ABSTRACT

Vitex negundo Linn. Is a plant of medicinal uses. It is commonly known as the five leaved chaste tree and in Sanskrit it is Nirgundi which is used in the indigenous system of medicine since a long time. The drug has been described in Ayurvedic texts as astringent, acrid, bitter, hot and it is useful in treating colic, oedema, dyspepsia, cough, asthma and eye diseases. One of the ancient uses of V. negundo leaves documented in Ayurveda is to provide mental peace. The tribal people of Northern India have been using various parts of this plant as anti flatulent. The present work highlights the phytochemical evaluation, microscopic studies, thin Layer chromatography (TLC) of the flower, since less literature is documentated on this useful flower. The present study is an attempt to highlight the beneficial effect of the flower, further study in this area is needed.
KEYWORDS: Nirgundi, Medicinal uses, Hepatoprotective, Ayurvedic treatment

INTRODUCTION

Medicinal plants have been a major source of therapeutic agents since ancient times to cure human diseases. The revival of interest in natural drugs started in last decade mainly because of the wide spread belief that green medicine is healthier than synthetic products.\textsuperscript{[1]} The plant is reported to have properties like expectorant, carminative, digestive, anodyne, antiseptic, alternant, antipyretic, diuretic, emmenagogue, depurative, rejuvenating, ophthalmic, vulnerary and used as tonic.\textsuperscript{[2]} \textit{V. negundo} Linn. belongs to family Verbenaceae, it is a large, aromatic shrub with quadrangular, densely whitish tomentose branchlets up to 4.5 m in height, or sometimes a small, slender tree, found throughout the greater part of the India, ascending to an altitude of 1500m. In outer Himalayas bark thin, grey, leaves 3-5 foliolate, leaflets lanceolate, entire or rarely crenate, terminal leaflets 5-10 cm x 1.6 x 3.2 cm, lateral leaflets smaller, all nearly glabrous above, white tomentose beneath, flowers bluish purple, small in peduncled cymes, forming large, terminal, often compound pyramidal panicles, drupes globose, black when ripe, 5-6 mm in diameter, invested at the base with enlarged calyx.\textsuperscript{[3]} According to Ayurvedic pharmacopoeia it has been reported that there are several varieties viz. śveta (whitish), nīla (bluish), ānūpa (aquatic), katurunika and (wild type) walnika.\textsuperscript{[4]}

2. REGIONAL NAMES \textsuperscript{[3]}


3. DISTRIBUTION AND DESCRIPTION

Found throughout India, fairly common in waste lands, on road side on the banks of streams or in moist place near deciduous forests.\textsuperscript{[5]} In addition to that in Siddhauadha nighantu mentions helanika (whitish) and nil nika (bluish) varieties.\textsuperscript{[4]} \textbf{Flowering time:} The plant is in flower during the greater part of the year but chiefly from March to May.\textsuperscript{[6]} \textbf{Bracteates:} bracts; 1.4 to 2.5 mm, long, lanceolate and randucous bisexual zygomorphic, bluish purple (lavender to blue). \textbf{Calyx:} small dark purple or violet to whitish or grey, 2 to 3 mm, long, gamosepalous campanulate (ringent Roxb.) shortly five lobed, with the lobes or teeth
triangular less than or about 1 mm long tomentose and valvate in bud, persistent. **Corolla:** small 8mm to 1cm long, gamopetalous, bluish purple, irregular two lipped tomentose outside hairy inside at the insertion of the stamens, upper lip about 2 mm long divided to the base into two obtuse lobes, lower lip large 4 to 5 mm long with the two lateral lobe short oblong and obtuse and the middle lobe large the longest (5mm) broadly obovate and crenulate. **Stamens:** Four, didynamous epipetalous usually exserted **Filaments:** hairy at the base anther cells at first parallel and pendulous later divaricate and often twisting so that the lower ends appear erect **Pistil:** bicarpellary, myocarpous **Ovary:** superior, globular, or oval small glabrous 2 or 4 called with four ovules on axile placenta. **Style:** filiform 7 to 9 mm long ending in a bifid or forked stigma. It is often cultivated in garden as a hedge plant.

4. **AYURVEDIC PROPERTIES OF V. NEGUNDO FLOWER AND USES**

According to Kaiyadeva Nighantu it is pungent, bitter in taste, hot in potency, catabolic in nature. Flowers are used in worm infestation, diseases of vata and kapha, abdominal tumors, spleen disorders, tastelessness, skin diseases, pruritis, inflammatory swelling. It is used in the treatment of jaundice in liver disorders. Ghee cooked with juice of nirgundi leaves alleviates cough caused by kapha. Decoction of nirgundi, guduci, haritaki and marica in equal parts mixed with salt alleviates cough and asthma. By taking cow ghee for three days and nirgundi juice for another three days severe guinea worms are destroyed.

