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## PHARMACOGNOSTICAL AND PHYSICO-CHEMICAL EVALUATION OF AN INDO-SRI LANKAN ETHNOMEDICINAL PLANT SPECIES *POLYALTHIA KORINTI* (DUNAL) BENTH. & HOOK. F.

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**ABSTRACT:** *Polyalthia korinti* (Dunal) Benth. & Hook. F. is a rare Indo - Sri Lankan species of Annonaceae family and are traditionally used by tribal people of Ghats region, India for treating several ailments. The number of mature plants and the area of its distribution are too low and this species is placed under the vulnerable critically endangered category. The present study deals with the pharmacognostic and physicochemical evaluations of the leafy part of this plant and was undertaken as an initial step to aid in documentation, quality assurance, proper identification *etc.* and appropriately to investigate the plant material for its ethnomedicinal claims. Macro and micro morphological parameters of leaf and dried leaves powder were set down following standard procedures recommended by Ayurvedic Pharmacopoeia of India. Macroscopic study of leaf revealed some of the characteristic features like size, shape and color and in microscopic study internal characteristic features like epidermis, cortex, xylem, phloem, trichomes, and stomata were noted. Physicochemical parameters like total ash value, water soluble ash, acid insoluble ash, sulphated ash, foreign organic matter content, moisture content and crude fiber content were determined. Fluorescence analysis with various reagents showed characteristic coloration at day (visible light) and under UV light. Extractive values showed that the methanol soluble phytoconstituents were high compared to other solvents. This study thus lays down the pharmacognostic and physicochemical standardization parameters of the *Polyalthia korinti* leaf for the first time to serve the identification and quality control purposes.

**INTRODUCTION:** The genus *Polyalthia* belonging to Annonaceae family is widely distributed in tropics and subtropics and consists of mainly shrubs and trees. Genus *Polyalthia* comprises about 120 species <sup>1</sup>, of which fourteen species are seen in India <sup>2</sup> and seven among them are indigenous to India <sup>3</sup>. *Polyalthia* plants are being used in folk medicine around the tropics.

*Polyalthia longifolia* is a common plant used by most of the folks and is considered a medicinal plant in India. It's antimicrobial, cytotoxic and hypotensive properties have been validated through scientific studies. Tribal natives of Eastern Ghats use *Polyalthia cerasoides* stem bark along with calcium and turmeric paste to apply on fractured bone <sup>4</sup>. The stem bark of this plant is also used by the Orissa tribes to treat diabetes <sup>5</sup>.

In tribal pockets of Gujarat, crushed stem bark and poultice of leaves is applied as antiseptic on cuts and wounds for fast healing <sup>6</sup>. *P. fragrans* is an ingredient of the medicine used in children for cough and anemia <sup>7</sup>. The genus is also well known in Chinese folk medicine for its applications

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against a number of ailments such as stomach ache, dysmenorrhea and of pharynx<sup>8</sup>. In Thailand, the plants of *Polyalthia* are used as galactagogue, to treat skin infections and for birth control<sup>1</sup>.

*Polyalthia korinti* is an Indo - Sri Lankan species. It grows as a shrub or small tree, 3 - 5 m tall, found spreading their young branches, with edible fruits<sup>9</sup> and is mainly confined to peninsular India. It is commonly called "Karuvalli" and "Korintipanel" in Malayalam, "Uluvintai" in Tamil and "Ulkenda" in Sinhala. Several researchers have reported the presence of these species in the Eastern Ghats<sup>10</sup> and the southern Western Ghats<sup>11</sup> of India. *P. korinti* has ethnomedicinal claims and is mostly used by tribes as these plants are highly confined and available to those areas. For instance, Tribes of Kambakam Hills, Eastern Ghats, India uses the root bark for the cure of stomach ache<sup>12</sup>. The village folk at Kadapa district of Andhra Pradesh, India use oral administration of root powder decoction as a good antidote for Russell Viper bite<sup>13</sup>.

In spite of the ethnopharmacological significance of this plant, literature review shows that this species is scientifically unexplored and the availability possess the limit to its exploitation. Thus there is an indispensable need to first set up parameters to identify and authenticate the plant specimen before any study is carried out. In this regard, the present work is aimed at standardization of the leafy parts of this pharmacologically important endemic species from peninsular India, in reference to its pharmacognostic and physicochemical characteristics.

