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PHARMACOGNOSTICAL STANDARDIZATION OF KAILASPATI

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ABSTRACT: Pharmacognostical parameters for the leaves including petiole of Kailaspati was studied with the aim of drawing the Pharmacognostical standards for this species. Macroscopical and microscopical characters of leaves and petiole, powder characteristics studies, fluorescence studies and thin layer chromatographic studies of the leaves powder included. The study also deals with the preliminary phytochemical screening of the leaves and petioles of Kailaspati with various extracts such as petroleum ether to water. Glandular, non-glandular trichomes and starch grains were identified. The qualitative analysis of some secondary metabolites, to ascertain medicinal claims of this widely used medicinal plant. The results showed that the moderate presence of oils, alkaloids, glycosides, flavonoids, ketosteroids, amino acids, phenols, and triterpenoids. The study includes pharmacognostical standardization of Kailaspati with petiole first time.

INTRODUCTION: Kailaspati botanical name *Couroupita guianensis* Aubl. Is grown in Indian gardens as an ornamental tree for its beautiful flowers. It is known as 'Cannonball' tree in English and 'Kailaspati' in Hindi, and it belongs to the family Lecythidaceace. *Couroupita guianensis* is a large deciduous evergreen tree growing to a height of 20 meters. It is a medicinal plant which is endowed with curative properties including antifungal, antibiotic, antiseptic, analgesic, antimalarial, stomachache, toothache, scabies, gastritis, bleeding piles, dysentery, and scorpion poison [1].

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The fresh fruit pulp is used in the preparation of cooling medicinal drink, and various parts are useful in skin disease [2, 3]. The leaf has been found to show antioxidant activity, anthelmintic activity, immunomodulator, and anti-nociceptive activity [4]. The pulp of the fruit of the cannonball tree is rubbed on the infected skin of the dog. It is claimed that when the dog licks its skin, this medicine will also work internally [5]. The flowers are used to cure cold, intestinal gas formation and stomach ache[6]. Barks are used to treat hypertension, tumors, pain, and inflammatory process [7]. Different extracts of the flower have been screened for immunomodulatory activity [8, 9]. In flowers, mainly eugenol, linalool, and stigmasterol were identified. Leaves of *C. guianensis* are widely used as an analgesics medicine by the Brazilian rural population [10, 11].

This study is intended to establish, conventional pharmacognostical and modern pharmacognostical

parameters of leaves of the plant. These will be used as diagnostic features in the identification, evaluation and monograph preparation of the plant.

MATERIALS AND METHODS:

Procurement of Plant Material: Fresh leaves *C. guianensis* was collected from Nashik road (Dist. Nashik), Maharashtra, India in Aug-Sep 2012. Plant sample was authenticated by Ministry of Environment and Forest, Botanical Survey of India; Pune (Voucher specimen number MOPCOG1).

Preparation of Plant Material: Fresh mature leaves are stored in formalin solution, and powder of leaves of *C. guianensis* was prepared by passing through sieve # 44 and kept in airtight container.

Chemicals and Instruments: Photomicroscope (OLYMPUS Pvt. Ltd., New Delhi; Model- CH 2OiBIMF) provided '3V-MICRO' video attachment eyepiece device (Version 8) with 10x, eyepiece (12 megapixels) with cells tracking function and 4x digital zoom camera was used. Solvents and reagents were procured from Loba Chemicals, Mumbai, India.

Macroscopical Examinations: The macro-morphological features of the plant leaves were observed under a magnifying lens and simple microscope [12].

Microscopical Examinations: Fresh leaves and herbaceous petiole of the species were studied using transverse sections. The different parts of leaf-like lamina and midrib were studied according to the methods of Brain and Turner [13]. For the microscopical studies, cross sections were prepared and stained as per the procedure of K. R. Khandelwal [14]. The different lens of photo-microscope as, OLYMPUS iNEA 5X, 10X/0.2; India, and 100X/1.25 oil India were used for capturing the photographs.

