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QUALITY CONTROL AND STANDARDIZATION OF VITEX NEGUNDO

Devi B[a], Reshma R Nair[b], Ajith Lal[c], Joel Blessing[d], Akhila Roy[e], Santhan Nehru Narkilli[f], Surabhi G S[g], Jiji Mohan Mu[h]

[a]*Student, Sree Krishna college of Pharmacy and Research centre, Parassala, Thiruvananthapuram*
[b]*Student, Sree Krishna college of Pharmacy and Research centre, Parassala, Thiruvananthapuram*
[c]*Student, Sree Krishna college of Pharmacy and Research centre, Parassala, Thiruvananthapuram*
[d] *Student, Sree Krishna college of Pharmacy and Research centre, Parassala,Thiruvananthapuram*
[e] *Student, Sree Krishna college of Pharmacy and Research centre, Parassala,Thiruvananthapuram*
[f]*Head, Department of Pharmacognosy,Sree Krishna college of Pharmacy and Research centre, Parassala, Thiruvananthapuram*
[g]*Assistant Professor, Department of pharmacognosy, Sree Krishna college of Pharmacy and Research centre, Parassala, Thiruvananthapuram*
[h]*Assistant Professor, Department of pharmacognosy, Sree Krishna college of Pharmacy and Research centre, Parassala, Thiruvananthapuram*

ABSTRACT

Vitex negundo is a woody and higher shrub plant belongs to family **Lamiaceae** horseshoe vitex, is a large aromatic shrub with quadrangular, densely whitish, tomentose branchlets. It is widely used in folk medicine, particularly in South and Southeast Asia. Nochi – (Nirgundi – Vitex negundo): Nochi or Nirgundi as it is commonly called is native to Eastern and Southern Africa and Asia. It is found throughout Indian Subcontinent and can be easily identified by its light purple flowers borne in panicle inflorescence. Nochi is called by different names in different parts of India.

Keywords: *Vitex Negundo, Botanical description, Pharmacological activity, Chemical constituents, Materials and methods*

1. INTRODUCTION

Vitex negundo, commonly known as Nochi or Nirgundi which belongs to family Lamiaceae horseshoe vitex, is a large aromatic shrub with quadrangular, densely whitish, tomentose branchlets. It is widely used in folk medicine, particularly in South and Southeast Asia and native to Eastern and Southern Africa and Asia. It is ethno botanically used in the treatment of anti-rheumatic, diuretic, anti-inflammatory, antipyretic, expectorant and skin disease.

1.2 Synonyms

- Vitex cannabifolia Siebold & Zucc.
- Vitex incisa Lam.
- Vitex incisa var. heterophylla Franchi.
- Vitex negundo var. heterophylla (Franch.) Rehder

1.3 Vernacular Names

Language	Name
Sanskrit	Nirgundika, Renuka, Nirgunda, Nilapushpi, Nilanirgundi,
Bengali	Nisinda, Samalu, Nirgundi, Nishinda, Sinduari
English	Five-Leaved Chaste Tree, Indian Privet

Tamil	Nirkunnchi, Nallanochi
Hindi	Samhalu, Saubhalu, Nirgandi

Table no. 01: Vernacular names

1.4 Botanical Description

Flowering season	June to December
Fruiting season	September to February.
Habit	Branched Shrub up to 5 m tall, or small, slender tree
Leaves	Palmately compound petiole 2.5-3.8 cm long; mostly trifoliate, occasionally Penta foliate; in trifoliate leaf, leaflet lanceolate or narrowly lanceolate, middle leaflet 5- 10 cm long and 1.6-3.2 cm broad, with 1- 1.3 cm long petiols , texture leathery.
Roots	Cylindrical, hard, tough with irregular fractures; external surface is rough due to longitudinal, narrow, cracks and small rootlets; middle region greyish-white, and xylem region cream coloured; bark thin, wood hard, forming a major part of the root.
Flowers	Bluish-purple,small, in forming large, terminal, often compound, pyramidal panicles
Fruit	The fruit is rounded, 1 to 3 mm in diameter, 1/3rd to 3/4th of its size surrounded by a dull grey cup-like, persistent calyx. calyx cup may show one or two vertical splits; fruit colour is light brown, locules two, each containing two seeds; texture is smooth, taste and odour not characteristic
Seeds	5-6 mm in diameter.

