INVITRO SCREENING OF ANTIFUNGAL ACTIVITY OF LEAF EXTRACT OF Vitex negundo

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ABSTRACT

Vitex negundo is a medicinal plant of family Verbenaceae which is commonly found throughout India and is used in traditional medicines. The present study was undertaken to screen potential antifungal activity of the extract of leaves of V. negundo against three dermatophytes i.e. Epidermophyton, Microsporum and Trichophyton. The leaf extract was prepared by crushing the leaves in methanol, ethanol and distilled water as a solvent. These extracts were evaluated for their antifungal activity against Epidermophyton, Microsporum and Trichophyton on Sabouraud Dextrose Agar media (SDA) plates by using agar well diffusion method. The plates were incubated at 35±2°C for 24 hrs. Then diameter of zone of inhibition was measured. Best antifungal activity was observed against Trichophyton with ethanol as a solvent and the least activity was observed against Epidermophyton with distilled water as a solvent.

Key Words: Epidermophyton, Trichophyton, Microsporum, Vitex negundo, Antifungal activity

INTRODUCTION

V. negundo is commonly known as Chinese chaste tree, five leaved chaste tree, horse shoe vitex or nirgundi is a large aromatic shrub with quadrangular densely whitish, tomentose branchlets. It has analgesic, antibacterial, antifungal and anti-inflammatory properties used by folk as medicines. It is the plant which is very widely grown and flourished. It is a woody aromatic shrub with tri or pentalolate leaves that gives rise to bluish purple color plant in branched cymes. It prefers to grow in humid places or along water courses in wasteland and makes open forest in India, Afghanistan, Sri Lanka, Pakistan, Thailand and Malaysia. It is used by local people for various diseases and treatments such as arthritis, rheumatism, malaria, toothache, bodyache and menstrual problems, premenstrual dysphoric disorder (PMDD), inflammatory conditions, diarrhea and flatulence. The ethanobotanical approach is to study the role of some medicinal plants such as Neem, Tulsi, Nirgundi, and Ginger etc, which are used for cure of various diseases. The practice of using these medicinal plants is not having particular prescribed or written source but these practices are followed at traditional level from ancient times till date. The tribes of Jhansi also use these plants and their parts as a substitute of medicines and heal themselves. Now a days global trend of interest in traditional systems of medicines have increased manifold times. India is very rich in medicinal plants. The forest in India is the principal repository of large number of medicinal and aromatic plants which are largely collected as raw materials for manufacture of drugs and perfumery products. Treatment of medicinal plants is considered very safe as there are no or minimal side effects. Use of herbal treatment is independent of any age groups and the sexes. Based on all these benefits, we decided to get the knowledge of some locally used medicinal plants. Vitex negundo also have medicinal value for prevention of skin diseases as it is reported as an antidermatophytic agent. The dermatophytes are a group of closely related fungi that have the capacity to invade keratinized tissue (spleen, hair and nails) of human and other animals to produce infection called dermatophytosis commonly referred to as ringworm. They are classified in three anamorphic genera: Epidermophyton, Microsporum and Trichophyton. Antidermatophytic activity of V. negundo was checked against these three
dermatophytes *Epidermophyton*, *Microsporum* and *Trichophyton*. Though all parts of *V. negundo* are used but leaves and barks are the most important ones in field of medicines. Leaves of *V. negundo* are considered as tonic, vermifused and are given with long pepper in catarrhal fever. Out of stem, root and leaf extract of *Vitex negundo* for antibacterial activity, best results were reported for leaf extract.

**MATERIAL AND METHODS**

**Collection of plant sample**

Fresh leaves of the plant *V. negundo* were collected from Notghat in Baruasagar, Jhansi district during October - November. Leaves were washed properly under running tap water then air dried, homogenized to fine powder and stored in air tight bottles at 4°C.

**Preparation of leaf extract**

Leaves extract were prepared by sequential cold maceration method. Using methanol, ethanol and distill water as a solvent. Five gram of *V. negundo* leaves powder was soaked in 50 ml of every solvent and incubated for 48 h at room temperature, and then it was filtered by whatman filter paper no-1 and stored at 4°C for further use.