## **MATERIALS AND METHODS:**

**Chemicals:** Solvents used for extraction viz. petroleum ether, chloroform, ethyl acetate and methanol were obtained from Merck India Ltd. The deionized water was used for reagent preparation. All other chemicals were of analytical grade.

### **Plant Material Collection and Authentication:**

Disease free fresh *Polyalthia korinti* leaves were collected from the Calicut University Botanical Garden (CUBG), University Campus, Kerala, India. The plant was authenticated by Dr. A.K. Pradeep, Department of Botany, University of Calicut and a voucher specimen (accession no: 6917) was deposited at the University herbarium.

The leaves were cleaned with distilled water, dried in shade and crushed into coarse powder using blender for physicochemical studies and extract preparation.

**Macroscopic and Microscopic Evaluations:** The studies were done following standard procedures recommended by Ayurvedic Pharmacopoeia of India. Various macroscopic characters of the leaf were noted for usual parameters like color and size. These parameters describe the shape and surface characters. The leaves were transverse sectioned through midrib and used for microscopic examination<sup>13</sup>.

**Physicochemical Analysis:** The determination of ash values (total ash, water soluble ash, acid insoluble ash, and sulphated ash) were carried out as per the methods of Indian Pharmacopoeia<sup>14</sup>. Assessment of foreign organic matter<sup>15</sup>, moisture content<sup>16</sup> and crude fiber content<sup>17</sup> were done following the methods of Ayurvedic Pharmacopoeia of India and Indian Standard.

**Fluorescence Analysis:** Fluorescence analysis is yet again another pharmacognostic procedure useful for the identification and authentication of samples from adulteration<sup>18</sup>. The coarsely powdered dried leaves of *P. korinti* were studied initially under day light and also under ultraviolet radiation. 0.5 g of the powder were taken in to each test tube and treated with 5 ml of various solvents like distilled water, acetone, benzene, chloroform, methanol, petroleum ether, and hexane; alkaline solutions like aqueous and alcoholic sodium hydroxide (1N) and potassium hydroxide (5%); acidic reagents viz. 1N hydrochloric acid, 50% sulphuric acid, 50% nitric acid, glacial acetic acid and picric acid; ferrous chloride solution, iodine solution and ammonia. All the tubes were properly mixed and allowed to stand for about 30 min. The solutions thus obtained were observed under visible light and UV light. The characteristic colors appeared were recorded<sup>19, 20</sup>.

### **Sequential Extraction using Organic Solvents:**

50 g of leaf powder was extracted sequentially using a rotary shaker with the solvents petroleum ether, chloroform, ethyl acetate and methanol in increasing order of polarity.

Extraction in each solvent was carried out thrice to completely extract all the phytoconstituents from leaves. Followed by each solvent extraction, the leaf residues were air dried and then the next solvent was added. Solvent extracts were filtered through Whatman no.1 filter paper and the filtrates were kept for complete evaporation of the solvents to obtain the dry extract. These extracts were weighed and percentage yields were calculated. Properties like color and consistency of the extracts were also noted.

**RESULTS:**

**Macroscopic and Microscopic Evaluation:** Leaf is oval in shape, entire, apiculate with rounded leaf base and short petiole. The adaxial surface is green and abaxial is dull green. The surface is smooth and shiny. Mature leaf is 7.5 - 9 cm long **Table 1** and **Fig. 1**.

**TABLE 1: MACROSCOPIC CHARACTERS OF POLYALTHIA KORINTI LEAF**

Parameters	Leaf
Color	Green
Shape	Oval
Length	7.5-9 cm
Breadth	3.5-5 cm
Base	Rounded
Margin	Entire
Apex	Apiculate
Texture	Shiny
Touch	Smooth

Microscopic analysis revealed the following characteristics. The transverse section of the leaf through the midrib showed the presence of upper and lower epidermis with trichomes. Epidermis is covered with cuticle. Collenchyma is seen below the epidermis. Stele is located at the center position. Stele consists of xylem and phloem. Vascular region is surrounded by lignified fibers called the bundle sheath.



