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Vijeta Gupta
Sharda University, School of Pharmacy, Knowledge Park III, Greater Noida, Uttar Pradesh, India

Dr. Vijender Singh
Sharda University, School of Pharmacy, Knowledge Park III, Greater Noida, Uttar Pradesh, India

Correspondence
Vijeta Gupta
Sharda University, School of Pharmacy, Knowledge Park III, Greater Noida, Uttar Pradesh, India

Pharmacognostic studies of the leaves of *Citrus limon* Linn.

Vijeta Gupta and Dr. Vijender Singh

Abstract
To study detailed pharmacognostic profile of leaves of *Citrus limon* Linn. (*C. limon* Linn.). (Rutaceae).
Methods: Leaf of *C. limon* Linn was studied by macroscopical, microscopical, physicochemical, phytochemical, fluorescence analysis of powder of the plant and other methods for standardization recommended by WHO.
Results: T.S of leaf shows a layer of upper and lower epidermis covered with thick cuticle on both surfaces; the cells of upper epidermis are large in size and devoid of stomata while the cells of lower surface are comparatively very small with stomata present. T.S passing through the lamina showed two layers of palisade cells which extended up to the Meristem leaving 2-3 cells of collenchyma in between, spongy mesophyll 3-4 cells broad, lateral vascular bundles are abundant in lamina arranged in the middle of mesophyll tissue. T.S passing through meristem showed slightly protuberated upper surface while the lower surface deeply protuberated with a notch in the middle of the margin. Multilayered collenchymatous hypodermis was present towards the lower side while 2-3 cells on upper surface, Merisel showed a large sized median, collateral vascular bundle surrounded by fibrous bundle sheath. Phloem was arranged in arc shaped towards the lower side, numerous mucilage canals and cluster crystals of calcium oxalate were present in midrib and laminar region. Stomata: Paracytic Shape of epidermal cells: Irregular and collapse. The investigations also included leaf surface data; quantitative leaf microscopy and fluorescence analysis. Physiochemical parameters such as LOD, swelling index, extractive values and ash values were also determined. Preliminary phytochemical screening of leaf mainly revealed the presence of triterpenoids, saponins, tannins & flavonoids. Carbohydrates, proteins.
Conclusions: The results of the study can serve as a valuable source of information and provide suitable standards for identification of this plant material in future investigations and applications.

Keywords: *Citrus limon* L inn, microscopy, Macroscopy, pharmacognosy, stomata, xylem, phloem, physicochemical

1. Introduction
Citrus limon Linn. (Rutacea) is commonly known as Lemon in English and Nimbu in Hindi. The lemon tree reaches 10 to 20 ft (3-6 m) in height and usually has sharp thorns on the twigs. The alternate leaves, reddish when young, become dark-green above, light-green below; are oblong, elliptic or long-ovate, 2 1/2 to 4 1/2 in (6.25-11.25 cm) long, [2] finely toothed, with slender wings on the petioles. The mildly fragrant flowers may be solitary or there may be 2 or more clustered in the leaf axils. It is found in India, China, Southeast Asia, New Guinea, Australia, especially southern Italy and Spain. It grows throughout the hotter parts of India, Eastern Himalayas and is abundant in Uttar Pradesh. Lemon juice helps to control Blood Pressure, purifies blood, reduces swollen spleen, and strengthens immune system as it has vitamin C, B, B2, calcium and iron. It protects your body against germs and bacteria. Drink lemon juice every day and its very good for health However, available literature revealed that no pharmacognostic study has been carried out on the plant except on leaves; hence the present investigation was under taken. The object of present study is to evaluate various Pharmacognostical parameters such as macroscopic, microscopy, physicochemical, fluorescence and phytochemical studies of the plant.

2. Materials and Methods
2.1 Plant material
Citrus Limon Linn. Plant was collected during the month of October, from local place of the Allahabad district and dried under shade. Care should be taken for selecting normal and healthy organs. The identity of the plant samples were confirmed by matching with the samples in the LWG herbarium of the National Botanical Research Institute, Lucknow whose reference no. is 97847.

