**Review Article**

Investigation on different parts of *Vitex negundo* for antipyretic and astringent activity in albino rats

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**Abstract**

**Objective:** The present pharmacological investigation was undertaken to study the effect of roots, fruits and flower of nirgundi plant on antipyretic and astringent activity by albino rat. **Materials and methods:** The pharmacognostic study of crude plant materials used and were performed and determined extractive values, ash values, loss on drying. The ethanolic extracts were prepared and phytochemical tests performed. The antipyretic and astringent activity of roots, fruits and flower of nirgundi was assessed by using in-vivo albino rat model. **Results:** The extractive values of *Vitex negundo* different parts used and were determined and found to be roots 12.40, fruits 15.80, and 10.71%. Phytochemical tests were performed and found to be ash values of roots3.27, fruits4.80 and 1.16% respectively. Extractive value ethanol soluble of extract values of *Vitex negundo* was discovered to be % that are Root extract values of *Vitex negundo* was 13.01, 14.30 and flower 10.06. Loss on Drying values of *Vitex negundo* was discovered to be % that are Root extract values of *Vitex negundo* was 2.01, 4.35 and flower 1.60. Phytochemical Screening tests were performed by using different parts of *Vitex negundo* ethanolic extract such roots, fruits and flowers and was found to be roots of plants had good phytocompounds. Astringent Property for different parts of *Vitex negundo* extracts (VNEE) was found that, VNEE contains carbohydrates, alkaloids and flavonoids. On the other hand, it has astringent and antipyretic properties. **Conclusion:** The evaluation of results confirmed that potential bioactive molecules of plant materials responsible for the antipyretic and astringent activity. The results suggest that plant extract can be further developed as nutritional supplements, preventive medicines, and topical products for cosmetic purposes.

**Keywords:** *Vitex negundo*, pharmacognostic study, Phytochemical tests, antipyretic and astringent activity

**Introduction**

One of the main sources of medications is thought to be plants (Thomson, 2010). Alkaloids, glycosides, tannins, and other phytochemical components with a bioactive nature aid in demonstrating this therapeutic activity (Edeoga et al., 2005). It has been demonstrated that oxidation reactions brought on by too many free radicals induce tumour formation, DNA, mRNA, protein, and enzyme damage; rapid ageing; Parkinson's and Alzheimer's illnesses; and rheumatic and pulmonary disorders (Baker et al., 2000). Therefore, it is essential to systematically check medicinal herbs for antioxidative activity. There is rising interest in plant-derived chemicals' antioxidative properties, which may be significant in regard to their nutritional prevalence and their involvement in health and disease. Natural antioxidants could be found in plants. The term "natural antioxidants" or "phytochemical antioxidants" refers to plant secondary metabolites. Antioxidants made by plants for survival include carotenoids, flavonoids, cinnamic and benzoic acids, folic and ascorbic acids, tocopherols and tocotrienols, among others. The most common antioxidants are alpha tocopherol, ascorbic acid, and beta-carotene. The antioxidant properties of plant extracts must be assessed by combining two or more distinct in-vitro assays due to the complexity of phytochemicals. A frequent condition known as cerebral venous sinus thrombosis (CVST) is associated with high rates of morbidity and mortality (Watson et al., 2002). Because of its effectiveness, safety, and practicability, heparin, an anticoagulating drug, is the first line of treatment for CVST (Biousse and Newman, 2004). In the treatment of individuals with CVST, thrombolytic medications such as tissue plasminogen activator (t-PA), urokinase, streptokinase, etc. are essential (Baruah et al., 1985).

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2455-2674/Copyright © 2023, N.S. Memorial Scientific Research and Education Society. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
Astringents are substances used internally to lessen the discharge of blood serum and mucous secretions in medicine. They promote constriction or contraction of mucous membranes and exposed tissues. With a sore throat, haemorrhages, diarrhoea, and peptic ulcers, this is possible. Astringents are used externally dry, stiffen, and protect the skin and may be the cause of a slight coagulation of skin proteins. Astringents are frequently used to treat acne in people with oily skin. Mild astringents soothe minor skin problems and irritations, including those brought on by superficial wounds, allergies, insect bites, anal haemorrhoids, and fungi like athlete’s foot. Procoagulants are substances that tend to enhance coagulation or are a precursor to a substance that is naturally produced to coagulate blood. The process through which blood transforms from a liquid to a gel and forms a blood clot is known as coagulation, often known as clotting. Hemostasis, the stoppage of blood loss from a damaged vessel, is aided by it, and then the vessel is repaired (Singh et al., 1999). Thrombin production during secondary hemostasis is significantly regulated by procoagulant and anticoagulant responses. The perennial plant known as Tagetes lucida is typically found in Mexico and Central America. It is both a culinary herb and has therapeutic qualities. The plant is thought to be abundant in phytochemical components and has therapeutic capabilities. This study’s objective is to identify the phytochemical components and their biological activities.

