Menthol is a monocyclic monoterpenoid obtained from volatile oil of *Mentha arvensis* or other species like *M. piperata*, *M. citrata*, *M. spicata*, and *M. pulgium* belonging to the family Labiatae. Menthol is used for treating insect bites, stings, itching, minor burns, sun burns, hemorrhoids, toothache, cold sores, sore throats, acne vulgaris, dandruff, seborrhea, corns, warts, athlete’s foot, in vaginal preparation to lessen irritation, to relieve pulmonary congestion in colds and allergy, muscle ache, post-coital antifertility, antiprotozoal, antispasmodic and cholorectic, carminative activity.

In view of these observations, present study has been done to synthesize its derivative so as to obtain therapeutically better compound. Therefore, Amino menthol was synthesized and its antifungal activity was carried out.

Menthol was first oxidized by chromic acid to Menthone, which on subsequent treatment with sodium nitrite and hydrochloric acid yield intermediate nitrozonium hydride to amino menthol. Thin layer chromatography was performed on glass plate coated with silica gel G preactivated at 110°C for 50 minutes. Amino menthol was dissolved in chloroform (CHCl₃) and chromatogram was developed in CHCl₃ 100%. Rₓ value was 0.532; spot was visualized as orange colour spraying with Dragendroff’s reagent. Few millilitre of it was dissolved in pure ethanol and an UV spectrum was recorded on JASCO Model IR 100 infrared spectrophotometer (λₓ max in cm⁻¹). The IR spectrum of the compound, displayed at a characteristic band at 3398.98 cm⁻¹ (-OH stretching), 2975 cm⁻¹ (1° NH₂ stretching), 2924.35 cm⁻¹ (-CH stretching), 1649.29 cm⁻¹ (1° NH₂ bending), 1550.91 cm⁻¹ (-CH₂ bending); 1458.32 cm⁻¹ (-CH₃ bending); 1371.51 cm⁻¹ (-CH₃ bending); 1371.51 cm⁻¹ (-CH₃ bending); 1371.51 cm⁻¹ (-CH₃ bending); 1371.51 cm⁻¹ (-CH₃ bending).

The structure of amino menthol synthesized was assigned on the basis of IR and NMR spectral data. The IR spectrum was recorded on JASCO Model IR 100 infrared spectrophotometer (λₓ max in cm⁻¹). The IR spectrum of the compound, displayed at a characteristic band at 3398.98 cm⁻¹ (-OH stretching), 2975 cm⁻¹ (1° NH₂ stretching), 2924.35 cm⁻¹ (-CH stretching), 1649.29 cm⁻¹ (1° NH₂ bending), 1550.91 cm⁻¹ (-CH₂ bending); 1458.32 cm⁻¹ (-CH₃ bending); 1371.51 cm⁻¹ (-CH₃ bending); 1371.51 cm⁻¹ (-CH₃ bending).

The value of λₓ max for amino menthol was found at 204.5 nm.

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**SYNTHESIS AND SCREENING THE ANTIFUNGAL ACTIVITY OF AMINO MENTHOL**

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**ABSTRACT**

Amino menthol have been synthesized and evaluated for antifungal activity. The synthesized compound is screened for antifungal activity against four fungal species i.e. Aspergillus niger (ATCC 16404), Aspergillus flavous (ATCC 9643), Candida albicans (ATCC 10231) and Alternaria Solani (ATCC 20476). The amino menthol showed good antifungal activity against all tested fungal stains at the concentration of 10-30 µg/disc. Miconazole nitrate (40 µg/ disc) were used as positive controls for fungi.

**Key words:** Antifungal activity and amino menthol.

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**Screening the Antifungal Activity**

Fungi were obtained from the stock cultures of the Central Drugs Laboratory, Kolkata (CDL) and of the Mycology and Plant Pathology Laboratory, Kolkata, India. The fungal stock cultures were maintained on Sabouraud-dextrose agar slants, which were stored at 4ºC. The fungi were maintained on Sabouraud dextrose agar, which is often used with antibiotics for the isolation of pathogenic fungi. Three to five similar colonies were selected and transferred to 5 ml broth with a loop and the broth cultures were incubated for 24 h at 37°C. For screening, sterile 6-mm diameter filter paper discs were impregnated with amino menthol.
and placed in Sabouraud-dextrose agar. The inoculum for each organism was prepared from broth cultures. The concentration of cultures was to 1 x 10^5 colony forming units/ml. The results were recorded by measuring the zones of growth inhibition surrounding the disc. Clear inhibition zones around the discs indicate the presence of antifungal activity. All data regarding antifungal activity are the average of triplicate analyses. Dimethylformamide (DMF) was used as a solvent. The control sample contained DMF with water. The antifungal compound miconazole nitrate (40 µg/disc) was used as reference standard, as recommended by the National Committee for Clinical Laboratory Standards. The results of the antifungal screening are given in Table -1.

Disc diffusion methods are used extensively to investigate the antifungal activity of natural substances. These assays are based on the use of discs as reservoirs containing solutions of the substances to be examined. In the case of solutions with a low activity, however, a large concentration or volume is needed. Because of the limited capacity of discs, holes or cylinders are preferably used (8). In this study, four fungal species were used to screen the possible antifungal activity of the amino menthol. Amino menthol showed a broad spectrum of activity against all the strains at the different tested concentration, as summarized in Table -1. The amino menthol showed good antifungal activity against all tested fungal stains at the concentration of 30 µg/disc. Miconazole nitrate (40 µg/disc) were used as positive controls for fungi. On the basis of the results obtained in the present study, we conclude that the amino menthol has significant amounts of antifungal activity.

Table -1: Antifungal activity of Amino menthol

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Species</th>
<th>Amino menthol</th>
<th>Zone of Inhibition (mm)</th>
<th>Miconazole</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>10 µg/ml</td>
<td>20 µg/ml</td>
<td>30 µg/ml</td>
</tr>
<tr>
<td>1</td>
<td><em>Aspergillus niger</em> (ATCC 16404)</td>
<td>5.5</td>
<td>9.5</td>
<td>16</td>
</tr>
<tr>
<td>2</td>
<td><em>Aspergillus flavous</em> (ATCC 9643)</td>
<td>6.2</td>
<td>9.8</td>
<td>16.6</td>
</tr>
<tr>
<td>3</td>
<td><em>Candida albicans</em> (ATCC 10231)</td>
<td>6.1</td>
<td>9.2</td>
<td>16.1</td>
</tr>
<tr>
<td>4</td>
<td><em>Alternaria Solani</em> (ATCC 20476)</td>
<td>5.9</td>
<td>9.5</td>
<td>15.3</td>
</tr>
</tbody>
</table>

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Structure of Amino menthol

[Diagram of Amino menthol structure]