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# PHARMACOGNOSTIC STUDIES OF *NERIUM INDICUM*

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## ABSTRACT

The study is aimed at development of physicochemical parameters and to investigate the active principle present in *Nerium indicum*. *Nerium indicum* (Apocynacea) commonly known as kanner is an important plant used against various disorders in indigenous system of medicine such as cardiotonic, antibacterial and antidiabetic. Thus from the extensive literature survey it was revealed that no reports were available on microscopic evaluation, standardization parameters and chemo profile of *Nerium indicum* to check the identity and purity of the drug. The present work embodies the investigations carried out to establish methods for quality control of drugs as per who guidelines: complete botanical evaluation which comprises macroscopic, microscopy physicochemical parameters like loss on drying extractive value, foaming index, ash value and to investigate the Phytochemical present the extract in the preliminary level with respect to thin layer chromatography were also carried out for the quality control of the drug. Thus, it was thought worthwhile to explore this plant on the basis of these standardization parameters. The study will provide referential information for the correct identification of the crude drug.

**INTRODUCTION**: The herbal medicines have wide therapeutic actions and safety profile. This makes the herbal therapies to be successful. One of these can be the use of *Nerium indicum,* the shrub commonly grows and has many therapeutic indications. It is in leaf all year, in flower from June to October. The flowers are hermaphrodite.[1] Leaves are powerful repellent. A decoction of the leaves has been applied externally in the treatment of scabies and to reduce swellings. The leaves and the flowers are cardiotonic, diaphoretic, diuretic, emetic, expectorant and sternutatory. It has also being reported to have antibacterial [2] and antidiabetic [3] activities. The objective of our study is to develop standardization and identification parameters for the crude drug as per Quality control methods for medicinal plant materials, World Health Organization Geneva.

**MATERIAL AND METHOD**:

**Plant material**: The leaves of *Nerium Indicum* were collected from the Herbal Garden, SPTM, NMiMS, Shirpur, in the month of January 2010 and were authenticated by Department of Botany, Doctor Hari Singh Gour Vishwavidyalaya, Sagar (MP) and herbarium number was provided as Bot/herb/2009/NI-345. The herbarium was prepared and kept in the pharmacognosy department at SPTM, NMiMS, Shirpur (MH). The collected leaves were washed; shade dried and was pulverized with mechanical pulverizer for the size reduction. It was then passed through # 60 and the fine powder was collected and used for the experiment and preparation of Extract. The fresh leaves were used for Microscopy Identification.

**Pharmacognostic Studies**: Morphological Studies were carried out by using simple determination technique, the shape, size, color, odor, margin and apex. Apex of the leaf .Microscopic Studies were carried out by preparing of thin hand section of leaf. The sections were cleared with alcohol and stained as per the Protocol. Histochemical reaction were applied with Concentrated Hydrochloric Acid and Phloroglucinol and were mounted in Glycerin for identification of Lignified Elements, Iodine Solution for Identification of Starch Grains, 60% Sulphuric Acid for Calcium Oxalate Crystals in the powdered leaf by reported methods [4, 5].

**Physico chemical parameters**: The parameter was done to evaluate the percentage of total ash, water soluble acid insoluble ash were calculated as per Indian Pharmacopoeia [6, 7]. The extract of the powdered leaves were prepared with the different solvents for the study of extractive value. Fluorescence analysis was also carried out for the powder as well as different extracts.

**Powder analysis:** Preliminary analysis of the powder of leaf of *Nerium indicum* were carried out with different chemical reagents [8, 9].

**Preliminary phytochemical analysis**: For the Preliminary phytochemical analysis, the extract was prepared by weighing 100gm of dried powdered leaf and were subjected to maceration with different solvents as per the Polarity, methanol, hydro-alcoholic, and finally with Aqueous. The extracts were filtered in each step, concentrated, and the solvent was removed by rotary evaporator. The extracts were dried over desiccator and the residues were weighed. The presence and absence of the primary and secondary phytoconstituents was detested by usual prescribed methods [10].

**RESULT AND DISCUSSION**:

**Macroscopic Characters of Leaf**: Leaf apex was pointed with symmetrical base and entire margin. Emerald green, odorless and bitter leaves of *Nerium indicum* with size having length 3.3-

20.5 cm and width 0.6-2.3 cm. The venniation was parallel, Petiole was slightly whorled; venniation was parallel and surface was smooth at the adaxcial and rough at abaxcial.

**Microscopic Features**: The transverse section of the leaf showed following characters. The leaf is generally dorsiventral in nature and it consisted of two major regions namely Midrib and the lamina portion. The lamina portion consisted of upper and lower epidermis, which was followed by the presence of unicellular uniseriate trichomes. Below the epidermis layer the presence of parenchymatous cells were observed which were 4 layered and palisade cells which were long and elongated in nature. Presence of calcium oxalate crystals were also observed in spongy mesophyll region. The midrib consisted of vascular bundles namely xylem and phloem. Below the vascular region there was parenchyma cells which were lossly packed with more intercellular space. Below the parenchymatous layer collenchymas cells were observed at the bottom part of the transverse section.

