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PHARMACOGNOSTICAL AND PHYTOCHEMICAL EVALUTION OF *VENTILAGO CALYCULATA*

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ABSTRACT

Ventilago calyculata (Rhamnaceae) commonly known as Pitti. *Ventilago calyculata* is present in hotter parts of India, Burma, Siam, China, in forest region. The plant is antimalarial, Antiviral, stomachic, skin disorder. Phytochemical studies had revealed the presence of flavonoids, triterpenoids, tannin, naphthoquinone, anthraquinone. Present study was carried out to determine, the morphological, microscopical and phytochemical profiles. Microscopy show thick unicellular covering trichomes, vein islet no.-3, vein termination no.-6, Anomocytic type stomata, lignifed, xylem, etc. The physical parameter such as moisture content, ash value and extractive value were evaluated.

INTRODUCTION: *Ventilago calyculata* (Rhamnaceae) commonly known as Pitti. *Ventilago calyculata* is present in hotter parts of India, Burma, Siam, China, in forest region. Shrubs are scandent. Young branches glabrous or sparsely glabrous or sparsely pubescent. Leaf blade oblong or ovate, lower and middle margins entire, upper margin irregularly remotely dentate. Mainly Bark, Leave for dye use. The juice of bark and young shoots is applied to the body as remedy for the pains which accompany malarial fever, yellow and green color dyes synthesis, as stomachic, tonic and stimulant, Antiviral Activity [1, 2].

Organoleptic evaluation of drugs refers to the evaluation of drugs by color, odor, size, shape, taste and special features including touch and texture etc. Organoleptic evaluations can be done by means of organs of special sense which includes the above parameters and thereby define some specific characteristics of the material which can be considered as a first step towards establishment of identity and degree of purity [3].

Phytochemical evaluation of drug refers to determine quality and purity, such as through Ash value it refers to the adulterants and impurity. Extractive value shows to estimation of specific constituents, soluble in that particular solvent used for extraction. Moisture content the moisture requirements for enzymatic activity and that which microorganisms demand, vary hot only with species, but also with other environmental factor [4].

MATERIALS AND METHODS: Leaves were collected from local area of Panna, Madhya Pradesh, India, in November-December. The plant was identified and authenticated by Dr. Zlia Ul Hasan, HOD**,** Department of Botany, Saifia Science College Bhopal. Prepared herbarium was submitted and the plant was certified as *Ventilago calyculata.* Specimen no.-231/Bot/Saifia /11.

Chemicals and instruments: Compound microscope, Camera lucida (mirror type), Stage and eye piece micrometer and other basic equipments and glass wares are used for the present study. Solvents like Methanol, Acetone, Petroleum ether, Water are used.

Pharmacognostical studies:

Morphological studies: The shape, size, color, taste and odor of leaves were determined.

Microscopical studies Microscopic studies were done by preparing free hand sections of leaf. The sections of the midrib of leaf was cleared with chloral hydrate solution, and stained with safranin and mounted in glycerin. Powders (#60) of the roots were used for observation of powder microscopical characters. The powdered drug was separately treated with Phloroglucinol, Hydrochloric acid solution, glycerin, to determine the presence of lignified cells etc.

Phytochemical studies: The powder leaves of *Ventilago calyculata* determined the Ash value, Extractive value, Crude fiber, Moisture content, Phytochemical screening [5, 6, 7, 8].

Determination of Ash Values:

Total Ash: To determined the total ash place about 2 gm of ground air dried drug, accurately weight in a previously ignited and crucible of silica. Spread the material in an even layer and ignite it by gradually increasing the heat to 500-600^{o}C until it is white, indicating the absence of carbon. Cool in a dessicator and weight. Then, we calculated the percentage of ash with reference to air-dried drug.

Acid Insoluble Ash: To determined the acid insoluble ash boil the ash with 25 of dilute HCL for 5 minutes, collected the insoluble matter in a sintered glass crucible, washed with hot water, ignited, cool in a dessicator and weight. Then, we calculated the percentage of acid-insoluble ash with reference to the air dried drug.

Water Soluble Ash: To determined water soluble ash total ash boil with the 25 ml of water for 5 minutes. Insoluble ash was collected in a sintered glass crucible. Washed with hot water and ignited in a crucible for 15 minutes at a temperature not exceeding 450^{o}C cool and weight. Then, we calculated the percentage of water soluble ash with reference to the air dried drug.

