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CHARACTERIZATION OF DIFFERENT SECONDARY METABOLITES FROM *TRIMATOSTROMMA SCUTELLARE* BY GC-MS.

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ABSTRACT

Filamentous fungi are remarkable organisms that readily produce a wide range of natural products there are often called as secondary metabolites.These compounds are very diverse in structure and functions. So for characterization of novel bioactives, the fungus *Trimatostromma scutellare* is isolated and identified. Thus the isolated fungus was extracted with variety of solvents of different polarity such as ethyl acetate, dichloromethane, chloroform and hexane for isolation of bioactive compounds by GC-MS. In hexane 1, 3, 5-Triazine, Benzophenone dimethyl ketal, Adipic acid, 1, 2-Benzenedicarboxylic acid were isolated.In chloroform extract 1-Cyclohexyl ethylamine, Pentanoic acid, 1-nonadecene, Hexanedioic acid, 1, 2-Benzenedicarboxylic acid. In Dichloromethane extract, 9-Eicosene, Anthraquinone,20-Methyl- heneicosane-1, 2diol. 1, 2-Benzenedicarboxylic acid were identified. In Ethyl acetate extract, 2-Acetyl 5-methyl thiophene, Pentanoic acid, 3-octadecene, Hexanedioic acid, Silane, 1, 2-Benzenedicarboxylic acid was identified.Thus the basic study is focused to identify the presence of bioactive compounds in *Trimatostromma scutellare* by GC-MS.

KEYWORDS: *Trimatostromma scutellare*, GC-MS, Ethyl acetate, Dichloromethane, Chloroform and Hexane.

INTRODUCTION

During mycological investigation of Melghat Forest of Amravati District, the authors collected and reported many rare and interesting fungal forms they arc new and rare to Maharashtra.[1,2] The anamorphic fungi are a group of microfungi that reproduced asexually.[3] The Hyphomycetes produce conidia directly from vegetative structures or on distinct conidiophores.

The genus was established by Corda . Hughes discussed and stressed the importance of conidia development in this rare genus. The present genus is also reported as a lichonicolous species[6] however later on Hawksworth and Cole separated lichonicolous species and raised it to genus level, because of lichonicolous character. Sutton and Ganpathi have described a new species of *Trimmatostroma excentricum* from New Zealand Fiji. *Trimatostromma* species produced colonies pulvinate, scattered, mycelium pale olivaceous, branched, septate. Conidiophores simple, short bearing conidia terminally. Conidia variable in shaped but mostly subspherical with cross and longitudinal septa.

The objective of the present study deals with morphological identification of the species of *Trimatostromma* as well as isolation of secondary metabolites by using different solvent (nonpolar, medium polar and highly polar).

MATERIALS AND METHODS

Morphology

Decaying leaves and stems of different plants were collected from Melghat forest. Samples were wrapped in butter paper and place in envelop and brought to the laboratory for examination. They were cut in small pieces and incubated in plastic containers lined with moist filter paper. Slides were prepared using lactophenol cotton-blue as mounting medium. Slides were observed and fungi identified on the basis of morphological characteristics and with relevant literature.[9]

Sample Preparation For Scanning Electron Microscopy

For Scanning Electron microscopic study, fungal samples obtained from pure culture were dehydrated in pure ethyl alcohol. The specimens were pasted using both sided adhesive tapes on the surface of aluminium stub and submitted to the metallization with platinum using sputter coater (Jeol Autofine coater), in order to increase its conductivity. Observations and microphotographs were made with a Jeol 6830A Scanning Electron Microscope. Scanning

Electron microscopic examination was conducted at the Metallurgical and Material Engineering Department of Visvesvaraya National Institute of Technology, Nagpur (India).

Gas Chromatography - Mass Spectroscopy Analysis

Preparation of Extract

After 8 days, colonics from PDA were transfer to Potato Dextrose broth for about 8-10 days. Mycelium mat was observed after10 days. Mycelium was removed from the conical flask, the media used for extraction of different metabolites.

For extraction of secondary metabolites three extraction solvents were used ethyl acetate, dichloromethane and chloroform (non polar, middle polar and polar). 25 ml of medium added for separating funnel to which 50 ml of ethylacelate was added and after shaking two layers of solvent observed. Transparent layer was separate out to which sodium sulphate was added to whole moisture get removed. Extract was kept in incubator at about 37^0C until solvents get evaporated. Compounds were then dissolved in pure methanol for GCMS analysis. The procedure was repeated for dichloromethane and chloroform.

