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Pharmacognostic and physicochemical evaluation of bulbs of *Eleutherine bulbosa* (Miller) Urban, a medicinal plant

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Abstract

Eleutherine bulbosa (Iridaceae) is an exotic ornamental plant, native to South America and now naturalized in Kerala and Southern parts of Tamil Nadu. The underground bulbous part of the plant is considered medicinal. The present study attempts to evaluate the pharmacognostical and physicochemical parameters of the underground bulbous part of *Eleutherine bulbosa* (Miller), Urban. The organoleptic studies of fresh bulbs revealed that the fresh bulb was purplish red in colour with a pungent odour and bitter taste. The microscopic sections showed epidermal cells filled with red shiny liquid and scattered vascular bundles. Some cells in the ground tissue showed the presence of styloid crystals. The different physicochemical parameters evaluated were the moisture content (9.9%) bulk density (0.25gm/mL) tapped density (0.29gm/mL) inter particle porosity (0.64), total ash value (3.12%), water soluble ash value (1.2%) and acid insoluble ash value (9.4%). Hausner ratio (1.188), Carr's Index (16%) indicated the food flow of the bulb powder. Fluorescence studies of bulb powder showed wide range of colours under visible light, long uv and short uv. These studies will impart the standardization of the bulb in fresh as well as in dry form.

Keywords: *Eleutherine bulbosa*, pharmacognostical, physicochemical parameters, styloid crystals.

1. Introduction

Eleutherine bulbosa (Miller) Urban (Iridaceae) is an exotic tropical American plant, now naturalized in the African and Asian continents. The plant has a scattered distribution in the Indian peninsula. It is a seasonal perennial, characterized by a bulbous root-stock; cauline, sheathing leaves and small, white, stellate flowers blooming only in the evenings once in a year around May-July (Fig 1). The fleshy bulbs, enclosed with reddish tunics have medicinal properties (Fig 2). In Kerala and southern parts of Tamil Nadu, underground bulbs of the plant were crushed and administered orally as an antidote to poisonous bites and applied topically in local oedema. When administered orally it is diluted with cow's milk or the juice of fresh coconut kernel [1]. Local medical practitioners revealed that the bulbs of the plant are used in the treatment of contusions caused by stones on the feet. The entire bulb heated on flame for some time and directly applied on the affected area, was found to be very effective. The other recorded report about the medicinal use of the plant is from the eastern part of India, especially in Odisha the tribals use the bulb to treat diarrhoea [2]. A perusal of literature revealed that pharmacognostic and physicochemical investigations on *Eleutherine bulbosa* were scarce. Scientific standardization techniques should be adopted for validation and quality control of herbal drugs and the process of standardization can be achieved by step-wise pharmacognostic studies [3].



Fig 1: Habit of *Eleutherine bulbosa*

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2. Materials and Methods

2.1 Collection of plant materials and identification

The plant samples of *Eleutherine bulbosa* were collected from Thiruvananthapuram and Kollam, Kerala, India. The plant was identified and authenticated by the Curator, Department of Botany, University of Kerala, Kariyavattom, Thiruvananthapuram. The plant material was also verified by comparison with specimens of *E. bulbosa* from Botanical Survey of India, southern circle, Coimbatore. A voucher specimen (Accession Number - KUBH 5802) has been deposited at the Kerala University Botanical Herbarium.

2.2 Drying and processing

The collected bulbs were cleaned and air dried in room temperature. The dried bulbs were homogenized using a mechanical grinder to obtain fine powder and stored in airtight containers for further analysis. The dried bulb powder was used for organoleptic and physicochemical studies, while fresh bulbs were used for the macroscopic and microscopic studies.



Fig 2: Underground bulbous part of *E. bulbosa*

2.3 Pharmacognostic studies

Organoleptic evaluation can be done by means of the organs of sense, and includes the macroscopic appearance of the plant material, its odour and taste. Organoleptic studies of fresh bulb as well as dried bulb powder were done and recorded. A thin section of the fresh bulb was cut by free hand sectioning and stained with Toluidine Blue O (TBO) (adjusted to pH 4.7) [4]. Thin sections without stain were also examined. Photomicrographs were taken with the image analyzer (Leica DM).

2.4 Physicochemical evaluation

Physicochemical parameters such as moisture content, bulk density and tapped density, Inter-particle porosity, Carr's Index, Hausner Ratio, pH value (of 5% aqueous solution), total ash, acid insoluble and water soluble ash were determined according to standard procedures [5, 6, 7, 8, 9].

