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Phytochemical, Pharmacognostic and Physicochemical Evaluation of Endangered Terrestrial Orchid- *Geodorum densiflorum* (Lam.) Schltr

P.A. Theng[1], A. N. Korpenwar[2]

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

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

Abstract: ***The present investigation deals with phytochemical, pharmacognostic and physicochemical evaluation of Geodorum densiflorum (Lam.) Schltr leaves. The phytochemical evaluation revealed that the leaves contain alkaloid, glycoside, steroids, saponins, carbohydrates, tannin and flavonoids. The ethanol having higher extractive value, then acetic acid, petroleum ether and chloroform respectively. In macroscopic evaluation, organoleptic parameters were studied like color, odor and taste. Leaves powder having light green colour, characteristic odour and slightly bitter taste. In microscopic study, transverse section of leaf and stomatal type were studied under microscope. In physicochemical evaluations total ash, acid insoluble ash, water soluble extractive value, alcohol soluble extractive value, loss on drying and foam index were calculated. These evaluations will provide valuable information for the identification of crude drug.***

Keywords: *Geodorum densiflorum*, phytochemical evaluation, physicochemical evaluation.

1. Introduction

Orchidaceae is a highly evolved and widely distributed monocotyledonous family with a large number of terrestrial, saprophytic and epiphytic species. Orchids are well known for their beauty and its medicinal use [(1)]. Orchids are natures most overgenerous group of flowering plants distributed throughout the world from tropics to high alpine [(2)]. The term orchid has its origin from the Greek word 'orchis' meaning testicle, referring to the paired underground tubers of European terrestrial orchids, compared with the testicle of a dog [(3)]. It is estimated that about 1,300 species (140 genera) of orchids are found in India with Himalayas as their main home and other scattered in Eastern and Western Ghats [(4)]. Orchids are well known for their economic importance and widely cultivated for ornamental purposes.

Geodorum densiflorum is belongs into family Orchidaceae. The genus *Geodorum* was described in 1811 by J. Jackson in Botanist's Repository [(5)]. *Geodorum densiflorum* is an endangered terrestrial orchid [(6)]. There is no report on phytochemical and physicochemical analysis of *Geodorum densiflorum* leaves. This study was carried out in order to secure some standards for standardization of these crude drugs.

2. Material and Methods

2.1 Plant Material Collection

The plant material was collected from Ambabarwa forest, Jalgaon Jamod, Dist. Buldana. The collected sample was authenticated and deposited at Shri Shivaji Science and Arts College, Chikhli, Dist. Buldana. The leaves were shade dried, powdered and stored in the air tight container for further use.

2.2 Extraction of Plant Drugs

The powdered plant material was subjected to extraction in a Soxhlet apparatus. The powdered plant material was successively extracted with petroleum ether, chloroform, ethanol and acetic acid as a solvent.

2.3 Phytochemical Evaluation: [(7, 8 & 9)]

For preliminary phytochemical evaluation powder of *Geodorum densiflorum* leaves was subjected to various qualitative chemical tests to determine the presence of various phytoconstituents like alkaloids, glycosides, steroids, tannins, phytosterols, proteins, amino acids, flavonoids, saponins. The percent yield was also calculated in different solvents.

2.4 Microscopic and Macroscopic Evaluation: [(10, 11)]

The macroscopic evaluation of *Geodorum densiflorum* was carried out. In organoleptic examination of leaves powder parameters like colour, odour and taste were observed. For microscopic study fresh leaves of *Geodorum densiflorum* was taken. In microscopic studies many free hand sections of leaf was taken and thin section was selected for staining. After staining and mounting process the photographs of the sections were taken using digital camera.

2.5 Physicochemical Evaluation: [(12, 13 & 14)]

Leaves powder of *Geodorum densiflorum* was subjected to physicochemical evaluation. In this study, parameters like total ash value, acid insoluble ash value, water soluble

extractive value, alcohol soluble extractive value and moisture contain were calculated.

2.6 Ash Values

Total ash value was obtained by incinerating leaves powder in Muffle furnace gradually to ignition temperature 650^0-700^0 C. Leaves powder was weighed in a silica crucible and incinerate at a temperature not exceeding 700°C until ash free from carbon. Weight of ash was taken and the percentage of ash was calculated with reference to air dried drug.

2.7 Water Soluble Ash

The total ash obtained was boiled with water and the insoluble matter was collected on ashless 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.

2.8 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.

2.9 Water Soluble Extractive Value

Leaves powder was macerated with chloroform water (2.5ml chloroform in 1000ml water) in a closed flask. It was filtered and 25 ml filtrate was evaporated to dryness at 105^0 C and weight. The percentage of water soluble extract value was calculated.

2.10 Alcohol Soluble Extractive Value

Leaves powder was macerated with alcohol in a closed flask. It was filtered and 25 ml filtrate was evaporated to dryness at 105^0 C and weight. The percentage of water soluble extract value was calculated.

