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Research Article

Pharmacognostic and Phytochemical Studies of Leaves of *Naringi crenulata* (Roxb.) Nicolson

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ABSTRACT

Naringi crenulata (Roxb.) Nicolson is an important medicinal plant of the family Rutaceae. It is commonly known as 'Mahavilvam' in Tamil. The present investigation deals with the pharmacognostic studies of the leaf of the said plant. Pharmacognostic studies include microscopic, physicochemical constituents (ash and extractive values), fluorescence analysis and preliminary phytochemical evaluations.

Key Words: *Naringi crenulata*, pharmacognostic features, stomata, fluorescence analysis.

INTRODUCTION

The world is blessed with natural and unique medicinal plants[1]. Medicinal plant research has now got a momentum among the scientists of the world. Medicinal plants contain some organic compounds which provide definite physiological action on human body and these bioactive substances include tannin, alkaloids, carbohydrates, terpenoids, steroids, and flavonoids[2, 3]. These compounds are synthesized by primary or rather secondary metabolism of living organisms. Secondary metabolites are chemically and taxonomically extremely diverse compounds with obscure function. They are widely used in the human therapy, veterinary, agriculture, scientific research and countless other areas[4]. A large number of phytochemicals belonging to several chemical classes have been shown to have inhibitory effects on all types of microorganisms *in vitro*[5]. The scientific evaluation of ethnomedicinally important plants is now being done thoroughly covering various aspects of their study like efficacy of the crude drugs, chemistry of active principles, different pharmacognostic parameters, etc. Chemical analysis and biological assays of medicinal plants are very important aspects in pharmacognostic evaluation of medicinal plants[6,7,8]. Use of micro morphology and anatomy is now also the recognized tool in the field of plant systematics. The main aim of the present study is to investigate the pharmacognostical and phytochemical properties of leaves of *Naringi crenulata*, an ethnomedicinally important plant. This investigation will be a useful marker for identification of the crude drugs obtained from the investigated taxa.

Naringi crenulata (Roxb.) Nicolson that belongs to Rutaceae family (subfamily- Aurantioideae) is a widespread species of the genus *"Naringi"*. It is commonly known as 'Mahavilvam' in Tamil. It has been used as folk medicine. The root extract is used for vomiting, dysentery and colic disorders. Fruit decoction is used as an antidote to insect poison. The bark juice is applied externally for getting speedy relief in sprain[9]. It is reported that its methanolic extract showed significant anthelmintic activity[10]. Pectic polysaccharides have been isolated from the fruits of *N. crenulata* by extraction with water[11]. However, biological activities such as anticancer[12], hepatoprotectivity[13], aphrodisiac activity[14], anti-inflammatory activities[15] of ethanol extracts of leaf and bark of *N. crenulata* have also been reported in our earlier studies.

The present research work is concerned with the leaves of the above mentioned Indian medicinal plant *Naringi crenulata*, which has reported folk-lore uses but yet not thoroughly explored so far for their exploitation in medicinal use. The first and foremost step, is the characterization of different pharmacognostical parameters, botanical identification, photomicroscopic study, powder characteristics and fluorescence study has been included here. A preliminary phytochemical screening of leaves has also been carried out.

MATERIALS AND METHODS

Botanical identification: The leaves were collected from well grown healthy plants inhabiting the natural forests of Agasthiarmalai Biosphere Reserve, Western Ghats, Tamil Nadu. The samples of *Naringi crenulata* were identified and authenticated by Prof.P.Jeyaraman, Plant Anatomy Research Centre, Chennai (PARC/2012/1382). The voucher specimen was deposited in the ethnopharmacological unit, Research Department of Botany, V.O.C.College, Tuticorin

Anatomical studies: For anatomical investigations, standard microtome techniques were followed[16]. Transverse sections of 10 to 12 µm thickness of leaf prepared. These microtome sections were stained with 0.25% aqueous Toludine blue (Meta chromatic stain) adjusted to pH 4.7[17]. Photomicrographs were taken with NIKON trinocular photomicrographic unit. The most

