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Antimicrobial Bioactive Compound Isolated from the Fungus *Myrothecium verrucaria*

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Abstract: Bioactive chemical compound M1 [3-(5,5-dimethylhexyloxy) 2,2,4,4-tetramethyl-cyclohexanone] was extracted, purified and identified from the fungal culture of *Myrothecium verrucaria* isolated from soil in southern Iraq. The identification of the compound by using GC-Mass and H^1NMR was confirmed. Solubility, toxicity, purity and the chemical formula and molecular weight of M1 compound were determined. The antimicrobial bioactivity of the purified compound against the bacterial strains *E. coli* and *S. aureus* and the dermatophytic fungus *Microsporum gypseum* was tested using a disc diffusion agar method. The minimal inhibitory concentration (MIC) was also performed. Purified M1 compound exhibited a good bioactivity against the tested bacteria as well as against the dermatophyte isolate.

Key words: Bacteria**;** bioactive compound, dermatophyte, isolation and purification.

Introduction

It is well known that fungi producing various metabolic chemical compounds. Hence, the research interest to explore new antimicrobial agents from fungi is continued. So far, several antibacterial and antifungal bioactive compounds were isolated from different fungal species [5,11,18]. Nonetheless, most of the previous investigations dealt with basidiomycetous fungal species that exhibit bioactive chemical components against pathogenic bacteria [1,2,8,9,11,13]. Among the Deuteromycetes, *Myrothecium* is a genus with several species inhabiting soils [4] and some members of this genus are capable of producing antibiotics such as Myrocin C and other bioactive compounds [6,10,15].

During our survey of soil fungi in southern Iraq, an isolate of *Myrrothecium verrucaria* was recovered by the dilution plating technique. In this report an attempt was made to examine the fungus culture metabolites parti-cularly the bioactive antimicrobial compounds and to be tested against a selected isolates of bacteria and dermatophytic fungus.

Materials and Methods

Fungal culture

The fungus *Myrothecium verrucaria* was isolated from soil samples collected from southern Iraq during spring 2008 using Malt Extract Agar (MEA) in Petri dishes. Plates were incubated at 25°C for seven days. After cultivation, the mycelium was removed from the agar medium surface and amended into a liquid culture medium consisted of (40 g glucose, 10 g malt extract, 4 g yeast extract in 1L DW). Then the

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mycelium culture was transferred into a fermentation medium in 2L volume conical flasks as described by Nakagawa *et al.* [15] and incubated at 25°C on a rotary shaker for 2 weeks.

Extraction, isolation and purification of bioactive compound

The fungal culture was filtered on Whatman No. 1 filter paper, the filtrate was extracted by ethyl acetate using a separating funnel. Thin Layer chromatography (TLC) was applied for the isolation of the extracted metabolite and Rf value was measured. Purification of the extract was made on Silica Gel Column Chromatography (GF243 Merck, Germany). A further purification of fraction compounds was made by a Column Chromatography method as shown in Fig. 1. The purity of the isolated compound was verified according to the described method [21].

Bioactivity Test

Discs diffusion agar method was used [3] to examine the antimicrobial activity of the purified compound. Two strains of bacteria; *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) were used for this purpose. Antifungal bioactivity of this compound was also tested against isolates of dermatophytic fungus *Microsporum gypseum*. Fungal cultures were obtained from the Basrah General Hospital, Dermatology Section.

The minimum inhibitory concentration (MIC) test

The MIC values were determined by the standard serial dilution assay [12]. The MIC values in this assay were indicated by the absence of bacterial or fungal growth at the minimal concentration of the compound. Emmons Sabourauds dextrose broth (ESDB) medium was used for this test.

Cytotoxic test

Cytotoxicity of the purified compound was examined by using human RBC following a previously described method [20].

Solubility tesi

The solubility of the bioactive compound in various solvents (ethyl acetate, ethanol, methanol, chloroform, hexane, dimethyl sulfuoxide DMSO and water) was carried out.