2.4.1. Determination of Moisture content

One gram of bulb powder was weighed and dried at 80 °C for 24h in a hot air oven. After 24h, the powder was weighed

again and the difference in the weight was determined. The percentage of moisture was calculated.

2.4.2. Bulk Density and tapped density

About 25g of bulb powder was weighed and poured in to a 100ml measuring cylinder and the volume noted. Then the cylinder was gently tapped and again the volume was noted. The initial volume gave the bulk density value and after tapping the volume reduced, it gives the value of tapped density. Bulk density (D_b) = $\frac{M}{V_b}$ where, M is the mass of particles and V_b is the total volume of packing. Tapped density (D_t) = $\frac{M}{V_t}$ where, M is the mass of the powder taken and V_t is the final volume after tapping. The interparticle porosity, (I_p) = $\frac{D_t - D_b}{D_t \times D_b}$.

2.4.3. Hausner ratio

Hausner ratio is related to inter particle friction and as such can be used to predict the powder flow properties. Hausner ratio = $\frac{D_t}{D_b}$ where, D_t is the tapped density and D_b is the bulk density. Values less than 1.25 indicate good flow and a value greater than 1.25 indicates poor flow.

2.4.4. Carr's index

Carr's index is another indirect method of measuring the powder flow from bulk density. Carr's index (%) = $\frac{D_t - D_b}{D_t} \times 100$ where, D_t is the tapped density and D_b is the bulk density. The bulk density and tapped density were expressed in grams per millilitre (g/ml). The value below 25% indicates good flow characteristics and a value above 25% indicates poor flow characteristics.

2.4.5. Determination of pH

About 5% (w/v) of the powder was kept on a shaker for 5h with 140rpm and filtered. The filtrate was analysed using the pH meter (Elico, India).

2.4.6. Determination of total ash content

A clean and dry crucible (silica) was weighed and its weight was noted. About 10g of powder was weighed in a crucible and the powder was kept in a muffle furnace and heated up to 300 °C for 3-4h until the whole powder turned into ash. The crucible was cooled and weighed again. The difference in the weight was noted and percent of total ash was calculated

2.4.7. Determination of water soluble ash

One gram of ash was weighed and 10ml of distilled water was added into it. The mixture was kept in a shaker at 140rpm for 8h and filtered through ash less filter paper (Whatman No.1). The ash remaining in the paper was kept in a crucible and burnt to ash in a muffle furnace for 3-4h. The weight of the ash obtained was noted and percent of water soluble ash was determined.

2.4.8. Determination of acid insoluble ash.

One gram of ash was weighed and 10mL of concentrated H_2SO_4 was added to it. The mixture was kept on a shaker at 140rpm for 8h and filtered through ashless filter paper

(Whatman No.1). The ash remaining in the paper was kept in a crucible (silica) and burnt to ash in a muffle furnace for 3-4h. The weight of the ash obtained was noted and percent of acid insoluble ash was determined.

2.5 Fluorescence study

Fluorescence study is an essential parameter for the first line standardization of crude drugs. The powdered material was treated separately with different reagents and exposed to visible and UV lights (short UV= 254nm and long UV = 365nm) in order to analyse their fluorescence behaviour [10, 11, 12, 13, 14].

3. Result and Discussion

3.1. Pharmacognostic studies

The organoleptic studies of fresh bulbs revealed that the fresh bulb was purplish red in colour with a pungent odour and bitter taste. The bulbs were 5-7 layered (Fig.3.) and had papery scale leaves as an outer covering in mature condition. The bulb powder was light red in colour, had a pungent smell and bitter taste. Bulb powder was composed of smooth fine particles which had a free flowing nature Transverse sections (TS) of the bulb showed the following layered bulbs (5-7 layers) in a coiled pattern (Fig.4). Some cells in the upper epidermis were filled with a shiny red coloured liquid (Fig 5). Styloid crystals were seen in the ground tissue (Fig.6). There were large and small vascular bundles and were scattered in the parenchymatous ground tissue (Fig.7) and were collateral and closed type. The organoleptic or macroscopic evaluation of the medicinally important part of the plants plays a crucial role in the construction of plant monographs.