Vitex negundo Linn., belonging to family Verbenaceae (which comprises 75 genera and nearly 2500 species), commonly known as simali in Nepali, and also called Five leaved chaste tree (Eng), Nirgandi (Hindi), Nirgundi (Sanskrit), is a deciduous shrub, occur in tropical to temperate regions (up to 2200 m from east to west) grows gregariously in wastelands and is also widely used as a hedge-plant. The reported chemical examination of Vitex negundo showed the Presence carbohydrates, carotene, fat, protein, phytin, amino acid, alanine, arginine, cystine, glutamic acid, glycine, hydroxyproline, leucine, serine, tryptophan, piperolic acid. And also presence of alkaloids, carotenoids and terpenoid, flavonoids, tannins, luflugfania, sapogenin, oleanolic acid, cucurbitatin B, E and antraithquinones. Leaves are a healthy food and contains good amount of fiber, types of vitamins such as Vitamin B2, Vitamin C, Calcium, phosphorus, iron and small quantities of iodine and fluorine. Seeds show presence of saturated and unsaturated fatty acid palmatic, stearic, oleic, linoleic and traces of lignoceric acid. Plant shows presence of oleanane type triterpene saponins- acutoside A, B, C, D, E, F, and G. Vitex negundo, commonly known as the “Five-Leaved Chaste Tree” or “Nirgundi,” is a medicinal plant that has been traditionally used in various cultures for its therapeutic properties. Native to Southeast Asia and parts of Africa, this plant has a long history of traditional use in Ayurvedic, Chinese, and folk medicine systems. Its leaves, bark, seeds, and roots have been utilized for treating a wide range of ailments. In this note, we will explore the traditional uses of Vitex negundo and the conditions for which it has been employed (Singh et al., 1999; Gupta et al., 1999). The present pharmacological investigation was undertaken to study the effect of roots, fruits and flower of nirgundi plant on antipyretic and astringent activity by albino rat.

Materials and methods

Collection and preparation of the plant material

The root, flower and fruits of Vitex negundo were collected from our college, herbal garden. These parts of plants were washed with water and were air dried for several days’ separately and then oven dried for 24 hours at considerably low temperature (not more than 40°C) for better grinding. The dried parts of plant materials were then ground to a coarse powder using high capacity grinding machine.

Preparation of plant extract

The plant parts were washed with clean water and subject to shade drying after which they were pulverized using an electric blender to obtain a fine powder. The finely powdered plant material was then extracted with suitable solvent using a soxhlet extractor. The extract was dried using a vacuum distillation to obtain a semi solid extract which was used for further analysis (Sumanta et al., 2009; Dubey et al., 2021; Shukla et al., 2018).

Phytochemical Parameters

Determination of Loss on Drying

To determine the loss on drying, 5-6 grams of powder were precisely weighed and placed into a tared vanishing dish. The sample was then dehydrated for 4 hours at 110°C. After cooling, the sample was dehydrated and weighed at hourly intervals until a constant weight was obtained. The calculation of loss on drying was based on the moisture content present in the sample and was determined using the following formula:

\[ \text{Loss on Drying} = \frac{\text{weight of powder after drying in crucible}}{\text{Initial weight of the powder in g}} \times 100 \]