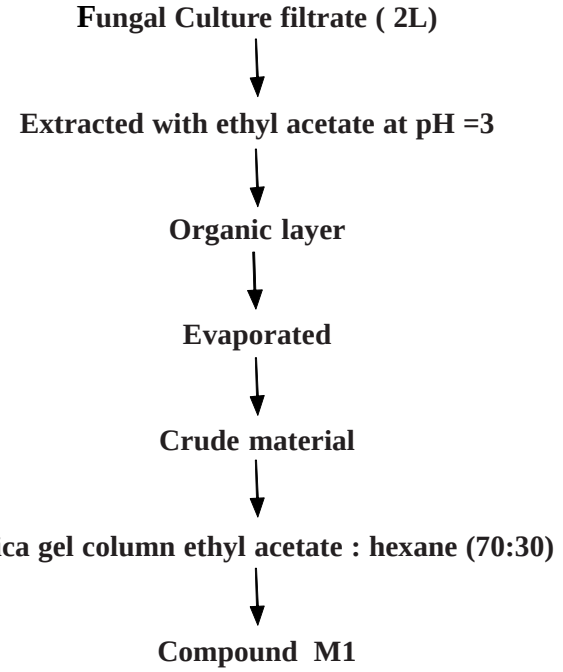

Fig. 1. Steps of extraction and purification of bioactive compound M1 from the fungus *M. verrucaria*

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Identification of the bioactive compound

Ultra violet (UV) spectrum (LKB-Sweeden UV), Infra-red spectrum (IR) (Pye-Unicam sp 3-3005 UK), Gas chromatography Mass (GC) and H^1NMR methods were applied for the identification and determination of the molecular weights and chemical formula and structure of the purified bioactive compound. .

Results

Fungal extract showed a single spot on TLC referred as M1 compound with Rf value of 0.90. Solubility test of M1 indicated that this component is insoluble in water and Methanol but it is soluble or partially soluble in the other examined solvents (Table 1).

Ultra violet (UV) spectra showed that the maximal absorbency beak of M1 compound was at 260 nm (Fig. 2).

IR spectrum revealed that M1 composed of various functional molecules structure (Table 2). The purified M1 compound exhibited a very strong spectra band at 2925 cm^{-1} representing a specific chemical functional group (Fig.3).

Based on GC- Mass and H^1 NMR methods, it appeared that the molecular formula of M1 compound is $C_{18}H_{14}O_2$ (Fig. 4), with a molecular weight 280 Kd. and its chemical structure is:[(3-(5,5-dimethylhexyloxy)2,2,4,4-tetramethyl-cyclohexanone)]. This indicated that M1 compound is more related to aromatic ketones group by comparing its spectroscopic data with available literature (Fig.5).

The MIC value of this bioactive compound is 6.25 µg/L for both *E. coli* and *S. aureus*, and 12.5 µg/L for the selected isolate of the dermatophytic fungus *M. gypseum*. A clear zone inhibition of 25 mm diameter was observed by using fungal crude extract against both bacterial strains *E. coli* and *S. aureus* and 15 mm against *M. gypseum*. However, the inhibition zones diameters revealed by the purified M1 compound were greater reaching to 28 mm and 27 mm for *E. coli* and *S. aureus*, respectively, and 21 mm for *M. gypsseum*

Table 1. Solubility test of purified compound M1 in different solvents

Purified component	Ethyl acetate	Methanol	Ethanol	Chloroform	Hexane	Water	DMSO
M1	Soluble	Non-soluble	Partially soluble	Soluble	Partially soluble	Non-soluble	Soluble

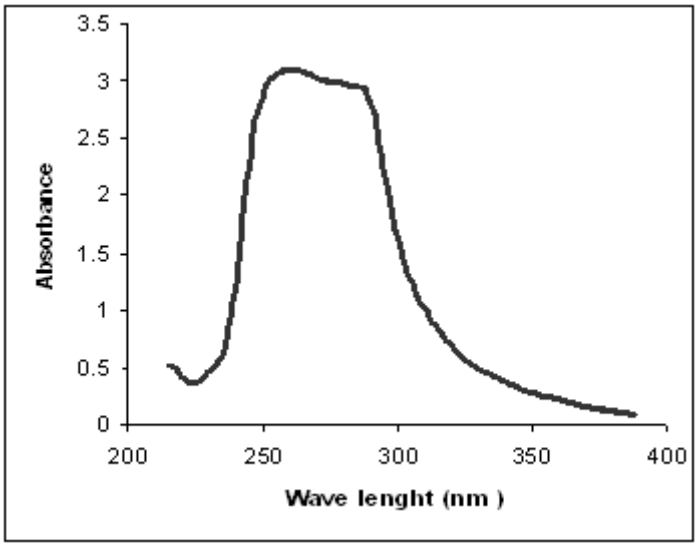

Figure 2. Absorbency of purified compound M1 from *M. verrucaria*

Table 2. Infra Red spectra showed the absorbency bands of different chemical functional groups composed the purified M1 component isolated from the fungus *M. verrucaria*

