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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.

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[1,2,3]. Menthol is used for treating insect bites, stings, itching, minor burns, sun burns, hemorrhoids, toothache, cold sores, sore throats, acne vulgaris, dandruff, seborrhea, calluses, corns, warts, athlete's foot, in vaginal preparation to lessen irritation, to relieve pulmonary congestion in colds and allergy, muscle ache, post-coital antifertility[4], antiprotozoal, antispasmodic and cholorectic, carminative activity[2,3].

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 ($CHCl_3$) and chromatogram was developed in $CHCl_3$ 100%. $R_f$ 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 by JASCO 7800 UV/ VISIBLE spectrophotometer**.** The value of $\lambda_{max}$ for amino menthol was found at 204.5 nm.

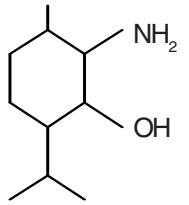
The structure of amino menthol synthesized was assigned on the basis of IR and NMR spectral data. The IR spectrum was recorded on JASCO Model IR 100 Infrared spectrophotometer ($\lambda_{max}$ in $cm^{-1}$). The IR spectrum of the compound, displayed at a characteristic band at 3398.98 $cm^{-1}$ (-OH stretching), 2975 $cm^{-1}$ (1° $NH_2$ stretching), 2924.35 $cm^{-1}$ (-CH stretching), 1649.29 $cm^{-1}$ (1° $NH_2$ bending), 1550.91 $cm^{-1}$ (-$CH_2$ bending); 1458.32 $cm^{-1}$ (-$CH_3$ bending); 1371.51 $cm^{-1}$ (-CH $\{CH_3\}_2$).

$^1$H NMR ($CDCl_3$) spectra on JEOLFX 90Q FOURIER TRANSFORM NMR SPECTROMETER using TMS as an internal reference. The chemical shift in $\delta$ ppm was 3.45 and 3.57 $\delta$ ppm double of doublet (CH at $C_2$); 5.20 and 5.33 $\delta$ ppm double of doublet (CH at $C_3$); 7.40 d ppm singlet ($NH_2$ and OH) [two proton of amino and one proton of alcoholic group]; 0.8- 3.0 d ppm {$CH_3$-, $(CH_3)_2$ CH, $CH_2$- and CH-}.

**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 Sabourauddextrose 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 105 colony forming units/ml. The results were




**Structure of Amino menthol**

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[5,6,7]. 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**

| S. No. | Species | Zone of inhibition (mm) Amino menthol 10 µg/ml | 20µg/ml | 30µg/ml | Control (Water+ DMF) | Miconazole 40 µg/ml |
|---|---|---|---|---|---|---|
| 1. | *Aspergillus niger* (ATCC 16404) | 5.5 | 9.5 | 16 | 0 | 19 |
| 2. | *Aspergillus flavous* (ATCC 9643) | 6.2 | 9.8 | 16.6 | 0 | 19 |
| 3. | *Candida albicans* (ATCC 10231) | 6.1 | 9.2 | 16.1 | 0 | 21 |
| 4. | *Alternaria Solani* (ATCC 20476) | 5.9 | 9.5 | 15.3 | 0 | 22 |

## REFERENCES

1. Knutson K. and Pershing L.K., Topical Drugs, In; Remington: The science and practice of pharmacy, 19th Edn, **2,** Mack publishing company, Easton Pennsylvania, 875 (1995)
2. Wallis T.E., Ed; Textbook of pharmacognosy. 5th Edn, CBS publishers and distributors, Delhi, 338-340 (1997)
3. Trease and Evans, Ed; Textbook of pharmacognosy. 14th Edn, W. B. Saunders Company limited, London, 259-262 (1996)
4. Kanganpothil D., Post coital anti-fertility effect of Mentha arvensis. Contraception, 559-567 (1981)
5. Seeley H.W. and Van Denmark P.J., Ed; Microbes in action, Freeman W.H. and Co., USA, 80 (1972)
6. Knuessel F. and Weirich F.G., Dermatologia, **145**(47), 233 (1972)
7. Mackeen M.M., Ali A.M., El-Sharkawy S.H., Manap M.Y., Salleh K.M., Lajis N.H. and Kawazu K., Antimicrobial and cytotoxic properties of some Malaysian traditional vegetables. *International Journal of Pharmacognosy,* **35:** 237-243 (1997)
8. Bartner A., Pfeiffer K.P. and Batner H., Applicability of disc diffusion methods required by the pharmacopoeias for testing antibacterial activity of natural compounds. *Pharmazie*, **49:** 512-516 (1994)