Ash Values determination

Total ash

To determine the total ash value, 2 grams of pulverized air-dried powder were accurately weighed and placed in a lighted crucible (typically platinum or silica) in an even layer. The crucible was then gradually heated up to 600°C until it turned white, indicating the absence of carbon. The material was cooled in a desiccator and weighed. If the ash was not in the sample, the sample was re-weighed and the process was repeated until no further weight loss occurred. The total ash value was calculated using the following formula:

\[ \text{Total ash} = \frac{\text{weight of ash}}{\text{weight of powder used}} \times 100 \]
contained carbon, it was not considered for further analysis. In such cases, the crucible was cooled and the deposit was moistened with 2 ml of water or ammonium nitrate solution. It was dried on a water bath and burned again to constant mass. The residue was allowed to cool in a desiccator for 35 minutes and weighed again. The total ash value was calculated as a percentage of the weight of the dried material using the following formula:

\[
\text{Total ash value} = \frac{\text{weight of empty crucible}}{\text{weight of drug taken}} \times 100
\]

**Acid insoluble ash**

In a container, the total ash was mixed with 25 mL of hydrochloric acid (HCl) and covered with a glass plate. The crucible was gently immersed in a water bath for 5-7 minutes. After that, the glass plate was rinsed with 5 mL of warm water and the resulting solution was poured into the container. The insoluble material was collected on an ash-less mesh and washed with warm water until the residue became neutral. The insoluble matter was then transferred to a new crucible from the filter paper. The crucible containing the material was placed on a hot plate and burnt until a constant weight was obtained. The excess was allowed to cool in a desiccator for 30 minutes and then weighed immediately. The acid-insoluble ash was calculated as a percentage with respect to the dehydrated plant material.

**Water soluble ash**

25 mL of purified water was added to a silica crucible containing total ash and heated for 5 minutes. The insoluble matter was then transferred to a sintered glass crucible and washed with hot water. The remaining insoluble matter was then moved to a new crucible and heated at 450°C for 15 minutes. The excess was allowed to cool for 30-40 minutes in a desiccator, and then weighed immediately. The water-soluble ash was calculated as a percentage with respect to the dehydrated plant material.

**Alcohol extractive value**

Approximately 5.0 g of coarsely powdered air-dried material was accurately weighed and transferred into a conical flask with a stopper. The powder was macerated with 100 mL of ethanol for 6 h with occasional shaking. After 18 h, the mixture was filtered quickly, and care was taken to avoid the loss of any solvent. Then, 25 mL of the filtrate was transferred to a flat-bottomed tarred dish and evaporated to dryness. The resulting extract was dehydrated for 6 h at 105°C, cooled in a desiccator for 30 min, and weighed immediately. The percentage of the extractive in terms of the air-dried powdered medicinal material was determined.

**Water extractive value**

Approximately 5.0 g of the drug substance was mixed with 100 mL of chloroform and allowed to macerate for 24 hours in a closed flask, with intermittent shaking for the first 6 hours, followed by an additional 18-hour rest. The resulting solution was rapidly filtered, and 25 mL of the filtrate was evaporated to dryness in a tared flat-bottomed dish, dehydrated at 105°C, and weighed. The percentage of the water-soluble extractives with respect to the dehydrated powder of the drug material was then calculated.

**Qualitative Phytochemical Analysis**

The dried ethanolic extracts were subjected to various color reactions to identify the nature of the phytoconstituents (Garg et al., 2015; Pandey et al., 2015; Yadav et al., 2020; Shukla et al., 2020).

**Test for Alkaloids**

a. Hager's test: Extract was treated with a few drops of Hager's reagent (saturated solution of picric acid) – formation of yellow precipitate indicates the presence of alkaloids.

b. Mayer's test: Extract was treated with Mayer's reagent (potassium mercuric iodide solution) – formation of cream precipitate shows the presence of alkaloids.

c. Dragendorff's test: Extract was treated with Dragendorff's reagent (potassium bismuth iodide solution) – orange precipitate shows the presence of alkaloids.


**Test for Steroids**

a. Liebermann – Burchard test: 10 mg of extract was dissolved in 1 ml of chloroform. 1 ml of acetic anhydride was added following the addition of 2 ml of concentrated sulphuric acid. Formation of reddish violet color precipitate at the junction indicates the presence of steroids.

b. Salkowski test: 1 ml of concentrated sulphuric acid was added to 10 mg of extract dissolved in 1 ml chloroform. A reddish brown layer exhibited by chloroform layer and green fluorescence by the acid layer suggests the presence of steroids.

c. Magnesium ribbon test: Few fragments of magnesium
ribbon were added and concentrated hydrochloric acid was added along the sides of the test tube. Magenta color formation indicates the presence of flavonoids.

d. Zinc-hydrochloride test: To the extract, a pinch of zinc dust was added followed by addition of concentrated hydrochloric acid along the sides of the tube. Magenta color formation indicates the presence of flavonoids.

