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PHARMACOGNOSTIC INVESTIGATION ON LEAVES OF *CITRUS MAXIMA* (BURM.) MERR. (RUTACEAE)

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ABSTRACT

Plant kingdom is one of the important sources of the natural product and food. In present era many lifesaving, nutritive chemical components are isolated from different plants due to both medicinal and economical values. In India Ayurveda provides major information about medicinal plants, but there are number of plants which is not studied totally. *Citrus maxima* is a perennial shrub commonly known as Papanus, distributed through India. Present Pharmacognostic research study was performed on leaves of *Citrus maxima*. Macroscopic photographs of transverse sections (T.S.) of *Citrus maxim* leaves shown distinct presentation of arrangement of palisade cells, vascular bundles, oil globules, parenchymatous cell & trichomes. Microscopy of the leaf shows abundant anisocytic stomata both surfaces of leaf, presence of vascular bundle in xylem vessels. Thick, ovoid, rectangular epidermal cells were present. Uniserate, multicellular, thin walled, unligified, covering trichomes were present. Spongy parenchyma cells were present. Calcium oxalate crystals were present in the parenchymatous cells. Starch grains were present except vascular bundle. Oil globules were present in leaf. Standardization of leaf was done with the help of extractive values [Water soluble extractives (18.8 % w/w), Alcohol soluble extractives (6.8 % w/w)], total ash value (4.66 % w/w), acid soluble ash value (0.316 % w/w), acid insoluble ash value (4.63 % w/w) and loss on drying (5.96 % w/w).

Keywords:

Citrus maxima, Pharmacognostic investigation, Microscopy, Extractive values

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INTRODUCTION: In the present scenario of pharmaceuticals there is day by day an increasing in demand of more active therapeutic agents. Apart from the synthetic route natural route is also major source for active therapeutic agents. In India Ayurveda provides major information about medicinal plants, but there are number of plants which is not studied totally.

Citrus maxima is a perennial shrub commonly known as Papanus, distributed through India. It shows different pharmacological activities. This work was

aimed at Pharmacognostic, investigation of the leaves of the *Citrus maxima*.

Plant Profile:

Botanical name: *Citrus maxima*

Toxonomical classification:

- Kingdom- Plantae
- Division- Magnoliophyta

- Class- Magnoliopsida
- Order- Rosidae
- Family-Rutaceae
- Common name-Pamelo
- Vernacular name-
 1. Sanskrit :- Madhukarkati
 2. Hindi :- Mahanimbu
 3. Marathi :- Panis, Papanas
- Botanical name-*Citrus maxima*
- Family- Rutaceae
- Plant type:- Tree
- Habitat:- Indigenous to East of India
- Height- A tree a 9-12 m height.

Leaves: large 15 to 23cm long, Ovate – oblong, frequently emarginated, Pubescent beneath. The leaves are said to be useful in epilepsy, chorea, and convulsive cough.

Petioles: Broadly winged

Flowers: Large, White

Stamens: 16-24

Fruit: large, pale yellow, globose or pyriform, rind thick, pulp varying in colour from crimson to pale pink or yellow. It is sweet, with a flavor nutritive, refrigerant. It is used in Leprosy, asthma, cough, hiccough, mental aberration, epilepsy.

Rind: Antiasthematic, Brain tonic, usefull in vomiting, griping in abdomen, diarrhea, headache and eye troubles.

MATERIAL AND METHODS:

Pharmacognostic Investigation ^{1, 2, 3}: Collection, authentication, processing & storage were done according to W.H.O. guidelines for the plant material.

Collection, identification & drying of plant material:

Citrus maxima (Burm) Merr. (Rutaceae) has been selected for dissertation work on the basis of literature review.

Collection: Leaves were collected from Kopergaon (Ahemadnagar, M.S.,India). Following were specifications of collected sample:-

- Season of collection :- Winter
- Month of collection :- January
- Plant stage: - fruiting.

Identification & Authentication: Collected plant material were subjected to preparation of Herbarium & sent for identification.