2.2 Pharmacognostic study
Fresh leaves was taken for morphological and histological studies. Coarse powder (60 #) was used to study microscopical characters, physicochemical parameters and phytochemical investigation. For the microscopical studies, transverse sections of leaves was prepared and stained as per standard procedure [5, 6]. The powder microscopy was performed according to the method of Khandelwal [5].

2.3 Physicochemical and phytochemical analysis
Physicochemical values such as percentage of ash values and extractive values were determined according to the well-established official method and procedure [8, 9]. Preliminary screening was carried out using the standard procedure described by Khandelwal [7].

2.4 Florescence analysis
Powdered leaf material was treated with various chemical reagents and exposed to visible, ultraviolet light (Short UV) to study their fluorescence behavior [10].

3. Results
3.1 Macroscopic characteristics
Macroscopically, the fresh leaf of C. Limon Linn. is5.5 -6.8 cm long, 7 to 12 cm wide and petiole 2-4 cm in length, twisted.in length, simple, glabrous, broadly obvate in shape, acute apex with crenate, dentate margin and green in color (Figure 1) Flower yellowish white, ill smelling, sessile; fruits large, round, green and fleshy; seed embedded in the fleshy pulp of the fruit. Bark dark grey exfoliating in thin strips.

Fig 1: Shape and Apex of *Citrus Limon* Leaf

3.2 Microscopical characteristics
3.2.1 Leaf microscopy
TS of leaf shows a layer of upper and lower epidermis covered with thick cuticle on both surfaces, the cells of upper epidermis are large in size and devoid of stomata while the cells of lower surface are comparatively very small with stomata present. T.S passing through the lamina showed two layers of palisade cells which extended up to the Meristem leaving 2-3 cells of collenchyma in between, spongy mesophyll 3-4 cells broad, lateral vascular bundles are abundant in lamina arranged in the middle of mesophyll tissue. T.S passing through meristem showed slightly protuberated upper surface while the lower surface deeply protuberated with a notch in the middle of the margin. Multilayered collenchymatous hypodermis was present towards the lower side while 2-3 cells on upper surface, Merisel showed a large sized median, collateral vascular bundle surrounded by fibrous bundle sheath. Phloem was arranged in a arc shaped towards the lower side, numerous mucilage canals and cluster crystals of calcium oxalate were present in midrib and laminar region. Stomata: Paracytic Shape of epidermal cells: Irregular and collapse. (Figure 2, 3, 4)

3.2.2 Microscopical study of Citruslimon petiole
TS of petiole was almost circular with circular surface. The outermost region consist of a thin layer of epidermis bearing trichome. Underneath the epidermis lies a continuous collenchymatous band of hypodermis embedded with mucilage canal followed by multilayered cortex region embedded with stone cells. Pith was located centrally encircled by xylem, phloem and Pericyclic fibers containing oil glands. Mucilage canals were absent in the xylem, phloem and pith region. (Figure 5)

3.2.3 Powder microscopy of *Citrus limon* Leaf
The powder microscopy of Citrus Limon leaf shows abundant fragments of upper and lower epidermis in surface view, upper epidermis devoid of stomata and lower epidermis containing stomata. Transversely cut fragments of the lamina showing a row of palisade cells lying underneath the upper epidermis, cluster crystals were scattered throughout and embedded in between the cells, fragments of medullary rays were present and unicellular unicellular trichome was present. (Figure 6).

