

PHARMACOGNOSTICAL EVALUATION OF BIJAPURA (*Citrus medica* Linn.) LEAVES

Kalpesh Panara^{1*}, Nishteswar K², Harisha CR³, Shukla VJ⁴

1. Ph.D. scholar, Dept. of Dravyaguna, I.P.G.T. & R.A., Gujarat Ayurved University, Jamnagar, Gujarat, India.
2. Professor & Head, Dept. of Dravyaguna, I.P.G.T. & R.A., Gujarat Ayurved University, Jamnagar, Gujarat, India.
3. Head, Pharmacognosy laboratory, I.P.G.T. & R.A., Gujarat Ayurved University, Jamnagar, Gujarat, India.
4. Head, Pharmaceutical laboratory, I.P.G.T. & R.A., Gujarat Ayurved University, Jamnagar, Gujarat, India.

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Abstract

Bijapura, botanically identified as a *Citrus medica* Linn. is an important plant of Ayurvedic material medica. Its various parts are widely used to treat many ailments in traditional system of medicine. Leaves are used to treat insomnia, skin diseases, dyspnoea, hiccough and vomiting. No reports are available on the pharmacognostic studies of the leaf of *C. medica*. Present study aimed to establish pharmacognostic standards and physicochemical analysis of leaf of *Citrus medica*. Macroscopic and microscopic characters, quantitative microscopy, histochemical analysis and physicochemical analysis were carried out to ascertain quality standards for leaf. Parameters were studied according to pharmacopoeial guidelines. Study showed that leaf of *C. medica* contains lysigenous cavities with the presence of oil globules, calcium oxalate crystals, paracytic stomata, and incomplete ring of sclerenchyma. Physicochemical analysis revealed loss on drying 12.29% w/w, total ash content 3.44% w/w, water soluble extractive 14.3% w/w, alcohol soluble extractive 25.6% w/w, and pH (5% aqua solution v/w) 6.0. These parameters will help for establishing identification of this drug for the future references.

Key words: Bijapura; *Citrus medica*; Pharmacognosy; Physicochemical analysis.

*Address for correspondence:

Dr. Kalpesh Panara,
Ph.D. Scholar, Dept. of Dravyaguna,
I.P.G.T. & R.A., Gujarat Ayurved University,
Jamnagar, Gujarat, India - 361 008
E mail: kbpanara@gmail.com

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INTRODUCTION

Pharmacognostic study gives the scientific information regarding the purity and quality of the plant drugs.^[1] The detailed pharmacognostical evaluation gives valuable information regarding the morphology, microscopical and physical characteristics of the crude drugs.^[2] *Citrus medica* Linn., commonly known as a Citron in English and Bijapura in Ayurvedic literature is member of *Rutaceae* family.^[3] Citron is a slow-growing shrub or small tree reaching up to 15 ft (4.5 m) in height with stiff branches and twigs and spines in the leaf axils. The flower buds are large and white or purplish. The peel is yellow, usually rough and bumpy and very thick. The pulp is pale-yellow or greenish divided into as many as 14 or 15 segments, firm, not very juicy, acid or sweet and contains numerous seeds.^[4]

Various parts of Bijapura are widely used in Indian traditional system of medicine. Leaves are useful to induce sleep.^[5] In ancient literature citron was mentioned as an antidote of every kind of poison.^[6] Both the leaves and juice of the citron are used by the people of South-Eastern Nigeria for febrile illness.^[7] Many pharmacological studies have been conducted to investigate the properties of *Citrus medica* L. in an attempt to authenticate its use as a multi-purpose medicinal agent. *Citrus medica* leaves possesses anthelmintic and estrogenic activities; fruit has analgesic, anticancer, insulin secretagogue and antiulcer activities; peel possesses many qualities including hypoglycaemic, anticholinesterase, hypocholesterolemic, hypolipidemic, antimicrobial and anthelmintic properties; seed has antidiabetic, hypocholesterolemic, hypolipidemic and estrogenic activities.^[8]

The macroscopic and microscopic description of a medicinal plant is the first step towards identification and determination of purity. However, such an attempt has not been made on *Citrus medica* so far. In the present

research, effort has been made to document the pharmacognostic features of the plant leaf to identify it in its crude form.