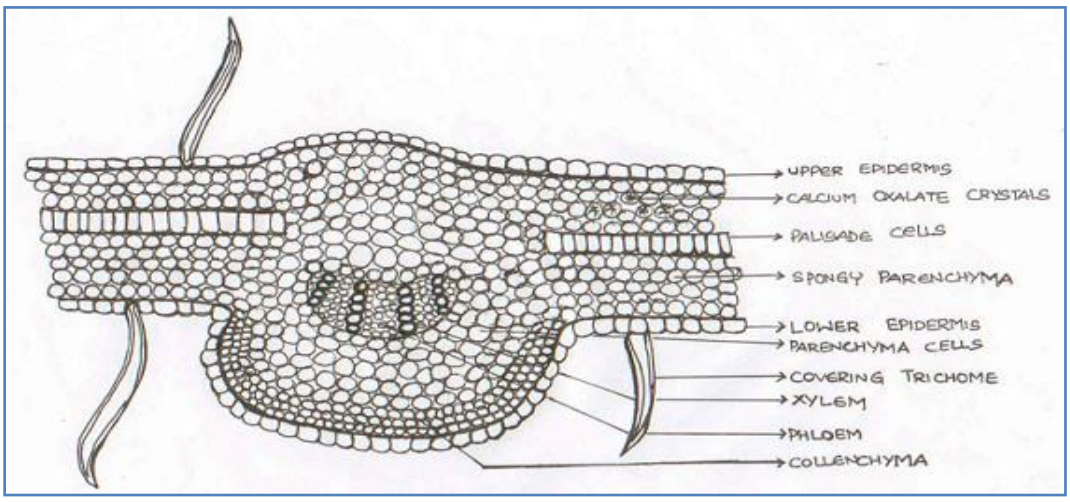


**FIG. 1: MICROSOCPY OF THE FRESH LEAF**

**Powder Microcopy**: The green colored powder was used for the study. The powder was stained with phloroglucinol and concentrated hydrochloric acid. It was mounted in glycerin and examined under 10 X and then magnified with 40 X. On microscopical examination it showed pyramidal calcium oxalate crystals. Trichomes were unicellular and uniseriate covering, vessels were annular to spiral in nature, epidermis showed parenchymatous cells with wavy walled, and fibers were long, slender and cylindrical in shape.

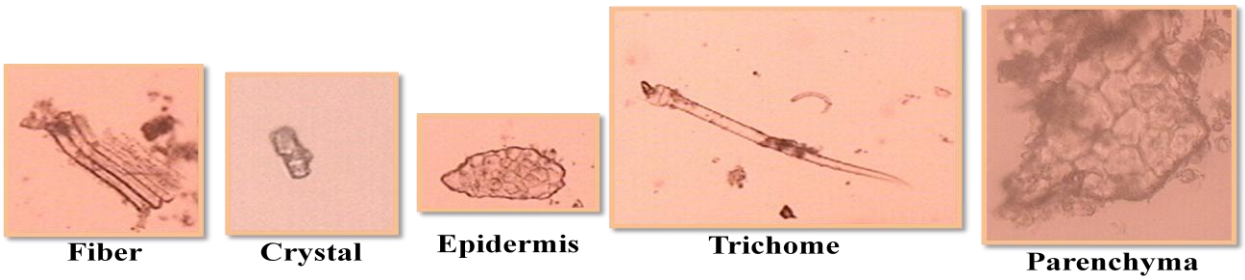


**FIG. 2: MICROSCOPY OF POWDERED LEAF**

**Fluorescence Analysis**: The powder drug and extracts were subjected to fluorescence analysis as per the standard procedure. The results are provided in the tables listed below;

**TABLE 1: RESULT OF FLUORESCENCE ANALYSIS OF POWDER**

| Drug | Short ultra violet (256 nm) | Long ultra violet (365 nm) | Visible ultra violet (400 nm) |
|---|---|---|---|
| Powder | Blackish Green | Green | Green |
| Powder + Water | Emerald Green | Brownish green | Light green |
| Powder + Conc. HCl | Green | Dark Green | Light green |
| Powder + Conc. $H_2SO_4$ | Black | Black | Brick red |
| Powder + Conc. $HNO_3$ | Green | Dark Green | Light green |
| Powder + NaOH | Light green | Dark Green | Light green |
| Powder + Acetone | Light green | Light green | Yellowish Green |
| Powder + Methanol | Light green | Dark Green | Light green |
| Powder + Acetic Anhydride | Light green | Light green | Greenish yellow |
| Powder+ (2M) HCl | Green | Dark Green | Greenish Brown |