Determination of Solvent Extractive Values: This method determines the amount of active constituents in a given amount of medicinal Plant material when extracted with solvents. For determination of solvent extractive values 5gm of the air dried, coarsely powdered macerated with 100 ml of water close flask for 24 hours, shaking frequently during first 6 hours and allowing stand for 18 hours. Thereafter, filter rapidly taking precautions against loss of solvent; evaporate 25 ml of the filtrate to dryness in a tarred flat bottomed shallow dish, at 105^{o}C and weight. The percentage of solvent soluble extractive with reference to air dried drug has to be calculated.

Phytochemical Analysis:

Test for alkaloids: Two grams of ground plant material was treated in a test tube with 10 ml of 1% HCl for 30 minutes in a water bath. The suspension was filtered through cotton into a test tube and was divided into two parts. Five drops of each of Dragendroff's, Wagner's reagent, Mayer's and Hager's reagent were added to the respective parts of the solution and the formation of different color indicated the presence of alkaloids.

Tests for carbohydrates: Molisch's, Fehling's and Benedict's tests were done to know the presence of carbohydrate.

Molisch's test: Aqueous or alcoholic solution of the extracted substance was added to 10% alcoholic solution of naphthol. Equal volume of concentrated H_2SO_4 was added along the side of the tube. A violet ring at the junction of two liquids confirms presence of carbohydrates.

Fehling's Test: 2ml of Fehling's solution A and 2 ml of Fehling's solution B was added to 2ml of extracts. After boiling, if bricked precipitation appears, then reducing sugars are present.

Benedict's Test: 5ml of Benedict's reagent and 3ml of test solution boiled in the water bath. If brick red precipitate appears at the bottom of the test tube then monosaccharides are present.

Test for Cardiac Glycosides:

Keller-Killiani test: To an extract of the drug in glacial acetic acid, few drops of ferric chloride and conc. H2SO4 were added. If a reddish brown color is formed at the junction of two layer and upper layer turns bluish green, confirms the presence of cardiac glycosides.

Legal Test: To a solution of glycoside in pyridine, sodium nitroprusside solution and sodium hydroxide solution were added. A pink to red color, confirms presence of cardiac glycosides.

Modified Borntrager's test: 0.1 g of the extract was added with 5ml of dilute HCI and 5ml of 5% solution of ferric chloride and boiled for 5 minutes, cooled and filtered. This filtrate was shaken with benzene. The benzene layer was separated and an equal volume of dilute solution of ammonia was added. Formation of pink color with the ammonical layer signifies presence of anthraquinone glycosides.

Test for Proteins and Amino Acids:

Biuret test: 2ml of extract was mixed with 2 ml of 10% NaOH solution and 2 to 3 drops of 1 % Cupper sulphate solution was added. Appearance of violet or purple color indicates the presence of proteins.

Ninhydrin Test: 2 ml of extract was added with 0.5 ml of Ninhydrin solution. The mixture was boiled for 2 minutes, and then the solution was cooled. Appearance of blue color shows the presence of amino acid.

Xanthoproteic Test: 2 ml. of extract was added with 1 ml of conc. HNO3, boiled and cooled. Then 40% NaOH was added drop by drop. Appearance of colored solution indicates the presence of proteins.

Test for Saponins:

Foam test: 1ml of alcoholic and aqueous extract was diluted separately with distilled water to 10 ml and was shaken in a graduated cylinder for 15 minutes and kept aside. One cm layer of foam after standing for 30 minutes indicates the presence of saponin.

Test for tannins and Phenolic Compounds:

With Ferric chloride: 5% w/v solution of ferric chloride was added in 90% alcohol. Appearance of blue color is the indication of presence of phenols.

With lead acetate: Extracts were mixed with lead acetate. Occurrence of precipitate is the indication of presence of tannins.

With gelatin solution: To a solution of tannins (0.5-1%) aqueous solution of gelatin (1%) and Sodium chloride (10%) are added. Appearance of white buff colored precipitated is the indication of presence of phenols.

Test for Steroid and Sterols:

Lieberman Burchard reagent: To a dry test tube, 2 ml of extract solution was mixed with 2ml of acetic anhydride and 2-3 drops of conc. H_2SO_4 was added.

The solution was mixed thoroughly. An emerald green color development is the indication of presence of steroids or sterols.

Salkowski's Test: 5ml of solution of extract was taken in a dry test tube with chloroform. Equal volume of conc. H2SO4 was added gently along the sides of the test tube. The acid layer develops a yellow color with a green fluorescence and the chloroform layer will give a play of colors first from bluish red to gradually violet red.