Table no. 02: Botanical description

1.5 Chemical constituents

Major chemical constituents in leaves of *Vitex negundo* Linn are volatile oil, triterpenes, diterpenes, sesquiterpenes, lignan, flavonoids, flavones, glycosides, iridoid glycosides and stilbene derivative. They are friedelin, vitamin c, carotene, casticin, artemetin, sabenine, globulol, α-terpineol, Spathulenol, β- Farnesene, farnesol, α-pinene, β-pinene, linalool

2. MATERIALS AND METHODS

2.1 Quality control

2.1.1 Morphological evaluation of fresh leaf.

Collection and authentication of specimens

The plant specimen (leaves) for the proposed study was collected from Parassala in Thiruvananthapuram district of Kerala. The collected leaves were examined and authenticated by the curator Dr.Remya Krishnan, Department of Botany, University of Kerala, Karyavattom Campus, Thiruvananthapuram. A voucher specimen (Voucher No: KUBH 10276) has been deposited for future reference.

Morphological characters

The morphological characters like colour, odour, taste, size, shape, extra features of the leaves were studied, by using sensory characters.

2.1.2Microscopical studies of fresh leaves

The fresh leaves were subjected to various anatomical studies. The sections of leaf specimen were taken using sharp blade and stained with various staining reagents like phloroglucinol- Hcl, iodine, saffranin and observed under microscope.

2.1.3Microscopical studies of dry powder

Powder microscopy

Majority of the crude drugs for commercial purpose are available in powder form. So, the powder microscopic studies give the anatomical characters of fragmented crude drugs. Glycerine mounted temporary preparations were made for powdered leaves.

2.1.4Determination of foreign matters

Methodology: Weigh 100g of original sample. Spread it out as a thin layer and separate foreign matter manually. Weigh and determine percentage of foreign organic matter from weight of drug taken.

2.1.5Phytochemical screening

Successive Soxhlet Extraction

Soxhlet extraction is also known as hot continuous percolation. Here the plant material is continuously flushed with fresh solvent which is obtained by evaporation and subsequent condensation of the solvent containing extracted materials. In successive soxhlation the plant material is extracted with solvents of increasing polarity and finally macerated with chloroform water.

Preparation of extracts

In the present study, 50 g of coarsely powdered leaves of *Vitex negundo* was packed in Soxhlet apparatus and extracted using solvents such as Benzene, Ethyl acetate, Ethanol and finally macerated with chloroform water. Before extracting with new solvent, the marc is pressed and dried at a temperature not exceeding 50^0 C. The filtrate was concentrated in rotary vacuum evaporator and the extracts were weighed and calculated the percentage yield in terms of air-dried material

Solvent	Polarity Index (p')
Benzene	0.11
Ethyl acetate	4.4
Ethanol	5.2
Water	

Table no. 3: Solvents and their polarity index

Preliminary phytochemical screening

Preliminary phytochemical screening was done to identify different constituents present in extracts i.e. carbohydrates, proteins, lipids, flavanoid, tannins, glycosides, alkaloids, essential oils etc. All the extracts of *Vitex negundo* leaves were subjected to preliminary phytochemical screening.

1. **Detection of alkaloids**

- Mayer's test: 2 ml of the extract was treated with 2 ml of Mayer's reagent.
- Dragendorff's test: 2 ml of the extract was treated with 2 ml of Dragondroff's reagent.
- Hager's test: 2 ml of the filtrate was treated with 1-2 ml of Hager's reagent.
- Wagner's test: 2 ml of the filtrate was treated with 1-2 ml of Wagner's reagent.
- Tannic acid test: 2ml Extract was treated with 2 ml of tannic acid solution.

