

Morphological and structural evaluation of the fruits of *Citrus limon* in India

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Abstract

Citrus limon (Linn) Burm. f. of Rutaceae family is an important plant for its medicinal uses in the treatment of worm infestation, abdominal pain etc. Though it is an important plant, until date no structural or morphological work is found on its fruits. Present study was carried out to evaluate morphological characters of the fruit. Fresh fruit of *C. limon* was collected from local vegetable market of Jamnagar and macroscopic and microscopic characters of fruit and its powder were studied. Rhomboidal crystals, oil globules, prismatic crystals of calcium oxalate, albido fragment, pitted vessels and scalariform vessels etc. were observed. Physicochemical and histochemical analysis was also carried out. It showed characteristics such as lignified cells, calcium oxalate crystals, tannin cells and oil. The current work appears to be the first of such approach and can be considered as a source of reference in future studies.

Key words: *Citrus limon*, Rutaceae, physico chemical analysis, histochemical analysis.

Introduction

Citrus limon (Linn) Burm. f. belongs to family Rutaceae. In Sanskrit it is known as Jambeer/Nimbu. Family Rutaceae contains about 150 genera and 1500 species in the world and India represents 25 genera and more than 80 species [1]. Among them about 12 species of *Citrus* are reported in India [2].

Citrus is a genus of evergreen aromatic shrub or small tree with spreading habit and thought to be native to India but not found growing in wild. *C. limon* is native to the foothills of the Himalayas, and is cultivated in throughout the tropical and temperate region [3]. It is distributed Western Ghats, Coorg, Malabar and Nilgiris as well as cultivated for its fruits and oil throughout India like the plains of Punjab, Sind and Baluchistan [4]. It is also distributed in Indo-Malaysian region, south-East Asia and China [5].

Citrus limon is a spinous shrub or a tree. It is 3-6 m tall. Leaves are 6.5-10.0 mm in length and elliptic to ovate in shape. Leaves contain serrulate margins and acute to acuminate leaf tip. Leaf is perfectly jointed to the petiole. Petiole is narrowly winged. Flowers are bisexual or male. Petals are white, tinged purple (Figure 1a). Stamens are 20-30.

Citrus limon is well known for its therapeutic and culinary purposes since *Samhita* period. Leaves, fruits, fruit juice and rind oil are used for these purposes. It is used as a rootstock for oranges and grapefruit. Further, it is commonly used for pickles [6].

Citrus limon has many biological properties. Fruit rind is stomachic and carminative. Fruit juice is anti-scorbutic, anti inflammatory, refrigerant and used in fever, scurvy, rheumatism, dysentery and diarrhoea. Oil is stimulant and rubefacient when applied externally. In Ayurveda, fresh juice of *C. limon* is used in excessive thirst, body ache, emesis, constipation, worm infestation etc. Ethno botanical studies reported usefulness of tree part in intestinal colic, bronchitis, emesis and heart diseases. Flower is used in cough and coryza. Fruit is useful in hypertrophy of spleen. Rind is used to treat malaria fever. Pericarp is used for the treatment for pimples. Fruit juice is used for diarrhoea, as a preventive measure against cataract, in piles, malaria and vomiting. Fruit and root juice is given to infants for stomach trouble. Seeds are used as vermicide and typhoid [7].

Data reveals that fruit morphology and microscopical structure including its powder has not been studied in detail till date which is an essential parameter for identification of a crude drug. Hence, the present work was undertaken to evaluate microscopic characteristics of *C. limon*.

Materials and methods

About 50 *Citrus limon* fresh fruit were collected from local vegetable market of Jamnagar, India. Healthy fruits

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were selected and Botanical identification was done with the help of various Floras and it was authenticated at Pharmacognosy lab, Institute for Postgraduate Teaching and Research in Ayurveda (I.P.G.T and R.A.), Gujarat Ayurved University, Jamnagar, India. Organoleptic characters like colour, taste, touch and odour were recorded. Stained and fresh thin free hand sections were studied (phloroglucinol and concentrated HCl). Powder microscopy was carried out for shade-dried powder which sieved through number 60 mesh [8]. Histochemical studies were carried out by obtaining free hand sections of fruit treated with various reagents to find tannin, mucilage, starch etc [9]. Photomicrographs were taken using Carl zeiss trinocular microscope attached to a camera. Physicochemical profile of shed dried powder was produced [10].

