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COMPARATIVE PHARMACOGNOSTIC STUDY AND ESTABLISHMENT OF QUALITY PARAMETERS OF LEAVES OF TWO SPECIES OF KAREN: *THEVETIA PERUVIANA* AND *NERIUM INDICUM*

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ABSTRACT

Background: Two species of Karen - *Nerium indicum* and *Thevetia peruviana* are found distributed throughout tropical parts of India. Their leaves are used as cardiotonic and diuretic. Objectives: The present investigation deals with the comparative pharmacognostical study of the leaves of the two species and establishment of its quality parameters.

Methods: Detailed morphological and qualitative as well as quantitative microscopic study was carried out. This was followed by phytochemical screening of the two species.

Results: Leaf surface microscopy of *N. indicum* leaf shows straight-walled epidermal cells and anomocytic stomata whereas *T. peruviana* leaf shows wavy-walled epidermal cells and anisocytic stomata. The chief powder characterisics of *N. indicum* powder are horse-shoe shaped and annular xylem vessels, pericyclic fibres, hooked-top covering trichomes and calcium oxalate rosette crystals, whereas *T. peruviana* powder shows mainly the presence of rosette crystals, thereby making it easy to differentiate the two leaves. Various quantitative microscopic parameters were also established. Phenolics, cardenolides, and sterols were detected in both the species, but alkaloids were detected only in *T. peruviana*.

Conclusion: The present work can serve as a useful tool in the identification, authentication and standardization of the plant material and distinguishing the two species from each other.

Keywords: Apocynaceae, *Nerium indicum, Nerium odorum, Thevetia neriifolia, Thevetia peruviana*

1. Introduction

Nerium indicum syn. *Nerium odorum* (Apocynaceae) is commonly known as 'Lal Karen' or Red Oleander because of its red flowers and is also found throughout India. The whole plant especially leaves are used as cardiotonic and for treatment of weak heart. Odorosides I to IV present in it are used in cardiac insufficiency, dropsy, arrhythmias and as diuretic.[1] *Thevetia peruviana* syn. *Thevetia neriifolia* (Apocynaceae) is commonly known as 'Pili Karen' or Yellow Oleander because of its yellow flowers and is found throughout India. Seeds are used as abortifacient and purgative, and in rheumatism and dropsy. Peruvosides present in it are used in treatment of cardiac insufficiency and weak heart. This drug shows relatively high degree of therapeutic index compared to digitoxin.[2] The present study deals with comparative pharmacognostic study of the leaves of the two species.

2. Materials and Methods

Leaves of *T. peruviana* and *N. indicum* were collected in the flowering stage from Herbal Garden of RK College of Pharmacy, Rajkot, Gujarat in July 2012. Herbarium and voucher samples of *N. indicum* (RKCP/COG/25/2012) and *T. peruviana* (RKCP/COG/26/2012) were deposited in the college laboratory. The fresh leaves were compared morphologically and used for transverse section, surface preparation and quantitative microscopy. The dried leaves were powdered, stored in airtight containers and used for powder study and phytochemical screening. For microscopical studies, safranin was used for staining. Photomicrography of the transverse sections and the powdered drug was performed using camera. Quantitative microscopic study was performed using camera lucida and stage micrometer scale (Table 1).[3] Phytochemical screening of both the species was performed using the appropriate extracts (aqueous and alcoholic) and appropriate chemical tests (Table 2).[4-8]

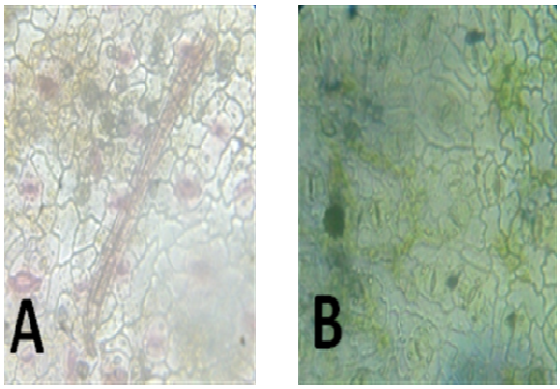
3. Results

3.1 Macroscopy: *T. peruviana* leaves are simple, linear lanceolate, 14-15cm X 1-2cm, glabrous, glaucous, having sub-acute apex, short petiole, decurrent base, entire margin with few notches and upper surface dark green and lower surface light green in color. *N. indicum* leaves are simple, lanceolate, 16-18cm X 2-3cm, glabrous, but not glaucous, having acute apex, 1-2cm long petiole, symmetrical base, entire margin and upper surface dark green and lower surface light green in color. Taste of both leaves is bitter (Fig. 1).

