



STUDIES ON PHYTOCHEMICAL, PHARMACOGNOSTIC AND PHYSICOCHEMICAL INVESTIGATIONS OF AN ENDANGERED ORCHID - *GEODORUM DENSIFLORUM* (LAM.) SCHLTR.

Pravin Ajabsing Theng^{1*} and AN Korpenwar²

¹Department of Botany, Shri Shivaji Science and Arts College, Chikhli, Dist-Buldana, (M.S.), India

²Rashtrapita Mahatma Gandhi Science and Arts College, Nagbhid, Dist Chandrapur (M.S.) India

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Abstract: The present study was carried out to describe some pharmacognostic, phytochemical and physicochemical evaluation of pseudobulb powder of *Geodorum densiflorum*. In macroscopic evaluation, organoleptic characters were taken like colour, odour and taste. The powder having dull white colour, characteristic odour and slightly bitter in taste. In microscopic study, pseudobulb having epidermis, cortex, and scattered vascular bundle. The preliminary phytochemical screening of pseudobulb was carried out in successive solvent like pet. ether, chloroform, ethanol and acetic acid by soxhlet extractor. Phytochemical screening revealed the presence of alkaloids, steroids, carbohydrates, saponins, phenol and tannin. The extractive values were also calculated. Physicochemical investigation was carried out by using parameters like total ash, acid insoluble ash, water soluble ash, loss on drying; alcohol extractive value and aqueous extractive value are 5.86, 9.89, 2.58, 9.9, 8.00 and 43.76 respectively. This study will provide information for the identification and standardization of the crude drug

Keywords: *Geodorum densiflorum*, pharmacognostic evaluation, phytochemical screening and physicochemical evaluation.

INTRODUCTION

Orchids are second largest group of flowering plant belonging to family Orchidaceae. It comprising about 778 genera and 18,500 species (Mabberley, 1997). The wild species of orchids have been used as indicators of regions that have a healthy ecosystem (Mishra, 2004). Orchids are well known for their economic values and widely cultivated for ornamental purposes. Most of the orchids have conspicuous storage organs like corms, rhizomes, or tuberosids are observed in terrestrial orchids while storage organs in epiphytic orchids are enlarged stems called pseudobulbs (Zimmerman, 1990; Arditti, 1992; Dressler, 1981). Pseudobulbs are also present in some terrestrial orchids like *Cymbidium*, *Eulophia* and *Spathoglottis*. *Geodorum densiflorum* is an herbaceous terrestrial orchid belongs to the family Orchidaceae and possess pseudobulbs as a storage organs. From Greek, *Geodorum* means 'gift from the ground' (*geo doron*). A common name in English is the ground gem orchid. It is widely distributed in India, Nepal, Australia, Bangladesh, Srilanka, China, Bhutan, Papua New Guinea, and Himalayas. In India it is distributed in Karnataka, Kerala, Tamilnadu and Maharashtra States.

Geodorum densiflorum is an endangered terrestrial orchid (Datta *et al.*, 1999). It is floricultural and medicinal important plant. The pseudobulb of *Geodorum densiflorum* is ethno medicinally used for the treatment of various diseases. The root paste is used as insecticide and wounds healing. The pseudobulb is used to regularize menstrual cycle (Dash, *et al.*, 2008),

for diabetes (Patil, *et al.*, 2005), applied externally to cure Carbuncles (Nath, *et al.*, 2011). It is also possess antimicrobial activity (Akhtar, *et al.*, 2010) and anti-diabetic property (Roy, *et al.*, 2002). The pseudobulbs have ability to store water, mineral and carbohydrates, so the pseudobulb has serious implications for survival in the harsh and nutrient limited condition. However, the present study is to evaluate various phytochemical, pharmacognostic and physicochemical properties of *Geodorum densiflorum* pseudobulb.

MATERIALS AND METHODS

Collection of Plant Material

The plant material was collected from Ambabarwa forest. The plant material was shade dried, powdered and stored in air tight container for further use.

Extraction of Plant Drug

The fine powdered plant material was subjected to extraction in soxhlet apparatus. The powdered Pseudobulb drug was successively extracted with Petroleum Ether, Chloroform, Ethanol and Acetic Acid.

Phytochemical Screening (Harborne, 1994; Khandelwal, 2006)

For preliminary phytochemical screening, powder of pseudobulb was subjected to various qualitative chemical tests to determine the presence of various phyto-constituents like glycosides, tannins, phytosterols, proteins, amino acids, flavonoids, saponins.