Results and discussion

Morphology

Fruit is a hemispermidium. It is 5-10 cm long, ovoid to globose in shape and nipple shaped at the end with rough or irregular or warted rind. It is rounded mamillate in shape and dark green in colour at unripe stage. It turns into yellow when ripe. Pulp is pale yellow in colour and tastes acidic and sweet. Thick thread like structures hold carpels forming a column at the center of fruit. It is in a leathery texture and aromatic with multi carpelled sacs nearly 9-10 and each sac contains 3-5 seeds (Figure 1b) [11].

Transverse section of fruit

Diagrammatic section of fruit has shown outer rind, middle segments and central axis.

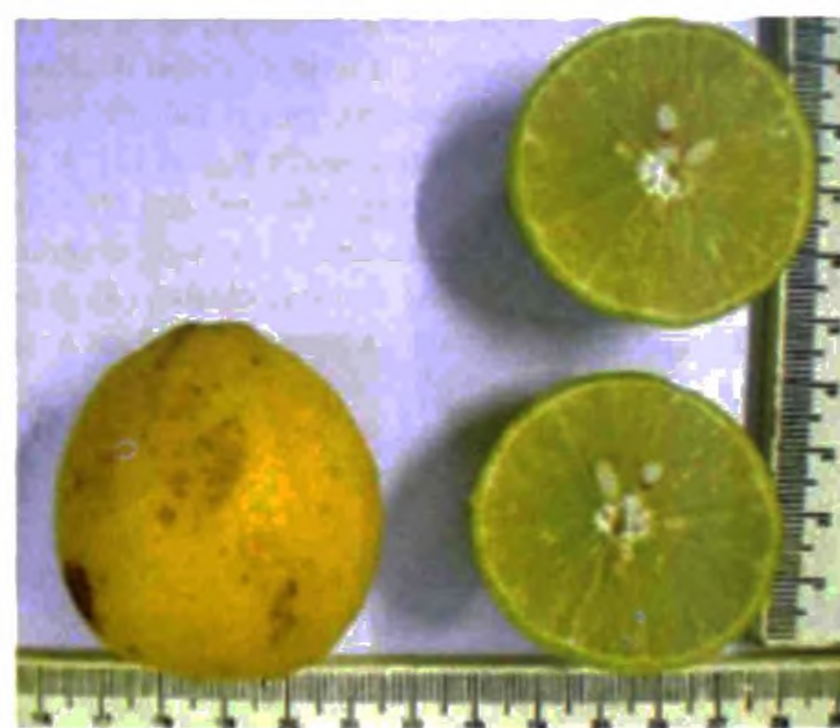
The detailed section has shown three distinct regions known as exocarp, mesocarp and endocarp. Exocarp is divided into inner albido (Figure 2a and 2b) and outer flavido (Figure 2c). Outer exocarp arranges as a flavido zone (Figure 2d) and the inner arranges loosely as an albido zone. Exocarp consists of single layered rectangular shaped cells with thin cuticle. Flavido zone is compactly arranged, some of the epidermal cells are interrupted by stomata. Below the epidermis, many celled layers contain parenchyma cells. Many lysogenous cavities (Figure 2d) are found all over the section with oil globules (Figure 2e). Some of the parenchyma cells also consist of prismatic crystals of calcium oxalate below the epidermis. Below the flavido zone, mesocarpal cells, loosely arranged parenchyma cells form a net like structure albido. Some of the vascular bundles (Figure 2f) are distributed in albido zone.

Annular, scalariform vessels of vascular bundle (Figure 3a) and rhomboidal crystals of mesocarp (Figure 3b) are present. *C. limon* is a multi-capillary fruit, where the endocarp consists of many chambered carpels. Each carpel consists of a juicy sac (Figure 3c) with 3-5 seeds. Each juicy sac (Figure 3c) is made up of outer compactly arranged 10-15 layers of elongated compressed parenchyma cells. Inner layer contains loosely arranged parenchyma cells having prismatic crystals of calcium oxalate. Central thick thread like structure is called central axis (Figure 3d and 3e) or column. It contains outer single layered barreled shaped cells and central pith comprises loosely arranged parenchyma cells. Xylem and phloem are shown in Figure 3f.