Figure 1. Leaves of *Thevetia peruviana* (L) and *Nerium indicum* (R)

3.2 Microscopy: Surface Preparation: Surface preparation of *N. indicum* leaf shows the presence of straight-walled epidermal cells and anomocytic stomata whereas *T. peruviana* leaf shows the presence of wavy-walled epidermal cells and anisocytic stomata (Fig. 2).

Figure 2. Surface preparation (x450)

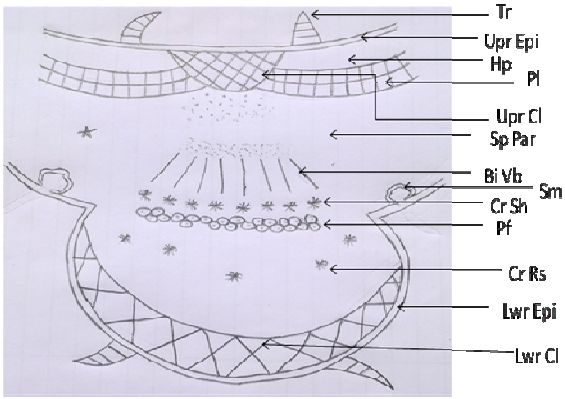


A: *N. indicum* leaf; B: *T. peruviana* leaf

3.3 Microscopy: Transverse Section: Transverse section of *N. indicum* leaf shows the presence of a thick cuticle, single layered epidermis composed of compactly arranged tabular cells and 2-3 layered hypodermis in the lamina, composed of round to polygonal parenchymatous cells. Bilayered palisade is present in the lamina which leaves a very small area for the spongy parenchyma. Lower epidermis shows the presence of sunken stomata, a characteristic of xerophytic plants. The mid-rib shows the presence of cup-shaped upper collenchyma and lower collenchymas. Rosette crystal sheath and groups of lignified pericyclic fibres are present beneath the bicollateral vascular bundles in the stele. Multicellular covering trichomes are observed predominantly on the lower surface (Fig. 3, 4, 7, 8).

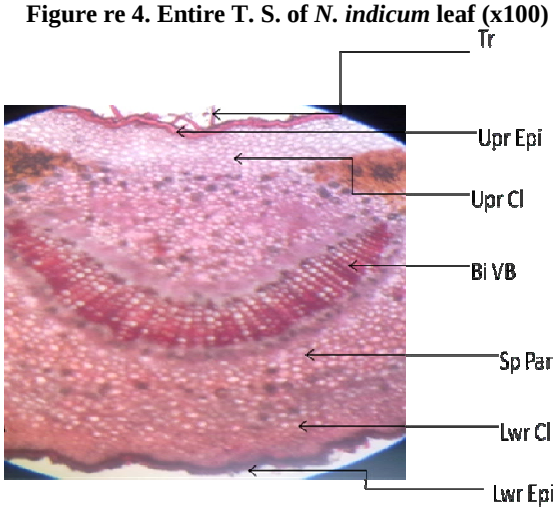
Transverse section of *T. peruviana* leaf shows the presence of a thin cuticle and single layered epidermis composed of compactly arranged tabular cells. Single-layered palisade is present in the lamina, due to which the spongy parenchyma is wider. The mid-rib shows the presence of upper and lower collenchyma. Bicollateral vascular bundles are present in the stele. Trichomes are rare. Rosette crystals are scattered in the lamina and ground tissue (Fig. 5, 6, 7, 8).

Figure 3. Diagrammatic T. S. of *N. indicum* leaf



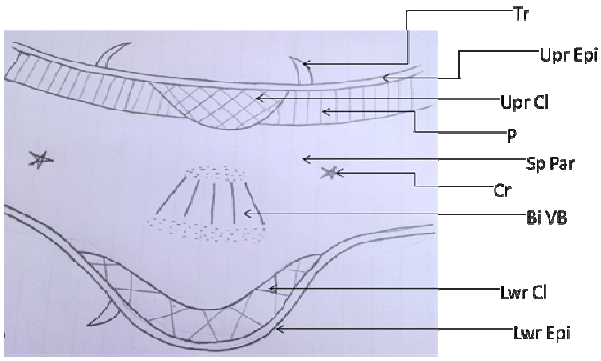
Tr: Trichome, Upr Epi: Upper epidermis, Upr Cl: Upper collenchyma, Hp: Hypodermis, Sp Par: Spongy parenchyma, Bi Vb: Bicollateral vascular bundle, Pf: Pericyclic fibers, Sm: Stomata, Cr Sh: Rosette Crystal sheath, Lwr Cl: Lower collenchyma, Lwr Epi: Lower epidermis