MATERIALS AND METHODS

Collection and Authentication and Drying of the Plant Material

Leaves of *Citrus medica* L. collected from its natural habitat during May 2013, light house, Jamnagar (Latitude 22°28'59.37" N, Longitude 70°03'28.91"E), Gujarat and identified and authenticated by the taxonomist, Jadeja BA, Associate professor, Department of Botany, M.D. Science College, Saurashtra University, Porbandar, India. Leaves was washed and dried in shade for several days then powdered coarsely with the help of mixer. Sample was deposited in the herbarium of Pharmacognosy lab, I.P.G.T. & R.A., Gujarat Ayurved University, Jamnagar with voucher specimen number: Phm. 2013/14/6103.

Pharmacognostical evaluation

Macroscopic Characters

The following macroscopic characters for the fresh leaves were noted: Size and shape, colour, surfaces, venation, presence or absence of petiole, the apex, margin, base, lamina, texture, odour and taste.^[9]

Microscopic Characters

Free hand transverse sections through petiole and midrib of fresh leaf were taken and washed with chloral hydrate solution. Sections were first observed in distilled water then stained with phloroglucinol and concentrated HCl.^[10] Surface study of lamina was carried out to observe its characters by leaf peeling off method. Powder microscopy of shade-dried leaves powder was also carried out. Photomicrographs were taken by Carl zeiss trinocular microscope.

Quantitative Microscopy

Quantitative leaf microscopy to determine palisade ratio, stomata index and vein-islet number Stomatal size, individual cellular characters were carried out. The leaf epidermal studies were carried out on fresh specimens. Peels were removed mechanically through epidermal peeling off and stomatal index (SI) was calculated. The vein islet number and palisade ratio of lamina were determined according to the standard method.^[11]

Histochemical analysis

Histochemical analysis for starch, tannin, oil, lignin and crystals also carried out.^[12]

Physico-chemical Evaluation

Physico-chemical Parameters like Loss on drying, total ash, alcohol soluble extractive (90% methanol) and water-soluble extractive values were determined as per the API guidelines for the powdered leaves.^[13]

RESULTS

Macroscopical Characters

Citrus medica has unifoliate compound leaf which is alternately arranged. Petiole was short (0.2-1 cm) and wingless or slightly winged. Leaflet was ovate elliptic with crenate to serrate margin, apex obtuse or rounded, rounded at the base, leathery surface and reticulate venation.

The average leaf size was 16 cm in length and 7 cm in breadth. Fresh leaves are dark green in colour, with an orange fragrant and bitter taste. (Figure 1)

Microscopical Characters

Petiole

T.S. of petiole showed thin single layer of epidermis with cuticle. Below the layer of epidermis many (12-16) layers of chlorenchymatous cells were situated. Upper 3 to 4 layers of chlorenchyma were arranged compactly which contain chloroplast while cells of lower layers were larger and pentagonal in shape with more intercellular space. Radially intermittently arranged 5 to 7 layer of lignified sclerenchymatous pericyclic fibres, situated between endodermis and vascular bundles covered the vascular bundles. Between two groups of this pericyclic fibre tissue cortex cells containing chloroplast were extended. These tissues provide strength to the vascular bundles. Vascular bundles were conjoint, collateral, discontinued ring situated at the centre. Xylem, radially arranged vessels, was composed by xylem fibres and xylem parenchyma. Central part was occupied with thin-walled polygonal pitted paranchymatous cells with intercellular space in between them. The lysigenous cavities were found in the upper lamina portion. (Figure 2)