Figure 1: Habit of *Citrus Limon*; (a) Vegetative features of fruit and flower (b) measurements of selected fruits.



a



b

Figure 2: Diagrammatic section of fruit; (a) Albido in 40 X (b) Transverse section of Albido (c) Transverse section of flavido (d) Transverse section of flavido and Lysogenous cavity (e) Oil globules (f) Stained vascular bundle.

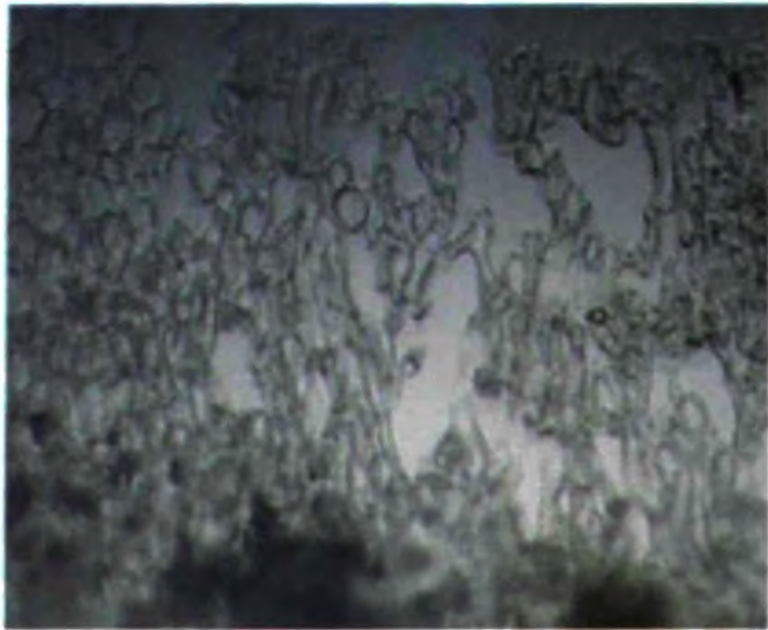
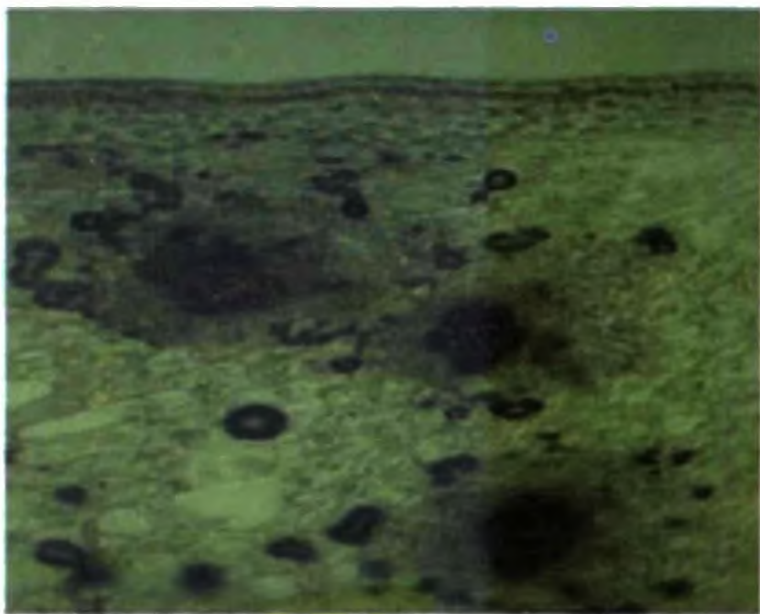
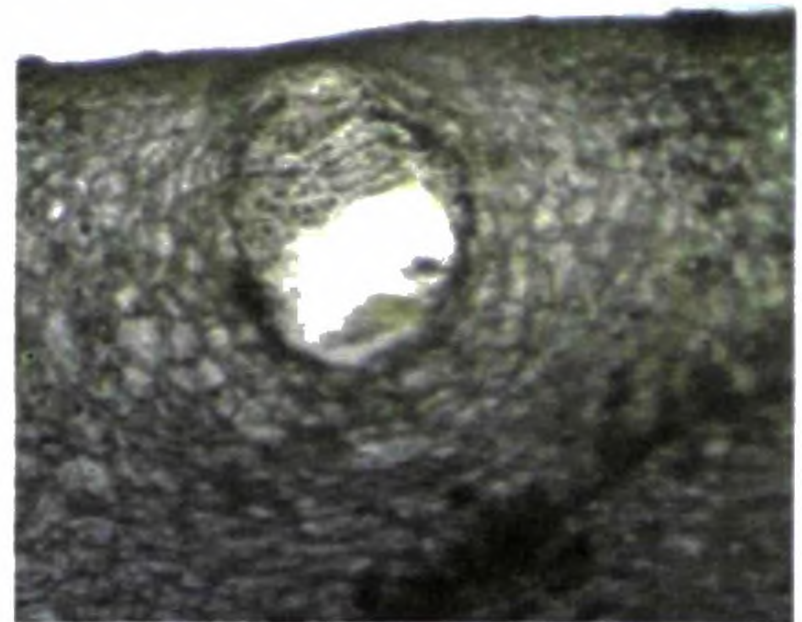
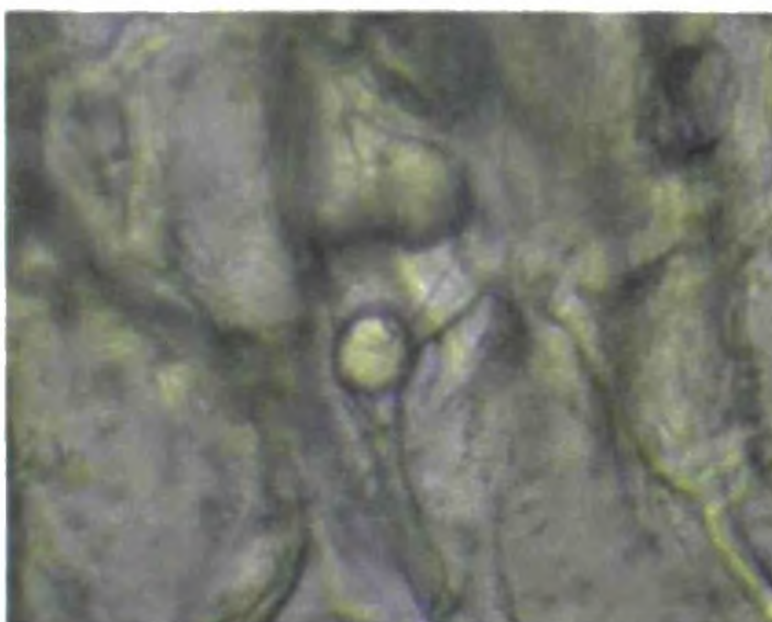
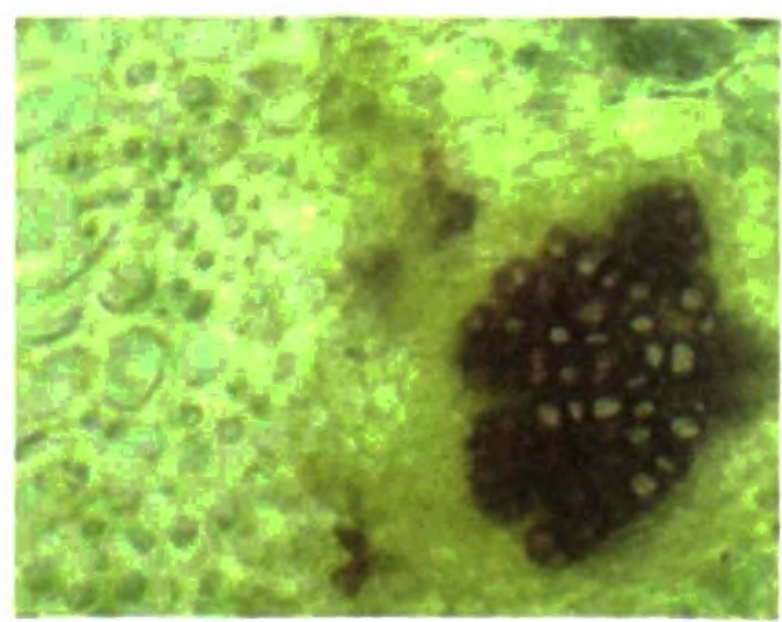
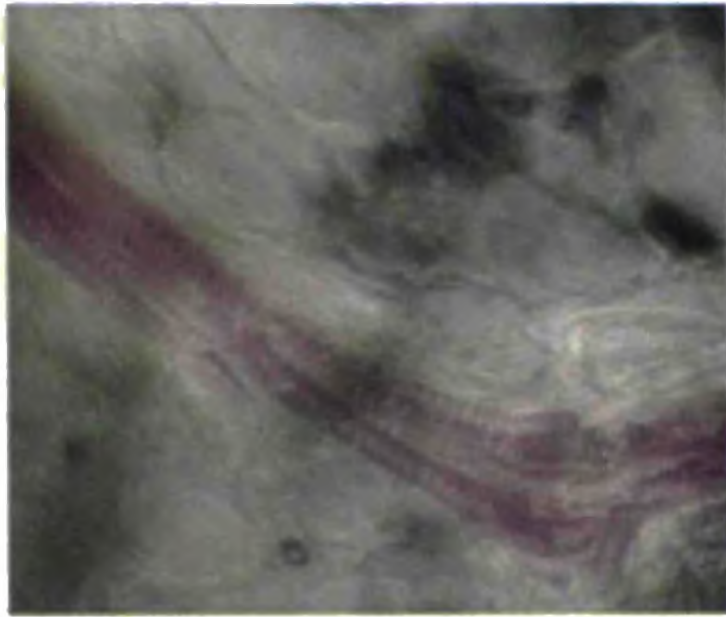