Test for Flavonoids:

With NaOH: The extract were dissolved in water, and then filtered. The filtrate was treated with sodium hydroxide. Yellow color is observed if flavonoids are present

Legal Test: To a solution of glycoside in pyridine, sodium nitroprusside solution and sodium hydroxide solution were added. A pink to red color, confirms presence of cardiac glycosides.

Modified Borntrager's test: 0.1 g of the extract was added with 5ml of dilute HCI and 5ml of 5% solution of ferric chloride and boiled for 5 minutes, cooled and filtered. This filtrate was shaken with benzene. The benzene layer was separated and an equal volume of dilute solution of ammonia was added. Formation of pink color with the ammonical layer signifies presence of anthraquinone glycosides.

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Ninhydrin test: 2 ml of extract was added with 0.5 ml of Ninhydrin solution. The mixture was boiled for 2 minutes, and then the solution was cooled. Appearance of blue color shows the presence of amino acid.

RESULTS: Macroscopical studies of leaves showed that dark green color, alternate, oblong, lanceolate or elliptic ovate to orbicular, base rounded, apex acute or sub acuminate, margins or crenate, ascending and covering near the margin, 3-5 cm of leaf length, characteristic, bitter.

Microscopical studies showed the leaf consists of a thin T. S of leaf showed dorsiventral nature. The following tissues were observed under lamina and midrib region.

Lamina: It consists of a thin layer of thick cuticle followed by a single layer of epidermal cells

Midrib: Below the upper epidermis & above the lower epidermis collenchymas cells were present. Below the upper epidermis 3 layers of collenchymas cells were seen and above the lower epidermis 5 layers of collenchyma cells were seen (**Fig. 1**)

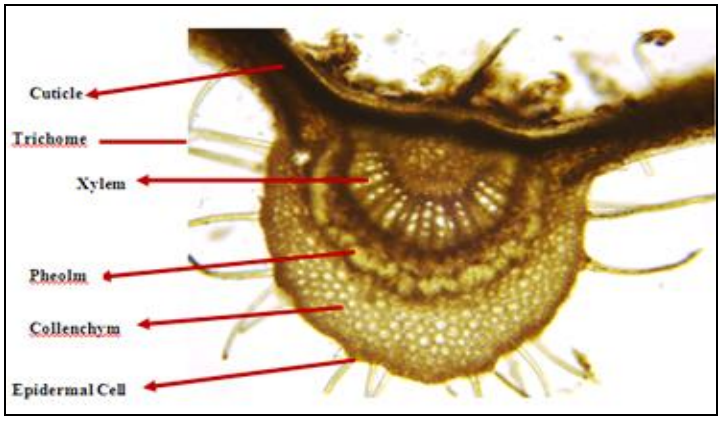

FIG. 1: TRANSVERSE SECTION OF MIDRIB *VENTILAGO CALYCULATA* LEAF

Vascular bundles: Arc shaped vascular bundles were present with which were surrounded by pericyclic fibers, Xylem towards the ventral surface and phloem towards the dorsal surface. Collateral type of vascular bundles was observed (**fig. 2**)

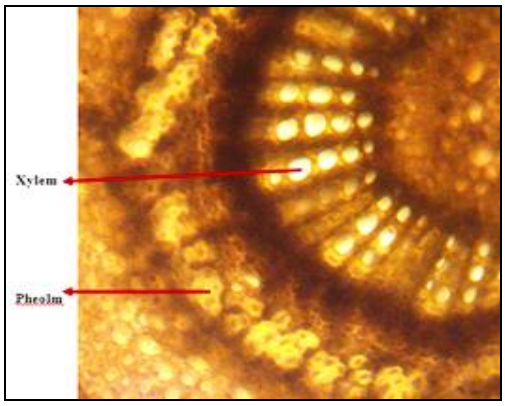

FIG. 2: VASCULAR BUNDLE OF *VENTILAGO CALYCULATA* LEAF

Trichomes: Thick unicellular covering trichomes with pointed apex were observed. Trichomes *in Ventilago calyculata* was unicellular non glandular covering trichome or plant hair, seen in **fig. 3**.