In Gandamala (cervical adenitis) one should use Nirgundi root pounded with water as snuff as nirgundi taila. Oil cooked with nirgundi juice added with rock salt, soot, and jaggery and honey checks purulent discharge from ear by filling. By taking castor oil and nirgundi juice separately lumbago is removed and the patient feels happy. Intake of warm decoction of nirgundi, lasuna and sunthi added with pippali powder destroys all disorders caused by kapha and vata in puerperal stage. The tribal of Northern India have been using various parts of this plant as anti flatulent. One of the ancient uses of *V. negundo* documented in Ayurveda is to provide mental peace.

5. **MEDICINAL IMPORTANCE OF THE FLOWER**

The flowers are astringent, used in fever, diarrhea and liver complaints. The flowers are used in cholera, fever, haemorrhages, hepatopathy and cardiac disorders. Flowers are used in pneumonia and folks of Rajouri used it in treatment of dysentery. Tribes of Garhwal used these flowers in the treatment of skin diseases, eczema, leprosy, syphilis. The plant is recommended for the treatment of the snake bite and scorpion sting. Phytochemical
constituents are essential oil of fresh leaves, flowers and dried fruits are δ-guaiene; guaia-3,7-dienecaryophyllene epoxide; ethyl-hexadecenoate; α-selinene; germacren-4-ol; caryophyllene epoxide; (E)-nerolidol; β-selinene; α-cedrene; germacrene D; hexadecanoic acid; p-cymene and valencene. [14]

6. PHARMACOLOGICAL STUDIES

Previous studies, shows that histo morphological effect of V. negundo extracts in rats, it was found that the stomach tissue were unaffected even by toxic doses while dose dependent changes were observed in the heart, liver and lung tissues. [8]

7. MATERIALS AND METHOD

Flower of V. negundo were collected and authenticated from Survey of Medicinal Plants Unit, Regional Ayurveda Research Institute for Metabolic Disorders, Bangalore. Flower were shade dried, pulverized by mechanical grinder, passed through 40 mesh sieves and stored in a closed tight container, to carry out powder microscopic studies, preliminary phytochemical analysis using Standard methods and results were recorded. Photomicrographs were captured with Catcam image analyzer. [15]

8. POWDER MICROSCOPY: The dried and powdered flower material was treated with chloral hydrate and stained with safranin to study the different fragments of tissues and observations were done through image analyzer (Plate 1). [16, 18]

9. PHYTOCHEMICAL ANALYSIS: The powdered drugs were extracted with different solvents and tested for various phytoconstituents. They are generally tested for the presence of alkaloids, flavonoids, tannins, phenols, steroids and saponins by using standard procedures and were recorded. [15]

10. THIN LAYER CHROMATOGRAPHY (TLC): The dried flower powder of V. negundo was extracted with petroleum ether (60-80°C), chloroform and ethanol by using soxhlet extraction apparatus. TLC studies of these extracts were carried out by using commercially available precoated plates with standardized adsorption layers, i.e. Silica gel 60 F254, (Merck, Germany) at room temperature as per the standard procedures . [19]

11. RESULTS

11.1 Powder is violet black in color, rough to touch, odorless, taste slightly bitter.

Powder Microscopy of V. negundo flower (Plate 1)
Fig 1: Different fragments of tissues
Fig 2: Epidermal cells in surface view
Fig 3: Single and group of fibers
Fig 4: Helical xylem vessels and xylem strands
Fig 5: Multicellular trichomes (Uni, bi and tri) cellular trichomes
Fig 6: Oil globules (10X x 10X)
Fig 7: Orange tannin content (10X x 10X)
Fig 8: Parenchyma cells (10X x 10X)
Fig 9: Trichomes and oil globules (10X x 10X)
Fig 10: Pollen grains and oil globules (10X x 10X)
Fig 11: Pollen grains and prism crystals (10X x 10X)
Fig 12: Pollen grains and trichomes (10X x 10X)
Fig 13: Pollen grains (10X x 10X)
Fig 14: Pollen sac (10X x 10X)
Fig 15: Stone cells (10X x 10X)
Fig 16: Prism shaped crystals (10X x 10X)
Fig 17: Rosette crystal (10X x 40X)
Fig 18: Starch grains and pollen grains (10X x 10X)

Plate 1: Powder microscopy of V. negundo flower

<p>| Fig 1: 10X x 10X  different fragments of tissue | Fig 2: 10X x 40X  Epidermal cells |
| Fig 3: 10X x 10X  Fibers | Fig 4: 10X x 10X  Helical xylem vessels in group |</p>
<table>
<thead>
<tr>
<th>Fig 5: 10X x 10X Multicellular trichomes</th>
<th>Fig 6: 10X x 10X Oil globules</th>
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<tr>
<td>Fig 7: 10X x 10X Orange tannin content</td>
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<td>Fig 9: 10X x 10X Trichomes and oil globules</td>
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</tr>
<tr>
<td>Fig 11: 10X x 40X Pollen grains and prism crystals</td>
<td>Fig 12: 10X x 10X Pollen grains and trichomes</td>
</tr>
</tbody>
</table>
### 11.2 Diagnostic Characters

- Presence of abundant Pollen grains.
- Presence of abundant Uni, bi and tricellular trichomes.
- Presence of helical xylem vessels and xylem strands in group
- Presence of single and groups of tracheids.
- Presence of rosette and prismatic crystals.
• Presence of orange tannin content and oil globules.
• Presence of Pollen grains in pollen sac.
• Presence of minute starch grains.