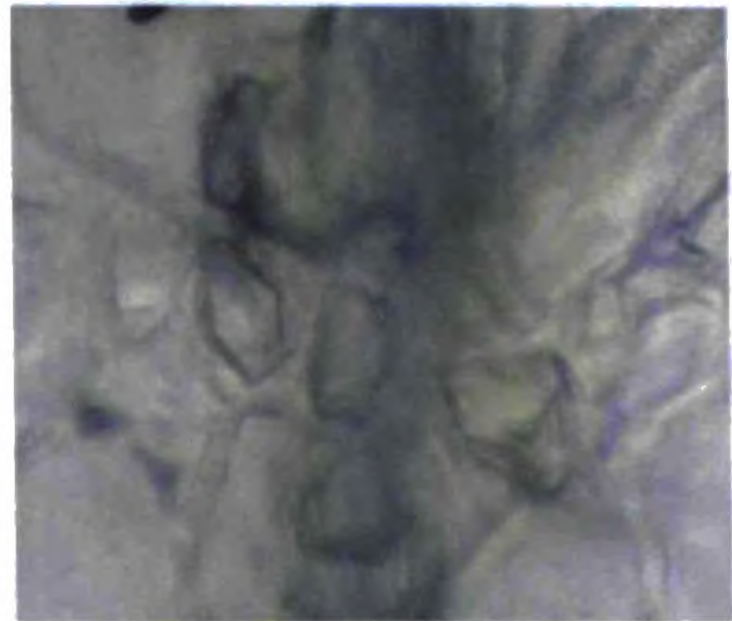
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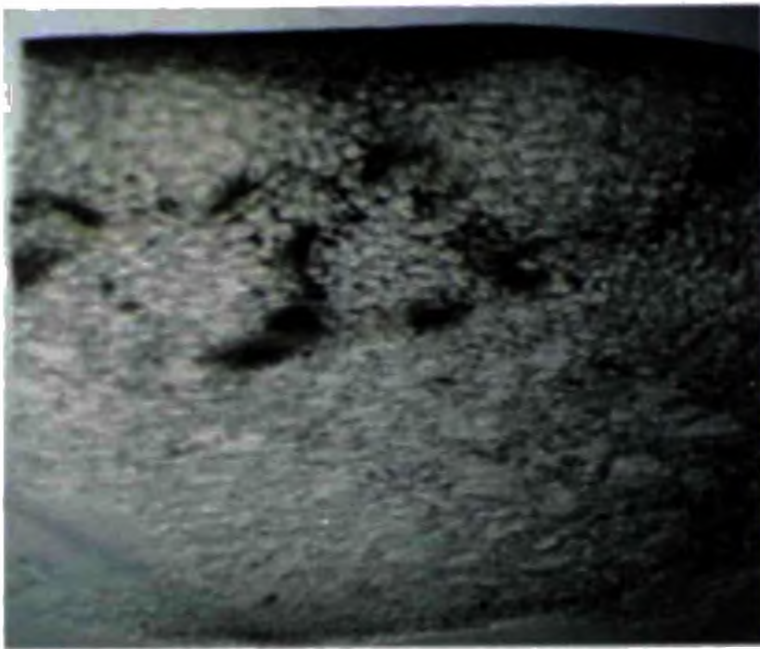
Figure 3: (a) Scaleriform vessels, (b) Rhomboidal crystals (c) Juicy sac (d) Central axis (e) Stained central axis (f) Xylem and Phloem.