Histochemical Studies: The dilute iodine solution, Dragendroffs reagent, dilute ferric chloride solution, Phloroglucinol + HCl, *etc*. The reagent treated a hard section of the plant tissue was observed and a microscope to detect the presence of histochemical components.

Powder Microscopy: A little quantity of leaves powder was taken onto a microscopic slide, 1-2 drops of 0.1% w/v phloroglucinol solution and a drop of concentrated hydrochloric acid were added and covered with a coverslip. The slide preparation was mounted in glycerol and examined under a microscope. The presence of starch grain was detected by the formation of blue color on the addition of 2-3 drops of 0.01 M iodine solution [15]. The characteristic structures and cell components were observed and their photographs were taken using photomicrography.

Physical Evaluation: The moisture content of the powdered determined based on the loss of drying method[16]. The ash values were determined, to find out about the physiological state and level of extraneous matter. Extractive values were determined according to the official methods prescribed in Ayurvedic Pharmacopoeia [17].

Phytochemical Investigation: The successive extractive values carry out as per the procedure of C. K. Kokate [18, 19].

Inorganic Elements and Fluorescence Analysis: Total ash of the drug was subjected to testing different inorganic constituents [20, 21]. Fluorescence analysis of successive leaf extract was done by the standard method of Chase and Pratt [22]. The behavior of drug powder with various chemicals was carried out as per Rathee [23].

TLC Finger Print Profile: Thin layer chromatography of the methanolic and ethanolic extract was studied, and R_f values were determined [24].

RESULTS AND DISCUSSION:

Macroscopic and Microscopic Examination: Macroscopically the fresh leaf of C. arborea is 14.5-18.0-21.6 cm in length and 3.85-5.34-7.90 cm in width and petiole 0.3-0.8-1.7 cm in length. The leaf is simple, glabrous, broadly obovate, acuminate apex with slightly toothed or entire, (tertiary veins subparallel, ± at right angles to midrib) margin and shiny dark green **Fig. 1**.

Trans-sections of Leaves: Lamina has a structure of dorsiventral type of leaf. The lamina shows upper and lower epidermises comprise uniseriate, polygonal to rectangle cells. The cuticle thickness is approximately the same on both epidermises in *C. guianensis*. There are covering and noncovering trichomes on both epidermises.

The palisade with a single layer of regular, long, columnar cells, beneath which, 2 to 3 layered mass of closely packed cells filled with chloroplast was present. Mesophyll is traversed by a large number of veins and is represented by groups of few spiral vessels.

Midrib shows concavo-convex outline in the basal and middle region. 3-5 layered collenchymas located below upper epidermises and continuous 2 to 3 layer collenchymas observed above lower epidermis. The vascular bundles are surrounded by a parenchymatic bundle sheath and spongy parenchyma cells. Scattered collateral vascular bundle as 5 numbers above the lower epidermis, 2-4 number below the upper epidermis and 2 numbers in middle surface were observed. The collateral vascular bundle is prominent, occupying the central portion of the midrib.

FIG. 1: MACROSCOPY OF *C. GUIANENSIS*

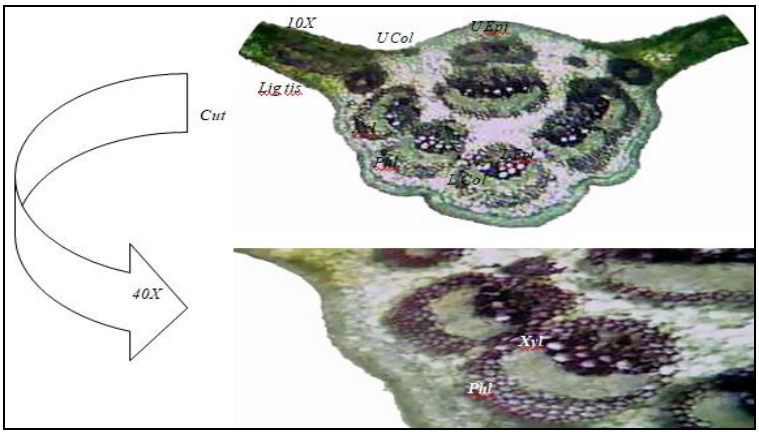


FIG. 2: TRANSVERSE SECTION OF *C. GUIANENSIS*

Petiole: Petiole anatomy of numerous arcuate or annular bundles in arcs. It shows two prominent grooves towards the upper side whereas lower side is round. The epidermis is composed of a single layer of cells. Few trichomes are observed on the lower epidermal cell which is identical with that of the leaf. Two to three layers of collenchymatous cells are found around the epidermal cell.