**Test for Saponins**

a. 1 ml of extract was diluted with 10 ml of distilled water and shaken in a graduated cylinder for 15 min. Formation of 1 cm layer of foam indicates the presence of saponins.

b. Haemolysis test: 2 ml of 1.8% sodium chloride solution was taken in two test tubes. To one test tube 2 ml of distilled water was added and to another tube 2 ml of extract was added. Blood was obtained by pricking the thumb and 5 drops of blood was added to each tube. The contents were gently mixed and observed under the microscope. Haemolysis indicates the presence of saponins.

c. Legal test: Extract was dissolved in pyridine. Sodium nitroprusside solution was added to it. Pink red or red color produced indicates presence of glycosides and sugars.

d. Barfoed's Test: Extract was treated with Barfoed's reagent (copper acetate in water and glacial acetic acid). Appearance of red color is a positive test for presence of glycosides and sugars.

e. Borntrager's test: Few ml of dilute sulphuric acid was added to the test solution. It was then boiled, filtered and the filtrate was extracted with ether or chloroform. The organic layer was then separated and ammonia was added. Pink, red or violet color was produced in the organic layer to indicate the presence of glycosides and sugars.

**Test for Phenolic Compounds & Tannins**

a. Ferric chloride: test 5 ml of extract was allowed to react with 1 ml of 5% Ferric chloride solution. Bluish black coloration indicated the presence of phenolic compounds and Tannins.

b. Gelatin test: When extract was treated with gelatin solution, white precipitate formed indicates the presence of phenolic compounds and Tannins.

c. Lead acetate test: 5 ml of extract was treated with 1 ml of 10% lead acetate solution in water. Yellow color precipitate shows the presence of phenolic compounds and Tannins.

**In vivo study**

**Animals**

Swiss albino mice (25–30 g) and albino wistar rats (130-170g) were used for the experiments. All animals were housed at room temperature (20–25°C) and constant humidity (40–70%) under a 12 h light–dark cycle in SPF grade laboratory at the animal house at VIT university after obtaining the necessary ethical clearance (clearance number: 1333/c/10/CPCSEA). The animal study was performed according to the international rules considering animal experiments and the internationally accepted ethical principles for laboratory animal use and care. Standard protocols were used to evaluate the pharmacological activities of phytochemicals in-vivo.

**Anti-pyretic activity**

The anti-pyretic activity of three plant ethanolic extracts such as root, fruits and flower were done by using digital thermometer. The albino rats used were of female gender and were approved from the research ethical committee. Experimental animals were divided into following groups i.e., negative control (1% of tween-80), positive control precipitate confirmed the presence of glycosides and sugars.

d. Barfoed's Test: Extract was treated with Barfoed's reagent (copper acetate in water and glacial acetic acid). Appearance of red color is a positive test for presence of glycosides and sugars.

e. Legal test: Extract was dissolved in pyridine. Sodium nitroprusside solution was added to it. Pink red or red color produced indicates presence of glycosides and sugars.

f. Borntrager's test: Few ml of dilute sulphuric acid was added to the test solution. It was then boiled, filtered and the filtrate was extracted with ether or chloroform. The organic layer was then separated and ammonia was added. Pink, red or violet color was produced in the organic layer to indicate the presence of glycosides and sugars.

**Test for Proteins and Amino Acids**

a. Millon's test: Extract was treated with Millon's reagent (mercuric nitrate in nitric acid). Red color formation indicates the presence of proteins and amino acids.

b. Biuret test: Extract was treated with sodium hydroxide and copper sulphate solution drop wise. Violet color shows the presence of proteins and amino acids.

c. Ninhydrin test: Extract treated with Ninhydrin reagent, ammonia and heated. Violet color is formed indicating the presence of proteins and amino acids.

d. Barfoed's Test: Extract was treated with Barfoed's reagent (copper acetate in water and glacial acetic acid). Appearance of red color is a positive test for presence of glycosides and sugars.

e. Legal test: Extract was dissolved in pyridine. Sodium nitroprusside solution was added to it. Pink red or red color produced indicates presence of glycosides and sugars.

f. Borntrager's test: Few ml of dilute sulphuric acid was added to the test solution. It was then boiled, filtered and the filtrate was extracted with ether or chloroform. The organic layer was then separated and ammonia was added. Pink, red or violet color was produced in the organic layer to indicate the presence of glycosides and sugars.

