Antifungal Activity of the Ethanolic Extract of *Nitella hyalina*

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Summary

The Antifungal activity of the ethanol extract of *Nitella hyalina* was evaluated at two different concentrations by the diffusion method. The ethanol extract of the *Nitella hyalina* shows antifungal activity at varied levels in Yeasts (*Candida albicans, C. parapsilosis) and* Dermatophytes (*Trichophyton rubru, T. mentagrophytes).* The yeast *Candida albicans* was found to be more active and *C. parapsilosis* was found to be less active in inhibition zone. **Dermatophytes** *Trichophyton rubrum and T. mentagrophytes* also shows Antifungal activity. *T. mentagrophytes* was found to be more active than reference inhibition zone.

Keywords *Nitella hyalina*, Antifungal activity, extract.

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Introduction

Marine medicine represents one of the most important fields of marine medicine all over the world. To promote the proper use of marine medicine and to determine their potential as sources for new drugs, it is essential to study medicinal bacteria. Over the past 20 years, there has been an increased interest in the investigation of natural materials as sources of new antifungal agents. Different extracts from marine medicinal bacteria have been tested to identify the source of the therapeutic effects. As a result some natural products have been approved as new antifungal drugs, but there is still an urgent need to identify novel substances that are active towards pathogens with high resistance. Recently, multiple drug resistance has developed due to indiscriminate use of commercial antifungal drugs commonly used in the treatment of fungal infectious diseases making it a global growing-problem. Isolation of antifungal agents less susceptible to regular antifungal and recovery of increasing resistant isolates during antifungal therapy is rising throughout the world which highlights the need for new principles. Natural products, a new source of antifungal agents shows possibly novel mechanisms of action.

Contrary to the synthetic drugs, antifungal of marine bacteria origin are not associated with many side effects and have an enormous therapeutic potential to heal many infectious diseases. For example, vincristine (an antitumor drug), digitalis (a heart regulator), and ephedrine (a bronchodilator used to decrease respiratory congestion) were all originally discovered through research on marine bacteria. This paper reports to study the antifungal activity of Indian marine bacteria, *Nitella hyalina* against an array of human pathogens. The ethanol extract of the *Nitella hyalina* was evaluated for the potential antifungal property. The selection of this plant for evaluation was based on its traditional usage. The marine bacteria posseses high content of active compound by HPLC methods and the Antifungal activity of the crude ethanol extract of *Nitella hyalina* was evaluated at two different concentrations by the diffusion method.

Materials and Methods

Marine bacteria

Nitella hyalina marine bacteria were collected in marine water from Konkan, Maharashtra, India. The dried marine bacteria were homogenized to fine powder and further subjected to extraction.

Crude Extraction

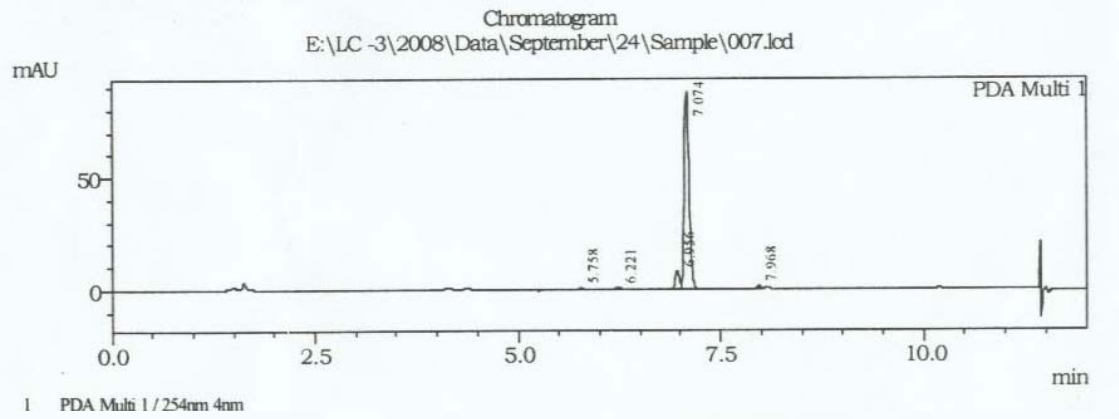
The crude ethanol extract was obtained by extracting 15gm of dried powder in 150 ml ethanol and was kept on a rotary shaker for 24hrs. The extract was filtered, centrifuged at 5000rpm for 15 min and was dried under reduced pressure. The yield obtained for ethanol extract was 40.10% with respect to the initial dry material. The extract was stored at 5^{o}C in airtight bottles.

HPLC spectrum of ethanol extract of *Nitella hyalina*

HPLC was applied for testing the presence of number of organic compounds available of ethanol extract of *Nitella hyalina* One of the major organic component with 90.274% and 7.074 retention time may have antifungal activity.