11.3 Phytochemical analysis: The phytochemical parameters in different solvents were tested for the presence of various phytoconstituents such as tannins, resins, carbohydrates, saponins, glycosides, volatile oils, flavonoid and found to be present and phenols, steroids were found to be absent by standard procedures and were recorded in (Table 1).

Table 1: Phytochemical analysis of V. negundo L.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Natural product group</th>
<th>Test for natural products</th>
<th>Presence(+) / Absence (-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>(a) Dragendorff’s test</td>
<td>_</td>
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<tr>
<td></td>
<td></td>
<td>(b) Hager’s test</td>
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<td>(c) Mayers’s test</td>
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<td></td>
<td></td>
<td>(d) Wagner’s test</td>
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<tr>
<td>2</td>
<td>Carbohydrates</td>
<td>(a) Anthrone test</td>
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<td></td>
<td></td>
<td>(b) Benedict’s test</td>
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<td>(c) Fehling’s test</td>
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<tr>
<td></td>
<td></td>
<td>(d) Molisch’s test</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Flavonoids</td>
<td>Shinoda test</td>
<td>++</td>
</tr>
<tr>
<td>4</td>
<td>Phenols</td>
<td>(a) Ferric chloride test</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(b) Lead acetate test</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Saponins</td>
<td>Foam test</td>
<td>++</td>
</tr>
<tr>
<td>6</td>
<td>Steroids</td>
<td>Liebermann Burchard test</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Tannins</td>
<td>Ferric chloride test</td>
<td>++</td>
</tr>
<tr>
<td>8</td>
<td>Glycosides</td>
<td>----</td>
<td>++</td>
</tr>
<tr>
<td>9</td>
<td>Volatile oil</td>
<td>----</td>
<td>++</td>
</tr>
<tr>
<td>10</td>
<td>Resins</td>
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</tbody>
</table>

11.4 Thin Layer Chromatography: TLC was carried out for three different solvent extracts that were Petroleum ether extract (PE) (60-80°C), chloroform extract and alcoholic extract. The mobile phase used in the preparation was saturated for 30 min and carried for TLC. Mobile phase used is toluene: ethyl acetate: formic acid in the ratio of (6: 3:1). After developing, plates were dried under room temperature for 5 to 10 minutes and sprayed with spraying reagents Anisaldehyde sulphuric acid and were observed under UV-254 and UV-366. Photographs were taken and the RF values were recorded in (Plate 2).
Under 244 nm: Petroleum ether (4 spots): 0.32, 0.38, 0.52, 0.68; Chloroform (7 spots): 0.13, 0.26, 0.30, 0.63, 0.68, 0.78, 0.89; Alcoholic extract (6 spots): 0.17, 0.42, 0.65, 0.71, 0.82, 0.91

Under 366 nm: Petroleum ether (3 spots): 0.20, 0.34, 0.82; Chloroform (6 spots): 0.13, 0.16, 0.21, 0.65, 0.67, 0.75; Alcoholic (6 spots): 0.31, 0.47, 0.61, 0.65, 0.80, 0.86

After spraying: Petroleum ether extract (7 spots): 0.50, 0.57, 0.61, 0.68, 0.75, 0.82, 0.89; Chloroform extract (2 spots): 0.73, 0.78; Alcoholic extract (4 spots): 0.39, 0.64, 0.68, 0.86

Plate 2: TLC Fingerprint of V. negundo flower

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>PE ext; Chloroform ext. and alcoholic ext. (Under 254 nm)</td>
<td>PE ext; Chloroform ext. and alcoholic ext. (Under 366 nm)</td>
<td>PE ext; Chloroform ext. and alcoholic ext. after spraying with Anisaldehyde Sulphuric acid solution</td>
</tr>
</tbody>
</table>

CONCLUSION

Above discussion on V. negundo L. flower conclude that this herb is having multiple uses which help in the treatment of various ailments. In Ayurveda and Unani systems of medicine, this herb is used in pharmaceutical industry for its various properties. Previous literature is mainly focused on its leaves, root and stem not on flower. This attempt will be beneficial for many people. Further research on this area can be taken and exploded.

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REFERENCES


