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Pharmacognostic Studies on Rudraksh (*Elaeocarpus angustifolius* Blume) Fruit

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Abstract: *Elaeocarpus angustifolius* Blume fruit is used traditionally for its medicinal properties by local people in Indian sub-continent for cure of various ailments and pharmacological activities like anti-convulsant, anti-hypertension, anti-inflammatory and antimicrobial activity of rudraksh as reported by researchers. In present work phytochemical screening, proximate composition, elemental analysis and microscopical studies of *Elaeocarpus angustifolius* Blume fruit were carried out. Phytochemical screening of fruit pulp extracts showed the presence of glycosides, flavonoids, saponins, alkaloids, steroids, tannins and phenolic compounds. The pulp proximate composition analysis showed percentual average value for moisture, protein, fat and ash as 4.2, 4.28, 1.9 and 1.55, respectively. The elemental analysis of fruit pulp showed carbon (44.78 %), hydrogen (4.54%), nitrogen (0.33%) and oxygen (35.66%). The C/N ratio was found to be 134.47, which support the proximate analysis indicating the protein content 4.28%. Anatomical characterization of the dried powder of *Elaeocarpus angustifolius* bead and pulp revealed important elements for their recognition and taxonomy, including the pattern of epidermal cells, crystals, stone cells, cork cells, vessels (xylem and phloem) and sclerenchyma, parenchyma and other characteristics. The anatomical study revealed key elements for the recognition of *Elaeocarpus angustifolius* fruit when reduced to fragments. These studies may further be useful in identification of fruit and elemental & proximate analysis revealed the nutritive importance of fruit as food supplements. The phytochemical screening strengthens the traditional use of fruit for its medicinal values.

Key words: *Elaeocarpus angustifolius* Blume · Elemental analysis · Microscopy · Phytochemical screening · Proximate composition

INTRODUCTION

Elaeocarpaceae family includes approximately 350 species, distributed in tropical and subtropical region of the world. In Indian subcontinent it is widely distributed around India (around 25 species), Bangladesh, Bhutan, Nepal, Pakistan and Sri Lanka [1, 2]. *Elaeocarpus sphaericus* (Rudraksh) is an evergreen tree and its geographical distribution is in central and north east states in India. It flowers mainly in the rainy season, the flowers being white and hermaphroditic. The plant bear fruits in the months of November and December. The outer husk of rudraksh fruit turns blue from green upon ripe. In India, Rudraksh (*Elaeocarpus angustifolius*; syn. *E. sphaericus*) is known as holy tree as belonging to Lord Shiva according to Hindu religion.

Traditionally the plant is used for medicinal properties by local people for cure of various diseases. Researchers have reported pharmacological properties like anticonvulasant, antimicrobial, antidiabetic, antihypertensive, antidepressant, analgesic & anti-inflammatory activity and anxiolytic effect of rudraksh [3-10]. Scientific practices on different prospective on rudraksh is in progress and phytoconstituents isolated from various plants showed potent biological activities [11-13]. Phytochemical screening has led to isolation of various indolizine alkaloids from *Elaeocarpus* family which are considered as potential chemotherapeutic agents for cancer, diabetes, viral infections and immunosuppressive deficiencies [14-18]. Medicinal and healing properties of this plant are reported in ancient literature [19]. Researchers are continuously exploring the

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plants and their parts by using different techniques including microscopy and other analytical techniques for their identification. The pharmacologically active phytoconstituents are further explored by using different drug design techniques [20-24]. The present work aimed to study phytoconstituents, proximate composition, elemental analysis and microscopy of *Elaeocarpus angustifolius* Blume fruit.

MATERIALS AND METHODS

Collection of Plant Material: The fruits of *Elaeocarpus angustifolius* Blume were collected from Dehradun, India, in June 2015. The plant material was authenticated by Botanical Survey of India, Northern Regional Centre, Dehradun, Uttarakhand, India (voucher specimen no. 115875). The fruit pulp was manually removed from the bead and dried at room temperature under the shade and lyophilised. Bead was also dried at room temp under shade and powdered. The proximate and mineral analyses of pulp powder were carried out to determine the moisture content, nutritional composition as crude protein, total fat& ash values and elemental composition.

Microscopical Analysis: Dried powdered samples of bead and fruit pulp of *Elaeocarpus angustifolius* were analyzed by using optical microscope. Anatomical and histochemical studies were carried out for after fixing samples in glycerol. Samples were stained with safranin (CI), iodine, Congo red, basic fuchsin (CI 42500), eosin, alcian blue (CI 74240) and haematoxylin. Slide assembly was carried out by applying glycerol after dehydration of the histological sections with ethanol as per standard procedure [25, 26].