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Plate 1: *Naringi crenulata*(Roxb.)Nicolson

Anatomy of the leaf

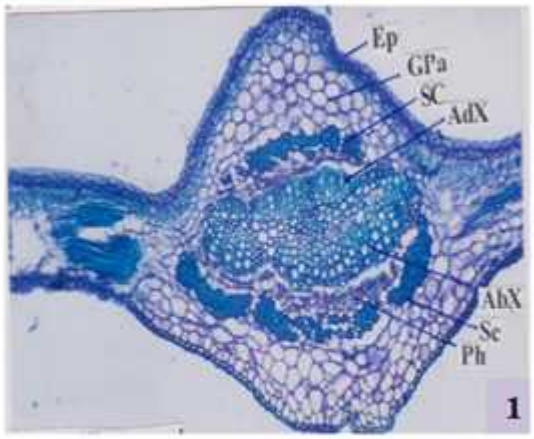

Fig.1: T.S. of leaf midrib-enlarged

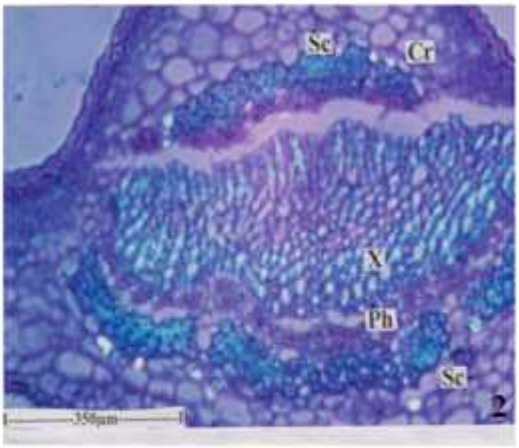

Fig.2: Crystals in the midrib (polarized light)

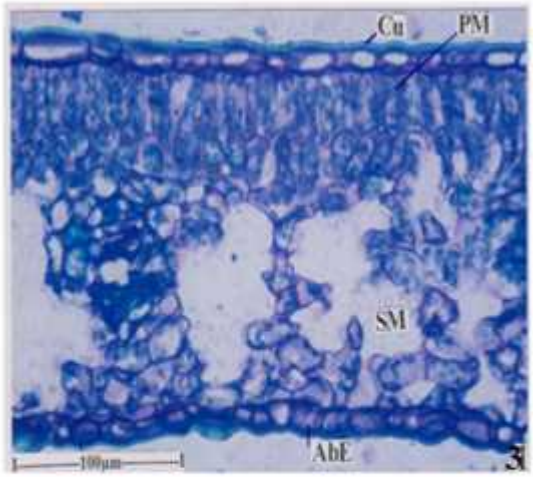

Fig.3: T.S. Lamina

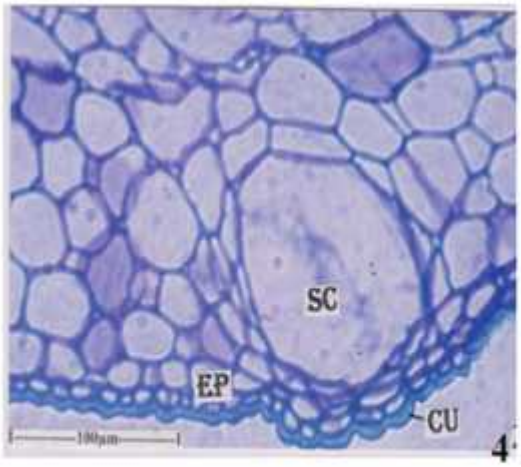

Fig.4: Secretory cavity - enlarged

Abx – Abaxial xylem; Adx – Adaxial xylem; Cr – Crystal; EP – Epidermis; GPa – Ground parenchyma; Ph – Phloem; SC – Secretory Cavity;X – Xylem; Sc – sclerenchyma;AbE – Abaxial epidermis; Cu – cuticle; VI – Vein Islet; VT – Vein Termination; PM – Palisade Mesophyll; SM – Spongy Mesophyll; SMV – Submarginal Veins