Herbarium of *Citrus maxima*:

1. Kingdom : Plantae
2. Division : Magnoliophyta
3. Class : Magnoliopsida
4. Order : Sapindales.
5. Genus : Citrus.
6. Species : *Citrus maxima*.
7. Family : Rutaceae
8. Locality : Kopergaon, Ahemadnagar Dist.
9. Habit : Tree
10. Vernacular name: Papanus
11. Remark : Tree
12. Collected by : Patil Ashwini V.
13. Identified by : Mr. P.G. Diwakar

The herbarium of the plant specimen has been deposited at Botanical Survey of India, Pune, the voucher specimen No. BSI/WC/Identi./Tech/2009/35.

C. Drying: - Leaves were spread in thin layer on aluminium tray & air dried for a week.

Macroscopic Study of *Citrus Maxima* Leaf^{4, 5}: 10 to 12 matured leaves were taken for macroscopic study. Size measured is mean of ten readings (Figure 1). Leaves were evenly distributed on branches of tree.

1. **Size:**
 - a. **Leaf**- 15-16cm long and 5.5-6.5cm width.
 - b. **Petiole**– 4-4.5 cm long (Winged) and 2.5-2.8cm width.
2. **Shape** : Ovoid.
3. **Apex** : Acute.
4. **Base** : Assymetrical.
5. **Margin** : Entire.
6. **Color** : Greenish.
7. **Surface** : Glabrous.
8. **Midrib** : Prominent on both surfaces of leaf.
9. **Odour** : Characteristic.
10. **Taste** : Acrid

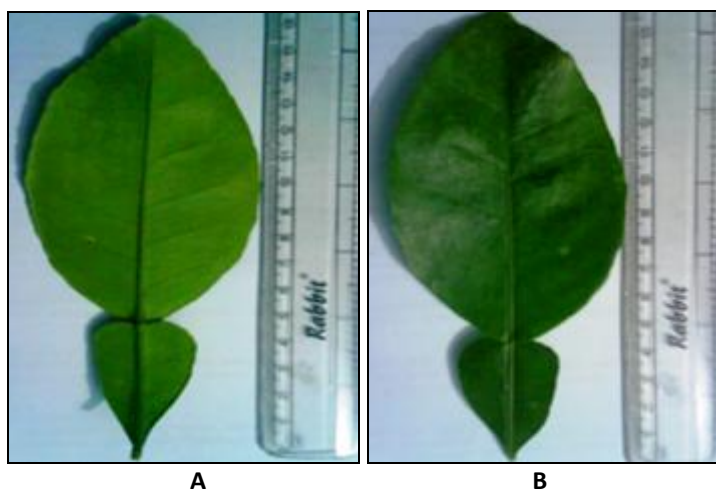


FIGURE 1: MORPHOLOGY OF *CITRUS MAXIMA* LEAF, A) DORSAL SURFACE, B) VENTRAL SURFACE

Leaf preparation for microscopy (Khandelwal; 2003, Evans; 1997): Fresh leaves were taken & boiled with chloral hydrate solution for clearing the section. Transverse section of leaf through midrib was taken & then photographs of section were taken (Figure 2, 3 & Following are some of the microscopic characters observed in sections;

Lamina: Leaf showed dorsiventral characteristics.

Epidermis: Lower and upper epidermis showed abundant Anisocytic stomata and uniseriate, multicellular, thin walled, covering trichomes on lower epidermis. It showed single layer of palisade cells on upper epidermis.

Mesophyll: It is middle part between lower & upper epidermis of leaf. It shows the sclerenchymatous cells covering to vascular bundle. Parenchymatous cells contain calcium oxalate crystals. Collechyma cells were present in mesophyll under the vascular bundle & lower epidermis.

Starch grain and yellow colored oil globules were present.

Midrib:

Vascular bundle: Vascular bundle present & showing xylem and phloem arrangement at middle of midrib.