Phytochemical Studies: Phytochemical investigations of hexane, ethanol and ethanol: water (1:1) extracts of fruit pulp of *Elaeocarpus angustifolius* were carried out for the presence of flavonoids with metallic magnesium and HCl, tannins with Ferric chloride reagent, alkaloids with Draggendorff's reagent, cardiac glycosides with Liebermann's test and Killer killiani test, anthraquinones with Borntrager's test, saponins with ability to form foam, reducing sugars with Fehling's reagent, triterpenes and steroids with sulphuric acid reagent according to standard methods [27, 28].

Proximate Analysis: Moisture content, protein, fat and ash values of *Elaeocarpus angustifolius* fruit pulp powder were studied according to standard procedure given in Indian Pharmacopoeia [29]. Total carbohydrate content of fruit was also determined.

Elemental Analysis: In present study, Thermo Flash 2000 CHNS/O Analyser (Thermo Scientific, US) available at SAIF Department of Panjab University, Chandigarh, India was used for elemental analysis. The fruit pulp of *Elaeocarpus angustifolius* (0.8mg) was weighed in tin sample container and stored in auto sampler. The sample is dropped in to the combustion reactor. The gas mixture was passed into the gas chromatographic column CC1 for separation of elements. The eluted gases were conveyed to the thermal conductivity detector TCD which generated electrical signals, which properly processed by the Eager 300 software, provided the Nitrogen, Carbon, Hydrogen and Sulphur percentages contained in the pulp powder of *Elaeocarpus angustifolius* [30-31].

Statistical Analysis: The statistical analysis of proximate and elemental composition data were carried out to draw the significance information. The analysis of data was carried out according to a completely randomized design, representing the mean values of triplicate analysis or more, depending on the availability of the data. Similarly data was also subjected to analysis of variance using the general linear models procedure of SAS [32].

RESULTS AND DISCUSSION

Microscopical Analysis

Microscopy of Bead Powder: Microscopy of *Elaeocarpus angustifolius* bead powder showed presence of lignified cork in surface view, tannin contents from cortex, lignified fibers of xylem, crystal fibers, simple starch grains, prismatic crystals of calcium oxalate, lignified parenchyma, stone cells and border pitted vessels of xylem at 40X (Fig. 1) and 100X zoom (Fig. 2). The bead powder showed elliptical cells in the uniseriate epidermis of abaxial face with periclinal walls of thicker and conspicuous cuticle. The epidermal detachment of this region revealed that the epidermis is formed by common polygonal cells with thick anticlinal walls and stomata. The presence of the parenchymal tissue, with smaller cells near the epidermal layers and larger cells in the middle region branched parenchyma cells and prominent intercellular spaces were observed. Various idioblasts containing starch grains or raphides of calcium oxalate were observed among the parenchyma cells.

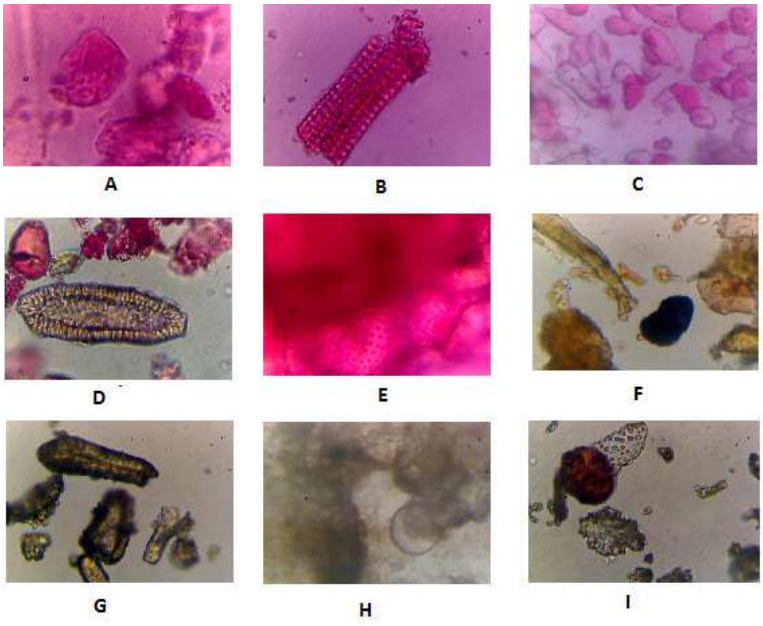

Fig. 1: Powder microscopy of bead powder of *Elaeocarpus angustifolius* Blume at 40X shows (A) Parenchymatous cells (stained) (B) Xylem fibres (C) Parenchymatous cells (stained and unstained) (D) Collenchymatous cell (E) Border pitted vessel (stained) (F) Starch grains (stained) (G) Stone cells (H) Oil globule (I) Border pitted vessels and Tannin-containing cell