2. **Detection of Carbohydrates**

- Molisch's test: 1 ml of the test solution was mixed with 2 ml of Molisch reagent, shaken the mixture and added 1 ml of concentrated sulphuric acid along the sides of the test tube.
- Benedict's test: Mixed 2 ml of the Benedict's reagent with 2 ml of the test solution. Boiled in a water bath.
- Fehling's test: Boiled 1 ml of test solution with 1 ml Fehling's solution A and 1 ml Fehling's solution B on a water bath.
- Barfoed's test: Mixed 2 ml of the Barfoed's reagent with 1 ml of the test solution and boiled in a water bath.
- Iodine test: Mixed 0.5 ml of Iodine solution with 1 ml of test solution.
- Seliwanoff's test: Boil 2 ml of Seliwanoff's reagent with 1ml of test solution.

3. **Detection of proteins and amino acids**

- Biuret test: About 2 ml of extract was mixed with 2 ml of Biuret reagent.
- Millon's test: 2 ml of the extract was mixed with 2 ml of Millon's reagent and boiled.
- Xanthoprotein test: 2 ml of the extract was treated with 1ml of concentrated Nitric acid and Sulphuric acid. Cooled the solution and made alkaline with 10% sodium hydroxide.
- Ninhydrin test: Boiled 2 ml of the extract with 1ml of 5% ninhydrin solution in a water bath for 5 minutes.

4. **Detection of tannins and phenolic compounds**

- Ferric chloride test: Mixed 2 ml of the test solution with few ml of 5% ferric chloride solution.
- Lead acetate test: Mixed 2 ml test solution with 1 ml of lead acetate solution.
- Dilute Nitric acid test: Mixed 2 ml of the test solution with dilute nitric acid.
- Bromine water test: Mixed 2 ml of the test solution with bromine water.

5. **Detection of flavonoids**

- Shinoda test: To 2 ml of the sample solution added magnesium powder or zinc powder and few drops of concentrated hydrochloric acid or sulphuric acid.
- Sulphuric acid test: Added few drops of concentrated sulphuric acid to few ml of sample solution.
- Lead acetate test: Mixed 2 ml of the test solution with lead acetate solution.
- Alkali test: Treated the test solution with increasing amount of sodium hydroxide.

6. Detection of steroids and triterpenoids

- Libermann test: Mixed 2 ml of test solution with 2 ml of acetic anhydride and boiled. Then added 0.5 ml of concentrated sulphuric acid.
- Libermann-Buchard test: Mixed 2 ml of the test extract with 1 ml of Chloroform and 1 ml acetic anhydride. Then added 1 drop of concentrated sulphuric acid.
- Salkowski test: Dissolved 1-2 mg of sample in 1 ml of chloroform and added 1 ml of concentrated sulphuric acid.

7. Detection of mucilage:

- Ruthenium red test: Treated the powder with ruthenium red.
- Swelling test: Dissolved the powder in water.

2.1.6Thin layer chromatography of all extract

Procedure: TLC plates were prepared by using silica gel G and the coated plates were allowed to dry in air and activated by heating in hot air oven at 105^0C for 1 hour. The extracts were dissolved in respective solvents and applied in TLC plates by means of capillary tube, a few centimetres above from the edge of plate. The plates were then developed in TLC chamber previously saturated with different solvent systems. By trial and error method appropriate solvent system were developed and different spots developed. The coloured substances were visual on the chromatogram. Colourless components were detected using visualizing agent, UV light and R_f values were calculated.

$$\text{Rf value} = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by solvent}} \quad (1)$$

1.2 Standardization

2.2.1Ash values

The ash of any organic material composed of their non-volatile inorganic components. Controlled incineration of crude drugs results in an ash residue consisting of an inorganic material (metallic salts and silica). Ashing involves oxidation of the components of the product. This value varies within wide limits and is therefore an important parameter for the purpose of evaluation of crude drugs. A high ash value is indicative of contamination, substitution, adulteration or carelessness in preparing the crude drug for marketing. In certain drugs, the percentage variation of the weight of ash from sample to sample is very small and any marked difference indicates a change in quality. Unwanted parts of drugs can raise the ash value. More direct contamination like sand or earth is immediately detected by the ash value.

2.2.1.1Total ash

Procedure: Incinerated about 3 g accurately weighed powdered drug in tared platinum crucible at a temperature not exceeding 450^0 C until free from carbon. Cooled and weighed, repeated to get constant value. Percentage total ash was calculated with reference to the air-dried drug.