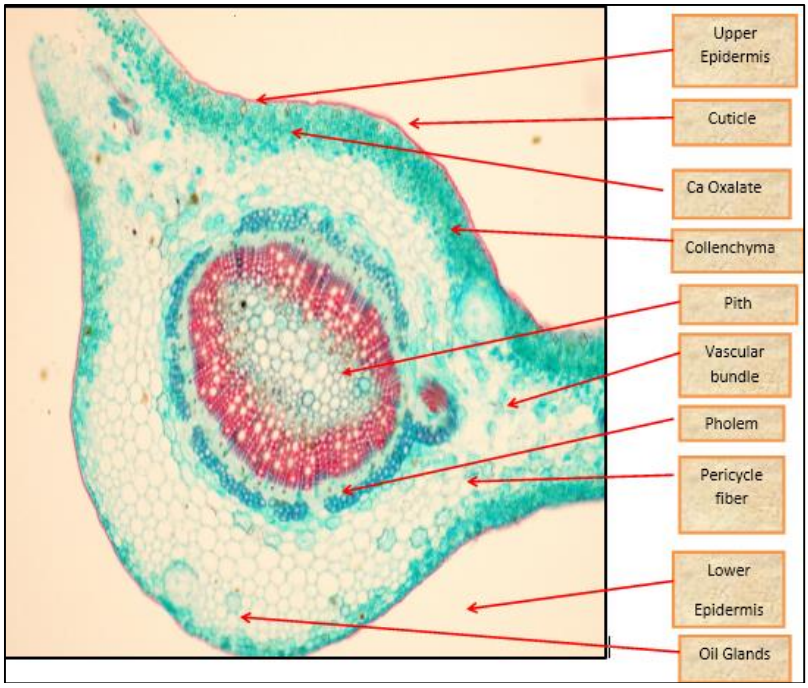

Fig 2: Transverse section from midrib Leaf (High Resolution)

