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Pharmacognostical Evaluation of Jeevak/*Crepidium acuminatum* (D.Don) Szlach. Pseudo bulb

Research Article

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Abstract

Background: Crepidium acuminatum (D.Don) Szlach. Syn. Malaxis acuminata D.Don belonging to the family Orchidaceae is a rare medicinal plant found in Himalayan region in temperate zone that has high therapeutic value. It is known as Jeevak in Ayurveda and is one among the astavarga (group of eight highly important vitalizing plants). It has been praised to be immunomodulatory, vitalizer, adaptogenic, muscle bulk promoting drug in Ayurveda. Although a very important plant in Avurveda proper pharmacognostical data is not available regarding this plant. Materials and Methods: The plant was collected from Chandravadani, Garhwal district, Uttarakhand and was authenticated from Botanical Survey of India, Kaulagadh road, Dehradun. The pharmacognostical, histochemical and micrometric evaluation was performed at the Department of Pharmacognosy, Institute for Post Graduate Teaching and Research in Ayurveda, Gujarat Ayurveda University, Jamnagar, Gujarat. Organoleptic features was observed with naked eyes. The transverse sections was stained with phloroglucinol along with hydrochloric acid. The photomicrographs were taken using Carl Zeiss trinocular microscope. Results: Jeevak was found in Himalayan region growing among stony soil in colonies together. The mucilaginous property in fresh sample was lost after getting dried. The pharmacognostical evaluation shows oval or irregularly shaped parenchymatous and mucilage cells with acicular raphides abundantly; acicular crystals of calcium oxalate scattered singly or in broken pieces or in bundles scattered in parenchymatous cells and simple starch grains with striations that help to distinguish it from other allied and nonallied drugs. Conclusions: The obtained results would be helpful for proper identification and standardization of Jeevak and hence provide the pharmacopeial standards and reference information for manufacturers and researchers.

Keywords: Ashtavarga, Ayurveda, Crepidium acuminatum, Jeevak.

Introduction

Crepidium acuminatum (D. Don) Szlach. is known as Jeevak and is used as an immunomodulatory, adaptogenic, aphrodisiac and vitaliser (1). It is mentioned in ancient Ayurvedic classics with its various uses and formulations (2). It is among the vital component of the formulations like Chyavanprasha avaleha (3), Bala taila (4), Prasarini taila (5), Shatavari ghrita (6), Baladi Choorna (7), Phalaghrita (8), etc. It is also one of the component of popular astavarga (9). The chemical constituents in Jeevak are Aluminium, Boron, Calcium, Chlorine, Iron, Copper, Barium, Potassium, Magnesium, Manganese,

* Corresponding Author: Maharjan Ramita Director, Rishicare Wellness and Research Institute, Bengaluru, Karnataka, India. Email Id: <u>ramitama@gmail.com</u> Molybdenum, Zinc, etc. like metals and minerals; fatty acids like Eicosadienoic, Eicosanoic, Alpha Linolenic, Terpenoids, Alpha-Tocopherol, Gamma- Tocopherol, Beta Sitosterol, ceryl alcohol, glucose, rhamnose, choline, limonene, eugenol, citronellal, 1,8-cineole, piperitone, p- cymene, Eugenol, Caryophyllene, Humulene, Phenol, 2, 4bis (1,1dimethylethyl), Caryophyllene oxide, 2,5 Octadecadiynoic acid, methyl ester (10,11,12,13). It possesses antioxidant (14,15), antimicrobial (16,17) and anti-inflammatory activity (18). It was a rare drug since sixteenth century (19). In this present era, it is substituted and adulterated. The review of literature also shows few pharmacognostical studies relating to it (20, 21,22). Considering it a preliminary pharmacognostical profile of Jeevak/ Crepidium acuminatum pseudo bulb was investigated which can be used for further research.

Materials and Methods

The plant was collected from Chandravadani, Garhwal district, Uttarakhand on 12th to 15th August, 2018. It was then authenticated from Botanical Survey Maharjan Ramita et.al., Pharmacognostical Evaluation of Jeevak/Crepidium acuminatum (D.Don) Szlach. Pseudo bulb

