. Pharmaceutical companies have spent lot of time and money in developing natural products extracted from plants to produce more cost effective remedies that are affordable to the population. Currently, plant derived bioactive compounds have received considerable attention due to their therapeutic potential as antimicrobial, anti-inflammatory, anticancer and antioxidant activities.

*Orchidaceae* is a highly evolved and widely distributed monocotyledonous family with a large number of terrestrial, saprophytic and epiphytic species. It comprises more than 30, 000 species in approximately 750 genera. It is estimated that about 1,300 species (140 genera) of orchids are found in India with Himalayas as their main home and others scattered in Eastern and Western Ghats. Many orchids are used in traditional system of medicine as a remedy for a number of ailments. Standardization of natural products is a complex task due to their heterogeneous composition, which is in the form of whole plant, plant parts or extracts. To ensure reproducible quality of herbal products, proper control of starting material is utmost essential. The first step towards ensuring quality of starting material is authentication. Thus, in recent years there has been a rapid increase in the standardization of selected medicinal plants of potential therapeutic significance. The process of standardization can be achieved by its morphological, anatomical and biochemical characteristics.

*Dendrobium* is a beautiful spray orchid which belongs to the largest family of plants, the Orchidaceae. It has thousands of genera and species.
native to all parts of the globe including the Pacific islands, Asia and Australia. The genus *Dendrobium* has more than 1,300 species and is among the most commonly encountered orchid in the retail trade today. In India, approximately 70 species of *Dendrobium* are distributed in North West, North East Himalayas and Western Ghats\(^4\). With this background, the present study was aimed to screen the phytochemical constituents of *D. panduratum* subsp. *villosum* Gopalan & A. N. Henry. These studies on phytochemical and fluorescence analysis will provide an account on correct identification and differentiation from other related orchids and it can act as a pharmacognostical tool in the pharmaceutical industry.

**Materials and Methods**

**Collection and preparation of plant material**

The whole plants of *Dendrobium panduratum* subsp. *villosum* (Plate 1) were collected from Upper Kothayar (Tirunelveli Hills, Western Ghats, Tamil Nadu, India). The collected plants were washed thoroughly with tap water and rinsed twice with distilled water. The plants were cut into small pieces and shade dried at room temperature for 20 days. The shade dried materials were powdered using the homogenizer.

**Plant sample extraction**

Powdered (20 g) plant materials (stem and leaves) were extracted by cold extraction method with 125 mL of various solvents, viz. acetone, benzene, petroleum ether, ethanol and distilled water with gentle stirring for 72 h separately. The extracts were filtered through Whatmann No. 1 filter paper and then transferred to glass vials and stored at 4°C.

**Phytochemical and Fluorescence analysis**

The physico-chemical and fluorescence analysis were carried out as per the standard procedure\(^11\). The preliminary phytochemical constituents were qualitatively analyzed using the method described by Brindha *et al.*\(^12\). In the present study, the leaf and stem powder was treated with acetone, benzene, petroleum ether, ethanol, distilled water, 1N sodium hydroxide, acids like 1N hydrochloric acid, 50% sulphuric acid and nitric acid. These extracts were subjected to fluorescence analysis in visible/daylight and UV light (254 nm and 365 nm). Various ash types and extractive values were determined by following standard method\(^13,14\). The ethanolic extracts were subjected to separation by using the thin layer chromatography (TLC). TLC studies were carried out by following Harborne method\(^15\).

**Results**

The colour of the extracts from organic and inorganic solvents was observed both under ordinary and UV light. There is little difference between different extracts and the light sources. In various organic solvents the extracts are generally light green or light yellow or yellowish green (Table 1). The colour of the extracts in inorganic solvents is usually light green or dark green or yellowish green (Table 1). Phytochemical studies of *D. panduratum* subsp. *villosum* revealed the presence of steroids, triterpenoids, alkaloids, tannins, phenols and flavonoids in different extracts. The results of the phytochemical analysis were tabulated in Table 2. Five different extracts were examined for the presence or absence of nine different organic chemical compounds. Anthraquinone, catechins and saponins did not show any positive result in all the five extracts. None of the chemicals screened is present in all the extracts. For TLC analysis, different compositions of the mobile phase were tested in order to obtain high resolution and reproducible peaks. The desired aim was achieved using Toluene: Ethyl acetate (93:7) and Chloroform: Methanol (27:3) as mobile phase for phenolics and steroids. Three distinct phenolic bands were observed with different RF values 0.34, 0.48 and 0.68. All the bands were golden yellow in colour and visualized only in the presence of iodine vapour. Six different steroidal...
bands with various Rf values 0.24, 0.36, 0.45, 0.54, 0.68 and 0.81 were also observed.