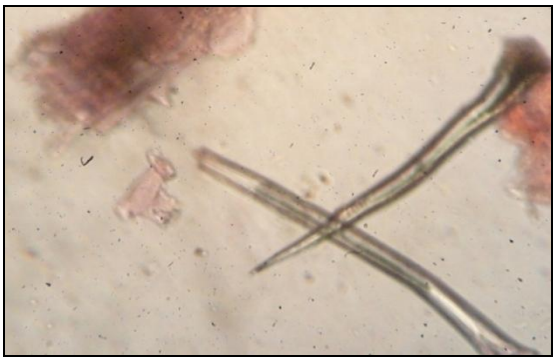

FIG. 3: TRICHOMES OF *VENTILAGO CALYCULATA* LEAF

Vein islet, Vein termination:

1. Vein islet Number: 3
2. Vein Termination Number: 6, seen in **fig. 4.**

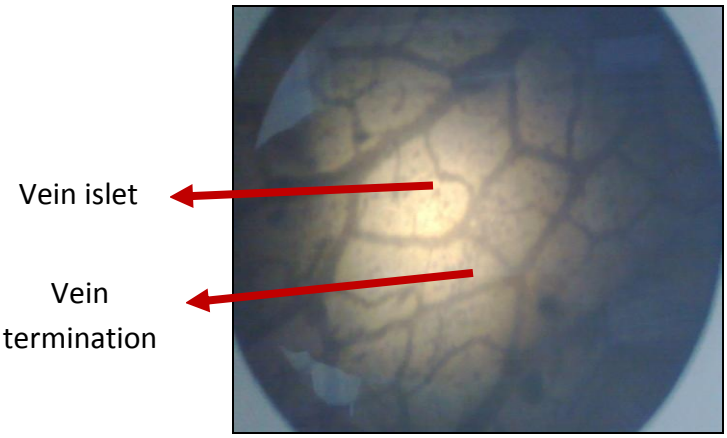

FIG. 4: VEIN ISLET, VEIN TERMINATION OF *VENTILAGO CALYCULATA* LEAF

Stomata: Anomocytic type stomata are present. The stomata are surrounded by varying number of cells, in no way differing from epidermis, seen in **fig. 5**.

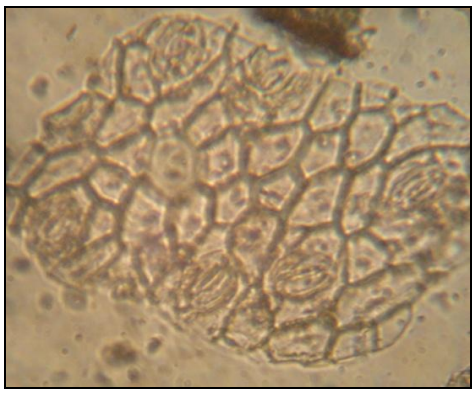

FIG. 5: STOMATA OF *VENTILAGO CALYCULATA* LEAF

Further analytical parameters like Ash value (**Table 1),** Extractive values (**Table 2**), phytochemical screening (**Table 3),** Moisture content (loss on drying) (**Table 4**), Crude fiber (**Table 5**) were carried out. The above studies enable the identification of the plant material for further investigations and form an important aspect of drug studies.

TABLE 1: ASH VALUES OF VENTILAGO *CALYCULATA*

Parameter	Determined value % w/w
Total ash	10.5%
Acid insoluble ash	5.1%
Water soluble ash	1.5%

TABLE 2: EXTRACTIVE VALUES IN FOLLOWING SOLVENTS

Parameter	Determined value %w/w
Petroleum ether	5.1%
Acetone	11.3%
Methanol	15.2%
Water	10.1%

TABLE 3: PHYTOCHEMICALS IN METHANOLIC, ACETONE PET. ETHER AND AQUEOUS LEAF EXTRACT OF *VENTILAGO CALYCULATA*

Phytochemicals	Petroleum ether	Acetone	Methanol	Water
Alkaloids	-	-	-	-
Tannins	-	-	-	-
Steroids	+	-	+	-
Flavonoids	-	+	+	-
Glycoside	-	+	+	-
Saponins	-	-	+	+
Carbohydrates	-	-	-	+
Amino acid	-	-	-	-

(+) Present; (-) Absent

TABLE 4: MOISTURE CONTENT

Parameter	Determined value %w/w
Moisture content	3%

TABLE 5: CRUDE FIBRE OF *VENTILAGO CALYCULATA* LEAVE

Parameter	Determined value %w/w
Crude fibre	8

CONCLUSION: The report of pharmacognostical evaluation of *Ventilago calyculata*. The Pharma-cognostical studies include macroscopical, microscopical, proximate analysis like ash values, extractive values and other analysis gives valuable information about the plant. It is helpful for correct identification of this plant for the future reference.

Further Research Scope: The plants have tremendous potential for further research.

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