

# Evaluation of phytochemical and antimicrobial activities of *Vitex negundo* L. leaf extracts.

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## ABSTRACT

*Traditional medicine in developing countries uses a wide variety of natural products in the treatment of some common infections. Vitex negundo L is a common plant in Indian Medicine belongs to verbenaceae, is a woody, aromatic and growing plant with 2-5 meters in height. In the present study extraction was done with different solvents like Methanol, Acetone, Chloroform, Water and Ethanol. Phytochemical Studies were also carried out with the Methanolic extract. Further antibacterial and antifungal activities were performed on Streptococcus pneumoniae, Staphylococcus aureus, Clostridium, Bacillus subtilis, Pseudomonas aeruginosa, Salmonella Sps, Klebsiella pneumoniae, E.coli, A.niger, Calbicans. Our results of the present study in phytochemical screening revealed the presence of carbohydrates, proteins, amino acids, steroids, glycosides (cardiac, anthraquinone and saponin), flavonoids, tannins and phenolic compounds. The methanolic extract showed strong antimicrobial activity than other solvents.*

**Keywords:** Anti bacterial activity, Anti fungal activity, Flavonoids, Glycosides, Phenolic compounds, Phytochemical studies, Steroids, Vitex negundo.

## INTRODUCTION:

Plants are considered not only as dietary supplement to living organisms but also conventionally used for treating many health problems and the medicinal value of many plants still remains unexplored. Investigations of plants are carried out to find new drugs or templates for the development of new remedial agents [1]. Over 60% of the world human population, 80% in developing countries depends directly on plants for their medicinal purposes [2]. Medicinal plants have a remarkable capacity to produce a wide variety of bioactive secondary metabolites, like alkaloids, terpenoids, glycosides, saponins, flavonoids, steroids, tannins, quinones and coumarins [3]. These biomolecules are the source of plant-derived antimicrobial substances [4]. Some natural products are highly efficient in the treatment of bacterial infections [5]. Which are obtained from a wide variety of natural resources like plant leaves, bark, berries, flowers and roots [6].

The word Vitex is derived from the Latin 'vieo' means to tie or bind, as its stems and twigs have the flexible nature. The Sanskrit word 'Nirgundi' for *V. negundo* means 'that which protects the body from diseases' [7]. In 1753 Linnaeus was established the genus Vitex and included it in the family of Verbenaceae. *V. negundo* is one of the most popular medicinal plants being used in all medicinal systems like Ayurveda, Unani, Siddha, Folklore, Chinese, Tibetan, Homeopathy, and Allopathy. Majority of the traditional medicines used in healthcare are obtained from plants [8]. In this context the *Vitex negundo*, commonly known as the five-leaved chaste tree is a large strongly scented deciduous large shrub with quadrangular branches with various ethno medicinal uses. Leaves are 3-5 foliolate, dark-green above and pale greenish tomentose beneath. Small flowers are bluish purple in color. It is commonly found to be in open-waste lands and deciduous forests near moist situations [9, 10]. It is native to India, Sri Lanka, Pakistan, Afghanistan, Thailand, Malaysia, Philippines, Burma, China and Japan [11, 12, 13, 14, 15]. It is commercially cultivated as a crop in parts of Asia, Europe, North America and the West Indies [16]. All parts of the plant (*Vn*), such as roots, bark, leaves, flowers, seeds and it's essential oil have the medicinal properties. They have been commonly used as a part or whole in medication to treat different ailments. Leaves and roots of the plant are regarded as febrifuge as tonic. Warm leaves are applied in rheumatoid arthritis. In the current investigation carried out, a screening of hydro alcoholic extracts of *Vitex negundo* Linn leaves against pathogenic bacteria and fungi is done in order to detect new sources of antimicrobial agents by extraction of the active molecules.

Phytochemicals or secondary metabolites usually occur in complex mixtures that varies among plant organs and different developmental stages [17, 18]. Phytochemical constituents of medicinal plants have been considered to be a basic requirement in the discovery of potent medicines and remedies on various diseases in Ayurvedic and nutraceutical research. Medicinal properties of any plant are depending

on the presence of phytoconstituents and nutritive elements as well as minerals. Hence, phytochemical screening, elemental and functional group analysis of plant extract is essential to evaluate medicinal properties of the plant. Therefore my aim of the research is to elucidate the phytochemical profile, soluble extractive percentage and in-vitro antimicrobial activity of the *Vitex negundo* leaves in order to identify potential bioactive compounds.