Table. 1a. Ash values of the powdered leaf of *Naringi crenulata*

S.No.	Type of Ash	Ash Value (%)[a]
1	Total Ash	12.98±1.21
2	Water soluble ash	14.89±0.08
3	Acid insoluble ash	3.75±0.01
4	Sulphated Ash	13.26±0.78

accepted descriptive terms were being used to describe the leaf anatomy[18].

Physicochemical constants and fluorescence analysis : These studies were carried out as per the standard procedures[19]. In the present study, the whole plant powder was treated with 1N aqueous sodium hydroxide and 1N alcoholic sodium hydroxide acids like 1N hydrochloric acid, 50% sulphuric acid, nitric acid, acetic acid, nitric acid with ammonia, ferric chloride, ammonia, benzene, petroleum ether, acetone, chloroform, methanol and ethanol. These extracts were subjected to fluorescence analysis in visible/daylight and UV light (254nm and 365nm). Various ash types and extractive values were determined by following standard method[20].

Preliminary phytochemical analysis: Shaded, dried and powdered whole plant samples were successively extracted with petroleum ether, benzene, chloroform, methanol, ethanol and water. The extracts were filtered and concentrated using vacuum distillation. The different

Table. 1b. Extractive values of the powdered leaf of *Naringi crenulata*

S.No.	Name of the extract	Extractive value (%)[a]
1	Petroleum ether	5.59±0.04
2	Benzene	10.39±0.11
3	Chloroform	11.8±0.32
4	Acetone	10.59±0.24
5	Methanol	15.59±0.36
6	Ethanol	8.79±0.17
7	Water	18.15±0.26

[a]All values are mean of triplicate determinations expressed on dry weight basis ±Standarderror