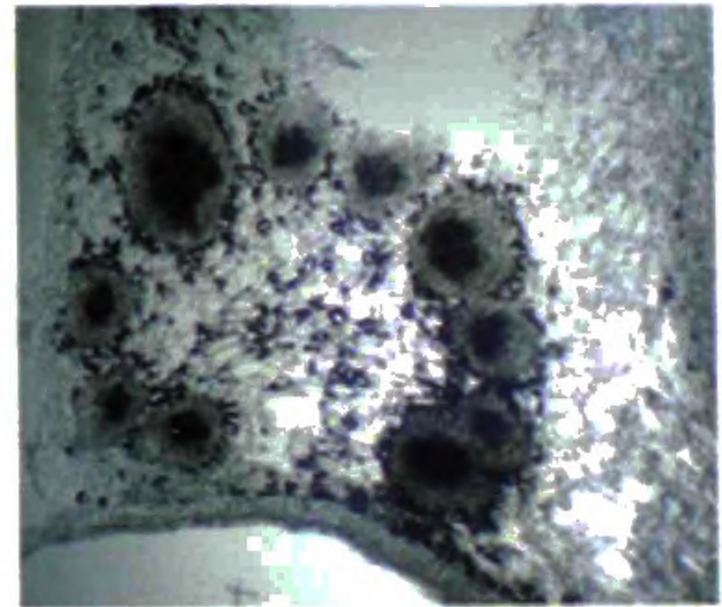
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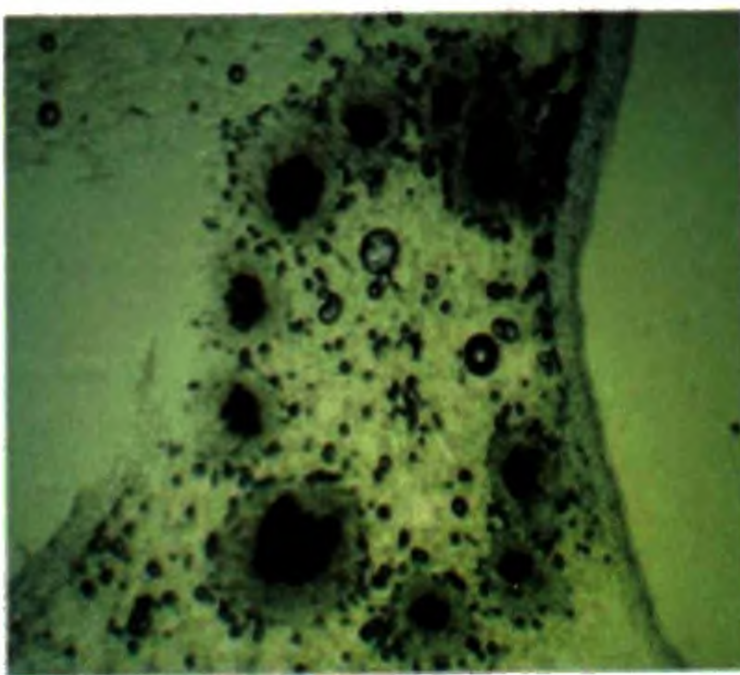
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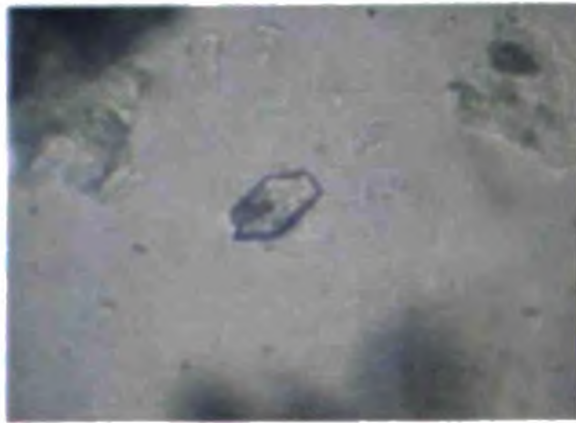