**Microbial culture**

Above genera were used as test cultures. They were grown on Sabouraud Dextrose Broth (Himedia) at 30±2°C for 48h and were maintained at SDA media slants (peptone- 10.0g, dextrose-40.0g, agar-15.0g, distill water-1000 ml, pH-5.6). These plates were then incubated at 30±2°C for 48h to 72h.

**Assay for antifungal activity**

To check the antifungal activity, stock cultures of above genera were revived. The agar plates of SDA media were prepared (peptone- 10.0g, dextrose-40.0g, agar-15.0g, distill water-1000ml, pH-5.6). Each plate was inoculated with 0.5 ml of fungal suspension which was spread uniformly on the plate. Wells of 5 mm diameter were made in plates by using sterile cork borer. Test samples of every solvent were filled in wells and all the tests were carried out in triplicates. All plates were incubated at 35°C for 3-4 days and then the diameter of zone of inhibition was measured in millimeters (mm). (Fig. 1)

**RESULTS AND DISCUSSION**

**Epidermophyton floccosum**

It is a dermatophyte that produces mycoses to human being

![Symptoms of Epidermophyton](image1)

![Epidermophyton observed under microscope(×45, ×10)](image2)

![Epidermophyton observed under microscope(×45, ×10)](image3)

![Antifungal activity of Epidermophyton with extracts of Vitex negundo in different solvents a=ethanol inhibition, b= methanol inhibition, c= distilled water inhibition](image4)

Fig. 1: Antifungal activity of *Epidermophyton* with extracts of *Vitex negundo* in different solvents a=ethanol inhibition, b= methanol inhibition, c= distilled water inhibition

475
**Microsporum canis**

It is pathogenic asexual fungus which infects dead layers of skin of human (Fig. 2)

![Symptoms of Microsporum](image1)

![Microsporum observed under microscope(×45,×10)](image2)

**Fig. 2:** Antifungal activity of *Microsporum* with extracts of *Vitex negundo* in different solvents

a=ethanol inhibition, b=methanol inhibition, c=distilled water inhibition

**Trichophyton rubrum**

It is a dermatophytic fungus that colonizes upper layer of skin and causative agent of athlete's foot, nail infection, ring worm etc. (Fig. 3)

![Symptoms of Trichophyton](image3)

![Trichophyton observed under microscope (×45,×10)](image4)

**Fig. 3:** Antifungal activity of *Trichophyton* with extracts of *Vitex negundo* in different solvents

a=ethanol inhibition, b=methanol inhibition, c=distilled water inhibition
Antifungal activity of *V. negundo*

The level of antifungal activity of leaves extract of *V. negundo* in different solvents against test dermatophytes is mentioned in table as observed by measuring the zone of inhibition in petri plates. The values given in Table 1 are the analysis of triplicate data (mean value). Zone of inhibition against *Epidermophyton* with solvents: distill water, methanol and ethanol is observed as 3.0 mm, 13.0 mm and 14.0 mm respectively. *Microsporum* growth was inhibited maximum (13.0 mm) by using ethanol as a solvent and minimum (5.0 mm) by distill water as a solvent. Zone of inhibition in case of *Trichophyton* was minimum (4.0 mm) with distill water and maximum (16.0 mm) with ethanol as a solvent. Best antifungal activity was observed against *Trichophyton* with ethanol as a solvent and the least activity was observed against *Epidermophyton* with distill water as a solvent.

**CONCLUSION**

It is concluded that *V. negundo* leaves can be used as good antidermatophytic agent as it has given positive results in all conditions. But the best action is in presence of ethanol solvent. Whereas the least activity was observed against *Epidermophyton* with distilled water as solvent. Therefore *Vitex negundo* leaves can be used as natural antifungal agent and be a good treatment against skin infection ringworms and mail infections.

**Table 1: Antifungal activity of leaf extract of *V. negundo* on dermatophytes**

<table>
<thead>
<tr>
<th>Strains</th>
<th><em>Epidermophyton floccosum</em></th>
<th><em>Microsporum canis</em></th>
<th><em>Trichophyton rubrum</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>3.0</td>
<td>5.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Methanol</td>
<td>13.0</td>
<td>11.0</td>
<td>11.0</td>
</tr>
<tr>
<td>Ethanol</td>
<td>14.0</td>
<td>13.0</td>
<td>16.0</td>
</tr>
</tbody>
</table>

**REFERENCES**