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## Phytochemical Analysis and Antifungal Activity of *Vitex negundo* Leaf Extracts Against Clinically Isolated Fungal Pathogens

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**Abstract:** The aim of this study is to examine the antifungal activity of plant *Vitex negundo* Linn. Disc diffusion method was used to assess the antifungal activity of these extracts against clinically isolated fungal pathogens, such as *Candida albicans*, *Aspergillus niger*, *Mucor sp*, *Cryptococcus neoformans*, *Penicillium sp*. The presence of phyto-compound in the extracts including, steroid, terpenoids, flavonoids, may be responsible for these activities. In ethanol extract of *Vitex negundo* showed the highest inhibition zone against *Candida albicans* and the growth of *Penicillium sp* were inhibited by methanol extract. The results clearly showed that ethanol and methanol extracts were specific in action against the fungal pathogens.

**Keywords:** *Vitex negundo*, fungal strains, Phytochemical analysis, antifungal activity

### Introduction

India is blessed with a fashionable wealth of medicinal Plants. The Medicinal Plants have created smart contribution to the event of ancient Indian systems of medicine or drugs are conjointly to native medicine among social group etc. India is represents as store house of genetic diversity of Medicinal Plant. Plants and plant derived agents have long history as supply of potential chemotherapeutical agents in Ayurvedic and Unani system of medication. The *Vitex* genus is members of Verbenaceae family ordinarily called Nirgundi, the Indic word nirgundi virtually means that protects our body from all diseases [1, 2, 3].

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*Vitex negundo* used as medication within the indigenous system of medication, the leaves square measure the foremost potent for medicative use. It's used for treatment of eye-disease, toothache, inflammation, leucoderma, enlargement of the spleen, skin-ulcers, inflammation fever, autoimmune disorder, sexually transmitted disease and respiratory disease. They're conjointly used as tonics, vermifuge, lactagogue, agent, bactericide, antipyretic and antihistaminic agents, acute and sub-acute inflammation [4-7].

Leaf extracts of *Vitex negundo* possess anti-oxidant potential and antifungal activities previously reported by Tiwari and Tripathi, [8], anthelmintic [9], Dysmenorrheal [10], medication and pain suppressing activity [11], anti-hyperglycemic activity [12], Anti-filarial [13], Anti-bacterial [14] and opposed plant activity [15]. The present study was undertaken to investigate the antifungal properties of the *Vitex negundo* Linn against fungal pathogens.

## Material and Methods

### *Collection and extraction of Vitex negundo Linn*

Fresh leaves of *Vitex negundo* Linn, were collected from Kolli Hills, Tamilnadu, washed several times with water to remove the dust particles and then sun dried to remove the residual moisture and grinded to form powder. Hundred grams of *Vitex negundo* Linn powder was used for extraction with ethanol, petroleum ether, chloroform, methanol and petroleum ether using a Soxhlet extraction apparatus at the boiling point of the solvent for 48-72 hours or until the extracted solvent become clear. After that extracts were filtered with the help of filter paper and solvent was evaporated from extract in rotary evaporator to get the syrupy consistency. Then extract was kept in refrigerator at 4°C for future experiments.

### *Phytochemical analysis of plant extracts:*

A stock concentration of 1 % (W/V) was prepared using the respective solvent in each case. These extracts along with positive and negative controls were tested for the presence of active phytochemicals. Preliminary phytochemical screening was carried out to find the presence of the active chemical constituents in extracts such as alkaloids, flavonoids, tannins, carbohydrates, phenolic compounds, terpenoids, glycosides, steroids, fixed oils and fats. In general, tests for the presence of phytochemical compounds involved the addition of appropriate chemical reagent(s) to the extract in test tubes. [16]