2.2.1.2Acid-insoluble ash

Procedure:

The total ash obtained was boiled with 25 ml of 2 M HCl for 5 minutes. The insoluble matter was collected on an ash less filter paper (Whatman-41), washed with hot water. Transferred the filter paper containing insoluble matter to the original crucible, ignited, weighed and calculated the percentage acid-insoluble ash with reference to the air-dried drug.

2.2.1.3Water soluble ash

Procedure:

The total ash obtained was boiled for 5 minutes with 25 ml of water; collected the insoluble matter on an ash less filter paper, washed with hot water, and ignited for 15 minutes at a temperature not exceeding $450\ ^0$ C. Subtracted the weight of the insoluble matter from the weight of the ash; the difference in weight represents the water soluble ash. Calculated the percentage of water-soluble ash with reference to the air-dried drug.

2.2.2 Extractive values

It is the number of active constituents in a given amount of medicinal plant material when extracted with a particular solvent.

The composition of phytoconstituents in that solvent depends upon the nature of the drug and solvent used. The use of a single solvent can be the means of providing preliminary information on the quality of a particular drug sample; for example, in a drug where the extraction procedure for the constituents commence with water as solvent, any subsequent aqueous extraction on the re-dried residue will give a very low yield of soluble matter.

Water soluble, alcohol soluble and ether soluble extractive values are an indicative of poor quality, adulteration with any unwanted materials or incorrect processing of the crude drug during the process of drying, storage etc.

2.2.2.1Alcohol soluble extractive

Macerated 5 g of air-dried, coarsely powdered drug with 100 ml of 90% ethanol in a closed flask for 24 hours. Shaken frequently during the first 6 hours and allowed to stand for 18 hours. Then it was filtered rapidly without any loss of solvent. Evaporated 25 ml of the filtrate to dryness in a tarred flat-bottomed shallow dish and dried at 105^0 C, to constant weight. Calculated the percentage of alcohol soluble extractive with reference to air dried drug.

2.2.2.2Water soluble extractive

Macerated 5 g of the air dried coarsely powdered drug with 100 ml of Chloroform water in a closed flask for 24 hours. Shaken frequently during the first 6 hours and allowed to stand for 18 hours. Then it was filtered without any loss of solvent. Evaporated 25 ml of the filtrate to dryness in a tarred flat-bottomed shallow dish and dried at 105^0 C, to constant weight. Calculated the percentage of watersoluble extractive with reference to air dried drug.

2.2.2.3Ether soluble extractive

Macerated 5 g of the air dried, coarsely powdered drug with 100 ml of Diethyl ether in a closed flask for 24 hours. Shaken frequently during the first 6 hours and allowed to stand for 18 hours. Then it was filtered, without any loss of solvent. Evaporated 25 ml of the filtrate to dryness in a tarred flat-bottomed shallow dish and dried at 105^0 C, to constant weight. Calculated the percentage ether soluble extractive with reference to air dried drug.

2.2.3Loss on drying

Procedure:

Accurately weighed about 1.5 g of fresh leaves of in a tarred porcelain dish and dried in an oven at 105^0 C to constant weight, cooled in desiccator and weighed.

From the difference in weights percentage loss on drying was calculated Morphological evaluation of fresh leaf.

3. RESULT

3.1 Morphological evaluation of fresh leaves

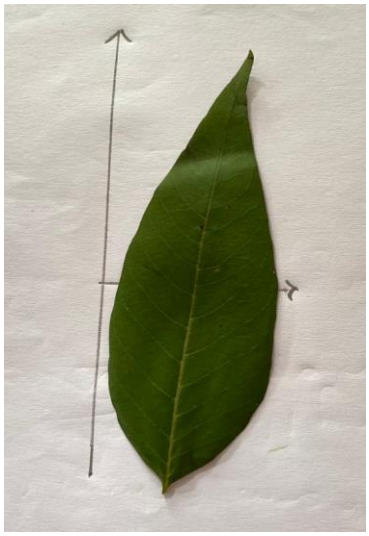

Fig. no. 01: Leaf of *Vitex negundo*

Sl.No	Features	Observation
1	Colour	Green
2	Odour	Characterstic odour
3	Taste	Bitter
4	Shape	Lanceolate
5	Petiole	Palmately compound 2.5-3.8cm
6	Texture	Leathery
7	Venation	Parallel

Table No.04: Morphological characters of *Vitex negundo*

Microscopical studies of fresh leaves.