3.2 Physicochemical evaluation

The various physicochemical constants such as moisture content, bulk density, tapped density, inter-particle porosity, Carr's Index, Hausner Ratio and pH value of 5% aqueous solution, total ash, water soluble ash, acid insoluble ash were determined and are provided in Table 1.

The moisture content was 9.9%. The general requirement for moisture content in crude drug is suggested not to exceed 14% [15, 16]. The moisture content of the bulb powder is 9.9%. A low amount of moisture is preferred in plant powders which are to be used as drugs since low moisture discourages bacterial, fungal, or yeast growth.

The bulk density (0.25gm/mL) and tapped density (0.29gm/mL) values were very close to each other. The inter particle porosity was 0.64. The Hausner ratio and Carr's index also indicated the free flowing nature of the bulb powder. The pH value of 5% aqueous solution was 8.03 and indicated that bulb powder had an alkaline nature. Total ash value was 3.2%. The low value indicated the purity of the dried bulb powder. Acid insoluble ash value (9.4%) of the bulb powder of *E. bulbosa* is higher than the total ash value (3.12%). The higher acid-insoluble ash value of the *E. bulbosa* bulb powder also confirmed the presence of styloid crystals which is chemically calcium oxalate.

3.3 Fluorescence studies

Fluorescence studies of *E. bulbosa* bulb powder in different solvents under visible, short UV (254nm) and long UV

(365nm) light are summarized in Table 2. It was found that the powder was red in visible light and different shades of red in short and long UV. In visible light with the different reagents the powder showed shades of orange and yellow colour. In short UV with almost all reagents the bulb powder showed shades of green. But in long UV, with different reagents, the bulb powder showed a wide range of colours.

Table 1: Physicochemical constants of *Eleutherine bulbosa*

Sl. no.	Constants	Values
1	Moisture content	9.9%
2	Bulk density(D _b)	0.25gm/mL
3	Tapped density(D _t)	0.29gm/mL
4	Inter particle porosity(I _p)	0.64
5	Hausner ratio	1.188
6	Carr's Index	16%
7	pH value of 5% aqueous solution	8.03
8	Total ash	3.12%
9	Water soluble ash	1.2%
10	Acid insoluble ash	9.4%

Table 2: Fluorescence studies of the bulb powder of *Eleutherine bulbosa*

Sl. No.	Sample +Reagent	Observations under		
		Visible light	Short UV (254nm)	Long UV (365nm)
1	Powder as such	Light red	Jasper red	Plum purple
2.	Powder + water	Mars orange	Spinach green	Ivy green
3.	Powder + 50% HCl	Cadmium orange	Spinach green	Current red
4.	Powder + 50% H ₂ SO ₄	Marigold orange	Schules green	Garnet brown
5.	Powder +50% HNO ₃	Lemon yellow	Cyprus green	Oxblood red
6.	Powder + 4N NaOH	Garnet brown	Parsley green	Garnet brown
7.	Powder + NaOH in methanol	Jasper red	Pod green	Delft rose
8.	Powder + Acetic acid	Citron green	Cyprus green	Garnet brown
9.	Powder + methanol	Chrome yellow	Cyprus green	Garnet brown