Functional group	Compound M1
N-H, O-H	3050W.Br
CH,CH2, CH	2925-2956
C=O	1730V. S.
C=C	1461 V. S.
C-O	1272 V. S.
N-HC-H	-

S.Br. = Strong Band
V. S. = Very strong Band
M.Br. = Medium Band
W.Br.= Weak Band

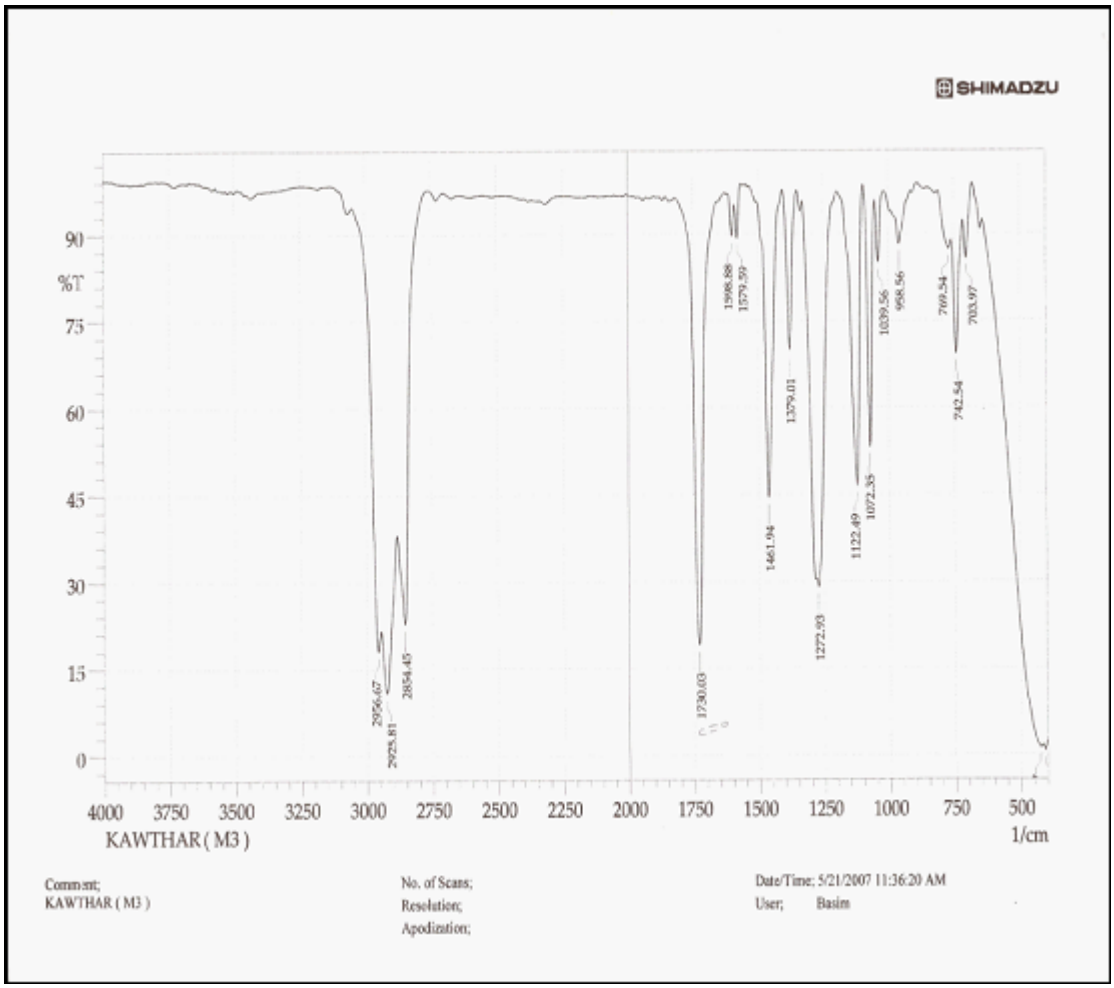

Fig. 3. Infra Red spectra of the purified M1 compound isolated from *M. verrucaria*