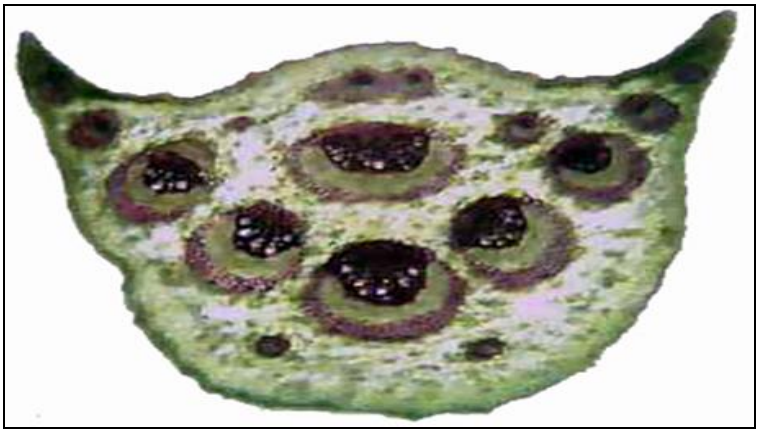
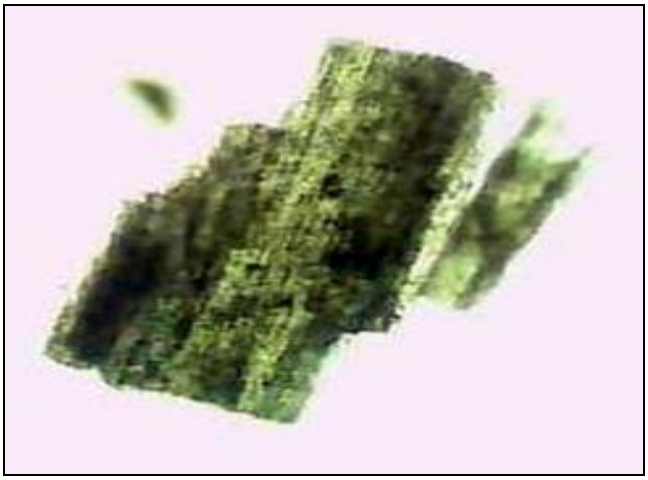


FIG. 3: TRANSVERSE SECTION OF PETIOLE OF *C. GUIANENSIS*

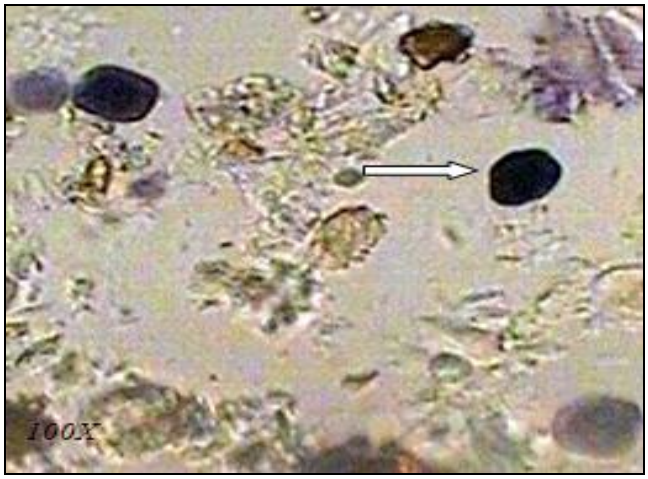
Eight to nine collateral vascular bundles are present in the ground tissue. The xylem is found towards the upper side and phloem lies towards the lower side.