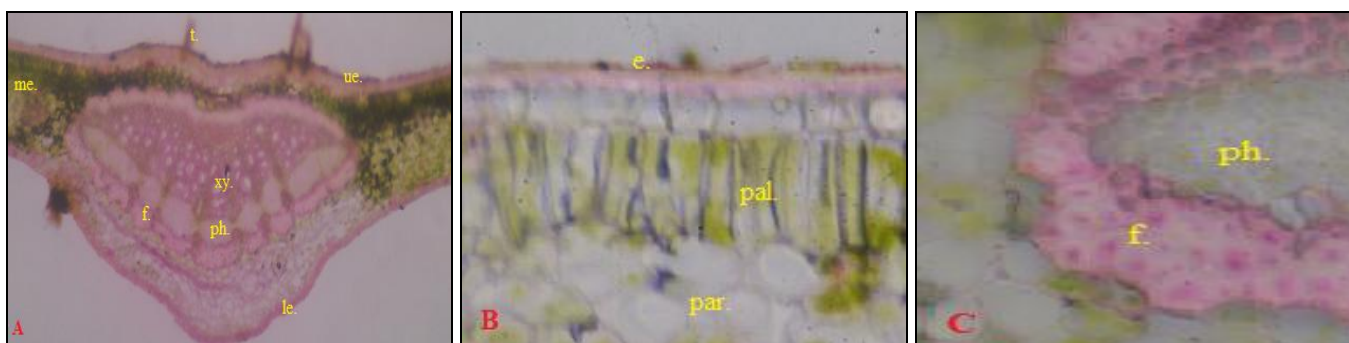
**FIG. 1: MORPHOLOGY OF P. KORINTI LEAF**

Leaf lamina is narrow, dorsiventral with upper and lower epidermis covered with cuticle and hairs. Stomata are present. Mesophyll cells with palisade and spongy parenchyma were observed **Fig. 2**. The leaf powder microscopy revealed the presence of fragments of trichomes, spiral vessels, lignified fibers, collenchyma cells *etc.* It also showed fragments of mesophyll in both surface and transverse view **Fig. 3**.

**Physicochemical Evaluation:** Physicochemical parameters aids in assessing the purity and quality of a preparation and can be employed to rule out the presence of any adulterants. The total ash value obtained by complete incineration of the powdered leaves indirectly indicates the amount of inorganic salts inherent to the plant. The physicochemical parameters of *P. korinti* leaves are represented in **Table 2**.

**TABLE 2: PHYSICOCHEMICAL PARAMETERS OF P. KORINTI LEAF**

Parameters	Yield (w/w) (%)
Moisture content	11.16
Total ash	4.34
Acid-insoluble ash	0.008
Water-soluble ash	2.67
Sulphated ash	6.22
Crude fiber	19.63
Foreign organic matter	absent



**FIG. 2: TRANSVERSE SECTION OF P. KORINTI LEAF SHOWING t.: TRICHOME; e.: EPIDERMIS; ue.: UPPER EPIDERMIS; le.: LOWER EPIDERMIS; ph.: PHLOEM; xy.: XYLEM; me.: MESOPHYLL CELLS; f.: FIBERS**