*Corresponding Author:

Mr. Pravin Ajabsing Theng,

Department of Botany,

Shri Shivaji Science and Arts College,

Chikhli, District Buldana, M.S. India.



Macroscopic and Microscopic Analysis (Evans, 2005; Kokate, 2002)

Macroscopic analysis of pseudobulb was studied. The organoleptic characters including colours, taste, odour of pseudobulb powder was observed. For microscopic studies many free hand section of pseudobulb and their root was taken and thin section was selected for staining. After staining and mounting process the photographs of the sections were taken using digital camera.

Physicochemical Evaluation (Brain, 1975; Kokate, 1986)

The powder of *Geodorum densiflorum* pseudobulb was subjected to evaluate total ash, acid insoluble ash, water soluble extractive value, alcohol soluble extractive value and moisture contain. Result was reported in table 4.

Ash Values

Total ash value was obtained by incinerating pseudobulb powder at temperature 650^o-700^o C in Silica crucible until free from carbon, weight of ash was taken and the percentage of it was calculated.

Acid insoluble ash:

The total ash obtained was boiled with 2N HCL, filtered and the insoluble matter was collected on ashless filter paper. It was washed with hot water, ignited in silica crucible. Cooled in desiccators and the residue obtained was weighed and the percentage of acid insoluble ash was calculated.

Water soluble ash

The total ash obtained was boiled with water and the insoluble matter was collected on ash less filter paper, washed with hot water and ignited. The difference in weight represents the water soluble ash. The percentage of water soluble ash was calculated.

Water soluble extractive value

Pseudobulb powder was macerated with chloroform water (2.5ml chloroform in 100ml water) in a closed flask. It was filtered and 25ml filtrate was evaporated to dryness at 105^oC and weight. The percentage of water soluble extract value was calculated.

Alcohol soluble extractive value

Pseudobulb powder was macerated with alcohol in a closed flask. It was filtered and 25ml filtrate was evaporated to dryness at 105^oC and weight. The percentage of water soluble extract value was calculated.

Loss on drying:

1.0 g of pseudobulb powder weight was taken. The sample was heated in oven maintained at 105-110^oC, for 3h, after which the sample was allowed to cool to room

temperature in desiccators. The percentage of moisture contain was calculated.

RESULTS AND DISCUSSION

Macroscopic and Microscopic analysis

Pseudobulb of *Geodorum densiflorum* subglobose in shape. The pseudobulbs were homoblastic in nature because they having two or more internodes of varying in length (Dressler, 1981). The size of new and old pseudobulb is 1.6 x 1.8 and 3.5 x 3.2 cm respectively. pseudobulbs were white when underground, but green after expose to environmental conditions. Each pseudobulb having 10-14 fleshy roots. pseudobulb are jointed to each other at the base to form continue bead like structure, So it was also known as 'Hargathi' by tribals. The new sprouting was formed at nodal segment.

Table 1: Macroscopic Evaluation of Powder drug

Sr. No.	Feature	Observation
1	Colour	Dull white
2	Odour	Characteristic
3	Taste	Slightly Bitter

Microscopic Evaluation

Pseudobulb: The T.S. of pseudobulb showed uniseriated epidermis. It is thin layered protective layering. Epidermis followed by cortex that having circular and some polygonal parenchymatous cells. These vascular bundles were scattered throughout the cortex. The vascular bundles which consisted of numerous collateral vascular bundles embedded in a slightly lignified parenchymatous tissue of cortex

Root: The fleshy root of pseudobulb shows uniseriated rhizoderm followed by velamen tissue. The velamen was formed by many polygonal cells in cross section. The size of the velamen could be related to condition of water and temperature. Therefore, species from dry environments have a many layered velamen and those grow in humid environments have a single layered velamen (Sanford and Adanlawo, 1973). Presence of velamen is a character feature of Orchidaceae. The exodermis cells showed U shaped pattern of secondary wall thickening. Bellow the exodermis many layered cortex was present. Variation was seen in shape of cortical cell. The cells towards endodermis were larger than towards exodermis. Uniseriated endodermis followed by cortex formed sheath around vascular tissues. Vascular cylinder includes xylem and phloem tissues.