Fig 3: Concentric layers of bulb



Fig 4: Transverse section of the bulb

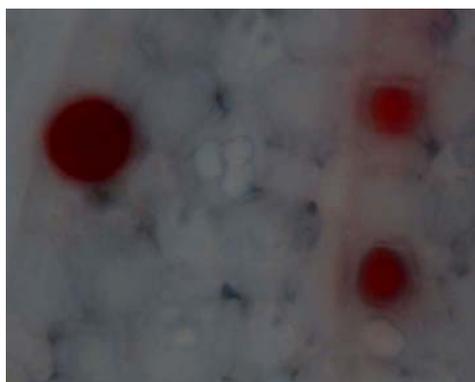


Fig 5: Red shiny material in epidermal cells

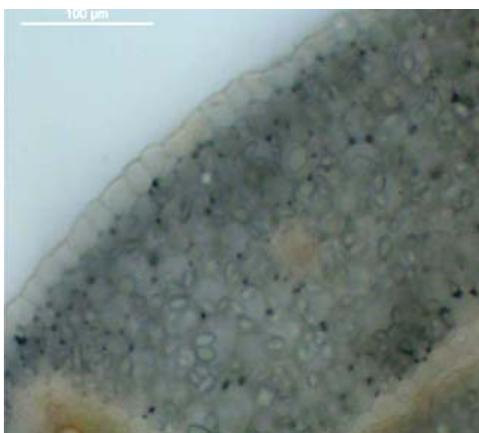


Fig 6: Ground tissue with styloid crystals

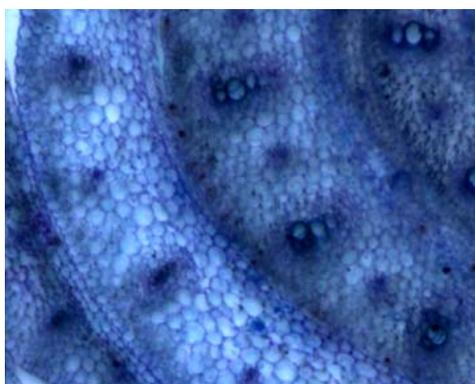


Fig 7: Scattered vascular bundles

4. Conclusion

The pharmacognostical standardization of *E. bulbosa* done presently would provide valuable foundation data for similar studies in future. The macroscopic, microscopic and physicochemical analysis will be useful in the proper identification, collection and investigation of the plant. The physicochemical investigation of crude drugs plays a crucial role in detecting adulteration. It is envisaged that the above information will serve as a standard data for the quality control of the preparations containing bulbs of this plant.

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6. References

1. Kuriachan PI, Cherian M. A preliminary study of the cytotoxic effects of the medicinal plant *Eleutherine bulbosa* using *Allium cepa* root tip meristem. Proceedings of the Kerala Science Congress, 1989, 188-189.
2. Panda SK, Patra N, Sahoo G, Bastia AK, Dutta SK. Anti-diarrhoeal activities of medicinal plants in Similipal Biosphere, Odisha, India. International Journal of Medicinal and Aromatic Plants. 2012; 2(1):123-134.
3. Lalitharani S, Mohan VR, Maruthupandian A. Pharmacognostic investigations on *Bulbophyllum albidum* (Wight) Hook f, International Journal of PharmTech Research. 2011; 3(1):556-562.
4. O'Brien TP, Feder N, dMc Cull ME. Polychromatic staining of plant cell walls by toluidine blue -O. Protoplasma 1964; 59:364-373.
5. *European Pharmacopoeia*. European Directorate for the quality of medicines, Council of Europe, Strasbourg, France, Edn 4, 2005, 37.
6. Iqbal D, Pawar RK, Sharma RK. Physico-chemical standardization of *Butea monosperma* (Lam) Kuntze (Palasha): An Ayurvedic drug. International Journal of Pharmaceutical Quality Assurance. 2010; 2(1):49-51.
7. Gupta AK. Quality standards of Indian Medicinal plants. Indian council for Medical Research, India, 2003, 177.
8. Indrayan AK, Sharma S, Durgapal D, Kumar N, Kumar M. Determination of nutritive value and analysis of mineral elements for some medicinally valued plants. *Current Science* 2005; 89:1252-1255.
9. Ahmad RV, Sharma RK. Evaluation of drug for standardization. Proceedings of WHO training cum workshop. Pharmaceutical lab for Indian Medicine. Ministry of Health and family welfare. Government of India, Ghaziabad, 2001.
10. Wilson RF. Horticultural Colour chart. Henry stone and son Ltd. Banbury, Great Britain 1938; 1:1-100.
11. Wilson RF. Horticultural Colour chart. Henry stone and son Ltd. Banbury, Great Britain 1939; 2:101-200.
12. Chase CR, Pratt RF. Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification, Journal of American Pharmacists Association. 1949; 38(6):324-331.

13. KoKoshi J, KoKoshi R. Fluorescence of powdered vegetable drug under ultra violet radiation. *Journal of American Pharmacists Association*. 1958; 47(10):715.
14. Kulkarni YA, Gokhale SB, Yele SU, Surana SJ, Tatiya AU. Preliminary phytochemical investigations on the bark of *Persea macrantha* (Nees) Kosterm (Lauraceae). *Indian Journal of Natural Products and Resources*. 2011; 2(2):211-217.
15. African Pharmacopoeia. General methods for analysis (OAU/STRC) Lagos 1986; 1(2):123.
16. Chand S. Importance of pharmacognostic study of medicinal plants: An overview. *Journal of Pharmacognosy and Phytochemistry*. 2014; 2(5):69-73.