**Test for Glycosides and Sugars**

a. Molisch's Test: 2 ml of concentrated sulphuric acid was added to 2 ml of extract solution. Then it was treated with Molisch's reagent with 15% ethanolic α-naphthol. Formation of a reddish violet ring indicates the presence of glycosides and sugars.

b. Fehling's Test: 5 ml of extract solution was mixed with 5 ml of Fehling's solution and boiled for 5 min. Formation of brick red precipitate demonstrated the presence of glycosides and sugars. (Fehling's solution A: 34.64 g of copper sulphate solution was dissolved in a mixture of 0.5 ml of sulphuric acid and sufficient water to produce 500 ml. Fehling's solution B: 176 g of sodium potassium tartrate and 77 g of sodium hydroxide are dissolved in sufficient water to produce 500 ml. Equal volumes of A and B solution are mixed at time of use)

c. Benedict's Test: - To 5 ml of extract solution, 5 ml of Benedict's solution (1.73 g of cupric sulphate, 1.73 g of sodium citrate and 10 g of anhydrous sodium carbonate were dissolved in water and the volume is made up to 100 ml with water) was added in a test tube and boiled for a few min. Development of brick red precipitate confirmed the presence of glycosides and sugars.

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standard drug), experimental group (extract dose 100mg/kg), experimental groups (extract dose 200mg/kg). After dividing them into the respective groups, they were fasted overnight and were given only access to drinking water. Normal rectal temperature of all rats was checked by using digital thermometer (Kumar et al., 2017; Okiemente Rosa et al., 2021). Pyrexia was induced in all rats by brewer's yeast with an aqueous concentration of 20% orally. After the 24 hours of induction of brewer's yeast, the rectal temperature of rats was recorded after every hour till 5th hour.

Astringent activity of *Vitex negundo* ethanolic extracts (VNEE)

Two Eppendorf tubes were taken and in 1st tube 5% plant extract was added in 1ml methanol. In second tube, 5% extract was added in 1ml distilled water. 100μL of milk was added in each tube and homogenized. After 3 minutes it was centrifuged for 1 minute at 3000 rpm. Presence or absence of pellets was noted (Watson et al., 2002).

Results and discussion

Morphological characters of plant material

The various morphological characters like colour, odour, taste, size, shape, etc. has been studied for all three plant materials i.e roots, fruits and flowers parts of *Vitex negundo*. All the plant parts are greenish in colour with Characteristic odour. The taste of leave as is mint in taste (Table 1).

**Extractive Values**

For ethanol solutions, the extractive values of the plant were assessed. All the compiled results are shown in table 2.

**Phytochemical parameters**

**Ash Values**

Water soluble ash of values of *Vitex negundo* was discovered %all the compiled results are shown in table 3.

**Water soluble extractive Values**

Water soluble extractive value ethanol soluble of Leaves extract of *Vitex negundo* was discovered to be % that are shown below under

**Loss on Drying**

The loss on drying of extract of *Vitex negundo* was discovered all the compiled results are shown in table 4.

