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Isolation and Characterization of New Antioxidant and Antibacterial Compounds from Algicolous Marine Fungus Curvularia Tuberculata

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***Abstract*—New trends in drug discovery from the natural sources emphasize further investigation of the marine ecosystem to explore numerous complex and novel chemical entities. These entities are the sources of new leads for treatment of many diseases like cancer, inflammatory conditions and a large variety of viral, bacterial and fungal infections. Because of extreme physical and chemical conditions in marine environment, the organisms produce a variety of molecules with unique structural features, which exhibit various biological activities. Twelve fungal strains were isolated from the Mandabam region of Tamilnadu Coast and the antioxidant activity was estimated. Antibacterial activity was tested against the Gram positve and Gram negative bacterial strains. The chemical structure of the most active Ethyl acetate extract was elucidated using IR and NMR. The analysis reveals its functional group to be similar to that of a macrolide which necessitates further study.**

Keywords-Marine fungi, Curvularia tuberculata, antioxidant, purification, 1D NMR analysis

I. INTRODUCTION

Marine-derived fungi are rich sources of chemically diverse products with a broad range of biological activities. Marine microorganisms, particularly marine fungi, have recently gained prominence as an important source of biologically active secondary metabolites [1]. Among marine fungi, those living in association with marine algae are of promising source of natural products due to the special ecological niche in which they exist. The association between algae and fungi appears to be highly developed since nearly one-third of all higher marine fungi described are so-called algicolous or algae-associated organisms [2]. Recently, unique biologically active metabolites such as, Apralactone A(1)-14 membered phenylaceticacid, macrolactone from *Curvularia* sp. [3], Curvupallides (alkaloids A, B and C) from *Curvularia pallescens* [4], Curvularin from *Curvularia oryzae* [5], Lunatin (1, 3, 8-trihydroxy-6-methoxyanthraquinone) from *Curvularia lunata* [6] are identified.

II. METHODOLOGY

A. Experimental section

Ethyl acetate soluble extracts were collected from extra cellular culture filtrates of *Curvularia tuberculata* and tested for their antibacterial activity against strains of *Staphylococcus aureus* (NCIM 5021), *Escherichia coli* (NCIM 2931) and *Pseudomonas aeruginosa* (NCIM 5029) obtained from the National Chemical Laboratory (NCL) Pune, India, were cultured in Luria-Bertani (LB) broth (Difco, Detroit, MI, USA, 0446-17-3) at 378C for 24 h. Sterile Mueller-Hinton agar plates were used for the bioassay. Extracts producing a clear zone in the turbid agar were recorded as positive

Hydroxyl radical scavenging assay and total reducing power assay were performed to determine the antioxidant potential of the fungus. Thin layer chromatography was performed to separate the compounds from the crude extract. The combined organic extracts were subjected to a silica gel column (100-200 mesh) as stationary phase and eluted with mobile phase (EA: H) under atmospheric pressure.

B. Characterization of the purified fraction (CT516 – E4)

The ^{1}H NMR, ^{13}C NMR and DEPT NMR data was recorded on a Bruker Avance III 500MHz NMR spectroscopy using $CDCl_3$ as solvent. An IR spectrum was obtained with the help of Perkin Elmer Spectrum on FT-IR spectrometer.

III. RESULTS

The antibacterial and antioxidant activities of the twelve strains are listed in Table 1. The crude ethyl acetate extract inhibited all three test bacteria. The 20µg of the crude compound (CT516-E4) showed 62.15 ± 4.97 % of inhibition in total reducing power assay, 11.69 ± 0.53 % of inhibition in Hydroxyl radical scavenging assay. CT516-E4 was further characterised by 1D NMR analysis and IR. Further 2D analysis has to be done for complete structural elucidation of the compound.

The proton NMR spectrum of compound 516-E4 shows that it has eight sets of protons (aliphatic). The ^{13}C NMR spectrum of compound CT516-E4 shows 11 carbon atoms. ^{13}C DEPT NMR shows a total of four primary and tertiary carbons.

The IR spectrum showed the functional group peaks at 3445 – NH, OH group, 2872 – C-H stretching, 1969- β lactone, 1668 – six or seven membered lactam ring, 1,3 diketone, 1506 – C-C or C=N stretching, 1456 - β -lactone, 1374 - Cyclic unconjugation anhydride, 1301 – C-O stretching or C-N stretching, 1252 – CH_2Br, 1112 – Free OH.

REFERENCES

[1] G.M. Konig and A.D. Wright, "Marine Natural Products Research: Current Directions and Future Potentia," Planta Med, 62, 1996, 193-211.

[2] J. Kohlmeyer and E. Kohlmeyer, In Marine Mycology, The Higher Fungi; Academic Press: New York, 1979; pp 54-69.

[3] H. Greve, P. Schupp J. Eguereva, E. Kehraus and S. Konig GM, "Apralactone A and a New Stereochemical Class of Curvularins from the Marine Fungus Curvularia sp," Europian Journal Organic Chemistry, 2008. 5085–5092.

[4] W.R. Abraham, and M. Meyer, "Curvupallides, A New Class of Alkaloids from the Fungus Curvularia pallescens," Tetrahedron, 51, 1995, 4947-4952.

[5] S. Busi, P. Peddikotla, S.M. Upadyayula and V. Yenamandra, "Secondary Metabolites of Curvularia oryzae MTCC 2605," Recent Trends in Natural Products. 2009. 3, 204-208.

[6] S. Jadulco, G. Brauers, R.A. Edrada, R. Ebel, S.S. Wray and B. Proksch, "New Metabolites from Sponge-Derived Fungi Curvularia lunata and Cladosporium herbarum,". Journal of Natural Products, 65, 2002, 730–733.

TABLE I. ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY OF THE TWELVE TESTED FUNGUS.

Fungus	**Hydroxyl Radical Scavenging assay (%)**	**Total reducing power assay (%)**	**Anti bacterial activity**		
			S. aureus	***E. coli***	***P. aeruginosa***
Nigrospora sp.1	11.992+0.9594	19.63+1.570	+++	+++	+++
Cladosporium sp.	70.69+3.66	22.84+1.72	+++	+++	++
Pestalotiopsis sp.1	63.7672+6.377	32.87+3.287	+++	+++	+++
Curvularia tuberculata	29.886+2.391	13.22+1.454	++	+++	+++
Cladosporium sp.	No activity	8.229+0.6583	++	-	++
Humicola sp.1	74.979+6.748	23.12+2.081	-	++	+++
Fusarium sp.	43.09+3.44	6.79+0.51	+++	+++	+++
Paecilomyces sp. 1	No activity	6.32+0.6320	+++	+++	+++
Curvularia tuberculata	**11.69+0.53**	**62.15+4.97**	+++	+++	+++
Fusarium sp.	1.07+0.04	18.02+1.10	+++	+++	+++

Aspergillus terreus	13.72+1.09	18.71+1.80	+++	+++	+++
Penicillium sp.	11.72+0.86	9.56+0.76	+++	+++	+++

Note:

+++ - Obvious inhibition zone

++ - Slight inhibition zone

\+ - Faint inhibition zone

\- - No inhibiton zone