Plate 2: *Naringi crenulata*(Roxb.)Nicolson

Epidermal morphology and venation pattern of the leaf

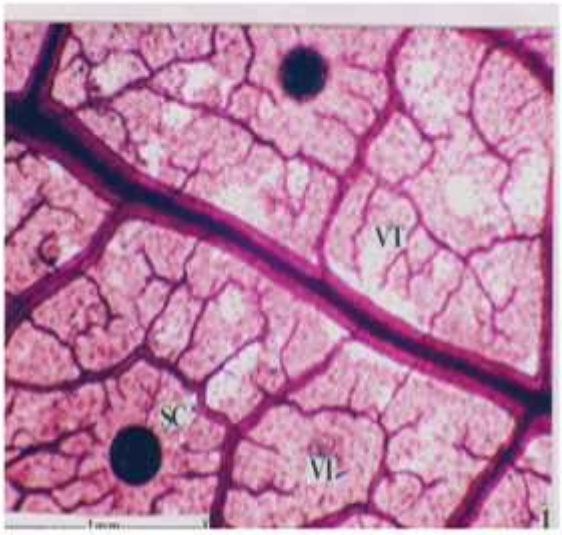

Fig.1: Lamina - cleared to show the venation

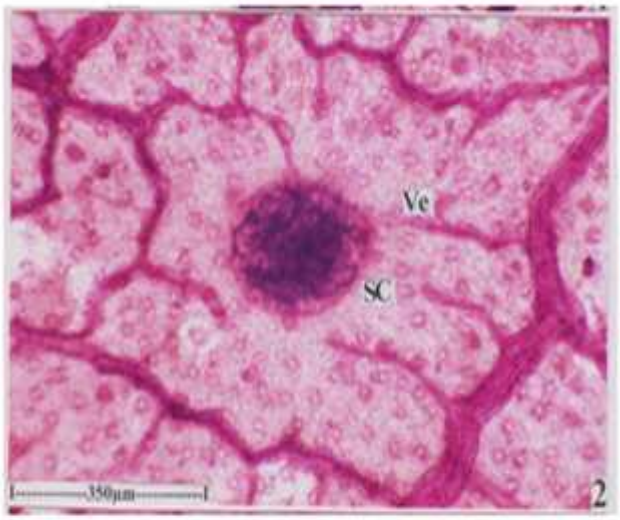

Fig.2: Vein termination - enlarged

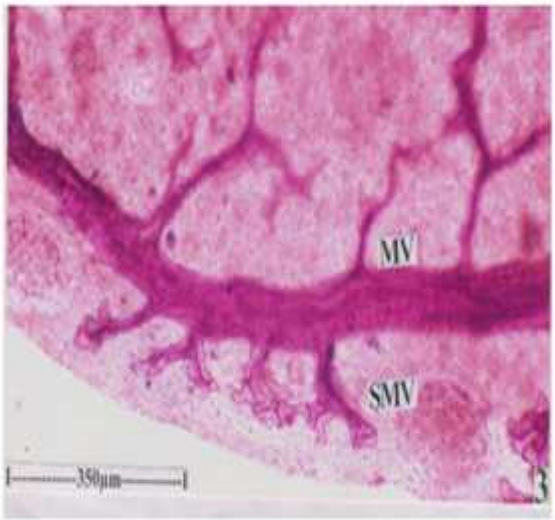

Fig.3: Intra marginal venation

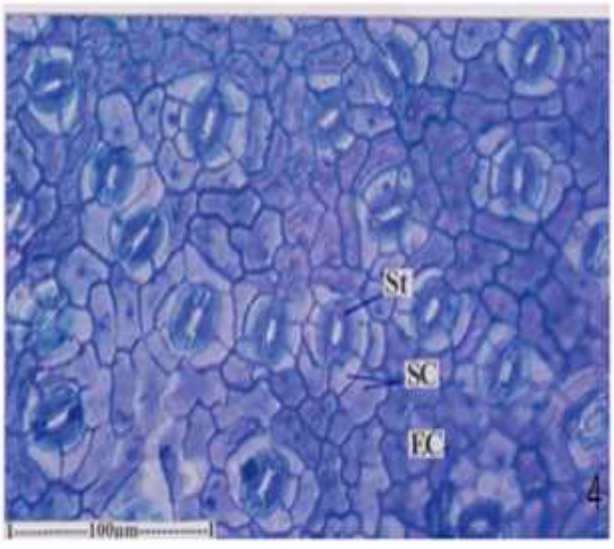

Fig.4: Paradermal section of the epidermis showing stomata

SC– Secretory Cavity; VI – Vein Islet; VT – Vein Termination; St – Stomata; SMV – Submarginal Veins; Ec – Epidermal cell; St – Stomata; Ve – Vein; MV – Marginal Veins;

extracts were subjected to qualitative tests for the identification of various phytochemical constituents as per standard[19, 21].

RESULTS

Pharmacognostic evaluation

Exomorphic features: *Naringi crenulata* is a deciduous tree found growing in low hills. It is an armed tree growing up to 12 m in height. The spines are straight and axillary. The leaves occur in cluster of 3-5. The leaf has three pairs of leaflets, which are oblong, ovate or elliptic up to 7×3 cm in size. The lamina is thin and coriaceous, glabrous, margins crenulate, rachis winged. Flowers are bisexual, tetra or pentamerous. Fruit – globose berry.

Microscopic Features

Leaflet: The leaflet exhibits prominent midrib, projecting equally on both adaxial and abaxial sides into thick cones; the lamina is uniformly even and thin. The midrib is 620 µm thick and the adaxial cone is 400 µm wide; the abaxial part is 550µm wide.

