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# Pharmacognostical analysis of *Naringi crenulata* leaves

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ABSTRACT

**Objective:** To analyse the pharmacognostical characters of an important medicinal plant *Naringi crenulata* (*N. crenulata*) (Roxb.) Nicols. **Methods:** The Pharmacognostic studies were carried out in terms of organoleptic or morphological characters, macroscopic studies, physico-chemical evaluation, phytochemical screening and fluorescence analysis of powdered crude drug were carried out. **Results:** The phytochemical studies of the ethanolic extract showed the presence of tannins, phenols, flavonoids, saponin, quinine, protein, lipid and Triterpenoid, Alkaloids Anthraquinones were not detected. The physico-chemical analysis of the leaves revealed a composition of 19.46% total ash value, 15.24% alcohol soluble extractive value, 61.02% water soluble extractive value, 51.49% acid insoluble ash value and 56.69% crude fibre content. **Conclusions:** These studies provide referential information for correct identification and standardization of *Naringi crenulata*. On the basis of various pharmacognostic parameters and the determination of these characters will aid future investigators in their pharmacological analysis of this species and the presence of phytocompounds, may be of use for developing plant based drugs for various ailments.

## 1. Introduction

*Naringi crenulata* (*N. crenulata*) (Roxb.) Nicolson belongs to Rutaceae family is known as Mahavilvam in Tamil. It is commonly known as Kattunarakam, Cheriyakuttunaregam, Malanarakam, Narinarakam in Malayalam, Kukka velaga in Telugu. It is wide spread species of the genus (Naringi) growing as understorey trees in evergreen forests upto 1 200 m. Trunk with branched thorns; Bark dark grey, smooth; blaze yellowish. Branchlets young are terete, glabrous, thorny. Leaves are compound, imparipinnate, 15cm long, alternate, spiral; rachis with oblanceolate wings, glabrous; leaflets 5-7, opposite, sessile 2.0-4.5 cm × 1.0-1.5 cm, elliptic to obovate, apex emarginate or obtuse, base acute, margin crenulate or irregularly serrulate, glandular punctuate, glabrous; secondary nerves 7-10 pairs, looped near margin tertiary nerves ademedially ramified. Inflorescence axillary subumbellate; pedicel 1 cm long. Fruit Berry, globose.

Seed 1-4[1]. It has been used as folk medicine. In survey of historical accounts on old folk medicines all parts of this tree viz. root, stem, bark, leaf and fruit are used in several ailments. Root is used as remedy for cobrabite[2], bodypain[3], colic[4], vomiting and dysentery[5].

Stem powder prevents acne and anti aging[6]. Bark is used as a remedy for puerperal fever [7] and pitta[8]. Bark juice is applied externally for getting speedy relief in sprain[9]. Leaves are used in offering pojas for Lord Siva and traditionally leaves paste administered orally along with milk to cure mental disorders[10], dysentery[7] fever[11] leaves are given with milk to children to cure digestive disorders[12], and remedy for epilepsy[8].

Fruit decoction is used as antidote to insect poison[4], and Intestinal worms[13] and also anthelmintic property[7]. Thus, the present study was carried out to study the physicochemical characters, phytochemical characters and pharmacognostical analysis of leaves of the *N. crenulata*.

## 2. Materials and methods

### 2.1. Plant material

The plant specimen was collected from

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Adiannamalai region of Tiruvannamlai, Tamilnadu, India. The taxonomic identification of the plant was confirmed by Botanical Survey of India (BSI), Coimbatore. Tamilnadu, India. (Certificate No. BSI/SRC/5/23/10-11/Tech.1134) and the specimens voucher were deposited in the Department of Botany, Government Arts College (Autonomous), Kumbakonam, Tamilnadu, India.

### 2.2. Preparation and extraction of plant material

The collected fresh plant parts were air-dried at room temperature. The dried plant material was then homogenized by electric mixer grinder to obtain coarse powder and stored in air-tight bottles for further analysis. The shade dried, powdered samples were extracted with 150 mL of solvent ethanol for 8-12 h by using the soxhlet apparatus[14].