In the leaf through the transverse section of midrib, following tissues were observed.

- Leaf is a dorsiventral leaf
- Single layer of epidermal cells covered externally within the cuticle. The epidermal cells (both upper and lower epidermis)shows the presence of large number of trichomes.
- There are 1-2 rows of palisade cells under upper epidermis which are not continuous over midrib
- Trichomes – unicellular to bicellular and sometimes multicellular covering trichomes are seen. Numerous bicellular trichomes with bulging basal cells and a dagger shaped upper cell is the significant chamber of Vitex negundo plant leaf..

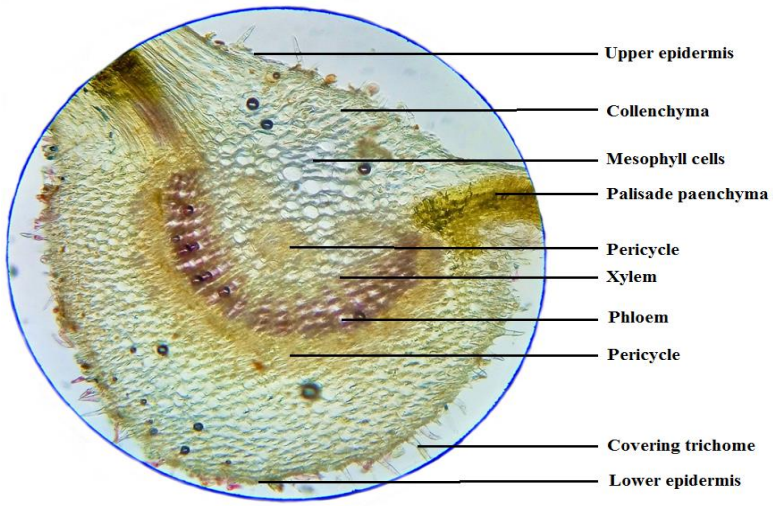

Fig no. 02: T.S of *vitex negundo*

The microscopy of *Vitex negundo* showed basic leaf tissues with specific characteristic features such as bicellular covering trichome, horse shoe shaped meristele region and gutter shaped stellar region followed by oval ring of pericyclic fibres.

3.3 Microscopical studies of dry powder.

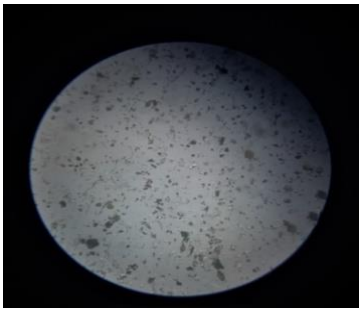

Fig no.03: Starch Grains

Fig no. 04: Fibre

3.4 Determination of foreign matters

The amount of foreign matters found in *Vitex negundo* is 2%.

3.5 Phytochemical screening

Sl.No.	Components	Benzene	Ethyl acetate	Ethanol	Aqueous
1.	**Carbohydrates** 1. Molisch's test 2. Fehling's test 3. Benedicts test 4. Barfoed's test a) Non reducing sugars- i. Fehling's test ii. Benedicts test b) Gum- i. Fehling's test	+ + + +	+ + +	+ +
2.	**Amino acid** 1. Cysteine		+ +		+
3.	**Steroid** 1. Libermann reaction	+			
4.	**Alkaloid** 1. Dragondroff's test		+		