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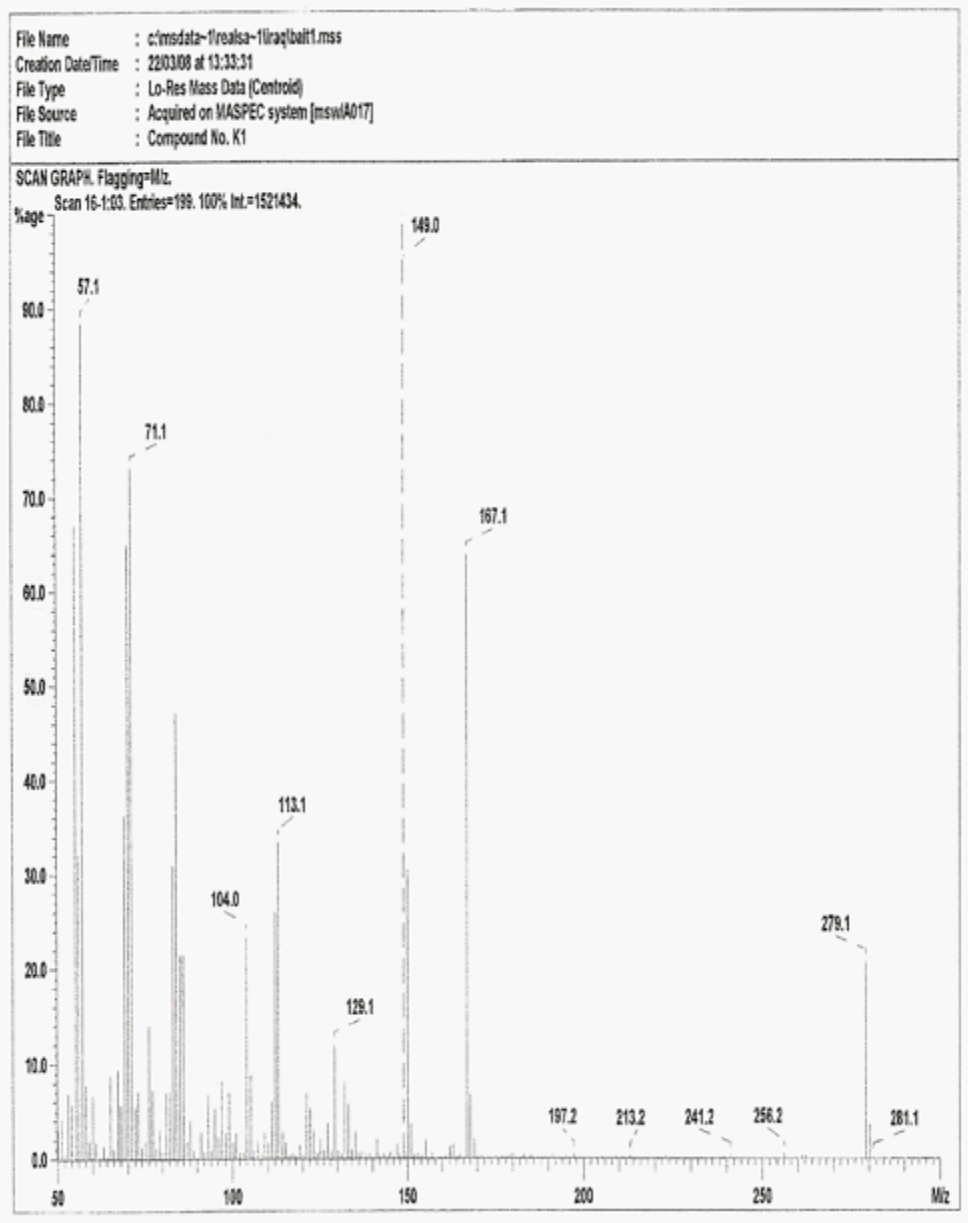
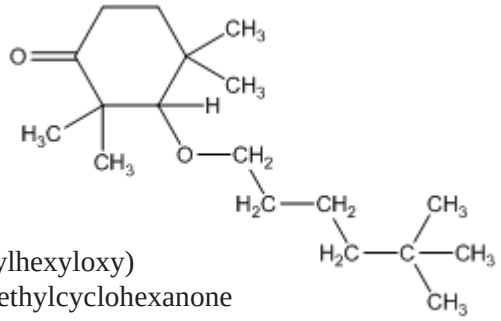


