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Pharmacognostic studies on *Neuracanthus sphaerostachyus* Dalz. (Acanthaceae) leaves

D.K. Dangar^a, N.J. Patel^{b, *}

^a Ganpat University, Ganpat Vidyanagar 384012, Mehsana, Gujarat, India

^b Shree S. K. Patel College of Pharmaceutical Education & Research, Ganpat University, Ganpat Vidyanagar 384012, Mehsana, Gujarat, India



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ABSTRACT

Background: *Neuracanthus sphaerostachyus* Dalz. species shows the spherically arranged flowering head. Mostly flowers are observed in purple colour. It is an annual herb with 30–60 cm high. Flowers arranged in rounded clusters without a stalk. Leaves are 5–10 cm in length, and are almost without a stalk. *N. sphaerostachyus* has been traditionally used to treat skin diseases, cough and asthma.

Objective: The present study was undertaken to study the Pharmacognostic parameters for the rapid identification and authentication of the plant.

Materials and methods: The macroscopic and microscopic characteristics with intensive quantitative microscopy of *N. sphaerostachyus* Dalz leaves were done using different chemicals and reagents.

Results: The plant leaves show single layered, wavy walled cells in upper epidermis. Powder study of leaves shows light greenish colour with multicellular covering trichomes and sessile glandular trichomes with fibres passing through parenchymatous cells.

Conclusion: The macroscopic and microscopic characteristics of *N. sphaerostachyus* Dalz leaves serves as a tool for low cost, rapid identification and authentication of this plant.

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1. Introduction

Neuracanthus sphaerostachyus Dalz. [1] belongs to the family of Acanthaceae (Ruellia family), known as Pin cushion plant due to its floral structure and commonly known as Putliyo (Hindi), Gogonda (Marathi) and Ganthera – Gandharo (Gujarati) (Fig. 1). It is native to Indian regions and widely distributed in Western Ghats (Goa), Deccan and throughout the Gujarat [1]. This plant is traditionally used in different areas of Western Ghats. The mixture of ash of whole plant with jaggery or honey is used for 2–3 times a day orally to cure cough and asthma [2]. Root paste is applied on ringworm [1]. *Neuracanthus sphaerostachyus* shows presence of Vanillic acid, Syringic acid, Melilotic acid and 6-OH Luteolin [3].

2. Materials and methods

2.1. Collection and authentication of plant

N. sphaerostachyus Dalz leaves were collected from Girnar forest region of Gujarat. Plant material is authenticated by NISCAIR-CSIR (National Institute of Science Communication and Information resources), New Delhi. (NISCAIR/RHMD/Consult/2016/2987-14)

2.2. Sectioning

Free hand sections of leaves were taken and cleared with chloral hydrate. The sections of plant materials were treated with phloroglucinol and a drop of concentrated hydrochloric acid to stain the lignified elements. The sections were also stained with dilute solution of iodine to study the starch grains. Photomicrographs were taken with a Motic Mono circular, Motic DM 111 Microscopic Unit. For study of the powder drug, the dried leaves were ground and passed through a sieve 60 mesh [4].

* Corresponding author.

E-mail: nileshcology127@yahoo.co.in

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Fig. 1. *Neuracanthus sphaerostachyus* plant.

2.3. Quantitative microscopy

Plant leaves were washed thoroughly and followed by staining with safranin for quantitative microscopic studies.

2.3.1. Stomatal number

It is the average number of stomata per square mm of the epidermis of the leaf. A minimum of ten readings were taken from different locations of the leaf and the average value was counted [4].

2.3.2. Stomatal Index

It is the percentage in which the number of stomata to the total number of epidermal cells, each stoma being counted as one cell. Stomatal Index was counted by using the following equation,

$$S.I = (S/E + S) \times 100$$

where, S.I = Stomatal Index, S = Number of stomata per unit area, E = Number of epidermal cells in the same unit area.

2.3.3. Vein-Islet and vein termination number

Counted the number of vein-islets in 4 sq.mm area in the central part between the midrib and the margin on leaf surface. Counted the number of vein terminations in 4 sq.mm area of the central part between the midrib and the margin on leaf surface.

2.3.4. Palisade ratio

It is the average number of palisade cells beneath each epidermal cells of a leaf. It is determined by counting the palisade cells beneath four continuous epidermal cells [5].

2.4. Histochemical colour reactions

The micro-chemical tests for histological zones were performed [6–8].