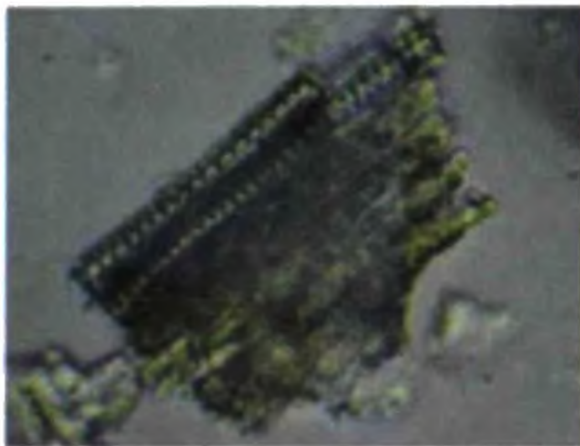
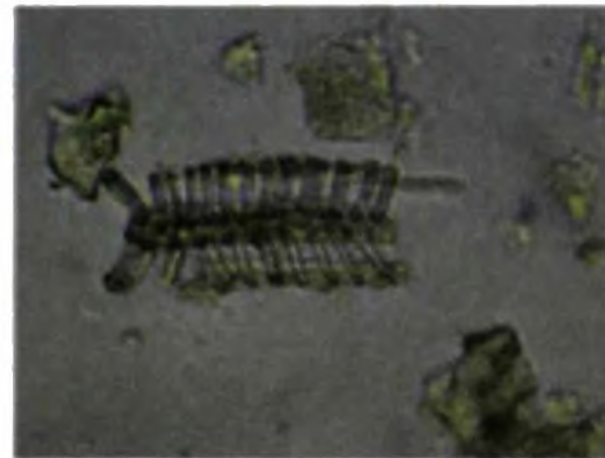
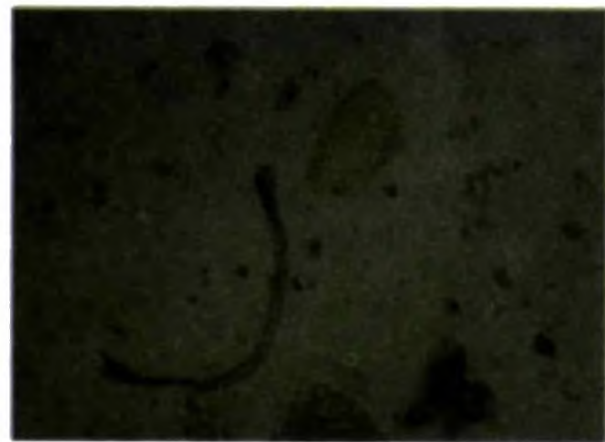
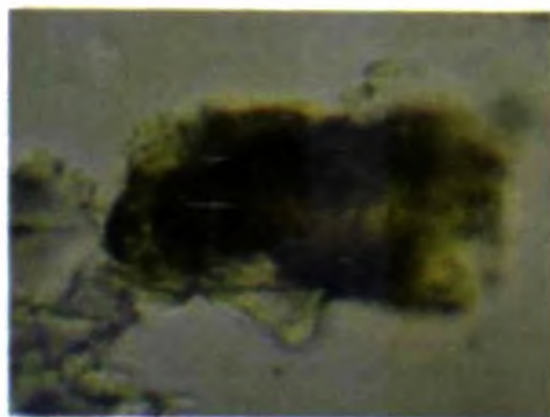
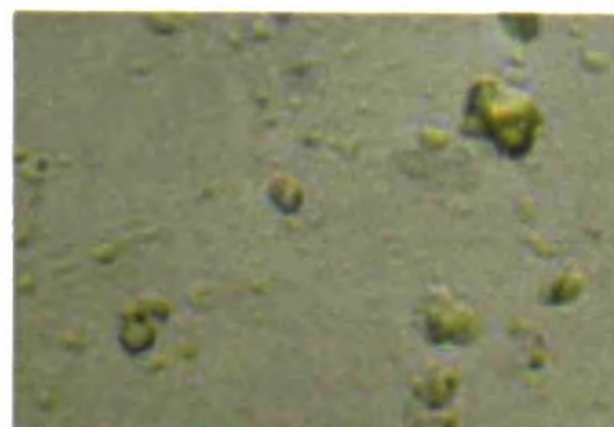