### 2.3. Macroscopic evaluation

Various macroscopic characters of fresh leaves of *N. crenulata* (such as colour, odour, taste, size and shape) were studied by macroscopic evaluation.

### 2.4. Physico-chemical evaluation

The physico-chemical analysis like total ash, acid insoluble ash, water soluble ash, ethanol soluble extractive and crude fibre content values were performed according to the methods prescribed in Indian Pharmcacopeia[15], Khandelwal and Kokate[16-18]. Then for the elementary analysis the leaf powder was acid digested and the digested material was used for elemental analysis.

### 2.5. Phytochemical screening

Freshly prepared leaf extracts were tested for the presence of various active Phytocompounds like Phenols, tannin, flavonoid, protein, reducing sugar, carbohydrates, lipids, saponin, triterpenoid, alkaloid, anthraquinone and quinone by the method of Kokate and Khandelwal[16-18].

### 2.6. Powder behaviour analysis

Behaviour of the leaf powder with different chemical reagents was studied to detect the presence of phytoconstituents, with colour changes[16-18].

### 2.7. Flourescence analysis

A small quantity of dried and finely powdered leaf was placed on a grease free clean microscopic slide and added 1-2 drops of the freshly prepared reagent solution, mixed by gentle tilting the slide and waited for 1-2 min. Then the slide was placed inside the UV viewer chamber and viewed in day light. The colours observed by application of different reagents in different radiations were recorded[19].

## 3. Results

### 3.1. Macroscopic evaluation

The leaf collected from the plant was given in Figure 1. And their macroscopic evaluations were tabulated in Table 1.

### 3.2. Physicochemical analysis

Acid insoluble ash is used to know the percentage of dirt and sand. Extractive values are primarily useful for the determination of adulterated drugs. Total ash values of a drug give information about inorganic compounds such as carbonated, phosphates, silica and silicates, which are naturally occurring in drug or deliberately added to it as a form of adulterant. The ash value, extractive value and crude fibre content were tabulated in Table 2.

### 3.3. Elemental analysis

The elemental analysis of the acid digested material was calibrated. Results were calibrated using standard calibrations and the mean of triplicate values were tabulated in Table 3 and 4.

**Table 1.**
Macroscopic evaluation of *N. crenulata* leaf.

| S.No. | Characters | Observation results |
|---|---|---|
| 1 | Colour | Fresh dark green and dried leaves are light green in colour |
| 2 | Odour | Aromatic |
| 3 | Taste | Bitter |
| 4 | Size | Leaves are compound, imparipinnate, 15 cm long, alternate, spiral; petiole and rachis jointed, with oblanceolate wings, glabrous; leaflets 5-7, opposite, sessile 5 cm. |
| 5 | Shape | Elliptic to obovate, apex obtuse rarely acute, notched at the tip, margin crenulate, glandular punctuate, glabrous, base acute joints of rachis obovate oblong crenulate. |

**Table 2.**
Physicochemical parameters of *N. crenulata* leaf.

| | Quantitative parameters | Values obtained (%) w\w |
|---|---|---|
| Ash values | Total ash | 19.46 |
| | Acid insoluble ash | 51.49 |
| | Water soluble ash | 61.02 |
| Extractive values | Ethanol | 15.24 |
| | Crude fibre content | 56.69 |

### 3.4. Qualitative phytochemical analysis

Freshly prepared leaf extracts were tested for the presence of phytoconstituents and the results were presented in Table 5.

**Table 3.**
Elemental analysis of *N. crenulata* leaf.

| Elements | Elemental content (ppm) |
|---|---|
| Ferrous | 0.474 |
| Zinc | 0.012 |
| Copper | 0.212 |
| Mangenese | 0.117 |
| Cobalt | 0.094 |

**Table 4.**
Heavy metal analysis of *N. crenulata* leaf.

| Elements | Elemental content (ppm) |
|---|---|
| Lead | 0.091 |
| Arsenic | 11.592 |
| Mercuey | 15.588 |
| Selenium | ND |

### *3.5. Quantitative analysis*

Quantitative analysis of ethanol leaf extract was carried out to analyse the presence of primary metabolites and secondary metabolites. The results are tabulated in Table 6 & 7.