**Discussion**

For fluorescence analysis, the powders of *D. panduratum* subsp. *villosum* stem and leaves were treated various chemical reagents to give different colours. This may be helpful to identify the purity of the drug. The present study on chemical parameters stem and leaves will helpful to identify the correct species of the plant when there is possibility of adulteration. Similar to the present study, Suganya *et al.*\(^6\) applied the fluorescence characters as tool to characterize the Selaginella tenera. Preliminary phytochemical analysis indicated presence of alkaloids, triterpenoids, glycoside and flavonoids. In the last four decades the scientists are keen to evaluate many plant drugs used in medicinal folklore. It is due to their specific healing properties, healthy action and non-toxic effects\(^7\). A number of studies were carried out for the phytochemical characterization using TLC\(^18\)-\(^20\). They employed the Rf values to distinguish the plants from other species and adulterant. In the present study also we developed the profile for *D. panduratum* subsp. *villosum*, which can be applied to distinguish this subspecies from other *Dendrobium* species. The phytochemical and TLC studies provide valuable information which may help in authenticating the genuine specimen along with the nature of phytoconstituents present in it.

In plants flavonoids can function as attractants to pollinators and seed dispersers, as antioxidants to protect plants against UV radiation, as insect feeding attractants in host-species recognition, as signal molecules to facilitate nitrogen fixation, in inducible defense against bacteria and fungal attack and as bitter or astringent taste attributes to repel birds and other animals\(^21\). Several health beneficial properties of dietary flavonoids are recognized for their antioxidant and antiproliferative effects which may protect the body from various diseases, such as cancers, cardiovascular disease and inflammation in humans\(^22\). Tannins are widely distributed in almost all plant foods. It is effective in protecting the kidneys and shows potential antiviral\(^23\), antibacterial\(^24\) and anti-parasitic effects\(^25\). It is believed that tannins isolated from the stem bark of *Myracrodruon urundeuva* are of neuroprotective functions\(^26\). Tannins also have the anti-inflammatory and antiulcer potency on rodents showing a strong antioxidant property for possible therapeutic applications\(^27\). Triterpenoids in plants are found as saponin glycosides which refers to the attachment of various sugar molecules to the triterpene unit. These sugars can be easily cleaved off in the gut by bacteria, allowing the aglycone to be absorbed\(^28\). This allows them insert into cell membranes, modify the composition, influence membrane fluidity and potentially affect signaling by many ligands and cofactors\(^29\). Alkaloids are the most

<table>
<thead>
<tr>
<th>Solvents</th>
<th>Stem</th>
<th>Ordinary light UV</th>
<th>Leaves</th>
<th>Ordinary light UV</th>
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<tbody>
<tr>
<td>Petroleum ether</td>
<td>Light yellow green</td>
<td>Light green</td>
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<td>Yellowish green</td>
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<tr>
<td>Acetone</td>
<td>Yellow Yellowish green</td>
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<td>Yellowish Yellowish green</td>
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<td>Distilled water</td>
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<tr>
<td>50% H₂SO₄</td>
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<td>1N HCL</td>
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<td>NaOH</td>
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<td>HNO₃</td>
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<th>Table 2—Preliminary phytochemical studies of <em>D. panduratum</em> subsp. <em>villosum</em></th>
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<td>Leaves</td>
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<tr>
<td>Steroids</td>
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A – Acetone, B – Benzene, PE – Petroleum Ether, E – Ethanol, Aq - Aqueous
important classes of natural products which are responsible for the antiplasmodial activity of many plant species used in traditional medicines for the treatment of malaria\textsuperscript{30}. Phenolic compounds which occur ubiquitously in plants are known to possess a variety of biological activities. These compounds showed linear correlations with free radical scavenging, no scavenging and total antioxidant activities\textsuperscript{31, 32}. Thus the results of the present study confirmed the presence of various phytoconstituents like triterpenoids, alkaloids, tannins, phenols, steroids and flavonoids which have varied therapeutic applications. The bioactive compounds detected in the extracts of this plant are documented to possess medicinal properties and health promoting effects such as antioxidant, antiproliferative, anti-inflammatory, antiulcer, neuroprotective, antiparasitic and antiplasmodial agents in the near future. As there is no record on phytochemical studies on \textit{D. panduratum} subsp. \textit{villosum}, the observations of the present study will surely help in the identification and standardization of the drug in the crude form and also to distinguish the drug from its adulterants.

**Conclusion**

Further research on this plant may help in the isolation of therapeutically potent compounds which can finally be subjected to pharmacological activities and clinical trials, thus leading to opening up a new path in the use of this plant as a natural products for therapeutic applications. The pharmacognostical characters reported in this work can serve as a valuable source of information and provide a suitable diagnostic tool for the standardization as well as identification of adulterants in future investigation or application. It will also be useful for carrying out further research and revalidation of its use.

**References**

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