2.5. Behaviour of powder with different chemical reagents

The dark brown fine powder was treated with different chemical reagents for the detection of the phytoconstituents with colour changes and observed under ordinary daylight by the standard method [9].

2.6. Loss on drying (gravimetric determination)

It is the amount of volatile matter of any kind (including water) that can be driven off. One gram of each of the samples was weighed accurately, spread on shallow Petri dish and heated at a regulated temperature of 105 ± 1 °C to constant weight. The samples were weighed immediately after removing from the oven. Loss on drying is expressed as the loss in weight in percent w/w [10].

2.7. Determination of pH

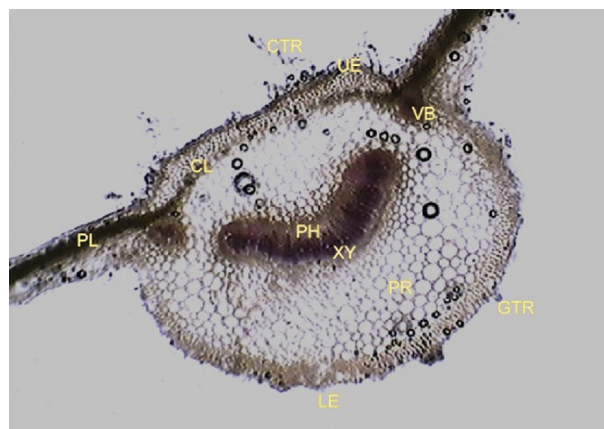
The pH value represents the acidity or alkalinity of an aqueous solution.

2.7.1. pH of 1% solution

Accurately weighed 1 g of air-dried coarsely powdered material dissolved in 100 ml of distilled water. The pH of the water soluble portion was measured with calibrated pH meter at 25 °C.

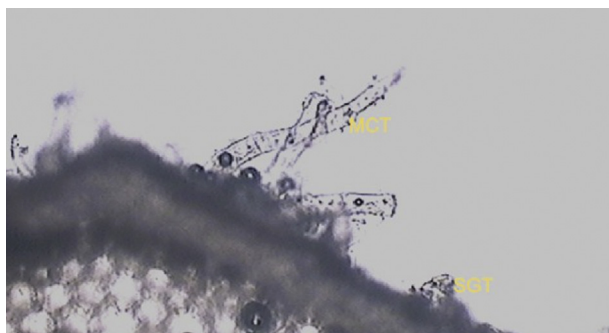


Fig. 2. External features of *Neuracanthus sphaerostachyus* leaves.



[UE: Upper epidermis, GTR: Glandular Trichome, CTR: Covering Trichome, PL: Palisade layer, CL: Collenchyma, VB: Vascular bundle, XY: Xylem, PH: Phloem, LE: Lower epidermis, PR: Parenchyma]

Fig. 3. Transverse section of leaf of *Neuracanthus sphaerostachyus*.



[MCT: Multicellular Covering Trichome, SGT: Sessile glandular Trichome]

Fig. 4. Enlarged portion of epidermis.

2.7.2. pH of 10% solution

Accurately weighed 10 g of air-dried; coarsely powdered material was dissolved in 100 ml of distilled water. The pH of the water soluble portion was measured with standardized pH meter at 25 °C [11].

2.8. Determination of ash values

2.8.1. Total ash

About 8 g of the ground air-dried material, accurately weighed, in a ignited and tared crucible and incinerated in a muffle furnace at a temperature not exceeding 450 °C until the formation of ash. The sample was then cooled overnight and transferred to desiccator and then weighed. The percentage of total ash was determined with reference to air dried sample [11].

2.8.2. Acid insoluble ash

To the crucible containing the total ash added 25 ml of 2N hydrochloric acid and boiled for 5 min. The insoluble matter collected on filter paper (ash less). Filter paper was washed with hot water until the filtrate became neutral. It was ignited to constant weight and allowed the residue to cool in a desiccator, then weighed immediately. The acid insoluble (percentage) ash was calculated in reference to air dried material.

2.8.3. Water soluble ash

To the crucible containing the total ash, added 25 ml of distilled water and boiled for 5 min. The insoluble matter was collected on

ash less filter paper and washed with hot water and ignited to constant weight. The residue was allowed to cool in a desiccator and then weighed immediately. The weight of insoluble ash was subtracted from the weight of total ash and the difference in weight indicated the weight of water-soluble ash. The percentage of water-soluble ash was calculated with reference to air-dried material [11].