of India, Kaulagadh road, Dehradun on 2018-09-07 with voucher number Tech./Herb (Ident.)/2018-19, Account number 118498. The collected pseudo bulb was washed with plain water thoroughly for removing physical impurities and other plant parts. The organoleptic evaluation of the plant Jeevak was performed at the Department of pharmacognosy, Institute for Post Graduate Teaching and Research in Avurveda, Gujarat Avurveda University, Jamnagar. Fresh and dried pseudo bulb along with the powder were evaluated for their organoleptic features i.e. size, shape, colour, odour, taste and texture with naked eyes. Thin free hand transverse section of different parts of pseudo bulb were taken. The sections were first observed in distilled water then stained with phloroglucinol (20 mg/ml of alcohol) along with hydrochloric acid (6N). It was again examined to assess different cellular structures and lignified elements like fibers, sclerides, xylem vessels, etc. The powder microscopy of powder 60 mesh was carried along with their physico-chemical profiling. The photomicrographs were taken using Carl Zeiss trinocular microscope attached to camera.

Results

Morphology

Crepidium acuminatum (D. Don) Szlach. is a terrestrial, lithophytic orchid 14-30cm tall (Plate 1.A). Leaves 3 or 4, thinly membranous, ovate-lanceolate, acute to acuminate, narrowed to sheathing base, petiolate, margins undulate, 5.5-12 x 2.5-6 cm; petiole 3-4 cm long (Plate 1.B). It has racemose inflorescence, laxly many-flowered. The flowers are 5-8 mm long, uniformly yellowish-green, yellow tinged with red-purple or pink-purple. The roots are fasciculate, slender and about 1mm wide. It has a glabrous, cylindrical, fleshy stem sheathed at base (Plate 1.C). The fruit is a capsule obovoid-oblong with many seeds (23).

Plate 1: A. Crepidium acuminatum (D. Don) Szlach. in wild B. Morphology of leaf of Crepidium acuminatum (D. Don) Szlach. C. Measurement of pseudo bulb of Crepidium acuminatum (D. Don)





Organoleptic characters

The organoleptic characters of fresh and dry sample of pseudo bulb of Jeevak/ *Crepidium acuminatum* (D. Don) Szlach. shows various features. There was marked change in colour of the pseudo bulb after drying which changed from dark green to brownish yellow. The smooth fracture of fresh sample was changed into hard, granulated and irregular fracture in dry sample. (Table no 1).

 Table 1: Showing organoleptic characters of fresh and dry sample of Jeevak/ Crepidium acuminatum (D. Don) Szlach, pseudo bulb

(D. Don) Szlach. pseudo bulb				
Observations	Fresh sample (pseudo bulb)	Dry sample (pseudo bulb)		
Colour	Dark green to light greenish yellow	Brownish yellow		
Odor	Characteristic	Characteristic		
Taste	Astringent, sweet; slightly mucilaginous	Astringent, sweet		
Fracture	Smooth	Hard, irregular; cut surface brownish yellow; granulated		

Macroscopic characters

The pseudo bulb were 3-8 cm in length and 1-1.5 cm in diameter in fresh state; when dried were 2-7cm in length and 0.5-0.7 cm in diameter. It was conical, smooth and shining, fleshy,

covered with translucent light green papery membranous cover in fresh state while conical, rough, covered with yellowish papery membrane with whitish yellow spots in dry state.

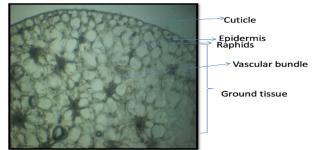
Dried pseudo bulb is conical in shape, straight or slightly curved, about 2 to 7 cm long and 0.5 to 1 cm thick, broader at the base with broken rootlets; surface dark brown to nearly black, wrinkled with 2 to 4 obliquely arranged rings of papery white remnants of dried leaf bases; taste mucilaginous; odourless.

Microscopic characters

Transverse section of pseudo bulb

Diagrammatic Transverse characters of cut surface of the pseudo bulb is circular to oval with irregularly crenate margins in out line and shows a layer of epidermis and wide parenchymatous ground tissue with scattered vascular bundles and mucilage cavities. (Plate 2.A) The detailed transverse section of pseudo bulb shows a layer of epidermis of tubular, tangentially running cells of irregular size with thin cuticle followed by ground tissue of parenchymatous cells traversed by wide air spaces, vascular bundles, numerous mucilage cells and cells containing bundles of acicular crystals of calcium oxalate; vascular bundles consisting of 3 to 5 xylem vessels and a narrow zone of phloem. (Plate 2.B)

