

# Estimation of Antifungal Activity of *Vitex Negundo* by Different Solvent Extracts

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**Abstract:** Natural substances for human health have long been found in plants. From the root to the fruit, every part of the plant has a number of secondary metabolites that are highly beneficial for treating a variety of ailments. The creation of costly antibiotics is a result of the ongoing rise in the number of microbe strains that are resistant to antibiotics. Compliance is challenging in the majority of developing nations due to the high cost of these antibiotics. This necessitates investigating alternate sources of antifungal agents. The medicinal shrub *Vitexnegundo*(Family: Lamiaceae) has been shown to have healing properties against a number of illnesses. Present work was carried out to assess the antifungal activity of *Vitexnegundo* against ten multidrug resistant pathogenic *Aspergillus* spp. *V. negundo* plant parts (leaves, stem, flowers) were collected, air dried, grinded and extracted by using different solvents (methanol, acetone, chloroform) through sequential extraction method. These extracts were then tested for antifungal activity using agar well diffusion method. Methanolic, acetonic and chloroform extracts exhibited moderate antifungal activity as compared to standard antibiotic flucanazole. From the above findings it is suggested that flower extracts of *V. negundo* has the potential to be developed as an antifungal agent

**Keywords:** *V. negundo*, solvent extracts, antifungal activity, bioactive compound

## I. INTRODUCTION

Herbal treatments have been used to treat a wide range of infectious disorders throughout human history. Many under developed nations still use plant materials as medicines in their primary healthcare systems (Zakaria, 1991). Finding therapeutic plants around the world is crucial for the fields of medicine and agriculture because it opens up new avenues for the spread of substitute medicinal crops with greater social and economic advantages. The use of plant preparation for such purposes has been documented (Herbal Medicine Research Centre, 2002). The control of bacteria and fungi infection has been remarkably effective since the discovery of antibacterial and antifungal drugs (Zaidan et al., 2005). Antimicrobial activity is the ability of a substance to kill or inhibit the growth of microorganisms, such as bacteria, viruses, fungi, and parasites. Plants produce a wide range of secondary metabolites, many of which have been shown to possess antimicrobial properties (Jain et al., 2019). These compounds may act by disrupting the cell membranes or cell walls of microorganisms, or by interfering with their metabolic processes (Yetginli., 2024). However some of the pathogens rapidly become resistant to many of the first discovered effective drugs. The development of drug resistance as well as appearance of undesirable side effects of certain antibiotics (WHO, 2002) has led to the search of new antibacterial agents in particular from medicinal plants. (Mitscher et al., 1987).

Several works have been done to examine the antifungal effects of herbal plants extracts, including roots, barks, stem, leaves or flowers. *Vitexnegundo* Linn. belongs to family Lamiaceae (which comprises 75 genera and nearly 2500 species), commonly known as 'Nirgundi' in marathi (Panda et al., 2009) 'Five leaved chaste tree' in English (Gautam and Kumar, 2012). It's a large aromatic, woody shrub, densely whitish, tomentose branchlets. Though all parts of *V. negundo* are used in the indigenous system of medicine, leaves and barks (Chandramu et al., 2003) are considered to be the most potent for medicinal use. The plant grows near water bodies, open forests and also planted as a hedge plant. The bluish purple flower is numerous; the fruit is succulent, rounded and about 4 mm in diameter (Mani et al., 2013). The decoction of leaves is used for treatment of inflammation, eye-disease, antibacterial, antipyretic etc. (Dharmasiri et al., 2003). This study was undertaken to carry out antifungal activity of *V. negundo* against some



infectious fungi. The evaluation of antifungal activity in *V. negundo* was investigated against ten fungal strains of *Aspergillus species* by using agar well diffusion method (Ansari et al., 2021).

## II. MATERIALS AND METHODS

### Plant Collection

*V. Negundo* leaves, stem and flowers were collected from Rais high school campus, Bhiwandi, Maharashtra. The plant parts were cut into small pieces and thoroughly washed under running tap water and then dried under shade. The dried parts were grinded into fine powder using mechanical grinder. Those powdered samples were kept in the air-tight containers to prevent moisture.

### Extract Preparation

Sequential extraction was carried out with the powdered sample using solvents of decreasing polarity by soxhlet apparatus. About 50 g of plant powder were extracted using 150 ml of solvents i.e methanol, acetone, chloroform for 1 h. The extracts were collected, allowed to cool and concentrated by evaporating the solvents at room temperature. The concentrated residue was dissolved in their respective solvents as a crude plant extracts.

### Microorganisms

The fungi employed in the current study were ten fungal isolates of *Aspergillus species* i.e., NH-07, NH-13, NH-25 NH-15, HA-96, HA-70, NH-07, NH-01, NH-18, NH-09. SDA (Sabouraud dextrose agar) was used for the isolation and maintenance of fungal cultures. Pure culture was again sub cultured to maintain the purity of the isolated strain (Priya et al., 2013).

### Antifungal Activity

Antifungal activity of plant extracts was examined by agar well diffusion method (Cakiret et al., 2004). About 20 ml SDA was poured in petri dishes and allowed to solidify for 30 minutes. Fungal inoculum (20 µl) was spread over SDA plates with a sterile spreader. A total of 8 mm diameter wells were punched with cork borer into the agar and filled with plant extracts 30 µl (1mg/ ml) and solvent blank. Standard antibiotic (Fluconazole, concentration 1 mg/ ml) was used as positive control. Plates were incubated at 37°C for 24 to 48 hours for maximum growth of the microorganism. After incubation, the plates were observed for distinct zone of inhibition surrounding the disc.

