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Pharmacognostic and Phytochemical Characterization of Leaves of *Citrus Maxima*

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ABSTRACT

Citrus maxima is an important drug mentioned in the traditional medicinal texts. Recent pharmacological findings indicate that leaves of *C. maxima* are used in convulsive cough, cholera, epilepsy and in treatment of haemorrhagie diseases. However, no conclusive pharmacognostic study or phytochemical investigation of these leaves has been performed yet. The present work deals with the qualitative and quantitative pharmacognostic evaluation of the leaf material of *C. maxima* and establishment of its quality parameters, including phytochemical evaluation. Diagnostic characters of powder include spiral xylem vessels, cystolithic unicellular and multicellular covering trichomes, lignified pericyclic fibres and prism crystals of calcium oxalate. Phytochemical analysis showed the presence of important classes of phytoconstituents like alkaloids, saponins and carbohydrates. This would pave the way for isolation of phytoconstituents, therapeutic investigations and standardization of formulations containing its leaf material.

Keywords: *Citrus grandis*, *Citrus maxima*, Madhukarkatika, Rutaceae.

INTRODUCTION

Citrus maxima Burm. syn. *Citrus decumana* Watt., *Citrus grandis* Osbeck. (Family – Rutacaea) is also known as (English) Chinese grape fruit, Pomelo, Jabong, Pummelo, (Hindi) Sadaphal and (Sanskrit) Madhukarkatika. Its leaves are traditionally used to produce sedative effect in cases of epilepsy, chorea and convulsive coughing. The essential oil from fresh leaves exhibits dermatophytic and fungistatic activity. The hot leaf decoction is applied on swellings and ulcers. Its leaves have anti-tumor activity (Anonymous, 1956; Khare, 2007; Kirtikar, 2005). The present investigation deals with the qualitative and quantitative microscopic evaluation of the leaf material and establishment of its quality parameters.

MATERIALS AND METHODS

Collection and authentication of leaves

Leaves of *C. maxima* were collected from the herbal garden of RK College of Pharmacy, Rajkot, Gujarat in July, 2011. Herbariums and voucher sample were prepared and deposited in Department of Pharmacognosy, RK College of Pharmacy (Voucher no. RKCP/COG/22/2011).

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Pharmacognostic studies

Morphology of fresh leaves of *C. maxima* was studied. Photomicrography of stained and unstained transverse sections of fresh leaves was performed. Leaf constants were established using camera Lucida. The leaves were dried under shade, powdered to 60#, stored in airtight containers and used for powder study and quantitative microscopy (Table.1) (Khandelwal *et al*., 1996).

Phytochemical study

5g powder was extracted with 50ml each of water and 80% methanol at 70°C for 15mins. Various phytoconstituents present in the leaves were detected by their respective chemical tests using the appropriate extracts (Table.2) (Feigl, 1956; Fishcher, 1952; Geissman, 1955; Harborne, 1973; Hodge *et al.*, 1962; List *et al.*, 1967; Robinson, 1964).

RESULTS

Pharmacognostic study

Macroscopy

Leaves are compound but appearing simple, 5-20cm X 2-12cm, alternate, ovate, ovate-oblong, or elliptic, apex acuminate, margin entire, surface leathery, texture glossy above, dull and minutely hairy beneath, base rounded to subcordate, venation reticulate, color of

upper surface dull-green and lower surface light green. Petiole is broadly winged and dull-green in color (Figure.1).

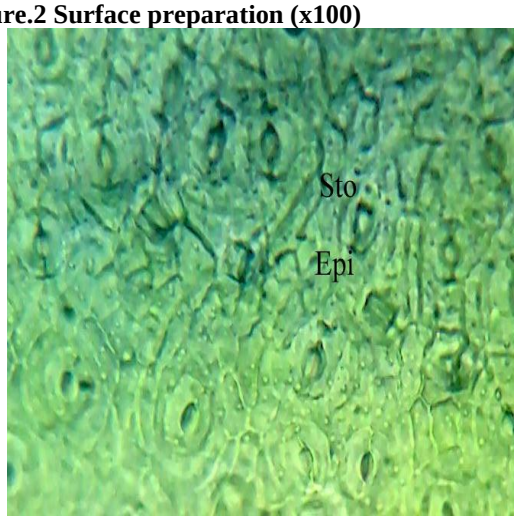
Figure.1 Leaf of *Citrus maxima*

Microscopy

Surface preparation

Epidermal cells are straight walled, having paracytic stomata and unicellular and multicellular covering trichomes (Figure.2).