2.11 Loss on Drying (Moisture content)

1g of leaves powder weight was taken and heated in oven maintained at 105-110°C for 3 h, after which the sample was allowed to cool at room temperature in desiccators. The percentage of moisture contain was calculated.

2.12 Foaming Index

About 1g of the powdered sample was transferred to a conical flask containing 100 ml of boiling water. Decoction was prepared and filtered. The decoction was poured into 10 stoppered test tubes (height 16 cm, diameter 16 mm) in successive portions of 1 ml, 2 ml, 3 ml etc. up to 10 ml and the volume of liquid in each test tube was adjusted to 10 ml with water. The test tubes were stoppered and shaken in a lengthwise motion for 15 seconds. The test tubes were allowed to stand for 15 minutes and the height of the foam was measured.

3. Result and Discussion

3.1 Phytochemical Evaluation

Extractive values and colour of successive solvent extract of *Geodorum densiflorum* leaves in the petroleum ether, chloroform, ethanol and acetic acid were depicted in figure 3 and table no.1. Ethanol having higher extractive value then acetic acid, petroleum ether and chloroform respectively. Phytochemical analysis revealed that, extract contains alkaloids, glycosides, saponins, carbohydrates, tannin and steroids. These phyto-constituents were active chemicals against different microbes and fungi, so drug having higher ethnomedicinal value. The phytochemical analysis of different extracts of *Geodorum densiflorum* leaves were reported in table no. 2.

3.2 Pharmacognostic Evaluation

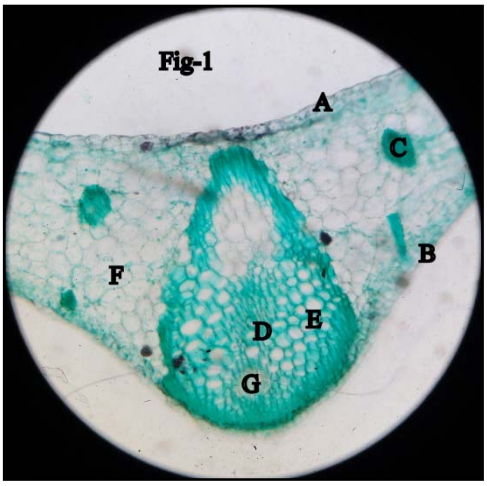

Figure 1: T. S. of leaf. A) Upper epidermis, **B)** Lower epidermis, **C)** Vascular bundle **D)** Phloem **E)** Metaxylem **F)** Mesophyll cell. **G)** Protoxylem

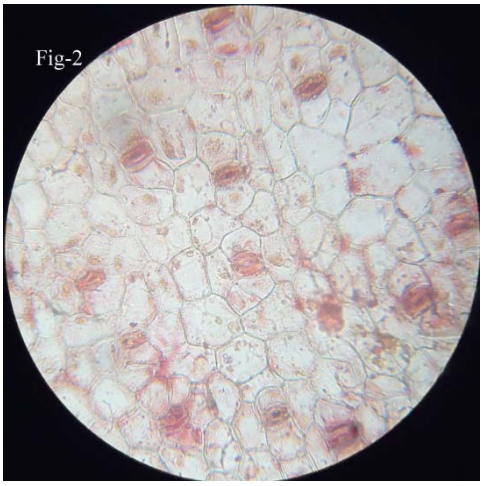

Figure 2: Anisocytic Stomata

In macroscopic (Morphological) study, *Geodorum densiflorum* having simple leaves with parallel veinations. It have 2-3 leaves per shoot, well developed at anthesis, lustrous elliptic, narrowly elliptic, or oblong-lanceolate, 15-35 x 3-9 cm, papery, apex acuminate or acute; petiole like stalk enclosed in several sheaths and forming a pseudostem and enclosed together in 2-4 common sheathing bracts. In

organoleptic evaluation of leaves powder, characters were taken like colour, odour and taste. Leaves powder is having green colour, characteristic odour and astringent in taste.

In Microscopic study, leaf lamina shows thin cuticle on uniseriated upper and lower epidermis with elongated cells. Parenchymatous cells of adaxial epidermis are larger in shape than abaxial epidermis. Thin and smooth cuticle was present on upper epidermis. Mesophyll not differentiated into palisade and spongy parenchyma. Mesophyll with oval shaped parenchyma cells. Midrib and lamina region are very distinct. Lateral veins vascular bundles were embedded in mesophyll. The vascular bundle of midrib is conjoint, collateral and close. Protoxylem and metaxylem present towards periphery and phloem present towards centre. Anisocytic stomata are confining to only lower epidermis. Structure and ontogeny of stomata are also considers as an important taxonomic character for many of the angiospermic taxa [15, 16]. The quantitative determination of some pharmacognostic parameters is useful for setting standards for crud drugs. The microscopic study also helps to differentiate closely related species in identification.