**Phytochemical Screening**

Plant material concentrates have shown that saponins,

<table>
<thead>
<tr>
<th>S. No</th>
<th>Parameters</th>
<th>Parts of Root</th>
<th>Parts of Fruit</th>
<th>Parts of Flower</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Colour</td>
<td>Brownish</td>
<td>Slight brown</td>
<td>Greenish</td>
</tr>
<tr>
<td>2</td>
<td>Odour</td>
<td>Characteristics</td>
<td>Characteristics</td>
<td>Characteristics</td>
</tr>
<tr>
<td>3</td>
<td>Taste</td>
<td>Characteristics</td>
<td>Characteristics</td>
<td>Characteristics</td>
</tr>
<tr>
<td>4</td>
<td>Size of leaves</td>
<td>5-10.1cm</td>
<td>4mm</td>
<td>8-17 cm</td>
</tr>
<tr>
<td>5</td>
<td>Shape</td>
<td>Broadly</td>
<td>Broadly</td>
<td>Broadly</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Name of The Plant Yield</th>
<th>Yield % w/w</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>root extract of <em>Vitex negundo</em> (VNERE)</td>
<td>12.40</td>
</tr>
<tr>
<td>2</td>
<td>fruit extract of <em>Vitex negundo</em> (VNEFRE)</td>
<td>15.80</td>
</tr>
<tr>
<td>3</td>
<td>flower extract of <em>Vitex negundo</em> (VNEFE)</td>
<td>10.71</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Name of the plant extracts</th>
<th>Ash Values (% w/w)</th>
<th>Soluble extractive (% w/w)</th>
<th>Loss on Drying Values (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Root extract of <em>Vitex negundo</em></td>
<td>3.27</td>
<td>13.01</td>
<td>2.01</td>
</tr>
<tr>
<td>2</td>
<td>Fruit extract of <em>Vitex negundo</em></td>
<td>4.80</td>
<td>14.30</td>
<td>4.35</td>
</tr>
<tr>
<td>3</td>
<td>Flower extract of <em>Vitex negundo</em></td>
<td>1.16</td>
<td>10.06</td>
<td>1.60</td>
</tr>
</tbody>
</table>
tannins, glycosides, and sugars are available. The concentrates not entirely set in stone to be absent any and all proteins. As indicated by this examination, the ethanolic separate has more parts. A preliminary study has reported that the leaves extract contained large number of bioactive secondary molecules like phenols, alkaloids, tannins, glycosides, carbohydrates, flavonoids. The presence of these components in this species is an indication that it may have some medicinal potential. Moreover, the restorative activities of the two unmistakable concentrates might be because of the presence of a few phytoconstituents.

Phytochemical Screening tests were performed by using different parts of *Vitex negundo* ethanolic extract such roots, fruits and flowers and was found to be roots of plants had good phytocompounds.

**Evaluation of anti-pyretic activity for different parts of Vitex negundo extracts (VNEE)**

**Anti-pyretic activity**

Anti-pyretic activity Screening tests were performed by using different parts of *Vitex negundo* ethanolic extract such roots, fruits and flowers and was found to be roots of plants had good phytocompounds and showed significant antipyretic compounds due to presence of isoquinoline alkaloid compound.

**Astringent Property for different parts of Vitex negundo extracts (VNEE)**

After undergoing centrifugation, the resultant outcome was observed, revealing the presence of pellets derived from the extract of VNEE. Consequently, within this experimental context, it was discerned that the fruits of VNEE exhibited better astringent properties than leaves and flower, inducing the contraction of cutaneous cells and other bodily tissues, akin to the effects of witch hazel on pores. Medical astringents are commonly employed to arrest or decelerate bleeding and facilitate wound healing. Illustrative instances encompass yarrow tincture and calamine lotion. Thus, based on the outcomes of this experiment, it can be deduced that the extract obtained from VNEE evinced astringent activity. From the study, it was found that, roots of VNEE contain carbohydrates, alkaloids and flavonoids. On the other hand, it has astringent and antipyretic properties. So, considering the potential bioactivity, this plant can be

### Table 4: Total ash, acid insoluble ash and water soluble ash values of Vitex negundo

<table>
<thead>
<tr>
<th>Crude drugs</th>
<th>Total ash value (% w/w)</th>
<th>Water soluble ash (% w/w)</th>
<th>Acid insoluble ash value (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roots extract of <em>Vitex negundo</em></td>
<td>4.7</td>
<td>11.14</td>
<td>7.32</td>
</tr>
<tr>
<td>Fruits extract of <em>Vitex negundo</em></td>
<td>4.9</td>
<td>12.72</td>
<td>6.71</td>
</tr>
<tr>
<td>Flower extract of <em>Vitex negundo</em></td>
<td>3.1</td>
<td>10.01</td>
<td>4.83</td>
</tr>
</tbody>
</table>

### Table 5. Phytochemical screening for extracts of Vitex negundo

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Chemical Tests</th>
<th>Root</th>
<th>Fruit</th>
<th>Flower</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Steroids and Triterpenoids</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Saponins</td>
<td>+</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>3</td>
<td>Alkaloids</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Glycosides</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Tannins and Phenolic compounds:</td>
<td>++</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>6</td>
<td>Flavonoids</td>
<td>++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>7</td>
<td>Proteins</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Carbohydrates</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+++: Excellent, ++: Good, +: Present

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further studied and evaluated extensively to explore its unexplored efficacy and to rationalize its medicinal use.

