RESEARCH ARTICLE

Pharmacognostic and Phytochemical evaluation of the bark 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 bark of the above said plant. Pharmacognostic studies include microscopic, physicochemical constants (ash and extractive values), fluorescence analysis and preliminary phytochemical evaluations. The results of the study are useful in setting some diagnostic indices for the identification and preparation of a monograph of the plant.

Keywords: Pharmacognosy, physicochemical, fluorescence, phytochemical

INTRODUCTION

The importance of plants is known to us well. The plant kingdom is a treasure house of potential drugs and in the last few decades there has been an exponential growth in the field of herbal medicine. It is getting popularize in developing and developed countries owing to its natural origin and lesser side effects.[1] Evaluation of plant materials and their derived products has always been an important part of the professional expertise workers in the field of discovery of phytopharmaceuticals. [2] A big quantum research works in the area of authentication of the correct plant source has been undertaken to provide means of differentiation among many available plant sources.[3] Chemical analysis and biological assays are very important aspects in pharmacognostic evaluation of medicinal plants.[4, 5, 6] Therefore, the investigations of micromorphological, phytochemical physicochemical characterization of bark of *Naringi crenulata,* an important ethnomedicinal plant have been carried out. This investigation will provide some useful markers for identification of crude drug obtained.

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. It is reported that its methanolic extract showed significant anthelmintic activity. Pectic polysaccharides have been isolated from the fruits of *N. crenulata* by extraction with water. However, biological activities such as anticancer,[7] hepatoprotectivity,[8] aphrodisiac activity,[9] anti-inflammatory activities[10] 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 bark 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, microscopic study, powder characteristics and fluorescence study has been included here, A preliminary phytochemical screening of leaves has also been carried out.

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MATERIALS AND METHODS

Botanical identification

The bark material was collected from the well grown trees found in the natural forests of Agasthiarmalai Biosphere Reserve, Western Ghats, Tamil Nadu. Small pieces of bark measuring about $3cm^2$ were chiseled out from the trunk of the tree at the breast level. 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.Chidambaram College, Tuticorin.

Anatomical studies

For anatomical investigations, standard microtome techniques were followed.[11] Both T.S. and T.L.S. of 10 to 12µm thickness of bark were prepared. These microtome sections were stained with 0.25% aqueous Toludine blue (Metachromatic stain) adjusted to pH 4.7.[12] Photomicrographs were taken with NIKON trinocular photomicrographic unit. The most accepted descriptive terms were being used to describe the root and stem anatomy.[13]

Physicochemical constants and fluorescence analysis

These studies were carried out as per the standard procedures.[14] In the present study, the trunk bark 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 methods.[15]

Preliminary phytochemical analysis

Shaded dried and powdered bark was successively extracted with petroleum ether, benzene, chloroform, methanol, ethanol and water. The extracts were filtered and concentrated using vacuum distillation. The different extracts were subjected to qualitative tests for the identification of various phytochemical constituents as per standard procedure[16, 14]

RESULTS

Pharmacognostic evaluation

BARK (Plate 1- Fig. 1, 2): The bark is 2-8mm thick. It includes two distinct zones- outer part periderm and inner zone of secondary phloem. The periderm is deeply fissured; the fissures are irregular; they are wide at the surface and narrow down towards the interior. The periderm consists of homogeneous phellem cells which are tabular in shape and suberised. Secondary Phloem includes two zones: outer collapsed phloem and inner non-collapsed phloem. The transition between collapsed and non-collapsed phloem zones is gradual.

Collapsed Phloem: The collapsed phloem consists of crushed phloem elements which appear as dark tangential lines and thick elliptical tangentially elongated blocks of sclereids.

Non-collapsed Phloem includes fewer amounts of sclerenchyma masses, intact sieve elements and other components. The sclerenchyma cells are angular and the inner walls are gelatinous (Plate I- Fig. 3).

Tangential longitudinal sections of the phloem (TLS): In TLS view, the phloem appears non storied, The rays are uniseriate, biseriate and 3-seriate. Biseriate and 3-seriate rays are more common. The rays are heterocellular; they consist of elongated marginal upright cells and middle procumbent cells. The rays are 100-200µm in height and 10-30µm thick. The ray cells are thick walled and compact. Ray frequency is 10-12/1mm. The sieve tube members are narrow and run straight. The sieve plate is oblique and compound. The sieve tubes are 150-250µm long and 20µm wide.

Calcium oxalate crystals are abundant in the stem and bark (Plate 2- Fig. 1). The crystals are exclusively prismatic type. In the stem they are located adjacent to the cortical fibres. In the phloem of the bark they occur in uniseriate vertical lines within the phloem parenchyma (Plate 2- Fig. 2) and rays cells.