The midrib consists of prominent epidermal layer of small hemispherical cells with thick cuticle. The cells are about 15µm thick. The ground tissue is parenchymatous, the cells being angular, thin walled and compact. The vascular strand is large and occupies the major area of the midrib (Plate I- Fig.1). It consists of a wide shallow abaxial arc and more or less flat adaxial plate. The abaxial arc of vascular strand consists of several short rows of xylem elements and fibres. The xylem elements are thick walled lignified and circular. A thick band of phloem occurs beneath the xylem which is abutting a thick arc of sclerenchyma cells. The adaxial cone is more prominent and it has thicker epidermal layer, wide, compact parenchymatous ground tissue and a thick plate of sclerenchyma followed by phloem and xylem segments. Calcium oxalate druses are sparsely distributed in ground parenchyma (Plate I- Fig. 2).

Lamina (Plate I- Fig. 3)**:** The lamina shows smooth surfaces and distinct dorsiventral symmetry. The adaxial epidermis is narrow with cylindrical cells and thick cuticle. The abaxial epidermis is thicker and the cells are rectangular to squarish. The adaxial epidermis is 10µm thick while the abaxial epidermis is 15µm thick.

The mesophyll is differentiated into adaxial band of palisade cells and adaxial spongy parenchyma. The palisade zone has two layers of short cylindrical cells; its zone is 50µm in height. The spongy parenchyma cells are in seven layers; they occur in vertical filaments with wide air chambers.

Secretory cavities: Wide circular secretory cavities are common in the mesophyll tissue (Plate I- Fig. 4). The cavity is 150µm wide. Epithelial cells are fairly distinct. No cell inclusions are evident in the cavity.

Leaf margin: The marginal part of the lamina is conical and possesses similar structures as the lamina. The epidermal cells become slightly thick walled; secretary cavity is frequently seen in the marginal part.

Venation type (Plate II- Fig. 1, 2)**:** The venation is reticulate. The major veins are thick; the secondary and tertiary veins are thin and slender.

Vein - islets are less distinct. When the islet is distinct, it is bounded by thin wavy veins (Plate II- Fig. 3). The vein – terminations are wide spread in the lamina. They are long, thin and wavy. They are simple or branched once. Intra marginal (sub marginal) veins are thick and it gives off short thick, lobed vein lets.

Epidermal tissue and stomata (Plate II- Fig. 4): In paradermal sections, the epidermal cells and stomata are visible in phase view. The epidermal cells are small, polygonal and random in orientations. The anticlinal walls are thick and slightly wavy. Stomata are densely distributed. They are cyclocytic type. The stoma is encircled by one or more commonly by two rings of subsidiary cells. The subsidiary cells are rectangular and are arranged parallel to each other. The stomata are circular and measure 20×22µm in size. The stomatal pore is wide and slit like.

Petiole (Plate III- Fig. 1, 2): In sectional view, the petiole is circular with two thick and long lateral wings. The petiole is 720µm thick. The wings 600 - 800µm long and 150µm thick. The epidermal layer is thin and comprises small thick walled cells. The ground tissue is homogeneous and parenchymatous; the cells are thin walled and compact.

The vascular strand is single large and biconvex in sectional outline. It includes an abaxial larger arc and an adaxial, more or less flat plate of vascular strands. The xylem element is long, narrow parallel lines; phloem occurs in thick segments on the outer portions of the xylem. The xylem elements are thick walled and wide; the phloem elements are arranged in parallel compact lines. Thick segments of fibres occur attached to the phloem.

Powder analysis of the drug

Physico-chemical evaluation: The physico-chemical parameters like ash and extractive values, fluorescence analysis of leaf of *N. crenulata* were determined. Preliminary phytochemical screening was also performed and results are presented below.

Ash and Extractive Values: The results of the ash and extractive values of the powdered leaf of *N. crenulata* are depicted in Tables 1a and 1b. The total ash content of the powdered leaf of *N. crenulata* is 12.98%. The extractive value of water is more than the other solvents investigated in the present study.