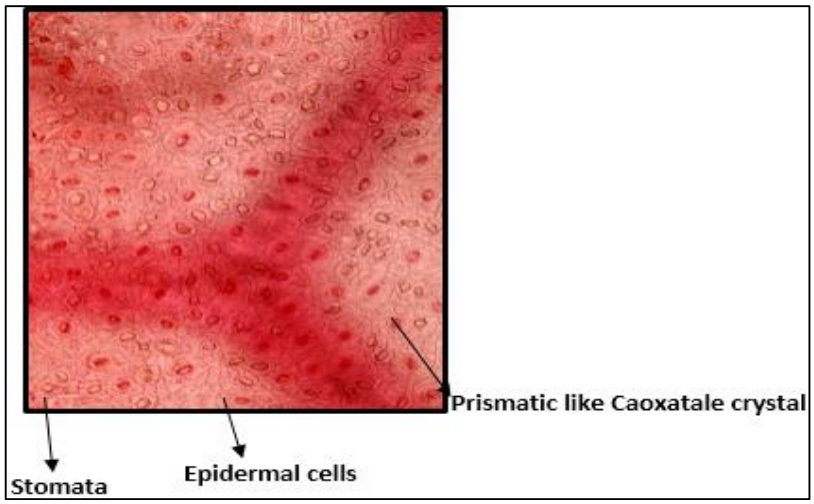

Fig 3: TS. Of leaf surface for quantitative analysis of *Citrus limon* Linn

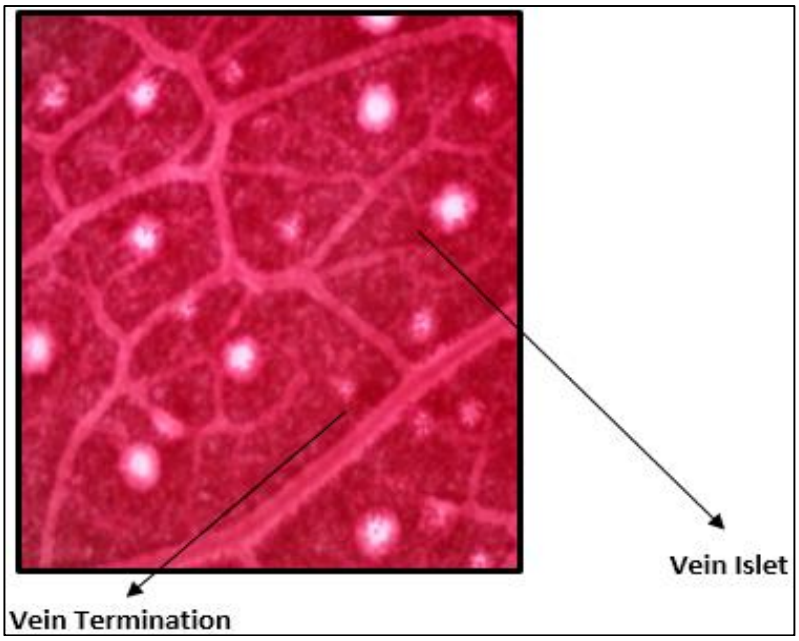

Fig 4: T. S. of leaf surface for quantitative analysis of *Citrus limon* Linn.

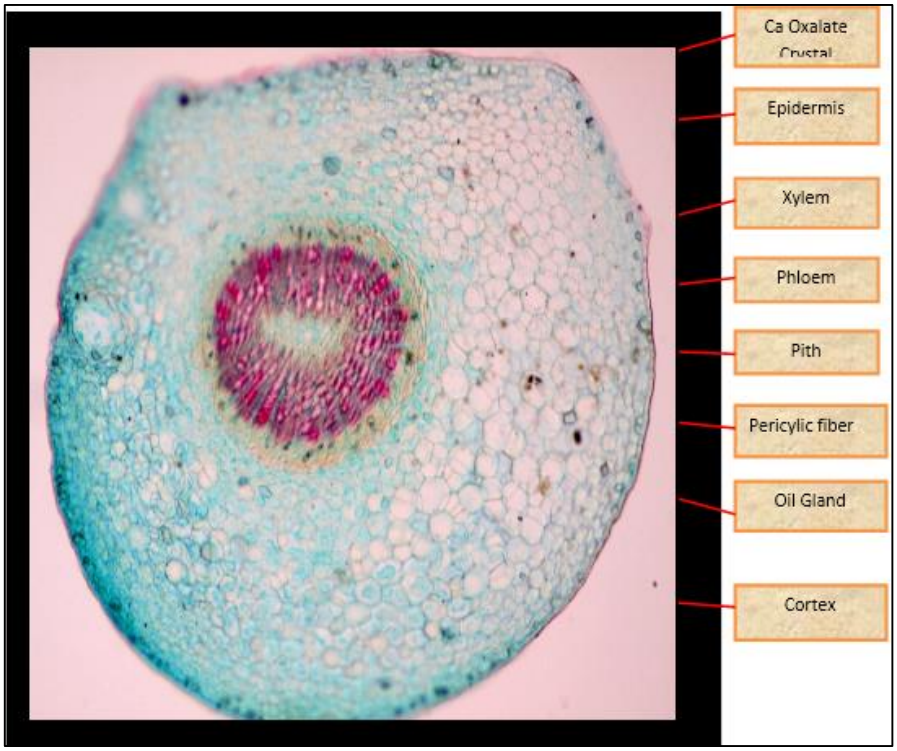

Fig 5: Transverse section of petiole Leaf (High Resolution)