3.3 Physicochemical Evaluation

In physicochemical evaluations, parameters such as total ash, acid insoluble ash, water soluble ash, alcohol soluble extractive value, water soluble extractive value and moisture contain of *Geodorum densiflorum* leaves were calculated. The total ash value, acid insoluble ash, water soluble ash, loss on drying was 13.64, 24.48, 17.30 and 9.9 respectively. The alcohol soluble extractive value and aqueous extractive value also calculated. The alcohol soluble extractive value and aqueous extractive value were 9.84 and 19.60 respectively. The foam index was over 1000.

The physicochemical analysis of leaves drugs is significant in detecting adulteration and improper handling of drug. The estimation of moisture content of the drug is important requirement in evaluation. Determination of ash value and acid insoluble ash value has the equivalent importance in the evaluation and identification of impurities in crude drugs.

Table 1: Color and percentage yield of extract obtained from the leaves of *Geodorum densiflorum* using different solvent.

Parameter	*Color*	*Values % (w/w)*
Petroleum Ether Extract	Yellowish green	3.6
Chloroform Extract	Dark green	2.4
Ethanol Extract	Yellowish green	9.84
Acetic Acid Extract	Dark green	9.12

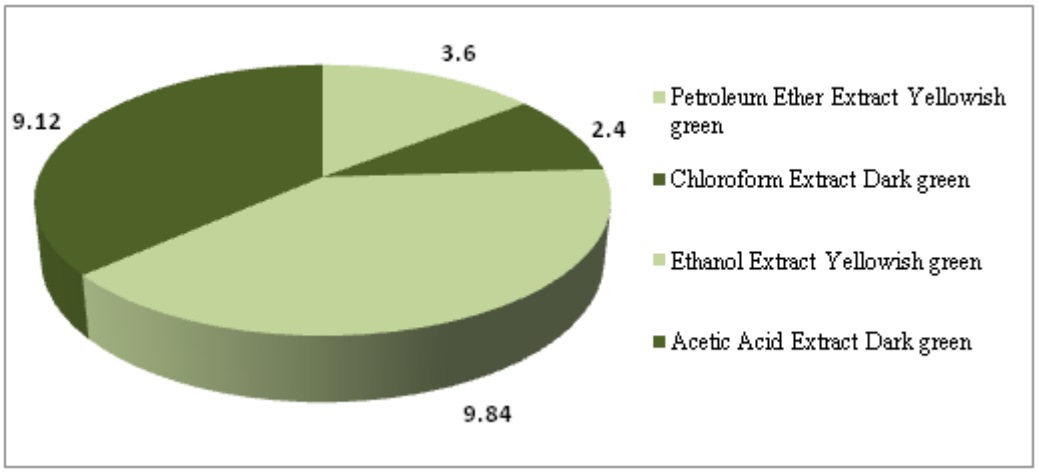

Figure 3: Successive extractive value of *Geodorum densiflorum* leaves.

Table- 2: Phytochemical Evaluation of *Geodorum densiflorum* Leaves Extracts.

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

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

Table 3: Physicochemical parameters of leaves of *Geodorum densiflorum* (Lam.)

Sr. No.	**Parameter**	**Values % (w/w)**
1	Total Ash	13.64
2	Acid Insoluble Ash	24.48
3	Water Soluble Ash	17.30
4	Loss on Drying	9.9
5	Alcohol Soluble Extractive Value	9.84
6	Aqueous extractive value	19.60
7	Foaming Index	over 1000

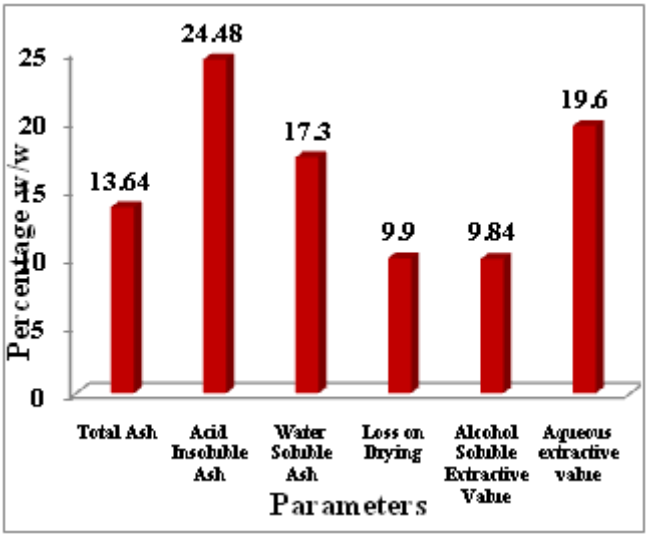

Figure 4: Physicochemical evaluation of *Geodorum densiflorum* Leaves

4. Conclusion

The present work carried out on phytochemical, pharmacognostic and physicochemical evaluation of *Geodorum densiflorum* leaf. The macro and microscopic study revealed a set of anatomical parameters which may facilitate identification of drug. Phytochemical evaluation was providing useful information for identification and Standardization of drug. Physicochemical evaluation is important to detect improper handling and adulteration of drug.

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