Figure.2 Surface preparation (x100)



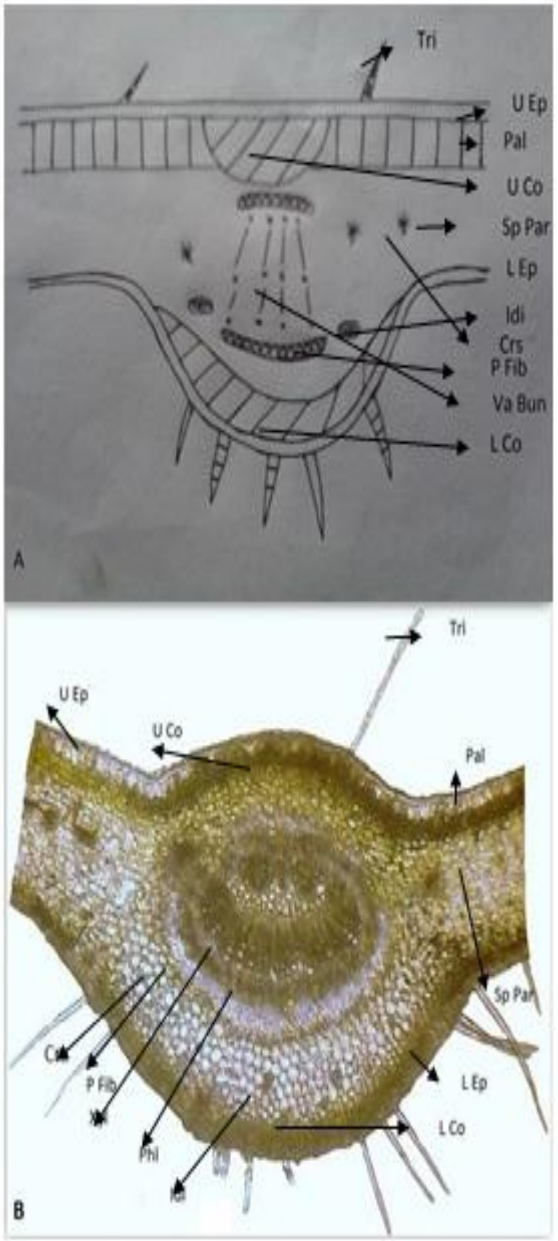
Transverse section

Lamina of transverse section shows large, cuboidal cells of upper epidermis covered by a thin cuticle. Unicellular and multicellular covering trichomes are present on both the epidermis, some of which are filled with cystolithic crystals. Underlying the upper epidermis is a bi-layered, compact, radially elongated palisade followed by spongy mesophyll composed of 5-8 layers of loosely arranged parenchymatous cells. Midrib consists of well-developed collenchyma beneath the epidermis. Vascular bundles are bicollateral and surrounded by discontinuous groups of lignified pericyclic fibres. Two small secondary vascular bundles are present above the primary vascular bundles. Ground tissue consists of loosely arranged polygonal parencymatous cells filled with cluster and prism crystals of calcium oxalate and some cystolithic idioblasts (Figure.3, 4).

Powder characteristics

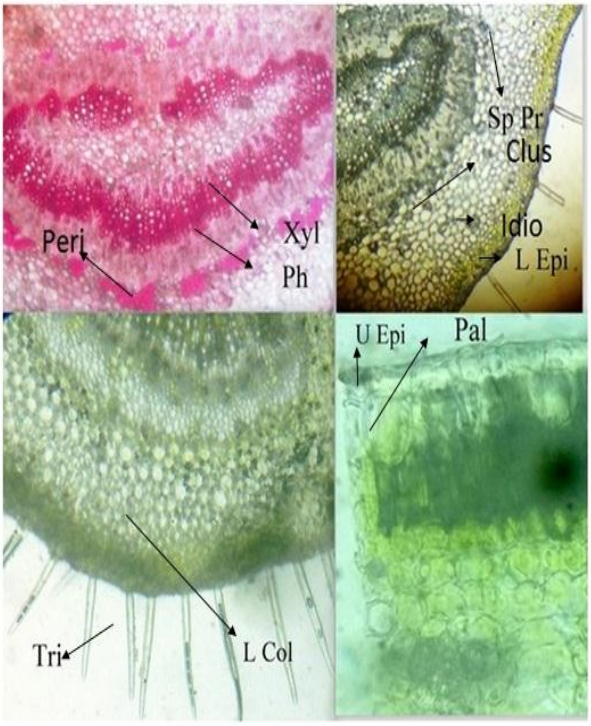
The powdered drug is dark green with no distinct odor or taste. The important diagnostic features of the powder include spiral xylem vessels, cystolithic unicellular and multicellular covering trichomes, lignified pericyclic fibres and prism crystals of calcium oxalate. (Figure.5).