Radial Longitudinal Section of the Phloem (RLS):

In RLS view, the phloem rays appear as horizontal ribbon like bands (Plate II- Fig. 3). In the middle part of the ray, the cells are horizontally oblong and rectangular. In the marginal part, the cells are vertically elongated. The body cells are called procumbent cells and the marginal cells are upright cells.

Powder analysis of the trunk bark

Physicochemical evaluation

The physicochemical parameters like ash and extractive values, fluorescence analysis of bark 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 bark of *N. crenulata* are depicted in Tables 1a and 1b. The total ash content of the powdered bark of *N. crenulata* is 7.37%. The extractive value of water is more than the other solvents investigated in the present study.

Fluorescence analysis

The results of fluorescent analysis of bark powder of *N. crenulata* are shown in Table 2. The powder from the bark of *N. crenulata* emitted light yellow under day light, sandle yellow under short UV light and yellowish green under long UV light. The bark powder shows the characteristic fluorescent green colour when treated with 50% H_2SO_4, 50% HNO_3 and 40% NaOH+10% lead acetate.

Preliminary phytochemical screening:

Petroleum ether, benzene, chloroform, methanol, ethanol and aqueous extracts of bark powder 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 bark of *N. crenulata* shows the presence of alkaloids, coumarins, flavonoids, saponins, steroids, tannins, glycosides, phenols quinines, terpenoids and xanthoproteins.

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 industries, rendering the herbal drugs undependable and invalid. This will lead to unpopularity of phytodrugs among the people. So, it is the 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 bark of *N. crenulata* is minimal. The present study attempts a modest comprehensive investigation of the bark of *N. crenulata*. Since the bark of *N. crenulata* as the folklore claims has therapeutic qualities. The present investigation has laid down a set of anatomical features of the trunk bark which can be employed for its botanical diagonosis. The salient features of identification of the fragmentary bark sample are as follows

- The stem exhibits normal, dicot type of structure. It is 2.2 mm thick and consists of

heavily circularised epidermal cells, wide parenchymatous cortex and discontinuous segments of sclerenchyma.

- The vascular cylinder is thick and hollow having wide continuous cylinder of phloem and secondary xylem comprising diffuse vessels, thick walled xylem fibres and thin straight xylem rays.
- Stem bark shows deeply fissured periderm; cells of periderm homogeneous and thin walled.
- Secondary phloem wide with several discrete, tangial lobes of phloem fibres and scleroids in the collapsed phloem zone.
- Prismatic calcium oxalate crystals are abundant in the collapsed phloem; the crystals occur in vertical strands associated.
- Phloem rays are non- storied; they are 1-3 seriate, heterocellular; short and spindle shaped
- Sieve tubes have simple oblique sieve plates.

Pharmacochemical characterization

Physiochemical constituents

Ash value

The physical constant evaluation of the drug is an important parameter in detecting adulteration or improper handling of drugs.[15] 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.[17]

The ash value of bark of *N. crenulata* is 7.37%. 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.[18] 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 bark 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.[19]

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* bark which could made the plant useful for treating different ailments as having a potential of providing useful drugs of human use.

Pharmacognosy implies a particular knowledge of methods of identification and evaluation of crude

drugs obtained from plants which include macromorphology, anatomy, phytochemical and pharmacological studies. The major problem of the commercial supply of crude drugs is the identification of genuine drug. Crude drugs may easily be adulterated or substituted by or confused for other ones because neither it has trade name printed on it nor it carries any identifying structure for the easy identification of it by the plant taxonomists, rather drug samples supplied are shrunked, rolled, twisted, deformed and discoloured.[20] So, pharmacognostic evaluation of crude drugs with macromorphology, micromorphology, ash value, fluorescence study and phytochemical screening will help in identifying genuine drugs because the tests are very specific for a particular drug. Thus for checking the adulteration or the use of substitutes can be easily detected to prove purity of the drugs.

CONCLUSION

Thus, it is evident that the present study of the plant material bark provides various resourceful information in relation to pharmacognostical identification of this plant material. Furthermore, information regarding physicochemical characteristics of trunk bark 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 polyherbal formulations

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Plate 1:Naringi crenulata (Roxb.) Nicolson