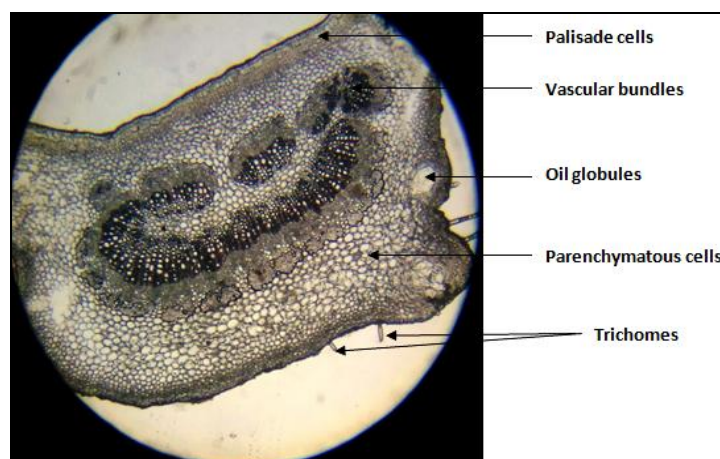


FIGURE 2: T.S. OF *CITRUS MAXIMA* LEAF (WITHOUT STAIN)

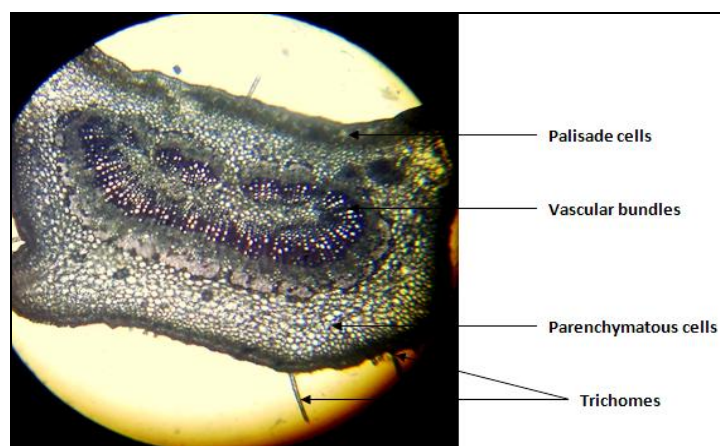


FIGURE 3: T.S. OF *CITRUS MAXIMA* LEAF (PHLOROGLUCINOL STAINING)

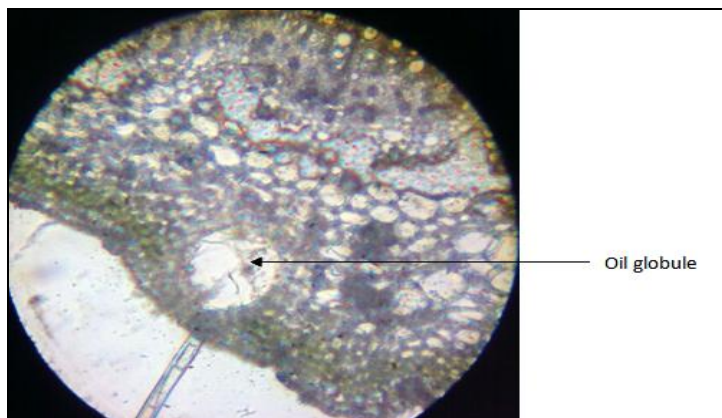
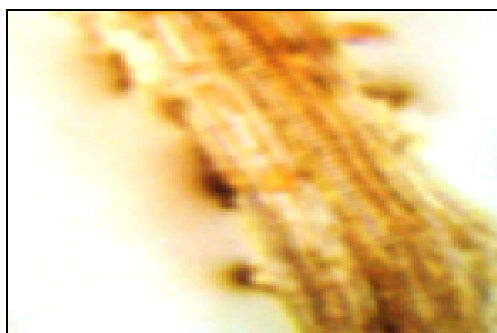


FIGURE 4: T.S. OF *CITRUS MAXIMA* LEAF SHOWING PRESENCE OF OIL GLOBULE

Powder characteristics of *Citrus maxima* leaf^{4, 5}: Leaf powder was boiled with sodium hypochlorite as clearing agent. Compound microscope was used for cellular observations (Figure 5).