Figure re 4. Entire T. S. of *N. indicum* leaf (x100)



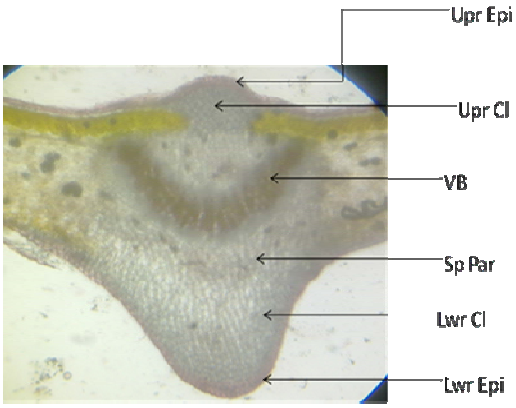
Tr: Trichome, Upr Epi: Upper epidermis, Upr Cl: Upper collenchyma, Hp: Hypodermis, Sp Par: Spongy parenchyma, Bi Vb: Bicollateral vascular bundle, Cr Sh: Rosette Crystal sheath, Lwr Cl: Lower collenchyma, Lwr Epi: Lower epidermis

Figure 5. Diagrammatic T. S. of *T. peruviana* leaf



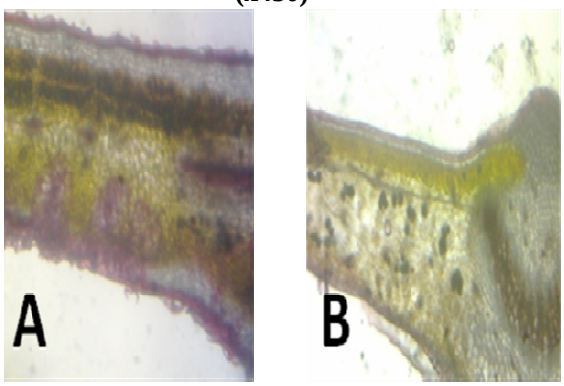
Tr: Trichome, Upr Epi: Upper epidermis, Upr Cl: Upper collenchyma, Pl: Palisade cells, Sp Par: Spongy parenchyma, Cr: Rosette Crystal, Bi VB: Bicollateral vascular bundle, Lwr Cl: Lower collenchyma, Lwr Epi: Lower parenchyma

Figure 6. Detailed T.S. of *T. peruviana* leaf (x100)



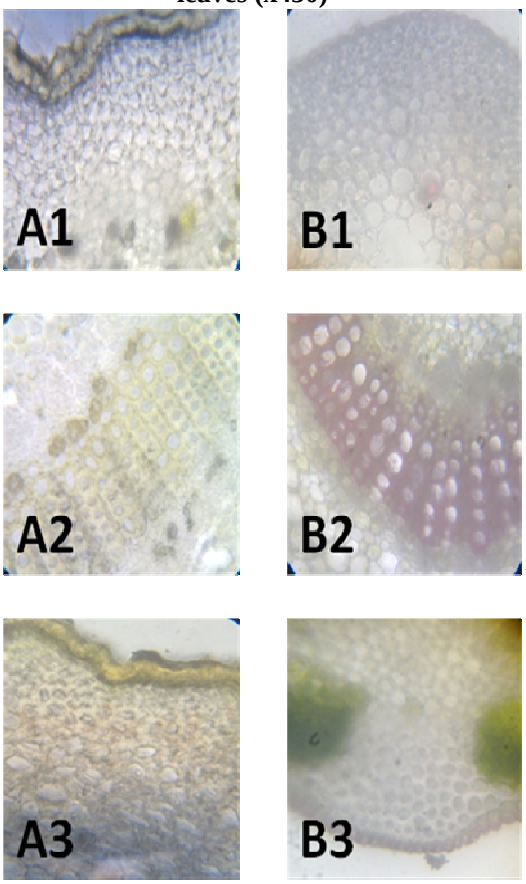
Tr: Trichome, Upr Epi: Upper epidermis, Upr Cl: Upper collenchyma, Pl: Palisade cells, Sp Par: Spongy parenchyma, Cr: Rosette Crystal, Bi Vb: Bicollateral vascular bundle, Lwr Cl: Lower collenchyma, Lwr Epi: Lower parenchyma