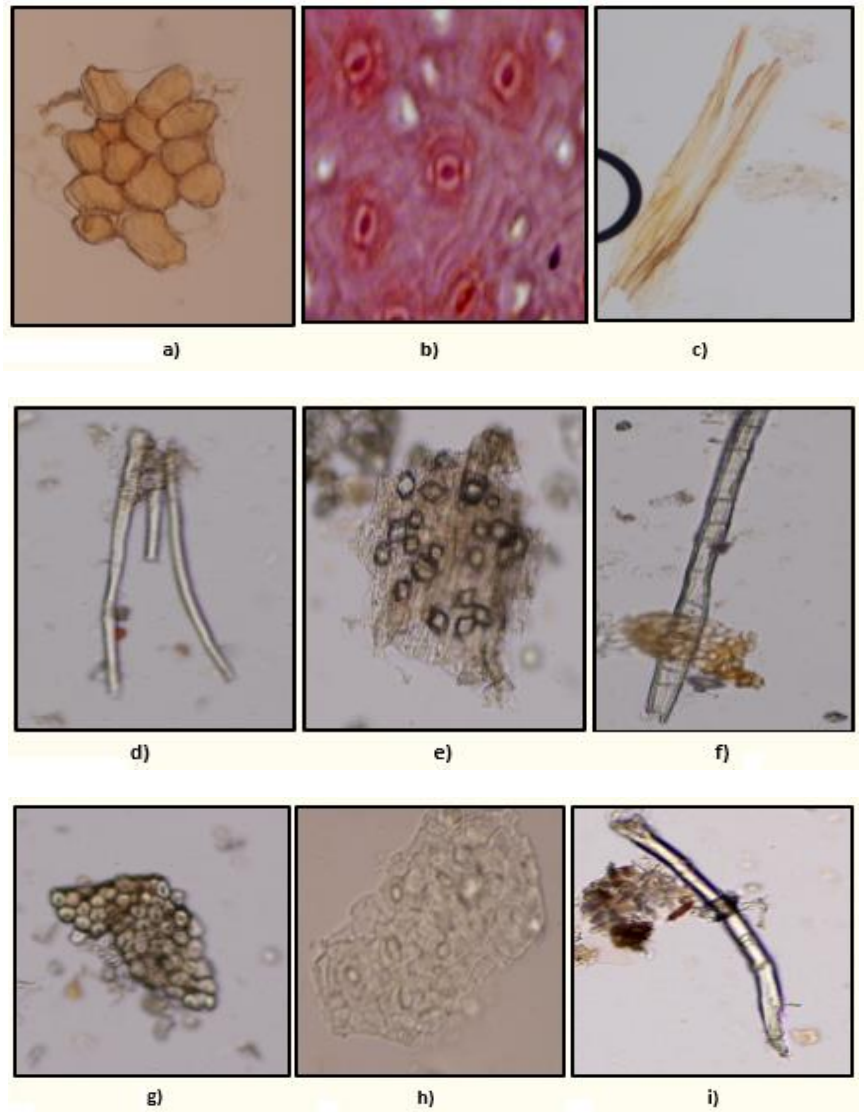

Fig 6: Powder microscopy of *Citrus limon* Linn. (Leaves)a)Sclerenchyma cells, (b) Stomata, (c)Fiber, (d)Fiber, (e)Calcium crystals (f) Vessels, (g) Scleroses, (h) Epidermal cells, (i) Unicellular Trichomes

3.3.3 Leaf surface of *C. limon* Linn

a: Stomata; b: Vein-islet and veinlet termination; c: Palisade cells.

Table 1: Leaf constants (at 100X)

S. No.	Parameter	Region	Valve (in 1mm^{3}area) Average
1	Stomata number, upper surface	Apex	606.66
		Midrib	711.66
		Basal	660.4
2	Stomata number, lower surface	Apex	290.00
		Midrib	306.66
		Basal	253.33
3	Stomatal Index, upper surface		16
4	Stomatal Index, upper surface		17.33
5	Vein-islet number	Apex	16
		Midrib	17.33
		Basal	17
6	Vein Termination No.	Apex	16
		Midrib	19
		Basal	16.6
7	Palisate Ratio		2-3 per cell

3.3 Preliminary Phytochemical screening

Preliminary phytochemical screening of leaf mainly revealed the presence of triterpenoids, saponins, tannins and flavonoids. Carbohydrates, proteins.

3.4 Physicochemical parameter

Physicochemical analysis of leaf powder *viz.* foreign matter, loss on drying, swelling index, ash value and extractive value are presented in Table 2. The fluorescence analysis of *C. Limon* leaf under day light and UV (Short, 254 nm) light is recorded in Table 3.