STOMATA: (40X) ABUNDANT ANISOCYTIC STOMATA WERE PRESENT ON BOTH SURFACES OF LEAF



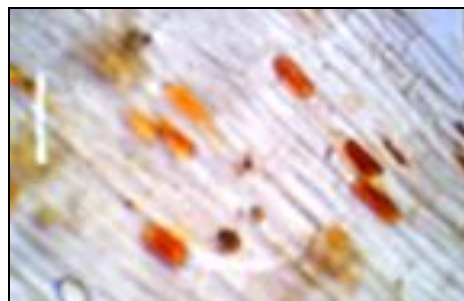
XYLEM VESSELS: (10X) XYLEM VESSELS PRESENT IN VASCULAR BUNDLE



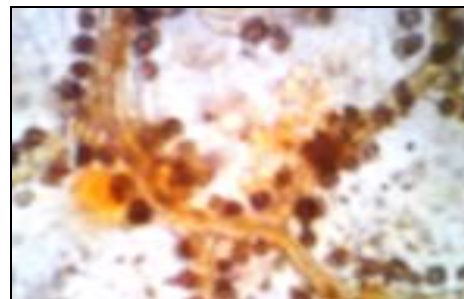
EPIDERMAL CELLS: (10X) THICK, OVOID, RECTANGULAR EPIDERMAL CELLS WERE PRESENT



TRICHOMES: (10X) UNISERATE, MULTICELLULAR, THIN WALLED, UNCLIGNIFIED, COVERING TRICHOMES WERE PRESENT



SPONGY PARENCHYMA CELLS: (10X) SPONGY PARENCHYMA CELLS WERE PRESENT



CALCIUM OXALATE CRYSTALS: (10X) CALCIUM OXALATE CRYSTALS WERE PRESENT IN THE PARENCHYMATOUS CELLS

FIGURE 5: POWDER CHARACTERISTICS OF *CITRUS MAXIMA* LEAF

Standardization of Plant Material^{6, 7, 8}:

Determination of Extractive Values: The determination of Water soluble or alcohol soluble extractive is used as a means of evaluating drugs the constituents of which are not readily estimated by other means. But as suitable as says become available, the extractive tests are no longer required as pharmacopoeial standards.

A. Determination of Water-Soluble Extractive Value:

Method: 5 gm of the air-dried, coarsely powdered drug was macerated with 100 ml of 0.01%v/v chloroform: water of the specified strength in a closed flask for 24 hours.

The flask was shaken frequently during the first 6 hours and after that allowed to stand for 18 hours. The content in the flask filtered rapidly and precautions were taken to prevent loss of water, dried in a tared flat-bottomed shallow dish, dried at 105°C & weighed. The percent of the water-soluble extractive value was calculated with reference to air-dried drug.

B. Determination of Alcohol-Soluble Extractive Value:

Method:- 5 gm of the air-dried, coarsely powdered drug was macerated with 100 ml of ethanol of the specified strength in a closed flask for 24 hours. The flask was shaken frequently during the first 6 hours and after that allowed to stand for 18 hours.

The content in the flask filtered rapidly and precautions were taken to prevent loss of ethanol, dried in a tared flat-bottomed shallow dish, dried at 105°C & weighed. The percent of the ethanol extractive value was calculated with reference to air-dried drug.

TABLE 1: DETERMINATION OF EXTRACTIVE VALUE (%W/W) OF THE LEAF WITH DIFFERENT SOLVENTS

Extractive	Extractive Value (%w/w)
Water soluble extractives	18.8
Alcohol soluble extractives	6.8

C. Determination of Total Ash Value: In the determination of total ash values, the carbon must be removed at as low a temperature (450°C) as possible because alkali chlorides, which may be volatile at high temperatures, would otherwise be lost.

If carbon is still present after heating at a moderate temperature, the water soluble ash may be separated and the residue again ignited or the ash may be broken up, with the addition of alcohol and again ignited.

Procedure:

Method: 2gm of the air - dried crude drug was weighed in a tared silica dish and incinerated at a temperature not exceeding 450°C until free from carbon. After incineration the material was cooled and weighed. The percentage of ash value was calculated with reference to the air - dried drug.