2.9. Chemicals and drugs

All the used chemicals were of analytical grade and were procured from Chemdyes Corporation, Rajkot, India.

2.10. Statistical analysis

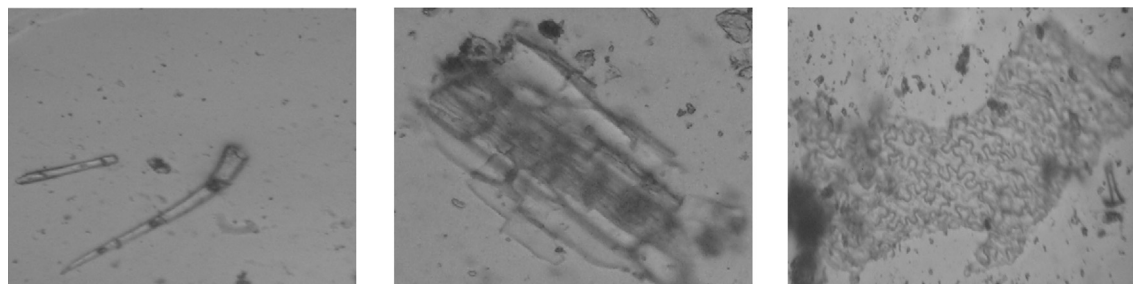
A result of pH values and Ash values has been expressed as results \pm Standard Deviation (S.D.).

3. Results

Macroscopic evaluation of *N. sphaerostachyus* leaves revealed that the leaves are simple, green, opposite and sessile about 10–12 cm in length and 5–7 cm wide with elliptic oblong Shape. The apex is acute, base is cordate. The leaves are having reticulate venation. The margin is wavy and Surface is pubescent. Odour is slight and Taste is acrid and mucilaginous (Fig. 2).

Microscopic studies of *N. sphaerostachyus* leaves shows Lamina with upper epidermis which is single layered, wavy walled cells covered with cuticle, containing 3–4 celled multicellular covering trichomes and sessile glandular trichomes. Mesophyll is dorsiventral and Palisade is single layered, compact and radially elongated cells (Fig. 3). Spongy parenchyma in 2–3 layered, loosely arranged with intercellular spaces and lower epidermis resembles the upper epidermis but numbers of trichomes were less. Midrib with collenchymas, thick walled cellulosic cells arranged in 3–4 layers below the upper epidermis and above the lower epidermis (Fig. 4). Vascular bundles with arc shaped, collateral and 3 in number with two lateral and one median, phloem were well developed and xylem consists of vessel elements with tracheides and tracheidal fibres.

Powder microscopy of *N. sphaerostachyus* leaves revealed trichomes, fibres and epidermal cells. Trichomes were multicellular covering trichomes and sessile glandular trichomes. Fibres passing through parenchymatous cells were present and epidermal cells



(a) Trichomes of Neurocanthus

(b) Fibres with parenchymatous cells

(c) Fragment of Epidermis showing wavy walled cells of Neurocanthus

Fig. 5. Powder characteristics of *Neurocanthus sphaerostachyus* leaves.

Table 1
Determination of leaf constant of *Neuracanthus sphaerostachyus*.

| Sr. no. | Parameter | Range | Average |
|---------|-------------------------|-------|---------|
| 1 | Stomatal number | 7–11 | 9 |
| 2 | Stomatal index | 2–5 | 4 |
| 3 | Vein-islet number | 16–19 | 17 |
| 4 | Vein termination number | 9–13 | 11 |
| 5 | Palisade ratio | 3–5 | 4 |

Table 2
Histochemical colour reactions of *Neuracanthus sphaerostachyus*.

| Reagent | Constituent | Colour |
|--------------------------------------|--------------------|--------------|
| Conc. H ₂ SO ₄ | Saponins or Lipids | Bluish black |
| Weak Iodine solution | Starch | – |
| Millons reagent | Protein | Red |
| Dragendroff reagent | Alkaloids | – |
| Phloroglucinol + HCl | Lignin | Faint pink |
| Mg + HCl | Flavonoids | Yellow |
| Iodine Solution | Cellulose | – |
| Caustic alkali + HCl | Calcium oxalate | Green |

Table 3
Behaviour of the powder with different chemical reagents on leaves powder of *Neuracanthus sphaerostachyus*.