Method

Column : YMC ODS 150 mm × 4.6 mm × 5 µ , ID: E-AC-1/06/COL/013
Mobile Phase:
A: 0.05 % TFA in Water /B: 0.05 % TFA in Acetonitrile
Inj. Vol: 10 µL , Col. Temp.: 30°C, Flow rate: 1.4 mL/min
Gradient:
5% to 95 % B in 8 min, Hold for 1.5 min ,9.51-12 min 05 % B



PeakTable

PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Area %	Peak Purity Index
1	5.758	896	0.239	Cannot be calculated
2	6.221	1788	0.476	0.99990
3	6.956	30592	8.143	0.56194
4	7.074	339130	90.274	0.99926
5	7.968	3260	0.868	0.99904
Total		375668	100.000	

Antifungal investigation

Antifungal activity of *Nitella hyalina* extract was tested by the agar diffusion method described in *European Pharmacopoeia* .Minimum inhibitory concentration (*MIC*) was determined using the serial two-fold broth dilution method described by Pepeljnjak *et al.* . For the agar diffusion method, 25 ± 2 mL of sterile and melted SGA at 45–50°C was inoculated with 1 mL of approximately 1to5 x 10^6 CFU mL^{-1} of yeast cells or conidia and mycelial structures of dermatophytes in sterile physiological saline in Petri dishes (9cm). Inoculum density was measured with McFarland's standard solution of freshly prepared barium sulfate in sterile water; density of 0.01% $BaCl_2$ in 1% H_2SO_4 solution equals approximately 3 x 10^8 cells mL^{-1} . After drying SGA at room temperature for a maximum of 30 minutes, holes of 6 mm in diameter were made with stainless steel cylinders and filled (60 µL) with the ethanol extract. One experiment was done in a Petri dish for one fungal strain. Plates were then incubated at +4°C for 1 hour and at 25 ± 2°C for 48hrs for yeasts and 7 days for dermatophytes. After the incubation period, inhibition zones were measured and expressed in mm. Minimum inhibitory concentartion (*MIC*) and minimum fungicidal concentration (*MFC*) were determined using the serial broth dilution method described by Pepeljnjak *et al.* . *MIC* was determined by the broth two-fold macro dilution method in Sabouraud 2% glucose broth starting with 50% (*V/V*) of the fluid extract or essential oil. *MIC* is defined as the lowest concentration of extract or essential oil that allows no more than 20% growth of the fungi, visualized as a reduced number of colonies after removing the loop with approx. 10 µL of each dilution on SGA and incubation at 25 ±2°C for 72 hrs for yeasts and 7 days for dermatophytes. *MFC* is defined as the lowest concentration of the extract that completely inhibited the growth of fungi.

Results and Conclusions

In the *Nitella hyalina* sample studied, HPLC was applied for testing the presence of number of organic compounds available of ethanol extract *Nitella hyalina*. Antifungal activity is shown in Tables I and II. It shows antifungal activity against all the species of dermatophytes investigated, with inhibition zones from 20 to 27 mm. The largest inhibition zone was observed against *T. mentagrophytes* (27 mm). The ethanol extract *Nitella hyalina* of extract had a well-balanced antifungal effect on the fungi species *Candida albicans, C. parapsilosis* with an inhibition zone of 14 to 16 mm. The ethanol extract *Nitella hyalina*of extract shows antifungal activity against all species of dermatophytes studied, with inhibition zones of 22 mm *Trichophyton rubrum* and 27 mm (*Trichophyton mentagrophytes*), All the fungi investigated are sensitive to the ethanol extract *Nitella hyalina*. inhibition of the growth of fungi was observed both on the surface and in the depth of the agar during the experiment when the ethanol extract *Nitella hyalina* was dropped into the hole (60 μL) of the Petri dish: concentration of the ethanol extract *Nitella hyalina* aerosol in the Petri dish was 0.9 μL cm^{-3}, with an incubation temperature of 25±2°C. Data on minimum inhibitory concentrations and mimimum fungicidal concentrations for the ethanol extract *Nitella hyaline*
The antifungal activity of the ethanol extract *Nitella hyalina* was testes invitro. Yeasts *Candida albicans, C. parapsilosis* and Dermatocytes *Trichophyton rubrum, T. mentagrophytes* showed more potent antifungal activity. . The extract inhibited the growth of Yeasts and dermatophytes in lower concentrations than the extract itself.

Table I. Antifungal activity of anise ethanol extract *Nitella hyalina* by the diffusion method

	Fungi	Inhibition Zone (mm)* Reference substance	ethanol extract
Yeasts	*Candida albicans*	18 mm	16 mm
	C. parapsilosis	18 mm	14 mm
Dermatophytes	*Trichophyton rubrum*	21 mm	22 mm
	T. mentagrophytes	24 mm	27 mm

a: Nistatin as reference substance (0.5 mg mL^{-1}) for yeasts and ketoconazole for dermatophytes.
b: GPA – growth promotion activity.
c: AE – fungicidal influence of aerosol (0.7 μL cm–3).

Table II. Antifungal activity of anise ethanol extract *Nitella hyalina* by the dilution method

	Fungi	Inhibition Zone (mm)* Reference substance (μg mL^{-1})		ethanol extract (%, v/v)	
		MICb	*MFCc*	*MIC*	*MFC*
Yeasts	*Candida albicans*	0.5	1	18	26
	C. parapsilosis	1	2	17	25
Dermatophytes	*Trichophyton rubrum*	0.25	0.5	1.4	4
	T. mentagrophytes	0.50	1	8.0	18

a Nistatin as reference antimycotic against yeasts tested and ketoconazole against dermatophytes tested.
b *MIC* – minimum inhibitory concentration.
c *MFC* – minimum fungicidal concentration.
d Significantly different from fluid extract ($p < 0.01$).
nt – not tested.

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