e



f

Figure 4: (a) Rhomboidal crystals (b) Albido fragment (c) Scaleriform vessels (d) Annular vessels (e) Fragment of fibers (f) Fibers and Tannin (g) Tanin (h) Oil globule.

**a****b****c****d****e****f****g****h**

Powder microscopy

Organoleptic characters

Organoleptic characters such as colour, odour, texture and taste are recorded by using sensory method as shown in Table 1.

Table 1: Organoleptic characters of *Citrus limon* powder

Serial No.	Character	Observation
1	Colour	Yellow
2	Nature	Smooth
3	Taste	Bitter, Sour
4	Odour	Aromatic (Citrus smell)

Diagnostic characters of powder of whole fruit shows rhomboidal crystals from albido region (Figure 4a) and prismatic crystals of calcium oxalate, albido fragment (Figure 4b), scalariform vessels (Figure 4c), annular vessels (Figure 4d), pitted vessels, fragment of fibres (Figure 4e, 4f), tannin content (Figure 4g), oil globules (Figure 4h), mesocarp cells, fragments of epicarp cells and fragments of column.

Histochemical evaluation

Thick sections were subjected to various histochemical tests to detect tannin and lignin etc. Results were as shown in Table 2.

Table 2: Histochemical evaluation of thick section of *Citrus limon*

Serial No	Reagent	Observation	Characteristic	Result
1	Phloroglucinol +Conc. HCl	Red	Lignified cells	++
2	Phloroglucinol +Conc. HCl	Dissolved	Ca Ox -crystals	++
3	FeCl ₃ solution	Dark blue	Tannin cells	++
4	Sudan III	Red	Oil	++

(++ Present)

Table 3: Physicochemical evaluation of *Citrus limon* powder

Parameters	Values obtained (%)
Loss on drying at 110°C	1.2 W/W
Ash value	1.8 W/W
Water soluble extract	58.4 W/V
Alcohol soluble extract	26.8 W/V

Table 4: Physico-chemical parameters of Juice of *C. limon* fruit (average of three batches)

Parameters	Values obtained
pH	3.5
Specific gravity	1.047
Total solid content	7.4% w/w

Conclusion

Mesocarpal cells, annular and scalariform vessels, rhomboidal crystals, prismatic crystals of calcium oxalate, fragment of fibre and albido, pitted vessels, tannin content, oil globules and fragments of column are found in *Citrus limon*. These observed parameters could be useful to establish certain botanical standards for identification and standardization of *C. limon*.

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