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HYDROCARBONS FROM *CURVULARIA LUNATA* - A NOVEL PROMISING ENDOPHYTIC FUNGI ISOLATED FROM *SOLANUM TRILOBATUM* LINN

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ABSTRACT

The recent discovery of microorganisms that produce fuel related hydrocarbon is the other valuable application of this era that replace expensive sugar and starch utilization. The endophytic fungi were reported to produce a variety of medium-chain and highly branched volatile organic compounds (VOCs) that have been highlighted for their potential as fuel alternatives and are collectively termed as myco-diesel. In the present study, endophytic fungal strain i,e; *Curvularia lunata* was selected and were grown on different glucose enriched medium for the production of hydrocarbons. Extraction of liquid cultures of the fungus revealed the presence of numerous fatty acids and volatile organic compound by GC/MS analysis. The chemical structures of compounds were extracted through ethyl acetate solvent extraction. The results were Glycine, N-(phenylacetyl)-,ethyl ester, 4-(6-Methoxypyridazinyl-3-amino)- 1,2–naphthoquinone, Hexadecanoic acid, 14-methyl-,Methyl ester, etc., The functional groups of compounds were characterized by FT-IR analysis and the results were major peaks with intensity of 3358.21cm-1 (sp C-H stretch, sp^2 C-H stretch, alcohol O-H stretch, carboxylic acid O-H stretch, 1^o N-H_2 stretch,2^o N-H stretch), 2944.46cm-1 (Sp^3 C-H stretch, aldehyde C-H stretch, carboxylic acid O-H stretch) respectively. All of these findings have implications in energy production and utilization.

Keywords: Endophytic fungi, *Solanum trilobatum* Linn., *Curvularia lunata*, Volatile Organic Compounds, Long chain fatty acids, GC/MS, FT-IR.

INTRODUCTION

The term “endophyte” originally introduced by de Bary (1866) refers to any organisms occurring within plant tissues, distinct from the epiphytes that live on plant surfaces. Endophytic fungi regarded as fascinating group of organisms colonize the living internal tissues of their host usually higher plants that do not cause any disease symptoms rather benefits plants in their secondary metabolite production (Azevedo *et al*., 2000).

Recently, a number of endophytic fungi produce a wide variety of secondary metabolites from which a low molecular organics were derived which are known as Volatile organic compounds (Korpi *et al*., 2009) such as mono-terpenoids, alkanes, cyclohexanes, cyclopentanes, and alkyl alcohols/ketones, benzenes and polyaromatic hydrocarbons. Many of these compounds are either identical to or are closely related to those specific classes of molecules that are found in diesel. Most importantly, these organisms make hydrocarbons while utilizing cellulosic and hemicellulose polymers found in all plant-based agricultural wastes, (Strobel *et al*., 2009). The microbial resources of endophytic populations are mostly unexplored. Thus, an investigation was carried out to characterize the endophytic fungi i.e: *Curvularia lunata* with the aim to explore their bioactive potential with reference to hydrocarbons.

MATERIALS AND METHODS

i. Collection of Endophytic Fungi:

The endophytic fungal strain of *Curvularia lunata* was obtained from Endophytic Fungal Culture Collection Centre (EFCCC), Department of Biotechnology, and Bannari Amman Institute of Technology. The screening of endophytic fungi from *Solanum trilobatum* Linn. was already published (Kannan *et al* 2016) and the cultures were preserved at (EFCCC).

ii. Preparation of fungal mycelial extract:

To screen and select the hydrocarbons producing endophytic fungi among purified fungal isolates, they were cultured in Potato Dextrose broth, Potato Carrot broth, Czepak dox broth and YEPD broth respectively. The mass culture were kept in shaker for 7 days at 150 rpm at 28^{o}C.The culture broths were filtered, using cheese cloth and the culture media and mycelia were separated. The mycelia were soaked in ethyl acetate and homogenized. Metabolite extraction were done by mixture of solvents Ethyl acetate, Chloroform, Dichloromethane with the ratio of 3:2:1. Then the extracted mixture was dried in rotary evaporator to yield the crude. Crude were stored at 4°C until analysis. (Tayung *et al*., 2011).

iii. Detection of Bioactive compounds

The extracts obtained were subjected to instrumental analysis such as UV, FTIR and GC-MS to identify the bioactive compounds present in the fungal extracts. The instrumental conditions and the compounds detected by various characterizations were discussed below.