## Test of Alkaloid

### *Mayer's test*

Alkaloids are basic nitrogenous compounds with definite physiological and pharmacological activity. Alkaloid solution produces white yellowish precipitate when a few drops of Mayer's reagents are added most alkaloids are precipitated from neutral or slightly acidic solution by Mayer's reagent. The alcoholic extract was evaporated to dryness and the residue was heated on a boiling water bath with 2% hydrochloric acid. After cooling, the mixture was filtered and treated with few drops of Mayer's reagent. The samples were then observed for the presence of turbidity or yellow precipitation. [17]

## **Test of Flavonoids**

### ***Lead Acetate Test***

Extracts were treated with few drops of 10% lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids. [17]

## **Test of Glycosides**

### ***Aqueous Sodium hydroxide:***

Extracts were treated with 1 ml water and 1 ml sodium hydroxide. Formation of yellow colour indicates the presence of glycosides. [17]

## **Test of Steroids**

### ***Salkowski Test***

Extracts were treated with few drops of chloroform and concentrated sulphuric acid. Formation of bluish red to cherry colour in chloroform layer green fluorescence acid layer indicates the presence of steroids. [17]

### ***Test of Terpenoids***

5 ml of each extract was added to 2 ml of chloroform and 3 ml of concentrated sulphuric acid to form a monolayer of reddish brown coloration of the interface was showed to form positive result for the terpenoids. [17]

### ***Test of Tannins***

To the extract 0.1% ferric chloride solution was added, formation of a dark blue or greenish black color showed the presence of tannins. [17]

## **Test of Phenols**

### ***Ferric Chloride Solution***

Extracts were treated with 3-4 drops of 5 % ferric chloride solution. Formation of bluish black colour indicates the presence of phenols. [17]

## **Detection of carbohydrate**

Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates. [17]

### ***Benedict's test***

Filtrates were treated with Benedict's reagent and heated gently in water bath 10 minutes. Brick red precipitate indicates the presence of reducing sugars. [17]

### ***Antifungal activity of Vitex negundo***

The antifungal activity was carried out by disc diffusion method. The sterile Muller Hinton agar plate was prepared. The clinically isolated fungal strains, *Aspergillus niger*, *Penicillium sp*, *Mucor sp*, *Candida albicans*, and *Cryptococcus neoformans* were spread over the Muller Hinton agar plates by using separate sterile cotton swabs. [18]

The sterile disc was prepared and coated with 30 µg concentration plant extracts. The prepared extracted discs were placed on medium with normal Ketoconazole disc with 30µg concentration. The plates were incubated 24 hours at 37°C. The zone of inhibition of microorganism growth was measured and compared with normal antifungal agents.

## **Results and Discussion**

### ***Phytochemical analysis of Vitex negundo***

Phytochemical analysis of *Vitex negundo* was carried out by the following solvents Methanol, Ethanol, Petroleum ether and Chloroform.

In Methanol extract of *Vitex negundo* contain all the components, carbohydrates, Alkaloids, flavonoids, tannin, terpenoids, glycosides, phenolic compounds and steroids are present. Whereas the Ethanol extract of *Vitex negundo* contain all the constituents except carbohydrates. Alkaloids, flavonoids, tannin, terpenoids, glycosides, phenolic compounds and steroids are present.

In Chloroform extract of *Vitex negundo* contain carbohydrates, Alkaloids, flavonoids, terpenoids, glycosides, and steroids are present. Tannin and phenolic compounds are absent. Petroleum ether extract of *Vitex negundo* contains Steroids, flavonoids, tannin and Sugars are present.

The predominant components present in all the extracts of *Vitex negundo* are flavonoids, Terpenoids and steroids. The results were plotted in Table No: 1

**Table: 1 Preliminary Phytochemical screening of various extracts of the leaves of *Vitex negundo***

Constituents	Ethanol	Methanol	Petroleum ether	Chloroform
Alkaloids	+	+	-	+
Flavonoids	+	+	+	+
Tannin	+	+	+	-
Carbohydrate	-	+	-	+
Terpenoids	+	+	+	+
Glycosides	+	+	-	+
Steroids	+	+	+	+
Phenols	+	+	-	-

+: Present    -: Absent

Chitra *et al.*, (2009) [19], reported that the preliminary phytochemical analysis carried out on the crude ethanol extract indicated the presence of alkaloids, glycosides, lignin, flavonoids and saponins.