## MATERIALS AND METHODS:

### Plant Material:

The *Vitex negundo* Linn. leaves were collected from Hyderabad and surrounding areas in Telangana state and the leaves were washed to remove external contaminants and shade dried then they powdered by using a electronic blender then it was stored in tightly closed glass containers and kept in the dark at room temperature.

### Extraction and Isolation:

500 gms of shade dried powdered *Vitex negundo* leaves were extracted with Methanol, chloroform, ethanol, acetone and water and concentrated using rotor evaporator. After evaporation of the solvents, concentrated extractions were collected and used for analysis of phytochemical screening and antimicrobial activities.

### Phytochemical Screening:

Phytochemical screening has been done for detection of carbohydrates, proteins, amino acids, steroids, glycosides, flavonoids, alkaloids, tannins and phenolic compounds [19,20].

### Antibacterial Activity:

Muller Hinton Agar was prepared according to the manufacturer's instructions. The medium was sterilized by autoclaving at 121°C for 15 minutes at 15psi pressure and was used to determine the antibacterial activity of pure compounds from *Vitex negundo* Linn. Sterile molten agar (45°C) was poured aseptically into sterile petriplates (15ml each) and the plates were allowed to solidify at room temperature in sterile condition. After solidification and drying, the plates were seeded with appropriate microorganisms by streaking evenly on to the surface of the medium with a sterile spreader. The bacterial strains used were *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Clostridium*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Salmonella Sps*, *Klebsiella pneumoniae*, *E.coli*. Wells (8mm diameter) were made in the agar plates using a sterile stainless steel borer and filled with *Vitex negundo* Linn Methanolic, Ethanolic, Chloroform, Acetone, and Water extracts of 5 different concentrations were filled in respective wells. Ampicillin, Streptomycin and distilled water were used as positive and negative control respectively. Then the plates were incubated at 37°C for 24hrs. After 24hrs, the zones of inhibition were measured with a measuring scale. This experiment was carried out and the results were read by the presence or absence of zone of inhibition.

### Antifungal Activity:

Anti-fungal activity of compounds were tested by well diffusion method with five concentrations of test compound (10, 25, 50, 75 and 100 µg ). The prepared PDA culture plates were streaked with different strains of *Aspergillus Niger* and *Candida albicans* fungi using streak plate method and filled with *Vitex negundo* Linn. Methanolic, Ethanolic, Chloroform, Acetone, Water extracts of 5 different concentrations were filled in respective wells. The plates were incubated at 25°C for 48 hrs. After 48 hrs, the plates were observed for zone formation around the wells and the zone of inhibition (mm) was measured with a measuring scale. The readings were taken and the values were tabulated.

## Results:

### Phytochemical Screening:

The phytochemical screening of five different extracts of *Vitex negundo* L. showed the presence of phytochemicals like carbohydrates, proteins, amino acids, saponins, flavonoids, tannins, anthorquinones and phenolic compounds. The results of present study are helpful for the discovery of potent remedies on various diseases. Methanol extract and Water extract of *Vitex negundo* leaves shows the presence of flavonoids, the present study also useful for preparing flavonoid-based drugs which have been most important in antimicrobial properties.

**Table 1: Presence phytochemicals in different solvent extracts of *Vitex negundo*.**

Secondary Metabolites	Phytochemical Tests	Methanolic Extract	Ethanolic Extract	Chloroform	Acetone	Water
Carbohydrates	Molisch's Test	+	+	+	-	+
Protein	Millon's Test	+	+	-	-	+
Amino acids	Ninhydrin Test	+	-	-	-	-

<b>Steroids</b>	Libermann-Burchard Reaction	+	-	+	+	-
<b>Glycosides</b>						
a)Cardiac	Legal's Test	+	-	+	-	-
b)Saponin	Foam Test	+	+	-	-	+
<b>Flavonoids</b>	Sodium Hydroxide Test	+	-	-	-	+
<b>Alkaloids</b>	Mayer's Test	-	+	+	-	-
<b>Tannins and Phenolic Compounds</b>	Dilute Nitric acid Test	+	-	-	+	+

'+' showed positive result and '-' showed negative result

**Anti-Bacterial Activity:**

The antibacterial activity of the *Vitex negundo* Linn different extracts was analyzed against both Gram-positive and Gram-negative bacteria, the results of which are presented in Figure and Table. From the results it is evident that Methanolic extract has showed anti-bacterial activity and there was no zones observed in the other extracts like Ethanol, Acetone, Chloroform and Water Extracts. Gram negative bacteria *Pseudomonas aeruginosa*, *Salmonella Sps*, *Klebsiella pneumoniae*, *E.coli* are more susceptible than the gram positive bacteria *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Clostridium*, *Bacillus subtilis*. In Gram Positive bacteria maximum zone of inhibition 29 mm was observed for *Clostridium* whereas Ampicillin showed 24mm of zone of inhibition. The maximum zone of inhibition for Gram Negative bacteria 26 mm was found for *Pseudomonas aeruginosa* while streptomycin showed 20mm zone of inhibition respectively.