## III. RESULTS AND DISCUSSION

According to the obtained results, *Vitexnegundo* extracts showed inhibitory properties against particular species of *Aspergillus*. Antifungal activity assessed in terms of zone of inhibition of the crude extracts. All the methanol, acetone, and chloroform extracts showed varied levels of antifungal activity against 10 fungal isolates i.e. NH-07, NH-13, NH-25 NH-15, NH-09, NH-18, NH-07, NH-01, HA-70, HA-96. It was observed that methanolic, acetonetic, and chloroform extracts of leaf showed higher inhibition zone ranging from 20–25 mm for NH-18 (Table 1). Likewise for NH-13, the zone of inhibition ranged from 15-20 mm in diameter. In contrast, NH-07, HA-96 and NH-09 were inhibited by the extracts with minimum zone of inhibition ranging from 10-15 mm. Antibiotics fluconazole showed moderate to highest inhibition zone than that of plant extracts ranged from 15-35 mm.

However none of the extracts were able to inhibit the growth of NH-25, NH-15, NH-07, NH-01 and HA-70. The methanolic and acetonetic extracts of leaf exhibited strongest antifungal activity as compared to chloroform extracts.

Furthermore antifungal activity of stem extracts against NH-18 and NH-13 exhibited highest zone of inhibition ranging from 20-25 mm in diameter (Table 2). Whereas, HA-96 was suppressed by methanolic, acetonetic and chloroform extract showing zone of inhibition ranging from 10-15 mm. Similarly acetonetic and chloroform extracts restricted the growth of NH-07 showing 16 mm and 18 mm zone of inhibition respectively. However none of the extracts were able to inhibit the growth of NH-25, NH-15, NH-07, NH-01 and HA-70. Hence, chloroform extracts of stem was proved to have the higher activity.

Moreover antifungal activity of flower extracts exhibited highest zone of inhibition of NH-18 by all extracts ranged from 21-28 mm in diameter. Similarly NH-07, NH-25 and HA-70 showed moderate inhibition by all the three extracts as shown in (Table 3) in between 10-20 mm range. Whereas on the other hand, NH-13 was only inhibited by methanolic and acetonetic extracts with 20 mm and 13 mm zone of inhibition. NH-09 and HA-96 was only inhibited by



chloroform extracts rather than methanolic and acetonc extracts. Hence, chloroform extracts of flower was proved to have the potent activity. Based on the aforementioned findings, it was determined that extracts from *Vitexnegundo* flowers effectively suppressed the growth of *Aspergillus* sp.

In the previous work done by Mahmud et al., (2009) Antifungal activity of *Vitexnegundo* fruits against *C. albicans*, *C. glabrata*, *A. flavus*, *M. canis* and *F. solani* showed positive result. Ethanol extract of fruit seeds showed significant activity against *F. solani* and moderate response against *M. canis* with no effect on *C. albicans*. The other investigation assessed by Bameta et al., (2019) the antifungal properties of *Vitexnegundo* leaf and stem extract against four fungal isolates: *A. niger*, *C. neoformans*, *S. cerevisiae*, and *C. albicans*. All fungal strains were susceptible to the antifungal effects of the leaf methanolic extract. According to reports, the maximum zone of inhibition against *C. neoformans* was 14.76±1.42 mm, followed by *A. niger* (8.33±1.33), *C. albicans* (8.33±0.57), and *S. cerevisiae* (6.26±0.73). The highest antifungal activity against *C. neoformans* alone was seen in the case of the stem for chloroform extract (9.93±0.90 mm), followed by ethanol extract (3.2±0.81) against *C. albicans* and methanolic extract against *A. niger* (3.1±0.42). Ababutain et al., (2021) demonstrated antifungal evaluation of ethanolic, methanolic, and aqueous extracts of *V. negundo* against *Candida* species.

**Table 1: Antifungal activity of leaf extracts of *Vitexnegundo***

Solvents	Zone of Inhibition (mm)									
	NH-07	NH-13	NH-25	NH-15	NH-09	NH-18	NH-07	NH-01	HA-70	HA-96
Methanol	--	20	--	-	--	25	--	--	--	13
Acetone	16	21	--	--	--	21	--	--	--	10
Chloroform	18	24	--	--	09	25	--	--	--	14

**Table 2: Antifungal activity of stem extracts of *Vitexnegundo***

Solvents	Zone of Inhibition (mm)									
	NH-07	NH-13	NH-25	NH-15	NH-09	NH-18	NH-07	NH-01	HA-70	HA-96
Methanol	12	18	--	--	--	25	--	--	--	15
Acetone	17	20	--	--	15	20	--	--	--	12
Chloroform	10	17	--	--	--	20	--	--	--	10

**Table 3: Antifungal activity of flower extracts of *Vitexnegundo***

Solvents	Zone of Inhibition (mm)									
	NH-07	NH-13	NH-25	NH-15	NH-09	NH-18	NH-07	NH-01	HA-70	HA-96
Methanol	15	20	10	--	--	28	--	--	10	09
Acetone	18	13	11	--	--	22	--	--	18	--
Chloroform	19	--	18	--	10	21	--	--	15	10

**Table 4: Antifungal activity of Flucanazole**

Standard Antibiotics	Zone of Inhibition (mm)									
	NH-07	NH-13	NH-25	NH-15	NH-09	NH-18	NH-07	NH-01	HA-70	HA-96
Flucanazole	15	35	30	15	25	26	15	27	21	20

## V. CONCLUSION

In conclusion, the *Vitexnegundo* chloroform flower extracts showed a broad spectrum of activity against the studied fungal isolates. It showed maximum activity against various *Aspergillus* spp. 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 diseases. More research would be needed to determine the phytochemical compounds which are present in leaves, flowers and stem.

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