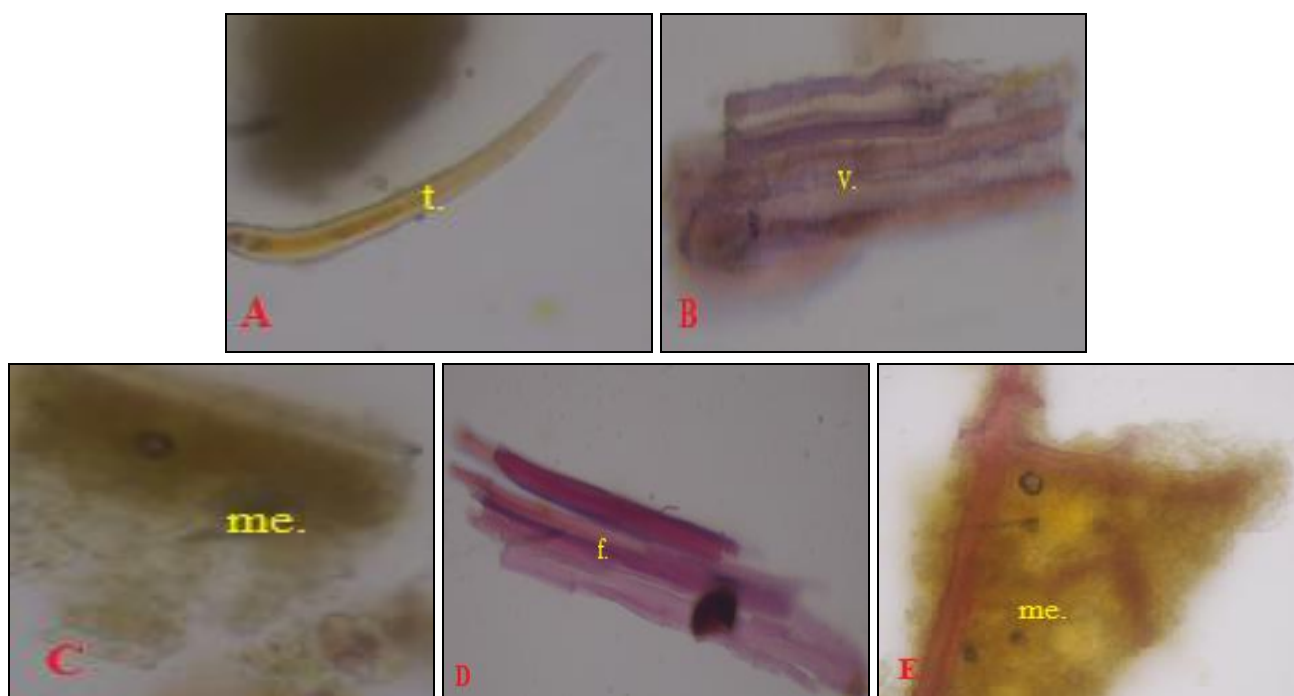


FIG. 3: POWDER MICROSCOPY OF LEAF POWDER SHOWING t.: TRICHOME; f.: FIBERS. v.: VESSEL; me.: MESOPHYLL CELLS

The extractive values and various properties of powdered leaves in different solvents obtained by successive extraction using rotary shaker are noted in **Table 3**.

**Fluorescence Study of Powdered Leaves:** This simple and rapid procedure makes it a highly valued aid in the identification and authentication of plant samples. The present study deals with the fluorescence analysis of the powdered leaves under ultraviolet radiation and also under day light. The observations are tabulated in **Table 4**.

TABLE 3: EXTRACTIVE VALUES AND PROPERTIES OF THE SUCCESSIVE EXTRACTS FROM *P. KORINTI* LEAVES

Extract	Extractive values (% w/w)	Consistency	Color under visible light
Petroleum ether	4.0	Sticky solid	Pale green
Chloroform	4.06	Sticky solid	Light green
Ethyl acetate	2.0	Sticky semi solid	Dark green
Methanol	14.0	Syrupy	Dark brown

TABLE 4: FLUORESCENCE ANALYSIS OF POWDER OF *P. KORINTI* LEAVES

S. no.	Powder Treatment	Color observed	
		Visible light	UV light (365 nm)
1	Powder as such	Dark green	Green and red tinted
2	Powder + distilled water	Yellowish green	Dark olive green
3	Powder + acetone	Greenish yellow	Orange
4	Powder + benzene	Yellowish green	Deep pink
5	Powder + chloroform	Green	Orange
6	Powder + methanol	Green	Sandy brown
7	Powder + petroleum ether	Pale yellow	Dark red
8	Powder + hexane	Very pale green	Bright red
9	Powder + glacial acetic acid	Yellow	Dark red
10	Powder + 50 % H <sub>2</sub> SO <sub>4</sub>	Brownish yellow	Sky blue
11	Powder + 50 % HNO <sub>3</sub>	Yellow	Yellow
12	Powder + 1N HCl	Pale yellow	Pale yellow
13	Powder + 5 % FeCl <sub>3</sub>	Black	Black
14	Powder + 5 % I <sub>2</sub>	Yellow	Pale yellow
15	Powder + picric acid	Bright yellow	Black
16	Powder + 1N aqueous NaOH	Bright yellow	Dark yellow
17	Powder + 1N alcoholic NaOH	Light green	Light pink
18	Powder + ammonia	Dark yellow	Pale green
19	Powder + 5% alcoholic KOH	Light green	Light pink
20	Powder + 5 % aqueous KOH	Dark yellow	Greenish yellow