a) UV of fungal mycelial extract

Fungal mycelial crude extract were subjected to UV analysis for absorbance of hydrocarbons. Ultraviolet Spectrum were run in JASCO-UV Model name V-630 were used the Data Array Type and Linear Data Array of Data Interval 1 nmof Measurement range 800 - 200 nm Wavelength UV/Vis bandwidth at 1.5 nm and received Absorbance at (Max-Min) at Scan speed of 400 nm/min.

b) Gas Chromatography (GC) analysis:

Hydrocarbon derivatives and other composition of the strain were extracted by acid were determined by GC/MS. 1µl of crude methanol extract of the plant was subjected to analysis of its constituents using Agilent Technologies 6890N GC system coupled with JEOL Mass

spectroscopy. The sample was injected to the Agilent J&W HP-5 capillary column (30 m × 0.2 mm × 0.25 μm) fused with silica. The injection temperature was maintained at 220°C. The oven temperature of GC was programmed with an initial temperature of 50°C and increased up to 250°C at the rate of 10°C per min. Helium (He) was used as the carrier gas system with the flow rate of 1 mL/min. GC-MS interface temperature was maintained at 250°C. Identification of compounds was based on the comparison of the spectral values with the National Institute of Standards and Technology (NIST) Chemical Web book database. In addition, the peak area percentage contributed by each of the compounds detected was calculated. (Meghan *et al.,* 2010).

c) Fourier Transform Infrared Spectroscopy (FT-IR) analysis

Fungal mycelial crude were subjected to Spectroscopic analysis for identification of the functional groups of hydrocarbons. The crude extracts of *Curvularia lunata* analyzed by FTIR to know the different functional groups present in the fungal extracts. The crude fungal extracts were subjected to characterize the functional groups in FTIR principle which diffuse reflectance technique was followed. The fungal crude powder was mixed with potassium bromide (KBr) to form a very fine mixture. This mixture was then compressed into a thin pellet which was characterized. KBr was also transparent in infra-red light. The sample was irradiated by a broad spectrum of infra-red light and the level of absorbance at a particular frequency was plotted after Fourier transformation of the data. The resulting spectrum was characteristic of the organic molecule present in the sample**.** (Devi *et al.,* 2014).

RESULTS

The endophytic fungal strain of *Curvularia lunata* was obtained from Endophytic Fungal Culture Collection Centre (EFCCC), Department of Biotechnology, and Bannari Amman Institute of Technology. The culture was sub cultured, and purified and taken for further study. (Fig: 1a, b and Fig: 3).