Figure.3 A - Schematic T. S. of leaf, B - Detailed T. S. of leaf (x100)



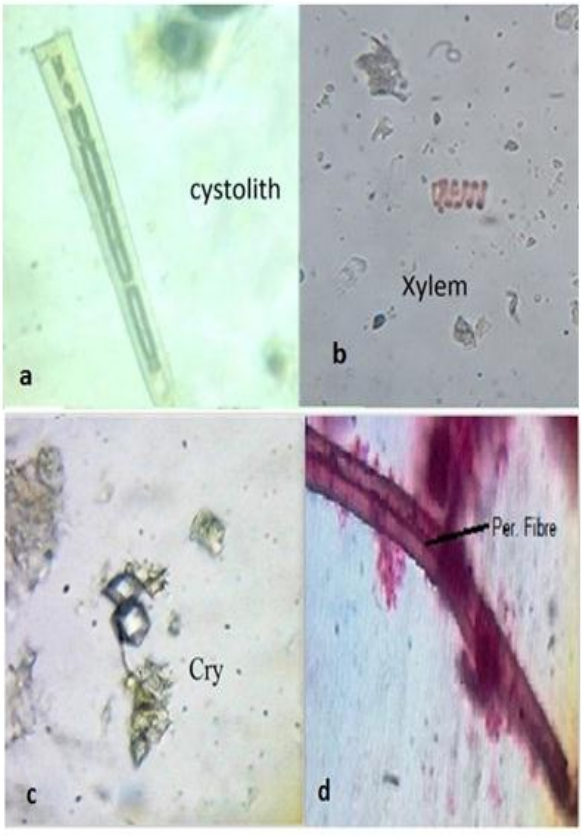
(U Co, Upper Collenchyma; U Ep, Upper Epidermis; L Co, Lower Collenchyma; L Epi, Lower Epidermis; Pal, Palisade; Xyl, Xylem; Phl, Phloem; Tri, Trichomes; Crs, prism crystals of calcium oxalate; Va Bun, Vascular bundle)

Figure.4 T. S. of leaf of *C. maxima* showing single enlarged portions (x400)



(U Co, Upper Collenchyma; U Ep, Upper Epidermis; L Co, Lower Collenchyma; L Epi, Lower Epidermis; Pal, Palisade; Xyl, Xylem; Phl, Phloem; Tri, Trichomes)

Figure.5 Powder study (x400)



(a, Cystolith trichome; b, Xylem vessel; c, Calcium oxalate prisms; d, Pericyclic fibre)

Table.1 Quantitative microscopy

Leaf constant	Mean value ± SD
Stomatal Number Upper surface Lower surface	 48±1 21±1
Stomatal Index Upper surface Lower surface	 68.6±0.5 34.28±0.5
Vein islet number	4±1
Vein termination number	3±1

Number of observations = 5
SD = Standard Deviation

Table.2 Phytochemical screening

Phytoconstituent	Test	Result
Alkaloids	Dragendorff's test Hager's test Wagner's test	+ve +ve +ve
Flavonoids	Shinoda test Lead acetate test	-ve -ve
Phenolics	Ferric chloride test Folin ciocalteu test	-ve -ve
Sterols and triterpenoids	Salkowski test Libermann-Buchardt test	-ve -ve
Cardiac glycosides	Legal test Baljet test	-ve -ve
Saponin glycosides	Foam test Lead acetate test	+ve +ve
Anthraquinone glycosides	Borntrager test Modified Borntrager test	-ve -ve
Carbohydrates	Fehling's test Molisch test	+ve +ve

DISCUSSION

The present work deals with the microscopical and phytochemical evaluation of the leaves of *Citrus maxima*. Diagnostic characters of powder include spiral xylem vessels, cystolithic unicellular and multicellular covering trichomes, lignified pericyclic fibres and prism crystals of calcium oxalate. Phytochemical analysis showed the presence of important classes of phytoconstituents like alkaloids, saponins and carbohydrates. This indicates that this plant can be useful for treating different diseases because the therapeutic activity of a plant is due to the presence of particular class of compounds. Development of such a monograph would help in isolation of phytoconstituents, therapeutic investigations and standardization of formulations containing its leaf material.

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