**TABLE 2: RESULT OF FLUORESCENCE ANALYSIS OF AQUEOUS EXTRACT**

| Drug | Visible ultra violet (400 nm) | Short ultra violet (256 nm) | Long ultra violet (365 nm) |
|---|---|---|---|
| Powder | Reddish brown | Green | Dark Green |
| Powder + Water | Light brown | Green | Dark Green |
| Powder + Conc. HCl | Brown | Light green | Dark Green |
| Powder + Conc. H2SO4 | Dark Brown | Light green | Black |
| Powder + Conc. HNO3 | Reddish brown | Green | Black |
| Powder + NaOH | Dark Reddish brown | Green | Black |
| Powder + Acetone | Greenish brown | Light green | Black |
| Powder + Methanol | Light brown | Green | Black |
| Powder + Acetic Anhydride | Light brown | Light green | Black |
| Powder + (2M) HCl | Brown | Green | Black |

**TABLE 3: RESULT OF FLUORESCENCE ANALYSIS OF HYDRO-ALCOHOLIC EXTRACT**

| Drug | Visible ultra violet (400 nm) | Short ultra violet (256 nm) | Long ultra violet (365 nm) |
|---|---|---|---|
| Powder | Yellow | Light Green | Black |
| Powder + Water | Light brown | Light Green | Black |
| Powder + Conc. HCl | Brown | Light green | Black |
| Powder + Conc. H2SO4 | Reddish Black | Dark green | Black |
| Powder + Conc. HNO3 | Yellow | Light green | Black |
| Powder + NaOH | Dark Yellow | Light green | Black |
| Powder + Acetone | Yellow | Light green | Black |
| Powder + Methanol | Dark Yellow | Light green | Black |
| Powder + Acetic Anhydride | Yellow | Light green | Black |
| Powder + (2M) HCl | Yellow | Light green | Black |

**Physicochemical Parameters:** The powdered drug was evaluated for its physico-chemical parameters like Ash values: Acid Insoluble ash, water soluble ash, water insoluble ash, extractive values (Alcohol and water soluble values) and loss on drying. All the results are tabulated in table no. 4

**TABLE 4: RESULTS OF PHYSICO-CHEMICAL PARAMETERS**

| Standardization Parameters | Results |
|---|---|
| Total Ash | 11% w/w |
| Acid Insoluble Ash | 6.167 w/v |
| Acid Soluble Ash | 5% w/v |
| Water Insoluble Ash | 3% w/v |
| Water Soluble Ash | 1.417% w/v |
| Water soluble Extractive value | 70.4% w/v |
| Alcohol soluble Extractive value | 12.8% w/v |
| Loss on Drying | 0.1399% w/w |

**Preliminary Phytochemical Analysis:** The ethanolic extract was subjected to preliminary phytochemical analysis for their presence of the constituents. It showed the presence of alkaloids, tannins and proteins were found to be present in aqueous extract where as Steroids and saponins were also found in Alcoholic extract. Now a day the standardization of crude drugs has become very important for identification and authentication of a drug. But due to certain problems the importance was not up to the mark.

Thus, the lack of standardization technique fails to identify the dug from its originality which there by exploits the usage of drug from its Traditional System of medicine. The plant *Nerium Indicum* is used widely for curing various diseases and gives a helping hand to the Humans. Thus a perfect protocol was designed for its Authentication and identification on the basis of Microscopy and chemical analysis. Thus the present investigation was aimed and the results were found to be significant and encouraging towards the goal for Standardization.

**REFERENCES:**


1. Kirtikar K.R., Basu B.D.: Indian Medicinal Plants. International book distributors, second Edition, Vol. 3, 2005, 2220.
2. Neumann W. and Lindner W., Arch. Exptl.Path.Pharmakol., 1937, 185,630.
3. Nuki B., Folia.Pharmacol. Japan, 1949, 45, 134.
4. Kokate C., Purohit A and Gokhale S: Practical Pharmacognocy. Vallabh Prakashan, New Delhi, tenth Edition, 1994: 112-120.
5. Trease GE and Evans WC: Pharmacognocy. Saunders Copant Limited, New Delhi, fifteenth Edition, 1996, 516-547.
6. Indian Pharmacopoeia, Controller of Publications, New Delhi, Vol 2, 1995, A-54.
7. Khandelwal K.R.: Practical Pharmacognosy. Nirali prakashan, Pune,Eighteenth edition, 2007.
8. Reddy YSR, Venkatesh S and Ravichandra T: Pharmacognostical Studies on Wrightia tinctoria bark, Pharmaceutical Biology, 1999; 37:291-295.
9. Pratt PR and Chase ER: Fluorescence powder vegetable drugs in particular to development systems of identification. Journal of American Pharmaceutical Association 1949; 38: 324-331.
10. Harbone JB. Phytochemical Methods-A guide to modern Techniques of Plant Analysis, Chapman and Hall, London,1998;42:129,189,203.


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