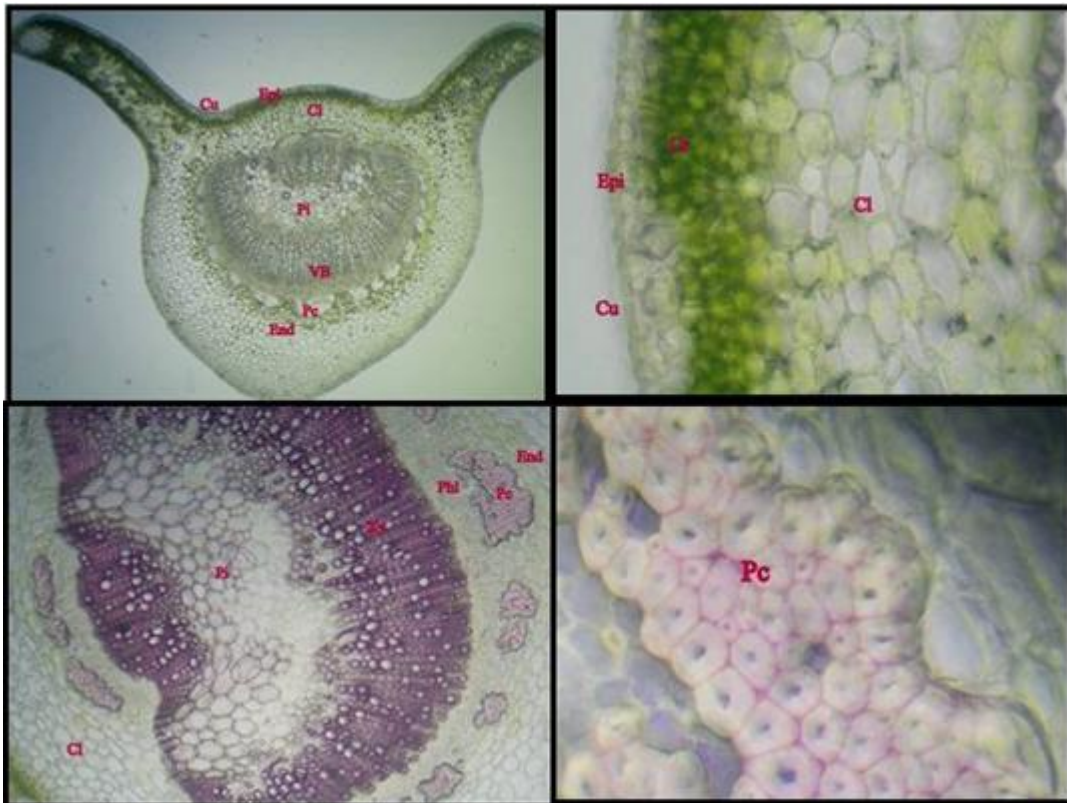
Midrib and lamina

Diagrammatic section through mid rib showed upper epidermis, lower epidermis and centrally located vascular bundle. Detailed Transverse section revealed epidermis, hypodermis with prismatic crystals, chloronchymatous cells which were continuous with palisade layer of lamina, chlorenchyma, fibrous ring of pericyclefibres, conjoint vascular bundles and pith centrally. Around the vascular bundle, the phloem tissues were surrounded (from the outside) with an incomplete ring of thick-walled fibres (sclerenchyma). This ring was composed of 1-5 layers of compact, donut-shaped fiber cells with very thick, apparently highly lignified, secondary walls.

Figure 1: Leaves of *Citrus medica*



Figure 2: Transverse section of Petiole



Cu – Cuticle; Epi – Epidermis; Cr – Chlorchyma; Cl – Cholenchyma; End – Endodermis; Pc – Pericyclic fibres;
Phl – Phloem, Xy – Xylem; Pi - Pith

The fibrous ring was wider (with more layers) in the center, especially on the lower side of the midrib, tapering towards the sides leaving a large gap (devoid of these fibers) on both sides of the vascular bundle. Several smaller gaps were also observed on both the lower and upper sides of the fibrous ring, and these gaps, in addition to the larger gaps on the sides, were occupied with much thinner-walled cells similar to those of the ground parenchyma. Central part occupied with thin-walled polygonal pitted paranchymatous cells with intercellular space in between them. The T.S. of lamina of leaf showed the presence of single layered epidermal cells; mesophyll differentiated into upper elongated compactly arranged palisade cells and lower oval to round shaped spongy parenchyma of 5 to 6 layers with intercellular spaces. The lysigenous cavities were found in the upper lamina portion and also in the ground tissue. Stomata were parasitic and present on lower surfaces only. Vascular bundles were conjoint, collateral, discontinued ring situated at the centre. Xylem radially arranged vessels, were composed of xylem fibres and xylem parenchyma. Phloem was present lower side of xylem consists phloem fibres and sieve elements. (Figure 3)