**DISCUSSION:** According to the world health organization's terms on establishing quality standards and specifications for herbal preparations, the samples should be pharmacognostically and physicochemically validated. Apart from being an endangered species, the lack of scientific information about this plant, has proved this study indispensable to establish its identity and purity. In this regard, the macroscopic and microscopic evaluations of the *P. korinti* leaves were carried out and documented. Macroscopic studies will help in the correct identification of the plant. The fresh leaves showed cuticle covered epidermis with trichomes on it. Microscopic methods thus detail the minute structures and are one among the simplest and cheapest methods to initiate the study on unexplored species.

The physicochemical evaluations were done on dried leaves powder and it dictates the presence of adulterants and other impurities which may be present due to improper handling of the samples. In the present study, the absence of foreign organic matter in the leaves powder clearly states that the plant was healthy and the specimen collection and processing was followed with cleanliness. The moisture content of dry powder was 11.16%, which is pretty less, and hence it would discourage the infection with microorganisms, insects *etc.* Low moisture content will even discourage deterioration following hydrolysis and help in increasing the shelf life.

The total ash value is primarily important in the evaluation of purity of drugs. The residue remaining after the incineration of plant material represents the inorganic salts naturally found in it or deliberately added to it as adulterants. The ash usually consists of carbonates, phosphates and silicates of sodium, potassium, calcium and magnesium. Thus it indirectly determines the presence or absence of foreign matter such as metallic salts and silica.

Acid-insoluble ash is a part of total ash soluble in dilute hydrochloric acid and measures the amount of silica present, especially as sand and siliceous earth. Water soluble ash is the water soluble portion of the total ash<sup>21, 22</sup>. The total ash of powdered leaves of *P. korinti* was 4.34%, acid insoluble ash was 0.008%, water soluble ash was 2.67% and sulphated ash was 6.22%.

The value of a plant as an herbal formulation is dictated by the phytochemicals present in it and the exploitability of these phytoconstituents in turn depends on their extractability from the source in to an easily used form. Thus the dried leaves powder was subjected to successive extraction with various solvents in the order of their increasing polarity so as to fractionate different phytoconstituents and to reduce the number of phytochemicals extracted in each solvent. Here, the methanol showed higher extractive values (12%) followed by petroleum ether and chloroform with 4% each and finally ethyl acetate with the least. These variations in the extractable matter suggests that the plant leaves contain high amount of alcohol soluble substances.

Fluorescence analysis, yet another important pharmacognostical parameter is used to qualitatively assess the crude sample. Some phytochemicals are naturally fluorescent like berberine and some others fluoresce under longer UV wavelength. Some phytoconstituents can even be converted in to fluorescent derivatives or decomposition products by reaction with different reagents. For instance, quinine in dilute sulphuric acid solution exhibit fluorescence when illuminated with UV light<sup>21</sup>. The colors thus shown by leaf powder with various reagents could even suggest the presence of specific phytoconstituents. In the study **Table 4**, reaction with  $\text{FeCl}_3$  shows the presence of phenolic compounds. Further, the presence of flavonoids was detected with ammonia and NaOH reagents. Thus every leaf powder has its specific fluorescent colors produced when treated with different reagents. Any deviation from these colors thus indicates the presence of contamination with adulterants, microorganisms and other impurities. These observations indicate that the powder contains active phytochemicals which attributes to the pharmacological quality of the plant material.

**CONCLUSION:** The present investigation focused on the pharmacognostic and physicochemical analysis of *P. korinti* leaves, provides valuable information useful for the proper identification and authentication of this unexplored, endangered and endemic species. The macroscopy, microscopy and physicochemical parameters used here are being reported for the first time for this plant.

This pioneer work would thus aid in preparation of a suitable monograph of this plant and also would emphasis on the conservation of such species. Further studies are in progress regarding isolation, identification, characterization, structure elucidation and therapeutic activity evaluation of various pharmacologically active phytoconstituents from *P. korinti*.

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**CONFLICT OF INTEREST:** The authors wish to declare no conflict of interest.

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