Figure 7. Enlarged portions of lamina of leaves (x450)



A: *T. peruviana*; B: *N. Indicum*

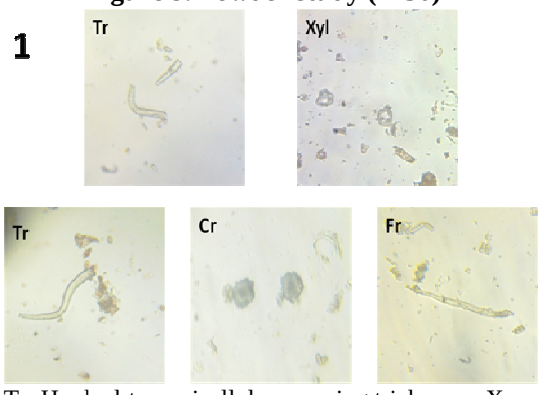
Figure 8. Enlarged portions of mid-rib of the leaves (x450)



A: *N. indicum* (1: Lower collenchyma, 2: Vascular bundle, 3: Upper collenchyma)
B: *T. peruviana* (1: Lower collenchyma, 2: Vascular bundle, 3: Upper collenchyma)

3.4 Microscopy: Powder Characteristics: The diagnostic features of *N. indicum* powder are horse-shoe shaped and annular xylem vessels, pericyclic fibres, hooked-top covering trichomes and calcium oxalate rosette crystals. *T. peruviana* powder shows mainly the presence of rosette crystals (Fig. 9).

Figure 9. Powder study (x450)



Tr: Hooked-top unicellular covering trichomes, Xy: Annular and horse-shoe shaped xylem vessels, Cr: Rossete crystals, Fr: Pericyclic fibre

Table 1. Quantitative microscopy

PARAMETERS	MEASURED VALUE	
	N. indicum	*T. peruviana*
Stomatal Index Upper surface Lower surface	 5.35±0.08 14.96±0.08	 4.75±0.08 13.83±0.08
Palisade ratio	8±1	7±1
Vein islet no.	13 ±1	12 ±1
Vein termination no.	28 ±1	22 ±1
Rosette diameter (μ)	12.88 - 23.39 – 37.2	11.35 - 24.72 – 36.85

Number of observations: 25

Table 2. Phytochemical screening

Phytoconstituent	Test	Result	
		N. indicum	*T. peruviana*
Alkaloids	Dragendorff's test Wagner's test Mayer's test Hager's test	-ve -ve -ve -ve	+ve +ve +ve +ve
Flavonoids	Shinoda test Lead acetate test	-ve -ve	-ve -ve
Sterols	Salkowski test Libermann Buchardt test	+ve +ve	+ve +ve
Cardiac glycosides	Legal's test Baljet test Keller Killiani test Kedde's test	+ve +ve +ve +ve	+ve +ve +ve +ve
Saponin glycosides	Foam test Lead acetate test	-ve -ve	-ve -ve
Phenolics	Ferric chloride test Folin ciocalteu test	+ve +ve	+ve +ve
Sugars	Fehling's test Molisch test	-ve -ve	-ve -ve

4. Discussion

A detailed comparative pharmacognostic study of leaves of *T. peruviana* and *N. indicum* has been performed. Surface preparation of *N. indicum* leaf shows the presence of straight-walled epidermal cells and anomocytic stomata whereas *T. peruviana* leaf shows the presence of wavy-walled epidermal cells and anisocytic stomata. Transverse section of *N. indicum* leaf shows the presence of unique characteristics like hypodermis in the lamina, sunken stomata, rosette crystal sheath and groups of lignified pericyclic fibres, which are absent in *T. peruviana*. The diagnostic features of *N. indicum* powder are horse-shoe shaped and annular xylem vessels, pericyclic fibres, hooked-top covering trichomes and calcium oxalate rosette crystals, whereas *T. peruviana* powder shows mainly the presence of rosette crystals, thereby making it easy to differentiate the two leaves. Various quantitative microscopic parameters were also established. Phenolics, cardenolides, sterols and triterpenoids were detected in both the species, but alkaloids were detected only in *T. peruviana*. The present study can be very useful in distinguishing *T. peruviana* and *N. indicum*, standardizing their formulations as well as serving as a guide for isolating novel phytoconstituents from them.

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