5.	**Glycoside** 1. Saponin glycoside				+

Tableno.05: Phytochemical screening tests

3.6 Thin layer chromatography of different extracts

Sl.No:	**Extracts**	**Solvent system**	**No. of spots**	**Color of spot**	**Rf value**
1	Benzene	Toluene: acetone (9:1)	2	Yellow Dark green	0.37 0.56
2	Ethylacetate	Toluene: ethyl acetate (9:1)	2	Yellow Light green	0.9 1
3	Methanol	Toluene:ethyl acetate (1:1)	3	Orange Yellow Bluish green	0.8 0.86 0.87
4	Ethanol	Toluene : ethyl acetate (1:1)	3	Orange Dark yellow Greenish blue	0.84 0.81 0.83
5	Water	Chloroform : methanol (4:1)	4	Brown Dark brown Brown Brown brown	0.2 0.21 0.18 0.19

Table no.06: Thin layer chromatography of extracts

3.7 Ash values

Sl.No.	**Ash value**	**Average**
1.	Total ash	8.2 ± 0.0014
2.	Acid insoluble ash	0.87 ± 0.0021
3.	Water soluble ash	5.62 ± 0.0010

Table no. 07: Ash values

3.8 Extractive values

Sl.No.	Extractive value	Average
1.	Alcohol soluble extract	16.87± 0.005771
2.	Water soluble extract	28.76± 0.055472
3.	Ether soluble extract	12.81 ± 0.042574

Table no. 08: Extractive values

3.9 Loss on drying

The percentage loss on drying of the powder drug was found to be 10%

4. DISCUSSION

4.1 Macroscopic evaluation

The leaves of *Vitex negundo* were observed to be fresh young leaves ,simple green with characterstic odour and bitter taste.It has a leathery texture ,lanceolate shape, parallel venation

4.2 Powder microscopy

The fine powder was mounted in glycerin and stained with Iodine ,Phlorolgucinol and concentrated HCL. The powder showed the presence of starch grains and fibre.

4.3 Phytochemical screening

The results of phytochemical screening of different extracts of leaves mainly reveal the presence of carbohydrate, amino acids, alkaloids, steroids, glycosides, in appreciable ,moderate and trace amount.

4.4 Thin layer chromatography

The resolution of different kind of chemical components was separated using TLC. Rf value was calculated to determine its identity, purity and strength. Among all chloroform: methanol (4:1) was found to be suitable mobile phase for aqueous extract and it revealed 4 spots.

4.5 Standardization

Physico-chemical parameters of the leaves of *Vitex negundo* are tabulated in table no.7 and 8. Deterioration time of the plant material depends upon the amount of water present in plant material.If the water content is high ,the plant can be easily deteriorated due to fungus.The loss on drying at 105ºC was found to be 10%w/w.

Total ash value of plant material indicated the amount of minerals and earthy material attached to the plant material. Analytical results showed total ash to be 8.2±0.0014, acid insoluble ash was found to be 0.87±0.0021 and water soluble ash to be 5.62±0.0010.The water soluble extractive value was indicating the presence of sugar, acids and inorganic compound. The water soluble extractive value in the drug sample was 28.76±0.05547,alcohol soluble extractive value was 16.87±0.005771.The alcohol soluble extractive value indicated the presence of polar constituents like phenols, alkaloids ,steroids, glycosides and secondary metabolite present in plant sample. Ether soluble extractive value was found to be 12.81±0.042574.

5. CONCLUSION

The plant *Vitex negundo* possesses numerous biological activities such as anticancer, antimicrobial, antifeedant, anti-inflammatory, anti-hyperpigmentation, hepatopropective, anti-histaminic, analgesic and related activities that have been proved by various experimental studies. It represents a class of herbal drug with very strong conceptual base for its use. The long list of uses of the drug suggests that each and every part of the herb can be used in different ailments which in turn reflect the importance of this herb. Scientifically explored exhaustive reports of the plant, their medicinal properties and active chemical constituents have a role in the management of various human ailments.

A number of pharmaceutical preparation, containing *Vitex negundo* makes it clear that this herb is contributing a lot not only in the field of Ayurveda but also in or modern system of medicine. So, this drug can still be explore a lot for the pharmaceutical purpose.

This study can be considered as the Pharmacopoeia standard and helpful to confirm the genuineness of the single plant drug and which can be used for the preparation of effective ayurvedic formulations in future.

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