The analysis was carried out using gas chromatography – high resolution mass spectrophotometer. All samples were worked out at Sophisticated Analytical Instrument Facility (SAIF), Indian Institute of Technology (IIT), Powai, Mumbai, Maharashtra, India. 2 µl of previously concentrated extract solution was employed for GC – MS analysis. The GC-MS analysis was carried out using Alegent Hp 7880 with column of 30 meter length, 0.25 mm ID, 0.32 thickness. Helium gas was used as carrier gas at constant flow rate of 1 ml/ minute. Injector temperature was set at 100^0C. The oven temperature was programmed from 50^0C to 280^0 C at 10^0 C/minute to 200^0C then 10^0C/3 minutes to 250^0C ending with a 5 minutes isothermal at 280^0C. The sample was injected in split mode as 50:1. Three solvents used for preparing the sample for GC-MS analyses namely ethyl acetate, dichloromethane and chloroform. Three samples of crude extract were analyze by Gas Chromatography-Mass Spectroscopy.

RESULTS

Morphology of *Trimmatostroma*

Trimmatostroma scutellare (Berk and Br.) Ellis

Colonies effuse pale brown to moderate brown, superficial 50-65mm diameter on PDA in two weeks. Mycelium subhyaline to pale brown, 1-2 µm thick. Conidiophore solitary,

smooth, simple, septate, subhyaline or brown, subcylindrical or occasionally strongly inflated, 30-50 µm long, 2-4 µm thick. Conidia born at the tip of conidiophores oblong, pyriform or obovate to subglobose, fertile hyphae often remain attached, multicellular 13.5-32.3µm × 29-39.5 µm, dark brown to almost black.

Matrix: On the twig of *Buchanania Lanzan* Spreng. (Anacardiaceae) collected from Ghatang, Semadoh (01/10/12), NFCCI No.- 3204.

Remark: The specimen understudy was found on comparison to be allied to a *Trimmatostroma scutellare,* hence assigned to the same, recorded as new for this region.

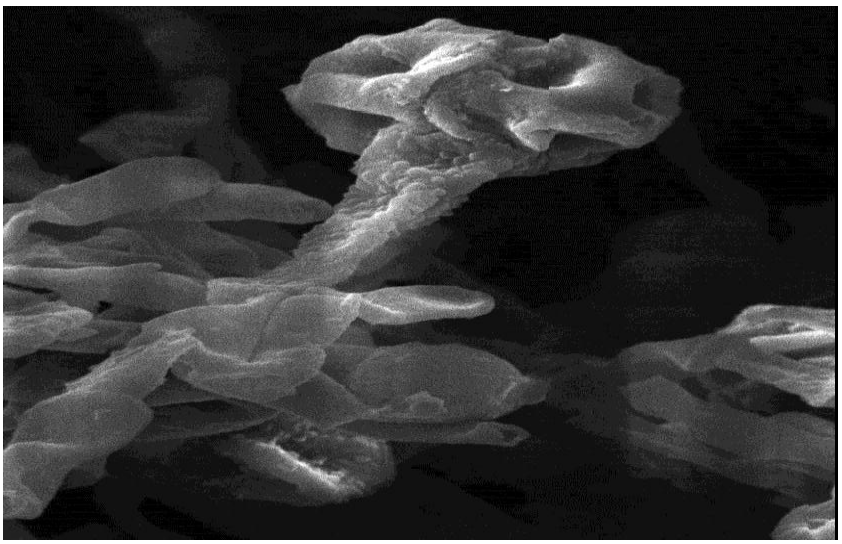

Figure 1: SEM microphotograph of *Trimatostromma scutellare* showing mycelium with conidia (4000 X).

Gas Chromatography – Mass Spectroscopy Analysis: The principle aim of this study was to develop a simple and reliable method for investigation of secondary metabolites excreted by the cultured *T. scutellare.* The fungus was culture and incubated to avoid oxygen limitation during fungus growth and then allow samples for extraction, chromatographic separation and spectra detection. The GCMS data were interpreted using NIST library, results were matched with entries in the library of chemical compounds.

Table 1: Identified compounds present in Ethyl acetate extract of *Trimmatostroma scutellare*.

Sr. No.	R.T.	Name of compound	Relative %	MF	MW
1	3.1	R-(-)-Cyclohexyletylamine	0.80	$C_8H_{17}N$	127
2	7.4	2-Acetyl-5-methylthiophene	1.87	C_7H_8OS	140
3	11.5	Pentanoic acid, 5-hydroxy- 2,4-di-t-butylphenyl ester	7.71	$C_{19}H_{30}O_3$	306
4	13.0	3-Octdecene	3.78	$C_{18}H_{36}$	252
5	15.7	1-Nonadecene	11.50	$C_{19}H_{38}$	266
6	18.3	3-Octadecene	6.18	$C_{18}H_{36}$	252
7	22.5	3- Eicosene	2.00	$C_{20}H_{40}$	280
8	24.1	1,2-Benezendicarboxylic acid mono,(2-ethylhexyl) ester	62.65	$C_{16}H_{22}O_4$	278

Table 2: Identified compounds present in Dichloromethane extract of *Trimmatostroma scutellare*.