TABLE 2: DETERMINATION OF TOTAL ASH VALUE

Reading	I	II	III
Weight of crucible [gm]	15.35	15.14	17.31
Weight of air dried drug [gm]	2	2	2
Weight of crucible + air dried drug [gm]	17.35	17.14	19.31
Weight of crucible + ash [gm]	15.44	15.24	17.40
Total ash [gm]	0.09	0.1	0.09
Percentage of total ash [% w/w]	4.5	5.0	4.5

D. Determination of Acid Soluble and Acid Insoluble Ash Value:

Method:-The total ash was boiled with 25 ml of 2M hydrochloric acid for 5 minutes. Insoluble matter was collected on an ashless filter paper. The collected insoluble matter was washed with hot water, ignited and cooled in a desiccator and weighed. Percentage of acid soluble and acid - insoluble ash was calculated with reference to the air - dried drug.

TABLE 3: DETERMINATION OF ACID-SOLUBLE ASH

Reading	I	II	III
Weight of Silica crucible [gm]	15.352	15.352	15.352
Weight of Silica crucible [gm] + ash	15.359	15.357	15.359
Total acid insoluble ash [gm]	0.007	0.005	0.007
Percentage Total acid insoluble ash (%w/w)	0.35	0.25	0.35

TABLE 4: DETERMINATION OF ACID-SOLUBLE ASH

Reading	I	II	III
Weight of Silica crucible [gm]	27.491	28.359	29.482
Weight of Silica crucible [gm] + ash	27.583	28.455	29.571
Total acid soluble ash [gm]	0.092	0.096	0.089
Percentage Total acid soluble ash (%w/w)	4.6	4.8	4.5

E. Determination of Loss on Drying: Although the loss in weight, in the sample so tested, principally is due to water, small amounts of other volatile materials will also contribute to the weight loss. For material which contains little volatile material, direct drying (100-105°C) to constant weight can be employed. The moisture balance combines both the drying process and weight recording.

Procedure:-Weigh a glass stoppered, shallow weighing bottle that was dried at 105°C. Transfer 2gm of sample to the bottle. Covered it & accurately weigh the bottle. The sample was distributed as evenly as practicable by gentle sidewise shaking to the depth not exceeding 10 mm.

The loaded bottle as placed in the oven .The stopper was removed & it was left in the chamber. The sample was dried to constant weight for the 3hrs. After drying was completed, open the drying chamber, close the bottle promptly & allowed it to cool to room temperature in a desiccator. The bottle was weighed. The percentage loss on drying was calculated with reference to the air dried drug.

Observation: The sample was heated at temp.105⁰C till constant weight of powder obtain.

TABLE 5: DETERMINATION OF LOSS ON DRYING

Reading	I	II	III
Weight of weighing bottle (g)	15.961	15.726	17.562
Weight of air dried material	2	2	2
Weight of weighing bottle + air dried material(g)	17.961	17.726	19.562
Weight after drying of weighing bottle + air dried material (g)	17.844	17.601	19.457
Percentage of loss of drying (%w/w)	6.05	6.25	5.60

RESULTS AND DISCUSSION: The present work was carried out on leaves of *Citrus maxima* family Rutaceae. The emphasis was given on Pharmacognostic studies of *Citrus maxima* leaves to find out their usefulness to human being. This plant was collected from Kopargaon, Ahemadnagar. The herbarium of the plant specimen has been deposited at B.S.I. Pune, the voucher specimen No. was BSI/WC/Identi./Tech/2009/35. Mr. P.G. Diwakar, Deputy Director, Botanical survey of India, Koregaon road, Pune, did the identification of the plant, by comparing morphological features.

In Pharmacognostic study of plant, macroscopy and microscopy of the leaf was performed. Macroscopic photographs of transverse sections of *Citrus maxim* leaves shown distinct presentation of arrangement of palisade cells, vascular bundles, oil globules, parenchymatous cell & trichomes. Microscopy of the leaf showed abundant anisocytic stomata both surfaces of leaf, presence of vascular bundle in xylem

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CONCLUSION: This pharmacognostic (macroscopic, microscopic & standardization) research on *Citrus maxima* leaves provides a ready database for pharmacy/herbal researchers. This information can be referred for future studies on *Citrus maxim* leaves.

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