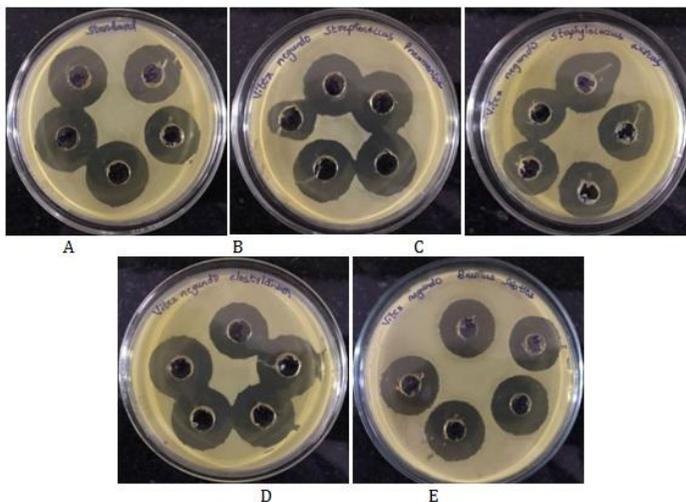


Figure 1: Zone of Inhibition shown Anti Bacterial Activity for Gram Positive Strains in Methanolic extract. A: Ampicillin; B: *Streptococcus pneumoniae*; C: *Staphylococcus aureus*; D: *Clostridium*; E: *Bacillus subtilis*

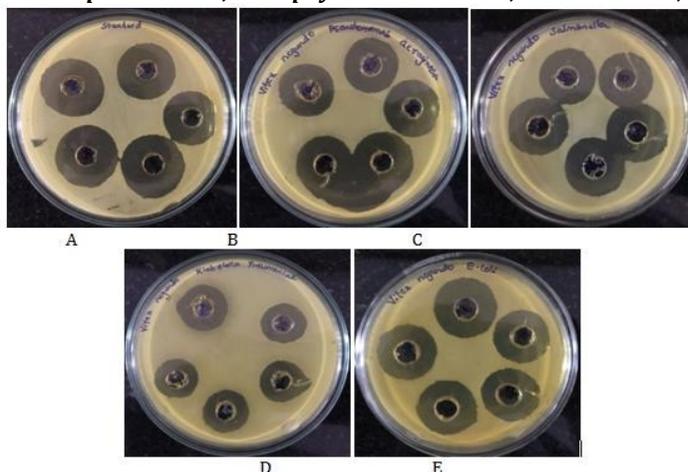
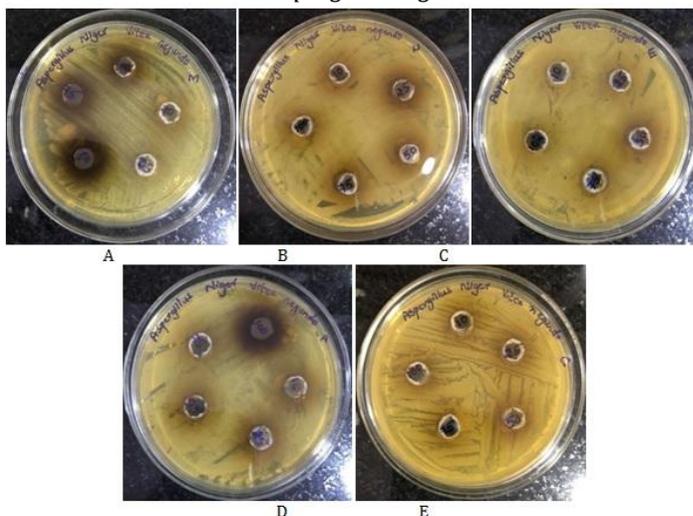