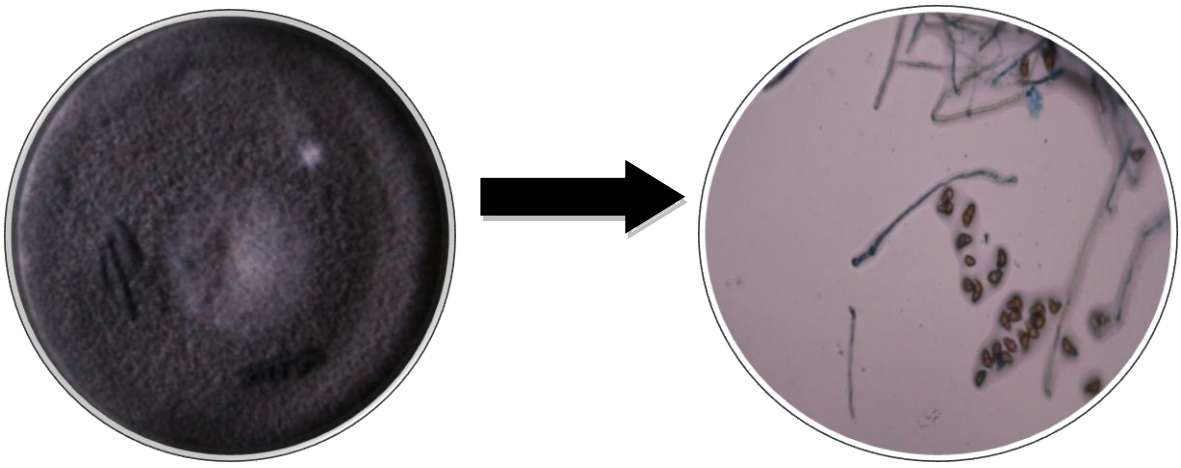

Figure 1 : *Curvularia lunata:* a. Pure culture b. Micrograph

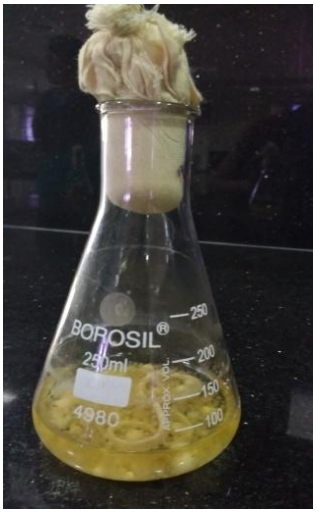

Figure 2. Mass culture of *Curvularia lunata in PDB medium*

UV analysis of crude extract from *Curvularia lunata*

The UV analysis of crude extract from *Curvularia lunata* shows maximum wavelength of absorbance at 0.049 λ Max. The minimum absorbance at the range of 800nm. The maximum absorbance at the range of 200nm is 7. This indicates the absorbance range of maximum in UV and less comparable in visible range (Fig: 3).

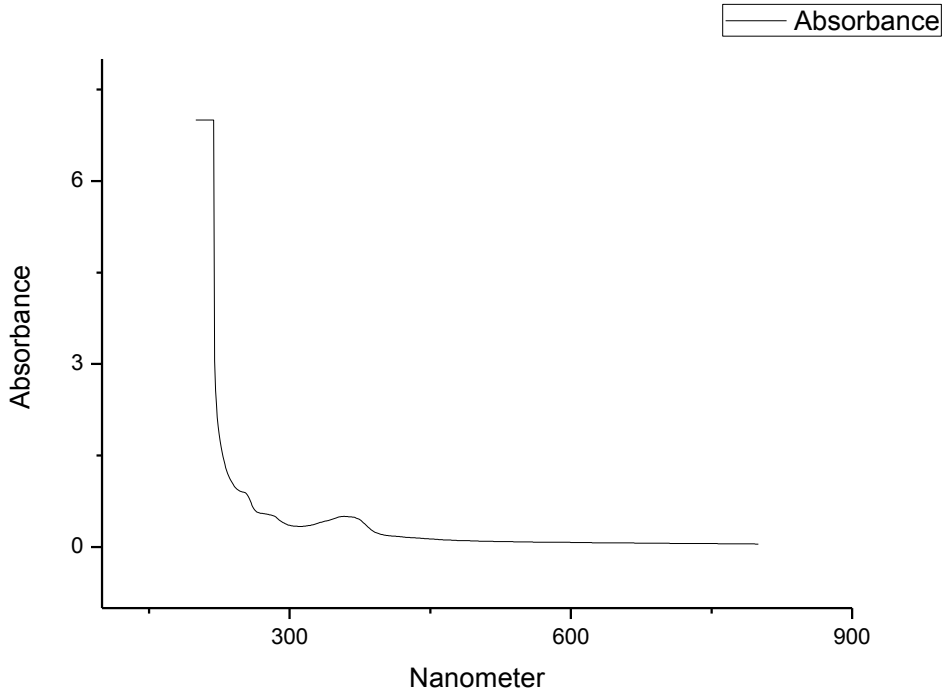

Fig 3: UV Analysis of Fungal strain *Curvularia lunata*

GC-MS analysis of *Curvularia lunata*

Interpretation on mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The chromatogram and the spectral analysis along with the name, molecular weight and structure of the components of the

endophytic fungus *Curvularia lunata.* were ascertained below in Table 2. Chemical structure of compounds present in ethyl acetate extract for *curvularia lunata* were given below (Fig: 4 and Table: 1)