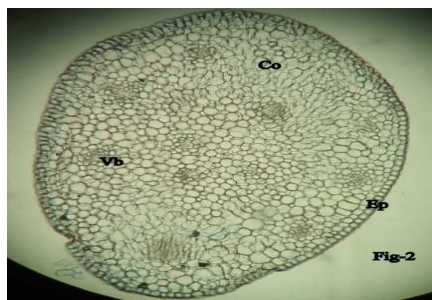
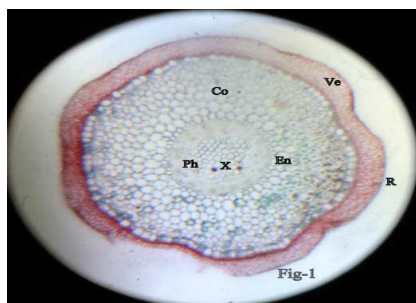


Fig.1: lateral root and **Fig.2:** Anatomy of pseudobulb (Co- cortex, En- endodermis, Ex- exodermis, Ph- Phloem, Vb-Vascular bundle, R- Rhizodermis, Ve- velamen, Ep- Epidermis, X- xylem, pi-pith).

Phytochemical Evaluation

The pseudobulb powder of *Geodorum densiflorum* was subjected to successive solvent extraction. Percentage yield of the selected successive extract were recorded in table 2.

Table 2: Successive extraction of the pseudobulb of *Geodorum densiflorum* (Lam.) Schltr.

Parameter	Values % (w/w)
Pet. Ether Extract	0.928
Chloroform Extract	2.988
Ethanol Extract	8.00
Acetic Acid Extract	6.288

Preliminary phytochemical screening of *Geodorum densiflorum* shows presence of alkaloids, steroids, carbohydrates, flavonoids, tannin and saponins in different extract. The result of phytochemical screening of petroleum ether, chloroform, ethanol and acetic acid extract of pseudobulb were reported in table 3. From this analysis it was clear that ethanol has higher extractive value, then acetic acid, chloroform and petroleum ether respectively. In pseudobulb, many chemicals were detected that helps for treating different diseases. Now day, tribal peoples were used pseudobulb and their roots to cure many diseases, so it has potential to provide valuable drugs for human beings.

Table 3: Phytochemical Screening of *Geodorum densiflorum* Pseudobulb Extract

Chemical Constituent	Tests	Pet. Ether	Chloroform	Ethanol	Acetic Acid
Alkaloids	Dragendroff's	+++	+++	+++	+++
	Mayer's	+	++	++	++
	Hager's	++	+++	+	++
	Wagner's	++	++	++	++
Carbohydrates	Molisch's	+	++	++	++
	Benedict's	+	+++	++	++
Saponins	Fehling's	+	+++	++	++
	Foam	+	-	++	+++
Tannin	Ferric Chloride	-	-	+++	++
Protein	Biuret	-	-	-	-
Flavonoids	Lead Acetate	+	++	+++	++
Steroids	Salkowski	++	++	++	-

(Low=+, Medium=++, High=+++; Absent=-)

Physicochemical Evaluation

Physicochemical evaluation of pseudobulb powder in silica crucible by using different parameters. The results of physicochemical characterization including ash value, extractive value and moisture contain were measured and mentioned in table 4.

Table 4: Physicochemical parameters of pseudobulb of *Geodorum densiflorum* (Lam.).

Sr. No.	Parameter	Values % (w/w)
1	Total Ash	5.86
2	Acid Insoluble Ash	9.89
3	Water Soluble Ash	2.58
4	Loss on Drying	9.09
5	Alcohol Soluble Extractive Value	8.00
6	Aqueous extractive value	43.76

Physicochemical evaluation was carried out by using parameters like total ash, acid insoluble ash, water soluble ash, loss on drying, alcohol extractive value and aqueous extractive value are 5.86, 9.89, 2.58, 9.9, 8.00, and 43.76 respectively. Physicochemical evaluation is important in identification of distinctive features of the drug and also helps to avoid adulteration. The ash values were used to examine quality and purity of drugs. Moisture contain was also important at the time of storage to avoid microbial contamination of drugs.

CONCLUSION

The pseudobulbs of *Geodorum densiflorum* (Lam.) have ability to store water, mineral and carbohydrates, so the pseudobulb has serious implications for survival in the harsh and nutrient limited condition. The study of macro and microscopic feature, physicochemical and phytochemical evaluation by using different parameters which may facilitate the identification of crude drug and also helps to avoid adulteration. The ash values were used to examine quality and purity of drugs. Moisture contain was also important at the time of storage to

avoid microbial contamination of drugs. This study may also be useful to standardization of crude drugs.

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