Table 2: Physico-chemical parameters

S. NO.	Physico Chemical Constant	Average Value
1	Foreign matter(%w/w)	0.20
2	Loss on drying (%w/w)	3.20
3	Moisture content (% w/w)	8.6
4	Total Ash (% w/w)	11.15
5	Water soluble Ash (% w/w)	3.69
6	Acid Soluble Ash (% w/w)	0.46
8	Alcohol soluble Ash (% w/w)	4.20
9	Swelling Index (%w/w)	6.83
10	Crude fiber	27.36
11	Extractive value (Hexane soluble) (% w/w)	7.33
12	Extractive value (Alcohol soluble) (% w/w)	13.7
13	Extractive value (Water soluble) (% w/w)	33.8

Table 3: Fluorescence analysis of leaf and stem bark powder of *C. limon* Linn.

S. No.	Reagents	Day light	UV light at 254nm	UV light at 366nm
1.	Powder as such	Mehandi green	Fluorescent green	Dark green
2.	Powder + 1N HCl	Greenish yellow	Yellow green	Dark green
3.	Powder + 1N HNO3	Greenish yellow	Light green	Dark green
4.	Powder + 1N H2SO4	Light green	Green	Green
5.	Powder + G.A. Acid	Greenish yellow	Green	Green
6.	Powder + 1N NaOH	Mehandi green	Dark green	Black green
7.	Powder + Aq. NaOH	Greenish yellow	Green	Dark green
8.	Powder + Meth. NaOH	Mehandi green	Dark green	Black green
9.	Powder + I2	Light yellow	Green	Black green
10.	Powder + 50% KOH	Greenish yellow	Green	Black green
11.	Powder + 50% HNO3	Greenish yellow	Light green	Dark green
12.	Powder + Conc H2SO4	Green	Black green	Black green
13.	Powder + Alcoholic FeCl3	Greenish yellow	Dark green	Black green
14.	Powder + Acetone	Light green	Light green	Black green
15.	Powder + Ethyl Alcohol	Mehandi green	Dark green	Black green

4. Discussion

Ethno medically, the leaves of this plant were used by local people in the treatment of various disease conditions without standardization. The standardization of a crude drug is an integral part for establishing its correct identity. Before any crude drug can be included in an herbal pharmacopoeia, pharmacognostic parameters and standards must be established. Microscopic method is one of the simplest and

cheapest methods to start with for establishing the correct identity of the source materials [20, 24]. The pharmacognostic standards for leaves of *C. limon* Linn. are carried out for the first time in this study. The macroscopical characters of the leaf can serve as diagnostic parameters. Microscopical studies indicated the presence of median large size vascular bundle and cup shaped xylem in leaf. Presence of cortical vascular bundle, patches of Pericyclic fibers and brown pigment containing cells are the characteristics of the plant. Ash values and extractive values can be used as reliable aid for detecting adulteration. These studies help in the identification of the plant materials [11]. Percentage extractives and ash analysis were carried out and results showed that total ash of leaf is about three times higher than water soluble extractive value of leaf was two times higher than alcohol soluble extractive value. Ash values of drug give an idea of earthy matter or the inorganic composition and other impurities present along with drug. Extractive values are primarily useful for the determination of exhausted and adulterated drugs. The result obtained showed that the drug sample contain significant amount of crude fiber and because of which it is used as roughages and fodder for animals. Extractive values are also useful to evaluate the chemical constituents present in the crude drug and also help in estimation of specific constituents soluble in particular solvents [12]. The fluorescent analysis under day light and UV light by treatment with different chemical reagents showed different color. This analysis suggests that, leaves extract of *C. limon* Linn. Probably contain active agent(s) and this provides the basis for their folkloric use as a cure for some human ailments.

In conclusion, these parameters which are being reported for the first time, could be useful in setting some diagnostic indices for the identification and preparation of a monograph of the *Citrus limon* L. inn plant.

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