Surface study

Leaf peelings from both abaxial (dorsal) and adaxial (ventral) side showed epidermal cells with prismatic crystals and lysigenous cavity with oil globules. Paracytic stomata where the stoma is surrounded by two subsidiary cells, the long axis of which is parallel to the stoma were present only in lower epidermis. (Figure 4 & Figure 5)

Quantitative Microscopy

The palisade ratio, stomatal index and vein-islet number were found 2.4, 17.64 and 7.3 respectively. The stomata were slightly elliptical measuring 6 X 5µm in size while

prismatic crystal with average 6 X 3 µm sized. (Table 1)

Organolaptic character of powder

Coarse fibrous powder of *C. medica* possesses yellowish green color, bitter taste and lemon odor. (Table 2)

Powder microscopy

The powder microscopy of *C. medica* showed epidermal peeling, isolated vascular strands, trichome, parenchyma cells, paracytic stomata and the guard cells containing prismatic crystal. Isolated xylem elements were seen united in a bundle. The bundle consists of vessels, fibres and parenchyma cells. Separated parenchyma cells of the mesophyll tissue, crystal fibres, simple fibre, oil globules and fragment of annular vessels were seen scattered in the powder. (Figure 6)

Histochemical analysis

The histochemical analysis of leaf powder confirms the presence of tannin, lignin, crystals and oil globules. (Table 3)

Physico-Chemical Evaluation:

Physicochemical analysis (Table 4) of powder of *Citrus medica* leaves revealed loss on drying 12.29% w/w, total ash content 3.44% w/w, water soluble extractive 14.3% w/w, alcohol soluble extractive 25.6% w/w, and pH (5% aqua solution v/w) 6.0.

DISCUSSION

Pharmacognostic study gives the scientific information regarding the purity and quality of the plant drugs. In the leaf of *C. medica*, it is observed that the thick-walled 'fibrous ring' (sclerenchyma fibers) with highest lignification in the cell corners and cell walls of the sclerenchyma fibers found surrounding the vascular tissue.

Table 1: Quantitative microscopic examinations of *Citrus medica* leaves

Determination	Value
Stomatal index	17.64
Palisade ratio	2.4
Vein-islet number	7.3
Size of stomata	6×5 μm
Size of prismatic crystal	6×3 μm

Table 2: Organoloptic characters of powder of *Citrus medica* leaves

Parameters	Results
Texture	Coarse fibrous powder
Color	Yellowish green
Taste	Bitter
Odor	Lemon flavor

Table 3: Histochemical analysis of powder of *Citrus medica* leaves

Reagent	Test for	Color change	Result
Iodine	Starch	No change	-
Ferric chloride solution	Tannin	Bluish black	+
Sudan iii	Oil	Red	+
phloroglucinol +HCl	Lignin	Magenta	+
phloroglucinol +HCl	Calcium oxalate crystal	Effervescence	+

‘-’ negative, ‘+’ positive

Table 4: Physicochemical parameters of powder of *Citrus medica* leaves

Sr. No.	Physicochemical Parameter	Powder of <i>Citrus medica</i> leaves
1.	Loss on drying	12.29 % w/w
2.	Ash value	3.44 % w/w
4.	Water soluble extractive	14.3 % w/w
5.	Alcohol soluble extractive	25.6 % w/w
6.	PH (5% aqua solution v/w)	6.0

It indicates that this lignification acts as a protective barrier for the vascular tissue. Lysigenous cavities (oil cavities) are internal secretory structure which is frequently found in leaf and peel of citrus plant. Secretory cavities are spaces that result from dissolution of cells (lysigenous spaces) partly disintegrated cells occur along the periphery of the cavity.