Sr. No.	R.T.	Name of compound	Relative %	MF	MW
1	15.7	1-Nonadecene	0.86	$C_{19}H_{38}$	266
2	18.3	3-Octadecene	1.57	$C_{18}H_{36}$	252
3	22.7	Antraquinone,1- (o- chlorophenyl)	3.02	$C_{20}H_{11}ClO_2$	300
4	24.1	1,2-Benezendicarboxylic acid mono,(2-ethylhexyl) ester	7.30	$C_{16}H_{22}O_4$	278
5	27.9	20- methyl-heneicosane-1,2-diol	14.04	$C_{25}H_{50}O_2$	382

Table 3: Identified compounds present in Chloroform extract of *Trimmatostroma scutellare*.

Sr. No.	R.T.	Name of compound	Relative %	MF	MW
1	3.1	R-(-)-Cyclohexyletylamine	0.96	$C_8H_{17}N$	127
2	11.5	Pentanoic acid, 5-hydroxy- 2,4-di-t-butylphenyl ester	8.37	$C_{19}H_{30}O_3$	306
3	15.7	1-Nonadecene	12.92	$C_{19}H_{38}$	266
4	18.3	3-Octadecene	14.29	$C_{18}H_{36}$	252
5	22.7	Anthraquinone,1-(o-chlorophenyl)-	4.91	$C_{20}H_{11}ClO_2$	300
6	24.1	1,2-Benezendicarboxylic acid mono,(2-ethylhexyl) ester	58.52	$C_{16}H_{22}O_4$	278

T.scutellare grown on PDB media produced a wide variety of metabolites. Near about 19 chromatographic signals could be detected and all compounds were identified. After analysis

of sample signals, the most significant signals with high abundance are listed in Table1, 2, 3. Numerous minor peaks were observed and could not be identified. Thus saturated and unsaturated hydrocarbons, alcohols, aromatic hydrocarbons, esters, aldehyde ketons,etc. could be detected. The compound classes of aldehyde, alcohols, ketones, esters, phenols from the fungal origin have been previously reviewed.

DISCUSSION

The present genus is also reported as a lichonicolous species[6] however later on Hawksworth and Cole (2002) separated lichonicolous species and raised it to genus level, because of lichonicolous character. Sutton and Ganpathi (1978) have described a new species of *Trimmatostroma excentricum.*

Morphology of *Trimmatostroma* shows variation in size of conidia *T. scutellare* was found on the dead twig of Nilgiri, which showed moderate brown to dark brown colony, mycelium subhyaline, conidiophore irregularly branched, septate, conidia obovate to subglobose, multicellular, dark brown, 13.5-32.3µm in diameter these observations were in agreement with earlier workers.[8,9]

The metabolites found in *Trimmatostroma scutellare* include R-(-)-Cyclohexyletylamine; 2-Acetyl-5-methylthiophene; Pentanoic acid, 5-hydroxy- 2,4- di-t-butylphenyl ester; 3-Eicosene; octadecene etc. in ethylactate extract(Table1) while, Antraquinone, 1-(o-chlorophenyl); 20- methyl-heneicosane-1,2-diol; 1,2-Benezendicarboxylic acid mono,(2-ethylhexyl) ester; 1-Nonadecene; 3- Octadecene found in dichloromethane extract (Table 2). 1,2-Benezendicarboxylic acid mono, (2-ethylhexyl) ester act as gelling agents, film formers, stabilizers, dispersants, lubricants, binders, emulsifying agents, and suspending agents. End-applications include adhesives and glues, electronics, agricultural adjuvants, building materials, personal-care products, medical devices, detergents and surfactants, packaging, waxes, paints, printing inks and coatings, pharmaceuticals, food products, and textiles[10] Siddiquee *et al.*(2012) reported the separation and quantitative determination of wide variety of organic component from *Trichoderma harzianum* by GCMS which are in line with the present studies.1,2-Benezendicarboxylic acid mono,(2-ethylhexyl) ester is Triterpene in nature and show antioxidant, antitumor, cancer preventive, immunostimulant, chemo preventive, lipoxygenase-inhibitor and pesticide.[12]

CONCLUSION

Chemical studies have revealed that morphologically different species may show similarity in the metabolic requirements or vice versa. The present study may prove to be of immense use in industry, it is well known especially used as plasticizer, it is also used as base in preparation of many different cosmetics. They are important plastics additives and when added to thermoplastics they increase their flexibility, transparency, and durability.

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