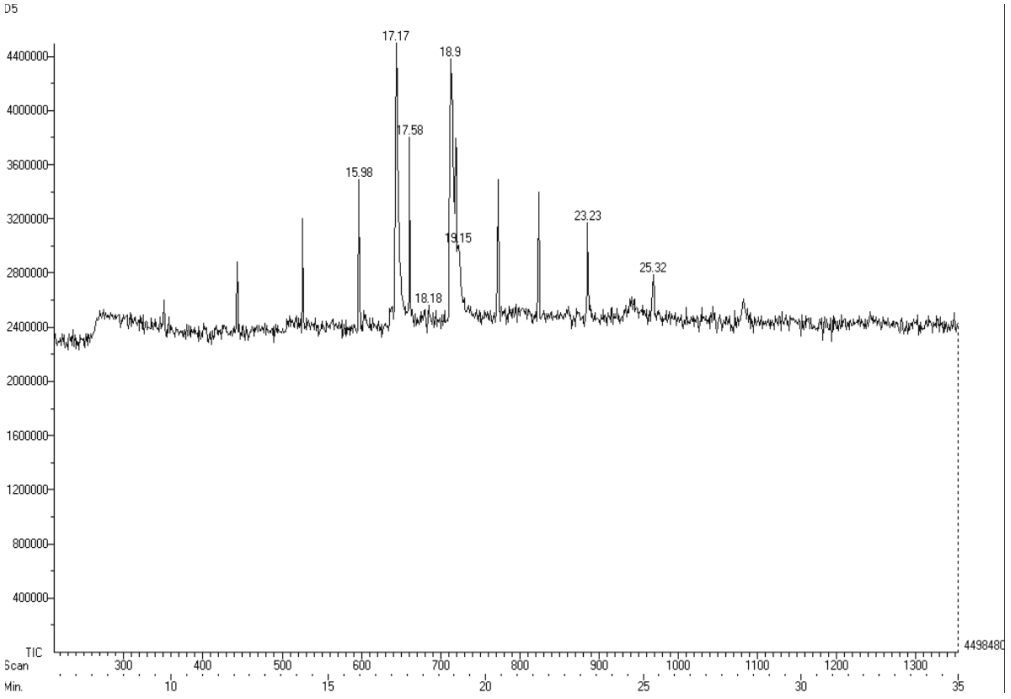

Figure 4: GC-MS analysis of endophytic fungal strain *Curvularia lunata*

Hydrocarbons present in GC-MS of fungal extract of *Curvularia lunata*

The chemical structures of compounds were extracted through ethyl acetate solvent extraction. The results were displayed in the Table 1 with retention time with the respective compound with molecular weight.

Retention Time	**Name of Compound (IUPAC)**	**Molecular Formula**	**Molecular Weight (Da)**
15.98	Glycine, N- (phenyl acetyl)-,ethyl ester	$C_{12}H_{15}NO_3$	221.252
17.17	Penta - decanoic acid,14-methyl-, methyl ester-	$C_{17}H_{34}O_2$	270. 4507
17.58	4-(6-Methoxypyridazinyl-3-amino)- 1,2 - naphthoquinone	$C_{15}H_{11}N_3O_3$	281.266
18.18	Hexa - decanoic acid, 14 – methyl -, Methyl ester	$C_{18}H_{36}O_2$	284.477
18.9	9,15-Octadecadienoic acid, methyl ester, (ZZ)-	$C_{19}H_{34}O_2$	294.472
19.15	6-Octa - decenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.4879
23.23	[3-Methyl- 2 - (4 -nitro phenyl) – 4 -oxo-1,2,3,4 tetrahydo-phthalazin-1yl], acetic acid, methyl ester	$C_{18}H_{17}N_3O_5$	355.345
25.32	Octa - decanedioic acid,3,16-dioxo-, dimethyl ester	$C_{20}H_{34}O_6$	370.48036

Table 1: Hydrocarbons present in GC-MS of fungal extract of *Curvularia lunata*

FTIR spectral analysis of *Curvularia lunata*

The FTIR spectral analysis of the ethyl acetate crude extracts of *Curvularia lunata* showed the major peaks with intensity showed below as peak and table (Fig: 4 and Table 2).