The remaining portion of the ground tissue is composed of parenchymatous cells **Fig. 3**.

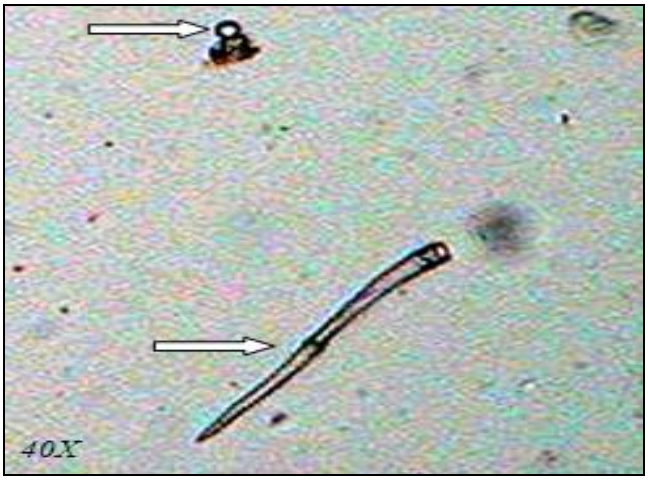
Powder Characteristics: The powder was green in color, on microscopical examination, the powder showed anisocytic stomata, epidermal cell, simple starch grains, glandular and non-glandular trichomes, lignified annular xylem vessel, lignified tissues and spongy parenchyma with veinlet **Fig. 4**.



Epithelial tissue

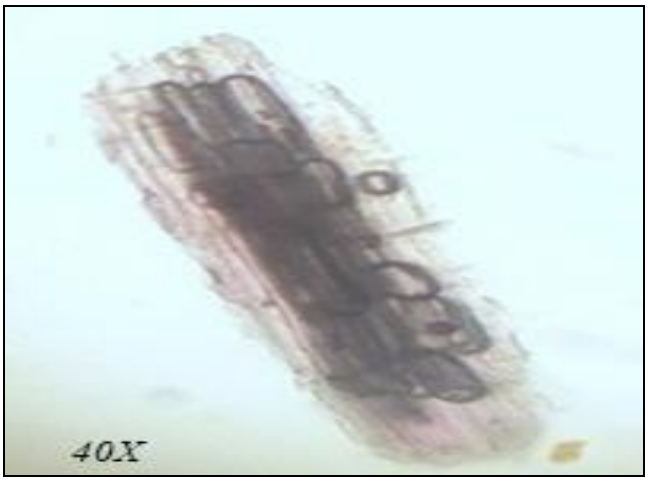


Starch Grains

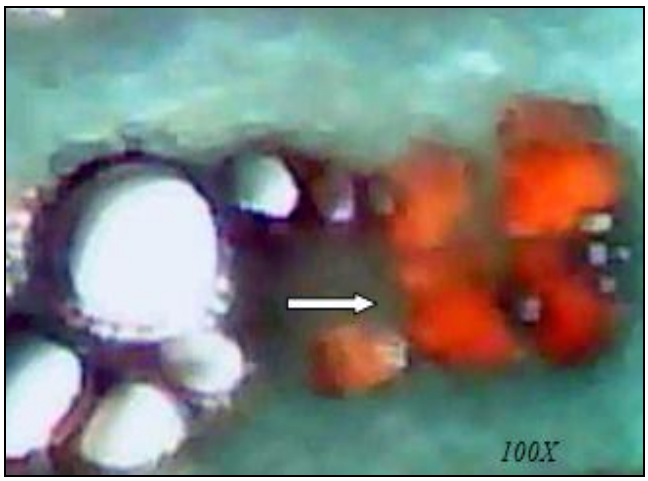


Trichomes

Vascular Bundle



Lignified Vascular Tissue



Lignified Tissue

FIG. 4: POWDER CHARACTERISTICS OF *C. GUIANENSIS* LEAVES

Histochemical Studies: The counter idea about the presence of phytoconstituent is obtained through this study like phenolic compound in palisade as indicated by the brownish black stain on ferric chloride solution treatment **Table 1.**