Fig. 4. H[1]NMR spectra of the purified compound M1 from *M. verrucaria*



3-(5,5-dimethylhexyloxy)
2,2,4,4-tetramethylcyclohexanone

Fig. 5. Chemical structure of M1 compound isolated from *M. verrucaria*

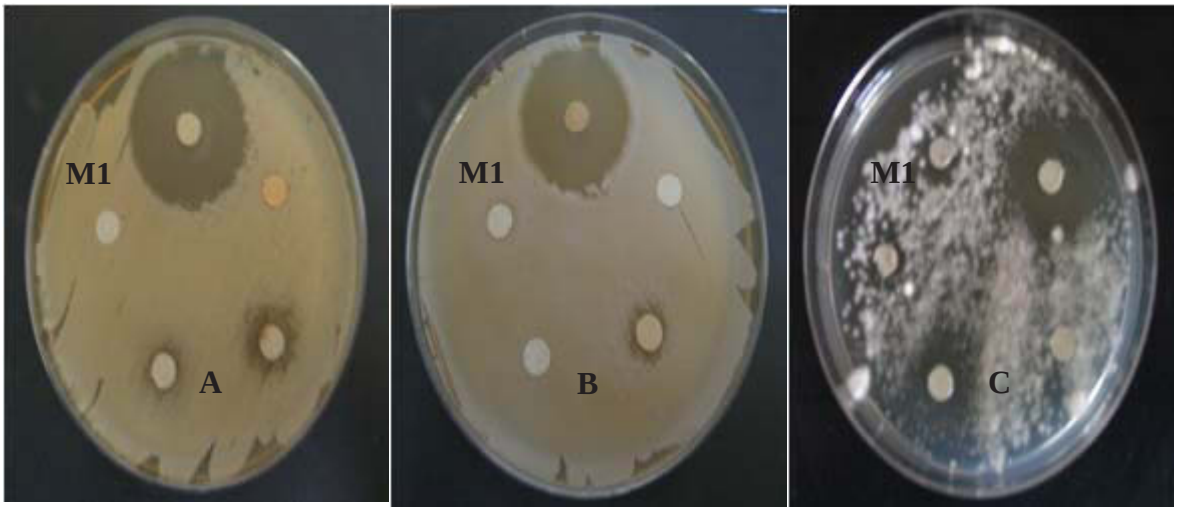


Fig. 6. Inhibition zones exhibited by the purified M1 compound against (A) *E. coli* (B) *S. aureus*, and (C) *M. gypseum*

(Fig. 6). The isolated M1 compound did not show any toxicity against human RBC.

Discussion

Fungi in general are a good source for antimicrobial agents [7,11]. Few reports on the bioactive secondary metabolites of *M. verrucaria* are available, for example, Myrocin C compound has been isolated from this fungal species and found to be effective against gram- positive bacteria [15] while the species *M. cinctum* produces an antifungal component which inhibits the formation of fungal cell wall [10]. The present study revealed that the purified extract (M1 compound) of *M. verrucaria* exhibited an inhibitory action against both bacterial strains *E. coli* and *S. aureus.* The production of metabolic substances by fungi, in general, is often affected by various growth conditional factors mainly the fermentation medium [19]. Nonetheless, a liquid state fermentation medium used in the present study is efficient for a mass production of metabolite by the selected fungus. These findings are in concomitant with the previous studies [7,16,17] examined some higher fungi. Meantime, the isolated M1 compound showed a good bioactivity against the dermatophyte *M. gypseum.* This dermatophytic species is frequently isolated from patients with skin infections in southern Iraq [14]. The purified M1 compound is more likely to be chemically related to ketone group [1,13] based upon their chemical structural verification by using H^1NMR and GC-Mass techniques.