### *3.6. Behaviour of leaf powder with various chemical reagents*

Treatment of leaf powder with different chemical reagents was studied to detect the presence of phytoconstituents with colour changes under daylight and the results were shown in Table 8.

**Table 5.**
Qualitative phytochemical analysis of *N. crenulata* leaf extract.

| Phytoconstituents | Ethanol extract |
|---|---|
| Protein | + |
| Lipid | ++ |
| Carbohydrate | – |
| Reducing Sugar | – |
| Phenol | +++ |
| Tannin | + |
| Flavanoid | + |
| Saponin | ++ |
| Triterpenoid | – |
| Alkaloid | – |
| Anthraquinone | – |
| Quinone | + |

+ present; – absent.

### *3.7. Fluorescence analysis of leaf powder*

The leaf powders were examined in visible light and ultraviolet light and the results were presented in Table 9.

**Table 6.**
Primary metabolites of N. crenulata leaf extract.

| Constituents | Values (%w\w) |
|---|---|
| Protein | 14.620±0.170 |
| Lipid | 1.240±0.012 |
| Carbohydrate | 0.500±0.010 |

**Table 7.**
Secondary metabolites of N. crenulata leaf extract.

| Constituents | Values (%w\w) |
|---|---|
| Phenol | 13.12 ± 0.00 |
| Tannin | 2.64 ± 0.03 |
| Flavonoid | 3.65 ± 0.07 |
| Vitamin E | 0.68 ± 0.08 |
| Vitamin C | 10.17 ± 1.21 |

## 4. Discussion

To ensure reproducible quality of herbal products, proper control of starting material is utmost essential. Thus in recent years there have been emphasis in Standardization of medicinal plants of therapeutic potential. Despite the modern techniques, identification and evaluation of plant drugs by pharmacognostical studies is still more reliable, accurate and inexpensive means. According to World Health Organization (WHO) the macroscopic and microscopic description of a medicinal plant is the first step towards establishing its identity and purity and should be carried out before any tests are undertaken[20].

Standardization is an important tool for herbal drugs in order to establish their identity, purity, safety and quality. In order to standardize a drug, various macroscopic, physicochemical analyses, phytochemical analysis, fluorescence analysis are done.Ash values or Ash content which simply represents inorganic salts, naturally occurring in crude drug or adhering to it or deliberately added to it, as a form of adulteration. The ash value was determined by three different methods, which measured total ash, acid-insoluble ash and water-soluble ash. The total ash method is employed to measure the total amount of material remaining after ignition.

This includes both 'physiological ash' which is derived from the plant tissue itself, and 'non-physiological ash' which is the residue of the extraneous matter adhering to the plant surface. Acid-insoluble ash is a part of total ash and measures the amount of silica present, especially as

sand and siliceous earth. Water-soluble ash is the water soluble portion of the total ash[21].

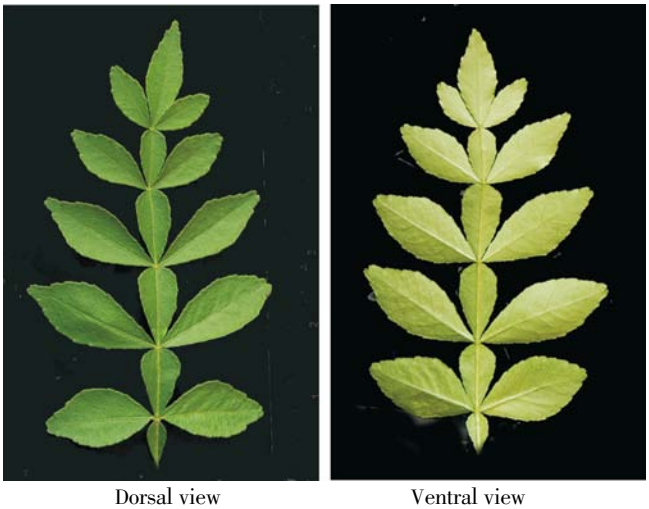
The physico-chemical evaluation of drugs is an important parameter in detecting adulteration or improper handling of drugs.