| Reagent | Colour/Precipitate | Constituent |
|--------------------------------------|--------------------|---------------------------------|
| Aq. FeCl ₃ | No change | Tannins absent |
| Iodine Solution | No change | Starch absent |
| Ammonia solution | No change | Anthraquinone glycosides absent |
| 5% Aq. KOH | No change | Anthraquinone glycosides absent |
| Aq. HgCl ₂ | No precipitate | Alkaloids absent |
| Aq. AgNO ₃ | White precipitate | Protein present |
| Conc. H ₂ SO ₄ | Reddish-pink | Lipids present |
| Mg–HCl | Yellow | Flavonoids present |
| Picric Acid | No change | Alkaloids absent |

found as a polygonal fragment with wavy walled epidermal cells (Fig. 5).

Leaf constant of *N. sphaerostachyus* were determined with different parameters and presented in Table 1. Transverse sections of leaves of *N. sphaerostachyus* using standard method were treated with different reagents and the results are given in Table 2. Behaviour with different chemical reagents was performed on the crude powder of Leaves of *N. sphaerostachyus* using different reagents to detect the phytoconstituents with colour changes under ordinary daylight by the standard method

Table 4
Fluorescence characteristics of powdered leaves of *Neuracanthus sphaerostachyus*.

| Sr. no. | Treatment | Under Ordinary light | UV light | |
|---------|---|-----------------------|----------------------|-----------------------|
| | | | Long | Short |
| 1 | Powder | Greenish | Dark Greenish | Light Brownish |
| 2 | Dry powder + Nitrocellulose | Black Greenish | Dark greenish brown | Greenish brown |
| 3 | Powder + 1M NaOH in Methanol | Yellowish green | Yellowish green | Dark blackish green |
| 4 | Powder + 1N NaOH in Methanol, mounted with Nitrocellulose in Amyl acetate | Dark Yellowish green | Dark Yellowish green | Brown |
| 5 | Powder + 1M HCl | Light green | Dark green | Brownish |
| 6 | Powder + 1M HCl, mounted with Nitrocellulose in Amyl acetate | Light green | Dark green | Brownish |
| 7 | Powder + 1M NaOH | Light yellowish green | Yellowish green | Light brown |
| 8 | Powder + 1M NaOH, mounted with Nitrocellulose in Amyl acetate | Greenish brown | Black | Bright greenish brown |
| 9 | Powder + 50% HNO ₃ | Greenish | Dark green | Dark brown |
| 10 | Powder + 50% H ₂ SO ₄ | Green | Dark green | Greenish black |

Table 5
Physicochemical values of *Neuracanthus sphaerostachyus* leave powder.

| Parameters | Results ± S.D. |
|---------------------------------|-----------------------|
| 1. Organoleptic characteristics | |
| Appearance | Powder |
| Colour | Greenish |
| Odour | Characteristics odour |
| Taste | Acrid |
| 2. Loss on drying | 7.8 ± 0.48 |
| 3. pH values | |
| pH of 1% aqueous solution | 5.43 ± 0.01 |
| pH of 10% aqueous solution | 5.92 ± 0.01 |
| 4. Ash values (%) | |
| Total ash | 4.72 ± 0.11 |
| Water soluble ash | 1.35 ± 0.11 |
| Acid insoluble ash | 2.47 ± 0.11 |

and characteristic changes observed are summarized in Table 3. Fluorescence characteristics of the powder as such and after treating with some chemical reagents were observed in daylight as well as under ultraviolet radiations. The results are recorded in Table 4. The standard physicochemical protocols developed from the Indian Pharmacopoeia were followed by calculating moisture content, pH and ash values. The values obtained are presented in Table 5.

4. Discussion

All medicinal systems have developed quality control and for herbal medicines, standardization is also required to assure the quality of the drug. These parameters help in the proper identification of plant with their substitute herbal materials which often found in the market. Human usage can be permitted after ensuring their identity, quality, purity and safety of drug. Various studies like microscopic analysis, macroscopic analysis and fluorescence analysis identify the particular drug and provide authenticity of raw material. Morphological and microscopical studies of leaves will be helpful in the identification of *N. sphaerostachyus* Dalz. Quantitative analysis of pharmacognostic characters may be helpful to establish quality standards of the plant. These simple, inexpensive, reliable standards can help society whenever using the drug as folk medicine. These studies will also be helpful for manufacturer for assessing the purity of raw material. The methods mentioned here can be considered as characteristics to identify and authenticate this drug.

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None.

Conflict of interest

None.

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