Fluorescence analysis: The results of fluorescence analysis of leaf powder of *N. crenulata* are shown in Table 2. The powder from the leaf of *N. crenulata* fluoresced light green, under day light, short UV light and dark green under long UV light. The leaf powder shows the characteristic fluorescent green colour treated with IN HCl, Conc.H_2SO_4, Conc.HCl and 40% NaOH+10% lead acetate under short UV light.

Preliminary phytochemical screening: Petroleum ether, benzene, chloroform, methanol, ethanol and aqueous extracts of leaf of *N. crenulata* are qualitatively analysed for the presence of different phytoconstituents and the results are presented in Table 3. The methanol and ethanol extracts of leaf of *N. crenulata* shows the presence of alkaloids, coumarins, flavonoids, saponins, steroids, tannins, glycosides, phenols and xanthoprotein.

Table. 2. Fluorescence analysis of the powdered leaf of *Naringi crenulata*

S.No	Experiments	Visible / Day light	UV light 254nm (shortwave length)	UV light 365nm (long wave length)
1.	Powder as such	Light green	Light green	Dark green
2.	Powder + 1N NaOH(aq)	Yellowish green	Yellowish green	Dark green
3.	Powder + 1N NaOH(alc)	Greenish yellow	Green	Brown
4.	Powder + 1N HCl	Light green	Fluorescent Green	Brownish black
5.	Powder + Conc.H_2SO_4	Reddish brown	Yellowish Green	Dark brown
6.	Powder + 50% H_2SO_4	Green	Fluorescent green	Dark Green
7.	Powder + Conc.HNO_3	Reddish brown	Yellowish Green	Dark brown
8.	Powder + 50% HNO_3	Light brown	Greenish yellow	Dark brown
9.	Powder + Conc.HCl	Dark green	Fluorescent green	Greenish brown
10.	Powder + 40%NaOH + 10%Lead acetate	Light green	Fluorescent Green	Dark brown
11.	Powder + Acetic acid	Greenish brown	Greenish yellow	Dark Green
12.	Powder + $FeCl_3$	Pale green	Greenish yellow	Dark brown
13.	Powder + HNO_3+NH_3	Reddish green	Green	Reddish brown
14.	Powder + NH_3	Light green	Greenish yellow	Dark Green
15.	Powder + Benzene	Greenish yellow	Green	Dark Green
16.	Powder + Pet. Ether	Light green	Leafy Green	Dark Green
17.	Powder + Acetone	Leafy green	Greenish yellow	Dark green
18.	Powder + Chloroform	Green	Green	Dark Green
19.	Powder + Methanol	Leafy green	Light green	Green
20.	Powder + Ethanol	Green	Greenish yellow	Dark brown

Plate 3: *Naringi crenulata*(Roxb.)Nicolson

Anatomy of the winged petiole

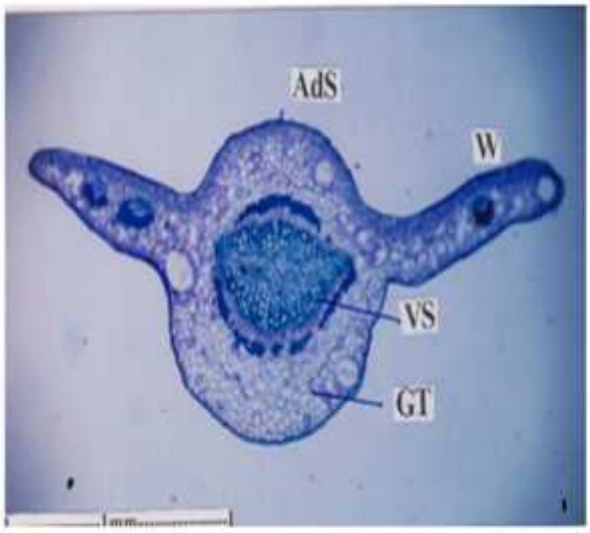

Fig.1: T,S. of winged petiole (Rachis)