**Table 8.**
Behaviour of *N. crenulata* leaf powder with different chemical reagents.

| Treatment | Observation |
|---|---|
| Powder as such | Pale green |
| Powder + 50% Sulphuric acid | Green |
| Powder + Conc. Sulphuric acid | Blackish brown |
| Powder + 50% Hydrochloric acid | Grey green |
| Powder + Conc. Hydrochloric acid | Dark green |
| Powder + 50% Nitric acid | Brown |
| Powder + Conc. Nitric acid | Orange brown |
| Powder + 10% Sodium hydroxide | Green |
| Powder + Sodium hydroxide + water | Greenish brown |
| Powder + 5% Ferric chloride | Green |
| Powder + 5% Potassium hydroxide | Light green |
| Powder + Water + shake | No change |
| Powder + Ethanol | Dark green |
| Powder + Acetic acid | Yellowish green |

**Table 9.**
Fluorescence analysis of *N. crenulata* leaf powder.

| Treatment | Visible light | Ultra violet light |
|---|---|---|
| Powder | Pale green | Ash |
| Powder + 1N Hydrochloric acid | Grey green | Black |
| Powder + 50% Hydrochloric acid | Grey green | Blackish green |
| Powder + 50% Sulphuric acid | Green | Dark green |
| Powder + 50% Nitric acid | Brown | Dark green |
| Powder + 1N NaoH + water | Fluorescence green | Dark green |
| Powder + 1N NaoH + ethanol | Pale green | Dark green |



Dorsal view Ventral view

**Figure 1.** Leaf of *N. crenulata*.

It can serve as a valuable source of information and provide appropriate standards to establish the quality of this plant material in future study or application.

The phytochemical screening of *N. crenulata* leaves reveals the presence of phytoconstituents like phenols, tannin, flavonoids, saponins, quinine, protein and lipids. These results expose that the plant has quite a number of chemical constituents, which may be responsible for the many pharmacological actions and will be useful in finding out the quality of the drug. Treatment of leaf powder with different chemical reagents had revealed the presence of different chemical constituents. Fluorescence is the phenomenon exhibited by various chemical constituents present in the plant material. Some constituents show fluorescence in the visible range in daylight.

The ultra violet light produces fluorescence in many natural products, which do not visibly fluorescence in day light. If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives or decomposition products by applying different reagents. Hence, some crude drugs are often assessed qualitatively in this way and it is an important parameter of pharmacognostical evaluation[22].

The knowledge of the chemical constituents of the plant is desirable to understand herbal drugs and their preparations. Most importantly, these studies will be helpful to isolate and characterize the chemical constituents present in those plant extract. In addition, the knowledge of the chemical constituents of plants would further be valuable in discovering the actual value of folkloric remedies.

**Table 6**
Primary metabolites of *N. crenulata* leaf extract.

| Constituents | Values (% w\w) |
|---|---|
| Protein | 14.620 ± 0.170 |
| Lipid | 1.240 ± 0.012 |
| Carbohydrate | 0.500 ± 0.010 |

**Table 7**
Secondary metabolites of *N. crenulata* leaf extract.

| Constituents | Values (% w\w) |
|---|---|
| Phenol | 13.12 ± 0.00 |
| Tannin | 2.64 ± 0.03 |
| Flavonoid | 3.65 ± 0.07 |
| Vitamin E | 0.68 ± 0.08 |
| Vitamin C | 10.17 ± 1.21 |

The presence of wide range of phytochemical constituents indicates that plant could serve as pilot for the development of novel agents for various pathological disorders. However, less information is available regarding chemical constituents and

bioactivity of this ethnomedicinally important species. Further studies regarding isolation and purification of active phytoconstituents with broad spectrum of antimicrobial activity is under study and these studies can serve as an important source of information to ascertain the identity and to determine the quality and purity of the plant material in future studies.

## Conflict of interest statement

We declare that we have no conflict of interest.

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