The secretions (oil) are formed in the thylakoids of plastids and appear in the cytoplasm as droplets resulting in degeneration of cell components.

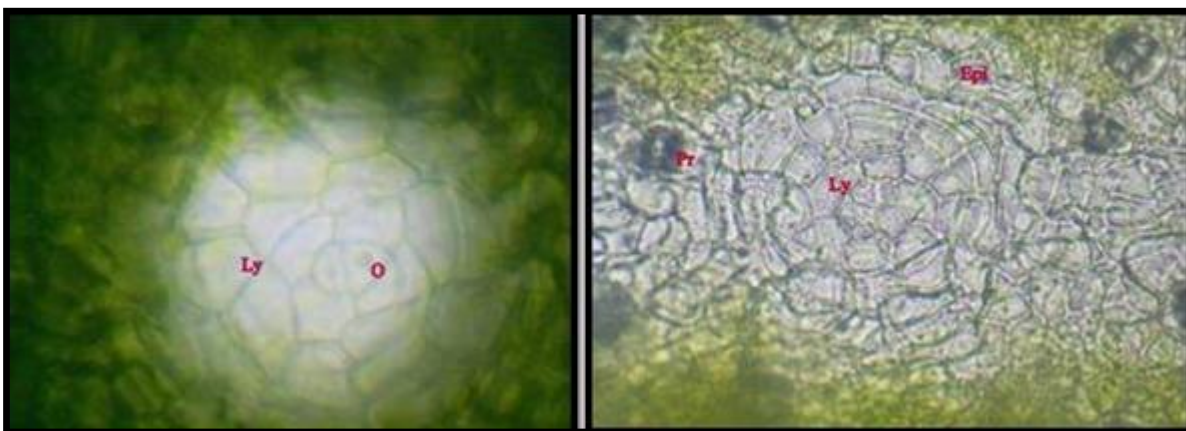
Among physicochemical analysis, less ash content indicates that leaves contain inorganic compound in lesser amount.

Figure 3: Transverse section of Midrib and Lamina



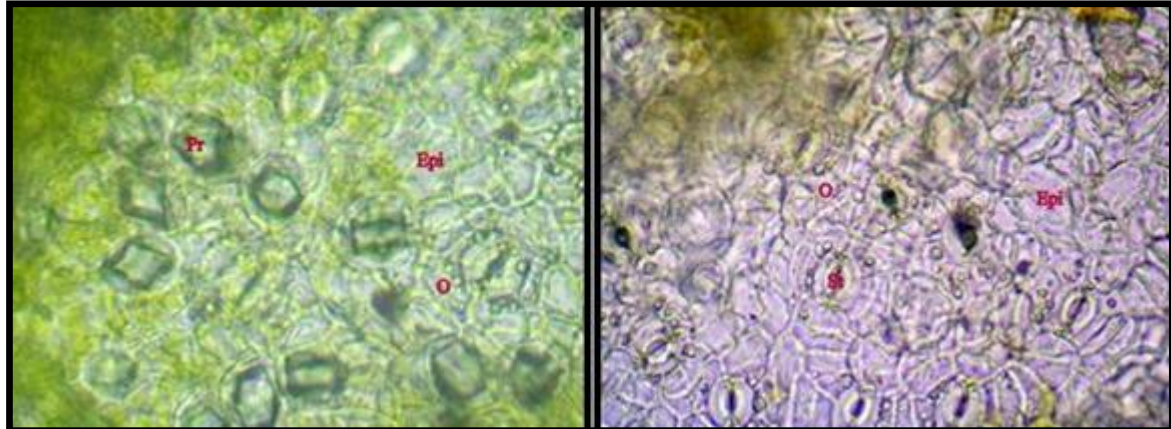
Cu – Cuticle; U Epi – Upper epidermis; L Epi – Lower epidermis; Hyp – Hypodermis; Cr – Chlorenchyma; End – Endodermis; Pc – Pericyclic fibres; Phl – Phloem; Xy – Xylem; Pi – Pith; Pr – Prismatic crystal; Pl – Palisade tissue; Gc – Ground tissue