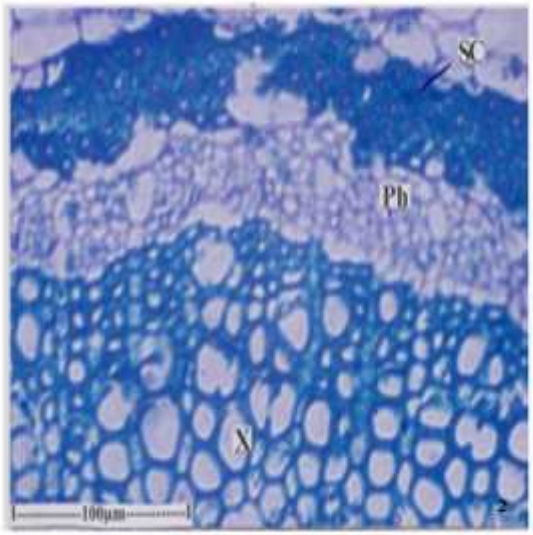

Fig.2: Vascular tissues of the petiole

Ads – adaxial side; SC – Secretory Cavity; GT – Ground Tissue; X- Xylem; W – Wing; Vs – Vascular strand; Ph – Phloem

DISCUSSION

It is globally accepted that herbal based drugs have many advantages over the synthetic drugs. However, one of the major problems in utilization of phytodrugs is correct diagnosis of the medicinal plants that are used either in the traditional systems or modern systems of preparation of the drugs. It is regrettable to note that most of the people involved in the manufacture or preparation of herbal drugs lack the basic background of botanical knowledge of the drugs. Consequently adulteration or substitutions of plants in the place of original ones permeate the pharmaceutical

Table. 3. Phytochemical screening of powdered leaf of *Naringi crenulata*

S. No.	Test	Petroleum ether	Benzene	Chloroform	Methanol	Ethanol	Water
1.	Alkaloids	-	-	-	+	+	+
2.	Anthraquinones	-	-	-	-	-	-
3.	Catechins	-	-	-	-	-	-
4.	Coumarins	-	-	-	+	+	+
5.	Flavonoids	-	-	-	+	+	+
6.	Phenols	-	-	-	-	-	-
7.	Quinones	-	+	+	-	+	-
8.	Saponins	-	-	-	+	+	+
9.	Steroids	+	+	+	+	+	-
10.	Tannins	-	-	-	+	+	+
11.	Terpenoids	+	+	+	+	+	-
12.	Sugar	-	-	-	+	+	+
13.	Glycosides	+	+	+	+	+	-
14.	Xanthoprotein	-	-	-	+	+	-

industries, rendering the herbal drugs undependable and invalid. This will lead to unpopularity of phytodrugs among the people. So, it is most essential that a medicinal plant, when found to be of high pharmacological potentials, should be subjected to thorough botanical standardization so that there wouldn't any ambiguity with respect to botanical identity of the plants. Identification of plants involves the study of the external features of the vegetative and floral parts. This study must be complemented with anatomical parameters which are very often useful to identify the fragmentary plant specimens. Raw drugs pose problem of identification and to establish their genuineness when they lack any external diagnostic features or any organoleptic clues. During such situations, the microscopic analyses of the specimen will offer a helping hand to establish the identity of the phytodrugs.

Literature dealing with anatomy of leaf of *N. crenulata* is minimal. The present study attempts a modest comprehensive investigation of the leaf of *N. crenulata*. Since the leaf of *N. crenulata* as the folklore claims has therapeutic qualities the present investigation has laid down a set of anatomical features of the leaf which can be employed for its botanical diagonosis. The salient features of identification of the fragmentary sample are as follows.