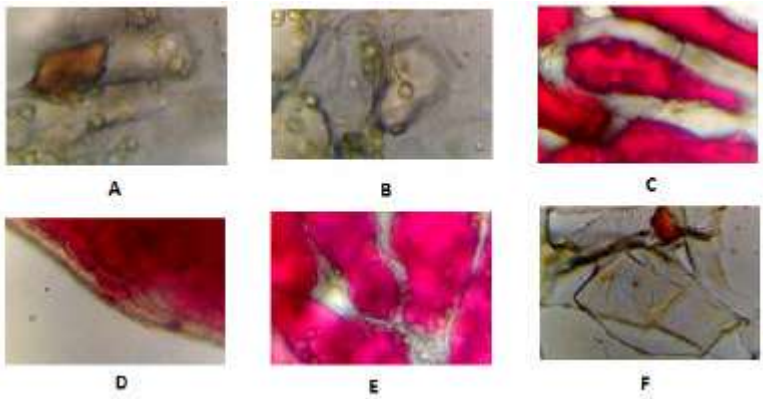

Fig. 2: Powder microscopy of bead powder of *Elaeocarpus angustifolius* Blume at 100X shows (A) Tanning containing cells (B) Parenchymatous cells (unstained) (C) Parenchymatous cells (stained) (D) Epidermal cells with cuticle (E) Starch grains (F) Calcium oxalate crystals.

Collateral vascular bundles and strands of sclerenchyma fibers were observed in the bead powder. The power also showed the presence of lignified parenchyma, tylosis, tannin containing material and border pitted vessels from xylem, stone cells.

Microscopy of Fruit Pulp Powder: Microscopy of *Elaeocarpus angustifolius* pulp powder showed presence of epidermal layer of epicarp. The epidermal cells were very narrowly oblong with prominent cuticle. Beneath the epidermis was a layer of sub-epidermal cells which were wider, tangentially oblong occurring parallel to the epidermis forming mesocarp. It consisted of several layers of parenchyma cells. These cells were homogeneous, thin walled, polyhedral in shape and compact. Several vascular strands found scattered in the parenchymatous mesocarp. The vascular bundles were found small and varied in size & shape. Xylem and phloem elements were observed in vascular bundles. Xylem elements were seen highly thick walled. Phloem elements were consisted of fairly large

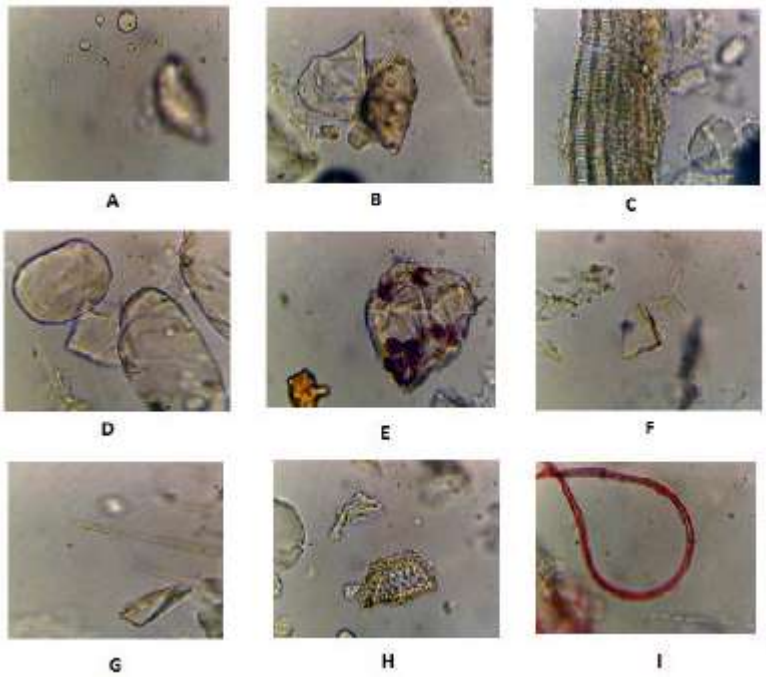

Fig. 3: Powder microscopy of pulp powder of *Elaeocarpus angustifolius* Blume at 100X shows (a) Oil globules (b) Tannin containing cells (c) Strip of vascular bundles (d) Parenchymatous cells (e) Starch grains (f) Calcium oxalate crystals (g) Dumble shaped stomata (h) Border pitted vessels (i) Stained fibre.

sieve tubes and companion cells. Powder microscopic characters of the fruit exhibit the masses of parenchymatous cells and thin pieces of epicarp or epidermal cells, dumble shaped stomata, lignified cells, starch grains (stained with iodine), calcium oxalate crystals, fibre etc. (Fig. 3).