Figure 4: Upper epidermis



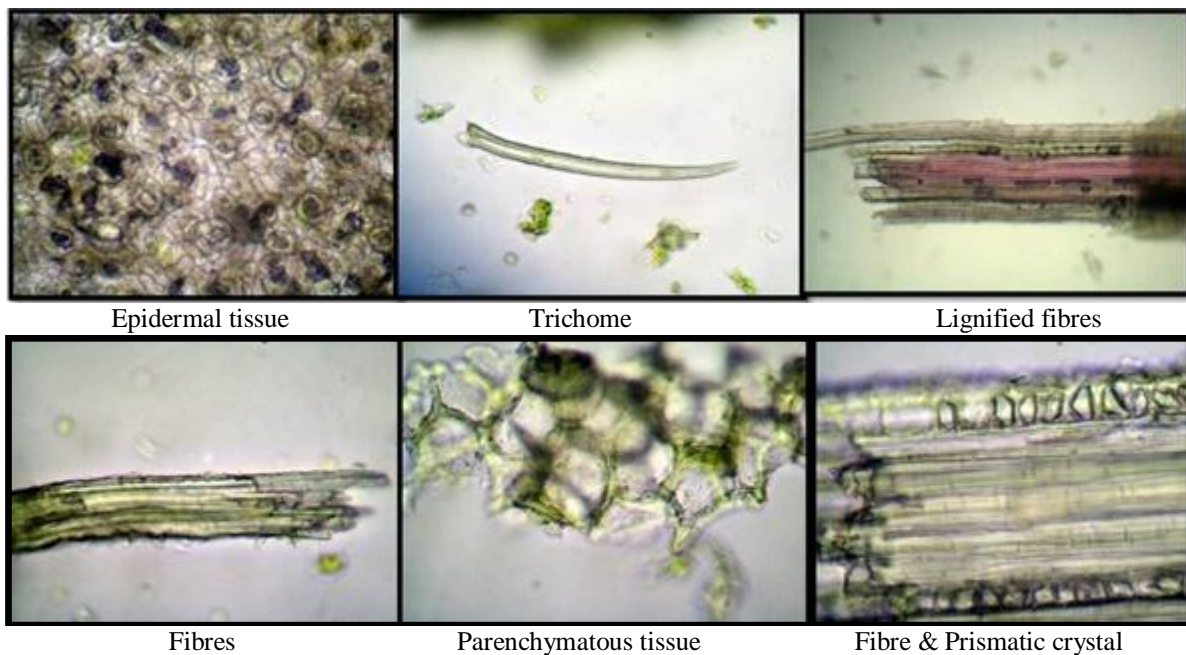
Ly – Lysigenous cavity; O – Oil globules; Pr – Prismatic crystal; Epi – Epidermal cells

Figure 5: Lower epidermis



Epi – Epidermis; O – Oil globules; Pr – Prismatic crystal; St – Stomata

Figure 6: Powder microscopy



Higher alcohol soluble extractive than water shows leaf of *C. medica* possesses more alcohol soluble constituents. pH indicates leaf is acidic in nature.

CONCLUSION

The analysis of the findings in the present study indicates that leaf of *C. medica* contains lysigenous cavities with the presence of oil

globules, calcium oxalate crystals, paracytic stomata, and incomplete ring of sclerenchyma. Histochemical analysis shows presence of tannin, lignin, crystals and oil globules. Physicochemical analysis revealed loss on drying 12.29% w/w, total ash content 3.44% w/w, water soluble extractive 14.3% w/w, alcohol soluble extractive 25.6% w/w, and pH (5% aqua solution v/w) 6.0. Pharmacognostical studies of the leaves from *Citrus medica*

provided a set of qualitative and quantitative standards that can serve as an important source of information to ascertain the identity and to determine the quality and purity of the plant materials for future studies. These parameters also will serve as standard data for quality control studies of pharmaceutical preparations which incorporate the leaves of *Citrus medica*.

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