**Conclusion**

The extractive values of different parts used and Vitex negundo were determined and found to be roots 12.40, fruits 15.80, and 10.71%. Phytochemical tests were performed and found to be ash values of roots 3.27, fruits 4.80 and 1.16% respectively. Extractive value of extract values of Vitex negundo was discovered to be % that are Root extract values of Vitex negundo was 13.01, 14.30 and flower 10.06. Loss on Drying values of was discovered to be % that are Root extract values of was 2.01, 4.35 and flower 1.60. Phytochemical Screening tests were performed by using different parts of Vitex negundo ethanolic extract such roots, fruits and flowers and was found to be roots of plants had good phytochemicals. Astringent Property for different parts of Vitex negundo extracts (VNEE) was found that, VNEE contains carbohydrates, alkaloids and flavonoids. On the other hand, it has astringent and antipyretic properties. So, considering the potential bioactivity, this plant can be further studied to explore its efficacy and to rationalize its medicinal use.

**Conflict of interest:** Not declared

**References**


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**Table 6.** Anti-pyretic effect of for different parts of Vitex negundo extracts (VNEE)

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Dose (mg/kg)</th>
<th>Rectal Temperature (°C)</th>
<th>Normal</th>
<th>After yeast</th>
<th>1 h</th>
<th>2 h</th>
<th>3 h</th>
<th>4 h</th>
<th>5 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control</td>
<td>10 ml/kg</td>
<td></td>
<td>38.12±0.04</td>
<td>38.12±0.05</td>
<td>38.93±0.15</td>
<td>39.02±0.16</td>
<td>39.11±0.16</td>
<td>39.01±0.17</td>
<td>39.03±0.16</td>
</tr>
<tr>
<td>PRA</td>
<td>150</td>
<td></td>
<td>38.18±0.03</td>
<td>39.15±0.04</td>
<td>38.77±0.18***</td>
<td>38.41±0.21***</td>
<td>37.78±0.12***</td>
<td>37.51±0.10***</td>
<td>37.12±0.07***</td>
</tr>
<tr>
<td>Roots</td>
<td>100</td>
<td></td>
<td>36.20±0.01</td>
<td>38.10±0.12</td>
<td>38.18±0.11**</td>
<td>38.18±0.12**</td>
<td>38.17±0.10**</td>
<td>38.13±0.10**</td>
<td>38.15±0.11**</td>
</tr>
<tr>
<td>Roots</td>
<td>200</td>
<td></td>
<td>37.70±0.08</td>
<td>38.50±0.18</td>
<td>38.62±0.16***</td>
<td>38.61±0.17***</td>
<td>38.61±0.16***</td>
<td>38.56±0.17***</td>
<td>38.50±0.15***</td>
</tr>
<tr>
<td>Fruit</td>
<td>100</td>
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<td>37.12±0.11</td>
<td>38.26±0.17</td>
<td>38.13±0.18**</td>
<td>38.03±0.21**</td>
<td>38.01±0.17**</td>
<td>38.02±0.18**</td>
<td>38.01±0.21**</td>
</tr>
<tr>
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<td>38.22±0.13</td>
<td>37.81±0.11***</td>
<td>37.81±0.11***</td>
<td>37.84±0.08***</td>
<td>37.61±0.11***</td>
<td>37.57±0.10***</td>
</tr>
<tr>
<td>Flower</td>
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<td>38.81±0.21</td>
<td>38.13±0.22***</td>
<td>37.51±0.08***</td>
<td>37.22±0.18***</td>
<td>37.20±0.04***</td>
<td>37.22±0.04***</td>
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<td>37.25±0.15***</td>
<td>37.21±0.15***</td>
<td>37.17±0.15***</td>
</tr>
</tbody>
</table>

Mean±SEM (n=5). *Significant values at P<0.0032. **Significant values at P<0.0021. *** Significant values at P<0.001. PRA: Paracetamol; Root: ethanol extract; Fruit: ethanol extract. Statistical Analysis Data were expressed as mean ±Standard error mean. The statistical significance of differences between groups was determined by analysis of variance (ANOVA) for multiple comparisons among groups.