TABLE 1: HISTOCHEMICAL STUDIES OF *C. GUIANENSIS*

Reagent	Phytoconstituent	Histological zone in leaves	Inference
Phloroglucinol + HCl (1:1)	Lignin	Vascular bundle	+
Aniline Sulphate + H_2SO_4	Lignin	Vascular bundle	+
Iodine Solution	Starch	Vascular bundle, lamina	+
Sudan III Solution	Oil globules	Vascular bundle	+
Aqs. $FeCl_3$ Solution	Phenolics	Pallisade cells	+
Dragendroff's reagent	Alkaloid	Lamina	+
Libermann-Burchardlt reagent	Steroids	Lamina	+
Millon's Reagent	Proteins	Midrib region	-

+ Positive; - negative

Physical Evaluation: The moisture content seems to be lower than necessary to support the growth of microbes to bring any change in the composition of the drugs. Physical constant as ash value of the drug gives an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. Extractive values are useful for the determination of exhausted or adulterated drugs **Table 2**.

TABLE 2: PHYSICOCHEMICAL PARAMETERS OF *C. GUIANESIS*

Parameter	% w/w Avg. ± S. D.
Ash Values	
Total	08.77 ± 0.063
Acid - insoluble	02.85 ± 0.103
Water – soluble	05.84 ± 0.091
Extractive Values	
Pet. Ether Soluble (40-60^o)	01.85 ± 0.017
Ethanol Soluble (95%)	13.57 ± 0.202
Water Soluble	08.43 ± 0.095
Moisture content	08.43 ± 0.130

Phytochemical Investigation: Revealed the presence of primary and secondary metabolites as oils, alkaloids, glycosides, flavonoids, steroids, amino acids, phenolic substances, and triterpenoids.

Inorganic Elements and Fluorescence Analysis: Various inorganic elements present in the plant are Na^+, K^+, Fe^{++}, Cl^- and NO_2.

In fluorescence analysis revealed that the powdered leaves of were treated various chemical reagents to give different colors **Table 3**.

The fluorescence color is specific for each compound. A nonfluorescent compound may fluoresce if mixed with impurities that are fluorescent.

TABLE 3: FLUORESCENCE ANALYSIS OF *C. GUIANESIS*

Powdered drug	Visible/Daylight	The short UV light (254nm)	The long UV light (365nm)
Powder	Dark Green	Greenish Black	Greenish
Powder + 1N HCl	Greenish brown	Light green	Golden yellow
Powder + 50% H_2SO_4	Reddish	Brownish	Blackish
Powder + 1N NaOH	Reddish	Yellowish green	Brown
Powder + 1N NaOH (alcoholic)	Greenish	Light green	Blackish

TLC Fingerprint Profile: Thin layer chromatography of the chloroform and ethanolic extracts was carried out using Chloroform: Glacial acetic acid: Methanol: Water (64:32:12:8) and Toluene: Ethyl acetate: Formic acid (7:3:1) as mobile phase respectively and the R_f were recorded in **Table 4.**

TABLE 4: TLC FINGERPRINT FOR *C. GUIANESIS*

Mobile phase	Extract	Number of spots and their R_f value
Chloroform: Methanol (8:0.6) Detection- 10 % Alcoholic H_2SO_4	Chloroform	0.03, 0.06, 0.35, 0.81 and 0.89
Toluene: Ethyl acetate: Formic acid (7:3:1) Detection- 365nm	Ethanolic	0.03, 0.08, 0.55, 0.71, 0.87 and 0.93

CONCLUSION: These data and parameters have been investigated for *C. guianesis* to lay down standards which could be useful to find the authenticity of this traditional medicinal system plant. These investigations may be useful to supplement existing information about distinguishing from substitutes and adulterants. In other words, the pharmacognostic features examined in the present study may serve as a tool for validation of the raw material and standardization of its formulations at herbal industrial level in the forthcoming days.

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CONFLICT OF INTEREST: Nil

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