Plate 2A: Diagrammatic photograph of transverse section of pseudo bulb



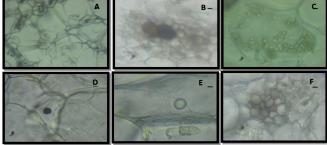
T.S. of Pseudo bulb



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Plate 2B: Descriptive photographs of transverse section of pseudo bulb.; A) Acicular crystal with starch grain; B)
Brown constant; C) Parenchyma cells with oil globules;
D) Iodine stained starch grain; E) Oil globule with

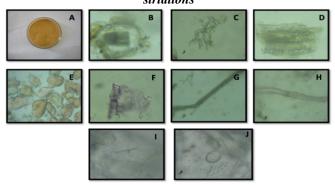
prismatic crystal F) Vascular bundle



Powder microscopic characters of dry sample Organoleptic and Microscopic characters

Diagnostic microscopic characters are abundant, oval or irregularly shaped parenchymatous and mucilage cells with acicular raphides (Plate 3 B); acicular crystals of calcium oxalate scattered singly or in broken pieces or in bundles scattered in parenchymatous cells and rarely reticulate vessels.

Plate 3: Powder microphotographs of Jeevak/ Crepidium acuminatum (D. Don) Szlach. A) Pseudo bulb powder ;
B) Raphids; C) Fragment of leaf part; D) Annular and spiral vessels; E) Fragments of Parenchyma cells;
F) Lignified leaf components; G) Simple fiber; H) Lignified fiber ; I) Acicular crystal; J) Simple starch grains with striations



Histochemical evaluation

The thick sections and powder was subjected to various reagents in chemical tests to detect the different chemical constituents.

S.N.	Reagent	Observation	Characteristic feature	Result		
1	Phloroglucinol + Concentrated Hydrochloric acid	Red	Lignified cells	++		
2	Iodine	Blue	Starch grains	++		
3	Phloroglucinol + Concentrated Hydrochloric acid	Dissolved	Calcium Oxalate crystals	++		
4	Ferric chloride/ FeCl ₃ solution	Dark blue	Tannin cells	++		
5	Ruthenium red	Red	Mucilage	++		

Micrometric evaluation

Micrometric measurements of T.S. of pseudobulb shows 6.2 μ m², other powder characters are given in table.

Table 3 :	Micrometry	value of	pseudobulb	powder
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Sr. No.	Character	Measurement (400×)	
1	Silica deposition	$1.5 \times 1.2 \ \mu m$	
2	Starch grain	$0.4 imes 0.6 \mu m$	
3	Annular vessel	$1.6\times0.9~\mu m$	
4	Brown constant	$1.3 \times 1.2 \ \mu m$	
5	Acicular crystal	$3.9\times0.1~\mu m$	
6	Fragment of fibre	$2.8~\mu m \times 0.2~\mu m$	
7	Raphides	$2.5\times3.9~\mu m$	

(× Magnification)

Discussion

Jeevak /Crepidium acuminatum (D. Don) Szlach. is one of the prized drugs of choice in Ayurveda found in Himalayan region used in various formulation ranging from powder, pills to oil or ghee based formulation. It was rare and often substituted as mentioned in Bhavaprakash nighantu (an Ayurveda textbook of 16th century) (24). It was a necessity to identify, authenticate and standardize it pharmacognostically. This study revealed the diagnostic microscopic feature includes abundant, oval or irregularly shaped parenchymatous and mucilage cells with acicular raphides; acicular crystals of calcium oxalate scattered singly or in broken pieces or in bundles scattered in parenchymatous cells and simple starch grains with striations that help it distinguish from other allied and non-allied drugs.

Most of the preparation with Jeevak in Ayurveda are ghee or oil based. Starch and oils present in Jeevak being non polar can get properly incorporated with ghee and oil preparations. Starch is known to increase body weight and promote bulk in the body. The presence of starch can be attributed to one of the reasons for the strength promoting and bulk promoting activity of Jeevak.

Conclusion

The present study of provides the pharmacognostical information for identification, authentication and standardization of Jeevak/*Crepidium acuminatum* (D. Don) Szlach. The microscopic features that distinguishes Jeevak is the presence of abundant, oval or irregularly shaped parenchymatous and mucilage cells with acicular raphides. The acicular crystals of calcium oxalate was found to be scattered singly or in broken pieces or in bundles scattered in parenchymatous cells and simple starch grains with striations. Very few published reports are available on this plant which are either vaguely described or incompletely arranged. Hence the results obtained in this study may be referred to as a standard for upcoming future studies.



Maharjan Ramita et.al., Pharmacognostical Evaluation of Jeevak/Crepidium acuminatum (D.Don) Szlach. Pseudo bulb

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