The various chemical constituents like flavonoids, flavone glycosides, volatile oil, triterpenes, tannins and lignin many others were identified and reported from *Vitex negundo* by Gautam *et al.*, 2008. [20]

**Antifungal Activity:**

The antifungal effect of different solvent extracts of *Vitex negundo* has been investigated against clinically isolated fungal strains. The disc was prepared in 30µg concentration and therefore the disc was placed on Muller-Hinton agar plates with standard disc containing reference antibiotic ketoconazole were used as a positive control.

The antifungal activity of the standard antibiotic ketoconazole disc at the 30µg concentration used, the zone of inhibition obtained against *Cryptococcus neoformans*, *Candida albicans* and *Aspergillus niger* as 17mm, 11mm and 10mm respectively.

In ethanol extract of *Vitex negundo* at 30µg concentration inhibit the growth of fungal culture. The inhibition zone was high (16mm) against *Candida albicans* followed by, *Aspergillus niger* (15mm) *Cryptococcus neoformans* (14mm), *Penicillium sp* (13mm), *Mucor sp* (11mm) respectively.

In chloroform extract of *Vitex negundo*, at 30µg concentration, the inhibition zone was recorded high (10mm) against *Penicillium spp.* and *Candida albicans*.

The antifungal activity of methanol extracts of *Vitex negundo*, at 30µg concentration the inhibition zone was high (11mm) against *Penicillium spp.* The results clearly showed that ethanol and methanol extracts were specific in action against the fungal pathogens (Table: 2).

Table: 2 Antifungal activity of *Vitex negundo* extracts against isolated organisms

Organisms	Concentration of extract and zone of inhibition (mm)				
	Ketoconazole (30µg)	Methanol Extract (30µg)	Ethanol extract (30µg)	Chloroform Extract (30µg)	Petroleum ether Extract (30µg)
<i>Aspergillus niger</i>	10	4	15	6	-
<i>Penicillium sp.</i>	-	11	13	10	-
<i>Mucor sp.</i>	-	9	11	9	-
<i>Cryptococcus neoformans</i>	17	-	14	-	-
<i>Candida albicans</i>	11	10	16	10	-

Aqueous and ethanolic extracts of *Vitex negundo* leaves has been shown to be restrictive against *Pyricularia oryzae* [20]. Similarly, chloroform leaf extract of *I. carneahas* been reportable to possess study antifungal activity against *Rhizoctonia solani* [23].

Crude extract of *Vitex negundo* Linn, showed glorious results against *Cryptococcus neoformans*, *Candida albicans*. *Candida glabrata* and *Asperillus flavus* strains were found to be ineffective against the plant extracts similar to the study reported by Shaukat Mahmud *et al* (2009) [21]. Binary compound and ethanolic extracts of *Vitex negundo* leaves has been shown to be restrictive against *Pyricularia oryzae* [22].

## Conclusion

In conclusion, the *Vitex negundo* ethanol and methanol leaf extracts showed a broad spectrum of activity against fungal strains. It showed maximum activity against *Candida albicans*, *Aspergillus niger*, *Cryptococcus neoformans* and *Penicillium sp.* The knowledge of extent and mode of inhibition of specific bioactive compounds which are present in plant extracts, may contribute to the successful application of such natural compounds for treatment of infection caused by fungal and bacterial diseases. The present status of medicinal plants and their products provide opportunity for the developing countries to benefit from the emerging marks as the developing countries possess most biodiversity of medicinal plants. Consideration of such problem with plants is also very important for availing the total beneficial effects of herbal drugs according to their relative bioactive components in our research. It is concluded that in coordinance of the chemical literature finding resistant strains of organism plant biodiversity may lead to unexpected research findings.

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