Anatomical data may be used for improving plant classification and identify different species. Most of the plants have been recognized on the basis of their distinctive anatomy. According to Dickson epidermis, trichomes and stomata, or inclusions in the parenchyma, have great importance in the identification of plants [33]. In the case of *Elaeocarpus angustifolius* bead and pulp powder, the constituents are difficult to detect by methods other than microscopy [34]. For this reason, the anatomical studies were carried out in order to characterize the *Elaeocarpus angustifolius* bead and pulp with the objective of improving recognition of this plant.

Phytochemical Analysis: The ethanol and ethanol: water (1:1) extracts of pulp powder of *Elaeocarpus angustifolius* showed the presence of glycosides, flavonoids, saponins, alkaloids, steroids, tannins and phenolic compounds. The phytochemical screening of fruit pulp extracts showed the presence of medicinally important class of compounds (Table 1).

Table 1: Phytochemical Screening of Fruit Pulp Extracts

Test	Hexane Ext.	Ethanol Ext.	Ethanol: Water (1:1) Ext.
Carbohydrates	-	+++	+++
Reducing Sugar	-	+++	+++
Monosaccharides	-	-	-
Non-Reducing Sugar	-	-	-
Gum	-	+	+
Protein	-	-	-
Steroids	-	+	-
Saponin glycosides	-	++	-
Flavonoids	-	+++	++
Alkaloids	-	-	-
Tannins and Phenolic Compounds	-	++	++

Proximate Composition: The results of proximate analysis of *Elaeocarpus angustifolius* pulp powder are presented in Table 2. The average (%) contents of moisture, protein, fat and ash in *Elaeocarpus angustifolius* pulp were 4.2, 4.28, 1.9 and 1.55, respectively. However, total carbohydrate content (81.8 g/100 g) was found to be above the average, probably due to dietary fibre present in *Elaeocarpus angustifolius* pulp [35].

Elemental Content: The elemental analysis revealed composition of fruit pulp as carbon (44.7863731%), hydrogen (4.5457015%), nitrogen (0.333039%) and oxygen

Table 2: Proximate composition of *Elaeocarpus angustifolius* Pulp

Chemical analysis	Content * (%)
Moisture	4.2 ± 0.58
Protein	4.28± 0.37
Fat	1.9± 0.23
Ash	1.55± 0.12
Carbohydrate	81.8± 0.42
TEV (Kcal/g)	362.05± 0.53

* Means of three determination ± SD.
TEV- Total Energetic Value

Table 3: Elemental analysis of *Elaeocarpus angustifolius* pulp

Element	Content [a] (%)
Carbon	44.786373
Hydrogen	4.545701
Nitrogen	0.333039
Oxygen	35.6692
Sulphur	0.0
C/N ratio	134.47

[a]Means of three determination ± SD.

(35.6692%). The C/N ratio was found to be 134.47, which supports the proximate analysis indicating the protein content 4.28%. The elemental analysis of *Elaeocarpus angustifolius* pulp is presented in Table 3.

CONCLUSIONS

Phytochemical screening of *Elaeocarpus angustifolius* Blume fruit pulp extracts confirmed the presence of glycosides, flavonoids, saponins, alkaloids, steroids, tannins and phenolic compounds. The pulp proximate composition analysis revelled percentual average value for moisture, protein, fat and ash as 4.2, 4.28, 1.9 and 1.55, respectively. The elemental analysis of fruit pulp showed carbon, hydrogen, nitrogen and oxygen as 44.78, 4.54, 0.33 & 35.66% respectively. The C/N ratio was found to be 134.47, which support the proximate analysis indicating the protein content 4.28%. The analysis of pulp powder demonstrated the considerable nutritional value and low caloric content. In view of the high nutritional value of pulp power, *Elaeocarpus angustifolius* fruit can be applied in diets in the form of dehydrated flour, easily incorporated into food. Based on the results of the present study, however, it was found that introducing rudraksh pulp into the human diet could have significant nutritive impact. Anatomical characterization of the dried powder of *Elaeocarpus angustifolius* Blume bead and pulp reflected important elements for their recognition and taxonomy, including the pattern of epidermal cells, crystals, stone cells, cork cells, xylem & phloem vessels and sclerenchyma, parenchyma and other characteristics. The anatomical study revealed key elements for the recognition of *Elaeocarpus angustifolius* fruit when reduced to fragments. These studies may be further useful in identification of fruit and elemental & proximate analysis indicated the nutritive importance of fruit. The phytochemical screening strengthens the traditional use of fruit for its medicinal values.

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