- *N. crenulata* **(**Roxb.) Nicolson (*Limonia crenulata* Roxb.) of Rutaceae is a deciduous tree with axillary straight spines, pinnately compound leaves, winged rachis and bisexual tetra or pentamerous flowers.
- The leaflet is dorsiventral with thick glabrous lamina and midrib projecting in to conical projections on both sides.
- The vascular system of the midrib comprises adaxial and abaxial plates of collateral vascular strands with sclerenchyma bands situated on both upper and lower parts of the vascular strands.
- The lamina is bifacial. The epidermal layers have spindle shaped cells with thick cuticle, single row of palisade cells and 7-10 layers of lobed spongy parenchyma
- Wide, circular secretory cavities of schizo – lysigenous origin are common in the mesophyll.
- The marginal part of the lamina is conical, straight, often with sub marginal secretory cavity and palisade and spongy mesophyll tissues.
- The venation system of the lamina is densely reticulate; vein islets are less distinct; vein terminations are simple or branch and wavy.
- Epidermal cells are polygonal outline with straight walls; stomata are cyclocytic type.
- The petiole is circular with thick and long wings; the midrib has vascular system similar to the midrib; these are adaxial and abaxial collateral juxtaposed vascular strands with sclerenchyma sheath.
- The external features coupled with the microscopic features are specific for *N. crenulata*. These features are discussed and proposed as microscopic diagnostic protocol for the taxon studied.

Pharmacochemical characterization

Physio-chemical constituents

Ash value: The Physio-chemical evaluation of the drug is an important parameter in detecting adulteration or improper handling of drugs[22]. Equally important in the evaluation of crude drugs, is the ash value and acid insoluble ash value determination. The total ash is particularly important in the evaluation of purity of drugs, that is, the presence or absence of foreign organic matter such as metallic salts and/ or silica[23]. The ash value of leaf of *N. crenulata* is 12.98%. The ash value is indicative of the impurities present in the drug and the value is also one of the diagnostic parameters of the drug. Total ash usually consists of carbonates, phosphatases, oxidases, silicates and silica. The total ash of crude drugs also reflects the care taken in drug preservation and purity of crude and prepared drug[24]. This value varies within fairly wide limits and is therefore an important parameter for the purpose of evaluation of crude drugs. In certain drugs, the percentage variation of ash from sample to sample is very small and any marked difference indicates the change in quality. A high value is indicative of contamination, substitution, adulteration or carelessness in preparing the crude drug for marketing. Samples have more water soluble ash than acid

soluble ash. The ash values are generally the index of the purity as well as identity of the drug.

Fluorescence analysis: The phytoconstituents present in the crude drug interact with the chemical reagents and may produce certain products which may be present inside the cell or may come out of the cell and react in the medium, thus resulting in a specific fluorescence pattern. This is the basis of the fluorescence analysis. The powder from the leaf of *N. crenulata* fluoresced light green under day light, short UV light and dark green under UV light.

Many phytocompounds fluoresce when suitably illuminated. The fluorescent color is specific for each compound. A non-fluorescent compound may fluoresce if mixed with impurities that are fluorescent. The fluorescent method is adequately sensitive and enables the precise and accurate determination of the analyze over a satisfactory concentration range without several time consuming dilution steps prior to the analysis of pharmaceutical samples[25].

Phytochemical studies: Presence or absence of certain important compounds in an extract is determined by color reactions of the compounds with specific chemicals which act as dyes. This procedure is a simple preliminary prerequisite before going for detailed phytochemical investigation. Various tests have been conducted qualitatively to find out the presence or absence of bioactive compounds such as flavonoids terpenoids, tannins, saponins, steroids and xanthoprotein are detected in *N. crenulata* which could made the plant useful for treating different ailments as having a potential of providing useful drugs of human use.

CONCLUSION

Thus, it is evident that the present study would provide various resourceful information in relation to pharmacognostical identification of this plant leaves. Furthermore, information regarding physicochemical characteristics of such plant leaves and nature of chemical constituents present in them would also be useful for standardization of such herbal drugs of folk medicinal practice of present era and enrichment of Ayurvedic Pharmacopoeia. It would also help scientists to utilize such needful information regarding the plants identity and characteristics in building new poly herbal formulations

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