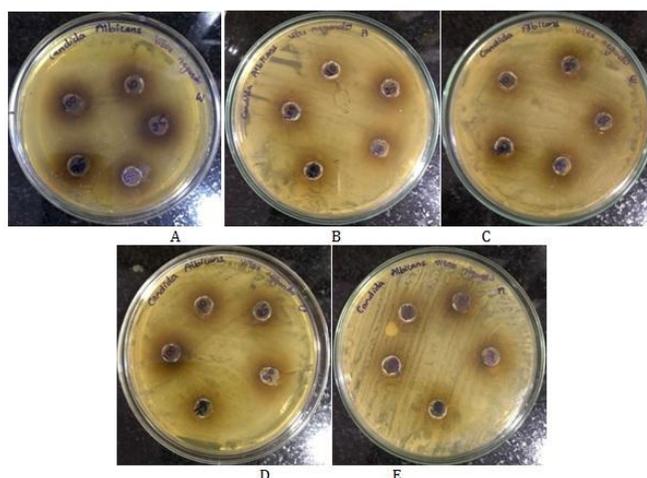
Figure 2: Zone of Inhibition shown Anti Bacterial Activity for Gram Negative Strains in Methanolic Extract. A: Streptomycin; B: *Pseudomonas aeruginosa*; C: *Salmonella Sps*; D: *Klebsiella pneumoniae*; E: *E.Coli*

**Anti Fungal Activity:**

The *Vitex negundo* Linn different extracts of Methanol, Ethanol, Acetone, Water and Chloroform was analyzed for the antifungal activity against *Aspergillus Niger* & *Candida albicans*. There was no zones observed either in the results of *C. albicans* nor *Aspergillus niger*.



**Figure 3 : Zone of Inhibition was not observed in Anti Fungal Activity of *Aspergillus Niger* in different extracts A: Methanolic Extract; B: Water; C: Ethanolic Extract; D: Acetone; E: Chloroform**



**Figure 4: Zone of Inhibition shown Anti Fungal Activity of *Candida albicans* in different extracts: A: Water; B: Acetone; C: Ethanol; D: Chloroform; E: Methanol**

**DISCUSSION:**

*Vitex negundo* has been traditionally used in ayurvedic medicine to treat several disorders such as catarrh, headache, neck gland sores, tubercular neck swellings, sinusitis, sexual debilities, nervous debility, liver complaints, inflammation of uterus, fever, diarrhoea etc [21]. The result of present research important to proves the medicinal properties of *Vitex negundo*. N. Nirmal kumar (2014) was reported aqueous soluble extractive value 22.45 % and alcoholic soluble extractive value 5.99% which indicated the maximum extractive percentage found in methanolic extract [22] and as per the present investigation similar results have been reported, maximum extractive value has been found in methanol than chloroform, water, ethanol and acetone extract. The phytochemical screening of *Vitex negundo* extracts revealed the presence of carbohydrates, proteins, amino acids, steroids, glycosides (cardiac, anthraquinone and saponin), flavonoids, tannins and phenolic compounds. The presence of these primary and secondary metabolites suggests that the *Vitex negundo* have curative ability against several diseases. Hence present results might be important to support the ethnomedicinal and traditional medicinal properties of *Vitex negundo*. Presence of tannins and phenolic compounds proves that plants may be having antimicrobial and anticancer activity [23]. Ramesh Mani et. al (2013) was revealed the presence of alkaloids, carbohydrates, cardiac glycosides, flavonoids,

glycosides, phenols, proteins, saponin and tannins in methanolic extract of *Vitex negundo* leaves [24]. The present study also focused on antimicrobial activity in which it is evident that Methanolic extract has showed anti-bacterial activity and there was no zones observed in the other extracts like Ethanol, Acetone, Chloroform and Water Extracts. Gram negative bacteria *Pseudomonas aeruginosa*, *Salmonella Sps*, *Klebsiella pneumoniae*, *E.Coli* are more susceptible than the gram positive bacteria *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Clostridium*, *Bacillus subtilis*. In Gram Positive bacteria maximum zone of inhibition 29mm was observed for *Clostridium* whereas ampicillin showed 24mm of zone of inhibition. The maximum zone of inhibition for Gram Negative bacteria 26mm was found for *Pseudomonas aeruginosa* while streptomycin showed 20mm zone of inhibition respectively. The different *Vitex negundo* leaves extracts of Methanol, Ethanol, Acetone, Water and Chloroform was analyzed for the antifungal activity against *Aspergillus niger* and *Candida albicans*. There was no zones observed either in the results of *C. albicans* nor *Aspergillus niger*.

**Conflicts of interest:** The authors declare that they have no conflict of interest.

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