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References

1. **Al-Fatimi, M.A., Julich, W.D., Janse, N R., Lindequist, U. (2006).** Bioactive components of the traditionary used mushroom *Podaixis pistillaris*. *eCAM*. 3: 87-92.
2. **Anke, T., Werle, A., Kappe, R., Sterne, R.O. (2004).** Laschiatrion, a new antifungal agent from a Favolaschia species (Basidiomycetes) active against human pathogen. J. Antibiot. 57: 496- 501.
3. **Casals, J.B. (1979).** Tablet sensitivity of pathogenic fungi. J. Clin. Pathol. 32: 719-722.

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4. **Domsch, H., Gams, W., Enderson, T. (1980).** Compendium of soil fungi. Academic Press, London,
5. **Gardiner, D.M., Waring, P., Howlett, B.T. (2005).** The epipolythioxopiperazine (ETP) class of fungal toxins: Distribution, mode of action, function and biosynthesis. J. Microbiol. 151: 1021-31.
6. **Greiner, U.H., Skrobranek, M.B., Estemaier, L.M., Kammerloher, W., Freund, C., Hartman, G.R. (1991).** Isolation and properties of a nitrite hydratase from soil fungus *Myrothecium verrucaria* that is highly specific for the fertilizer **c**yanomide and cloning of its gene. Proc Nat Acad Sci. 88: 4260-64.
7. **Janes, D., Kreft, S., Jurc. M., Seme, K., Strukelj, B. (2007).** Antibacterial activity in higher fungi: Mushrooms and endophytic fungi from Slovenia. Pharma. Biol. 45: 700-7006.
8. **Jonathan, S.G., Fasidi, I.O. (2003).** Effect of carbon, nitrogen and mineral sources on growth of *Psathyerella atroumbonata.* Afric. J. Biomed. Res. 6: 85-90.
9. **Kettering, M., Valdivia, C., Sterner, O., Anke, T., Thines, E. (2005).** Heptemerone A- G, seven novel diterpenoids from Coprinus heptemerus; producing organism, fermentation, isolation and biological activities. J. Antibiot. 58: 390-396.
10. **Kobyashi, M., Kanasaki, R., Ezaki, M., Sakamoto, K., Takase, S., Fujie, A., Hino, M., Hori, Y. (2004).** FR227244, a novel antibiotic from *Myrothecium cinctum* No. 002, 1. Taxonomy, fermentation, isolation and physic-chemical properties. J Antibiot. 57: 780-87.
11. **Lindequist, U., Niedermeyer, T.H.J., Julich, W. (2005).** The pharmacological potential of mushrooms. eCAM 2: 285-299.
12. **McGinnis, M.R. (1980).** Laboratory Handbook of Medical Mycology, Academic Press, New York.
13. **Mothana, R.A., Jansen, R., Julich, W.D., Lindequis, U. (2000).** Ganomycin A and B, a new antimicrobial farnesly hydroquinones from the basidiomycetes *Ganoderma Pfeiffer.* J. Nat. Produc. 63: 416-418.
14. **Muhsin, T.M., Al-Duboon, A.H., Al-Rubiay, K.K. (1997).** Characteristics of dermatophytes in Basrah, Iraq. Mycoses 42: 335-39.
15. **Nakagawa, M., Hsu, Y.H., Hiroto, A., Shima, S., Nakayama, M. (1989).** Myrocin C, a new diterpene antitumor antibiotic from *Myrothecium verrucaria*. 1. Taxonomy of the producing strain, fermentation, isolation and biological properties. J. Antibiot. 23; 219-22.
16. **Roberts, L.M. (2004).** Australian *Ganoderma* identification, growth and antibacterial properties. Ph.D.Thesis, Swinburne University of Technology. Australia.
17. **Stamets, P. (2002).** Novel antimicrobials from mushrooms. Am Bot. 54: 155-65.
18. **Strohl, W.R. (1977).** Industrial antibiotics: Today and the future. In: Strohl WR, (ed) Biotechnology of antibiotics. Marcell Dekker NY.
19. **Vahidi, H., Kobarfard, F., Namjoyan, F. (2004).** Effect of cultivation conditions on growth and antifungal activity of *Mycena leptocephala*. Afic. J. Biotech. 3: 608-9.
20. **Xian-guo H., Ursula, M. (1994).** Antifungal compound from *Solanum nigrescens.* J. Enthopharm. 43: 173-177.
21. **Zure, E. (2001).** Isolation, structure determination and biological activity assessment of secondary metabolites from marine derived fungi. Ph.D. Thesis, Von Claudia Osterhage. 186 pp.

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