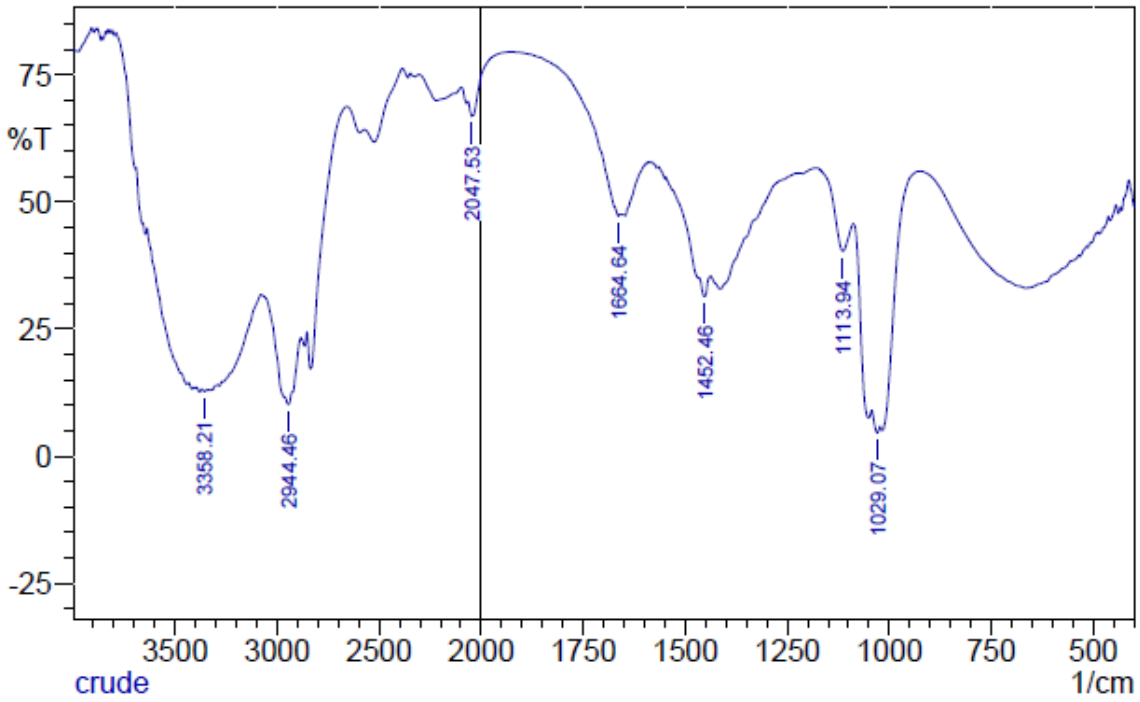

Figure 5: FT-IR analysis of selected endophytic fungal strain *Curvularia lunata*

Functional groups of Hydrocarbons present in *Curvularia lunata*

The functional groups of compounds were characterized by FT-IR analysis and the results were showed major peaks with intensity of 3358.21cm-1 (sp C-H stretch, sp^2 C-H stretch, alcohol O-H stretch, carboxylic acid O-H stretch, 1^o N-H_2 stretch,2^o N-H stretch), 2944.46cm-1 (Sp^3 C-H stretch, aldehyde C-H stretch, carboxylic acid O-H stretch) respectively (Table 2).

S. No	Wave No	Functional group
1	1029.07	alkoxy C-O, C-N
2	1113.94	alkoxy C-O, C-N, acyl C-O phenol C-O
3	1452.46	C=C stretch aromatic
4	1664.64	C=O stretch, C=N stretch, C=C stretch alkene, C=C stretch aromatic, N-H bend, nitro
5	2047.53	thiol S-H stretch
6	2944.46	Sp^3 C-H stretch, aldehyde C-H stretch, carboxylic acid O-H stretch
7	3358.21	Sp C-H stretch, sp^2 C-H stretch, alcohol O-H stretch, carboxylic acid O-H stretch, 1^o N-H2 stretch, 2^o N-H stretch

Table 2: Functional groups of Hydrocarbons present in *Curvularia lunata*

DISCUSSION

Endophytes are capable of producing biologically active compounds. In the present study the selected *Curvularia lunata* when provided with essential nutrients to explore the production of hydrocarbon derivatives showed positive results as by UV, FT-IR and GC-MS analysis.In the report, In order to determine which volatile compounds were produced by the fungus, the volatile organic compounds (VOCs) found in the GC-MS analysis of controls were removed from the list of VOCs appearing in the flask supporting fungal growth as done previously The above results were confirmed that fungal endophyte *Curvularia lunata* can produce a variety of hydrocarbon derivative similar to myco-diesel (Strobel *et al*., 2001,2006,2008). However, an examination of this approach has revealed that it was inaccurate for the study. The automated library search results generated from the NIST 2005 database spectral search were used as the only means of compound comparison between fungal products and those of the control. Due to the similarity of many alkane fragmentation patterns the automated search is not always reliable (Schulz and Dickschat, 2007).

CONCLUSION

Our findings emphasizes that other endophytes could be explored for hydrocarbons for fuel production. Further investigation on this fungus for myco-diesel production and commercial enhancement will be our future goals.

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