Anatomy of the bark

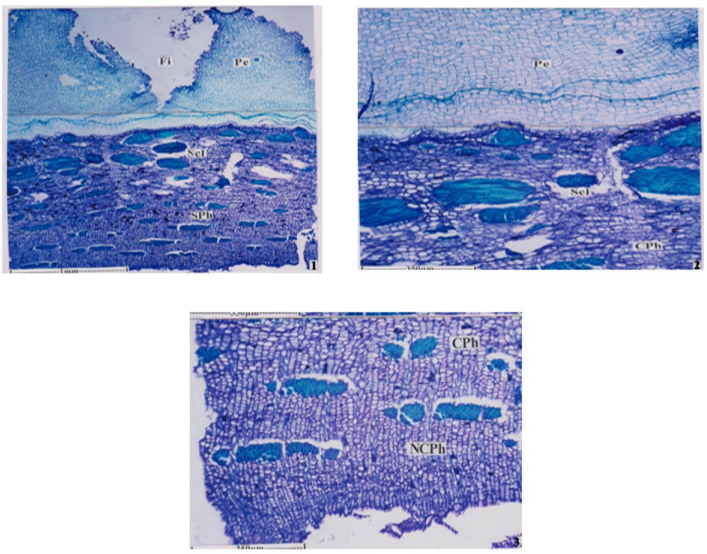

Plate 1

Anatomy of the bark

1. T.S. of bark – entire view
2. T.S. of outer bark (periderm and collapsed phloem)
3. T.S. of Non-collapsed phloem

Cph – Collapsed phloem; Fi – Fibre; GFi – Gelatinous Fibres; Ncph – Non-collapsed phloem; Pe – Periderm; Scl – Sclerenchyma; Sph – Secondary phloem

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

Anatomy of the bark

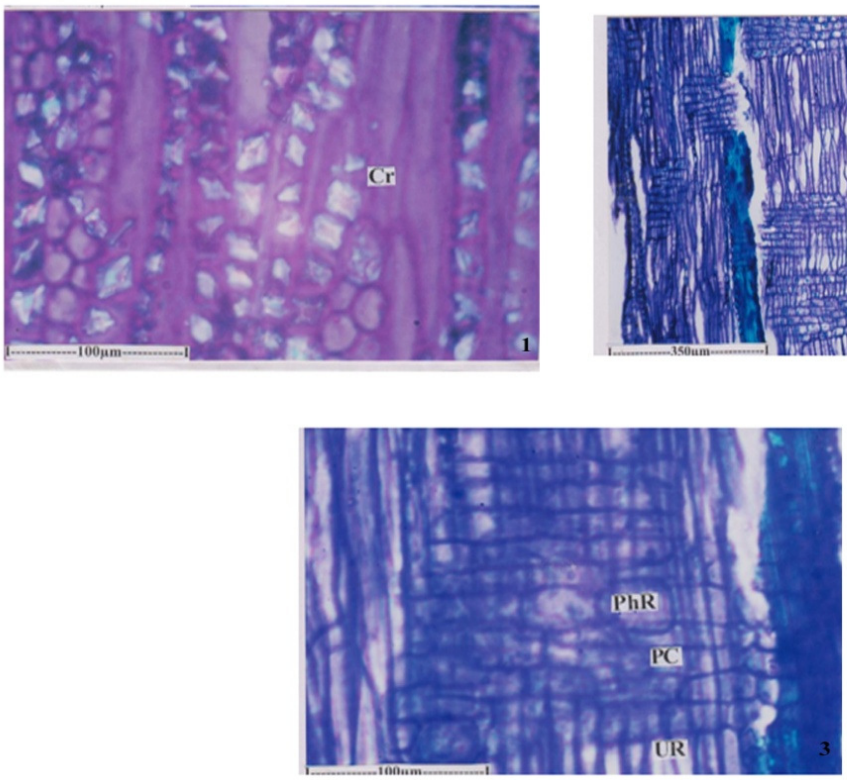

Plate 2
Different views of phloem

1. Prismatic crystals in the parenchyma cells and rays
2. RLS of phloem
3. RLS Phloem ray - enlarged

Cr – Crystal; PC – Procumbent cell; PhR – Phloem Ray; SE – Sieve Element; UR – Upright cells

Table 1a: Ash values of the powdered bark of *Naringi crenulata*

S. No.	Type of Ash	Ash Value (%)[a]
1	Total Ash	7.37±0.68
2	Water soluble ash	3.15±0.03
3	Acid insoluble ash	2.55±0.01
4	Sulphated Ash	8.41±0.65

Table 1b: Extractive values of the powdered bark of *Naringi crenulata*

S. No.	Name of the extract	Extractive value (%)[a]
1	Petroleum ether	4.36±0.06
2	Benzene	6.74±0.07
3	Chloroform	4.79±0.03
4	Acetone	27.90±0.12
5	Methanol	10.36±0.31
6	Ethanol	7.20±0.15
7	Water	15.80±0.17

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

Table 2: Fluorescence analysis of the powdered bark of *Naringi crenulata*

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

Table 3: Phytochemical screening of powdered bark 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	-	-	-	+	+	-

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