

## GC-MS And FTIR Analysis Of Endolichenic Fungus *Penicillium* Sp. Isolated From *Cryptothecia Striata* Of Arunachal Pradesh, India

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### ABSTRACT

In the present study GC-MS and FTIR analysis of an endolichenic fungus *Penicillium* sp. isolated from *Cryptothecia striata* was done to identify the bioactive compounds present in it. Our study indicated the presence of many functional groups as identified through FTIR analysis and GC-MS analysis of methanolic extract of *Penicillium* sp. (CRP 3a) shows the presence of eight compounds such as Phenol 3,5-Bis(1-1 Dimethyl ether), Pentanoic acid, 5-hydroxy-2,4-di-*t*-butyl phenyl esters, Pentanedioic acid, (2,4-Di-*T*-Butylphenyl ester), ethyl caryophyllate, Carissanol Dimethyl ether, Vanillin and Diethyl, 2,6-Pyridine-dicarboxylate. Some of the compounds exhibited strong antimicrobial activity and it has been hypothesized that the antimicrobial activity of lichen might be due to the associated endolichenic fungi.

**Keywords**-Endolichenic fungus, bioactive compounds, GC-MS and FTIR

### INTRODUCTION

Lichen is a symbiotic association between fungi (mycobiont) and at least chlorophyll-containing photosynthetic organism (photobiont) such as microalga, a cyanobacterium etc (Lutzoni and Miadlikowska, 2009). In addition to the mycobiont, lichen is also a home to numerous cryptic microfungi which live in close association with the lichen thallus (Arnold et al. 2009). This group of fungi, which lives inside the lichen thallus, is called as endolichenic fungi. (Arnold et al. 2009). Endolichenic fungi doesn't cause any harm to the lichen thallus, they are thus asymptomatic in nature (Arnold 2001, 2007; Petrini 1991). Many works have been done to isolate endolichenic fungi from lichen thallus. It has been found that most of the endolichenic fungi belong to Ascomycetes, Pezizomycetes etc. (Arnold et al. 2009; Girlanda et al. 1997; Kannangara et al. 2009; Li et al. 2007; Petrini et al. 1990; Suryanarayanan et al. 2005; Tripathi and Joshi 2015; U'Ren et al. 2010, 2012). Endolichenic fungi possess strong antimicrobial, antibacterial, anticancerous, anti-

inflammatory properties due to the presence of several bioactive compounds. Due to this reason research on endolichenic fungi is a highlighted area (Kellogg and Raja 2016; Suryanarayanan et al. 2017).

*Cryptothecia striata* is a crustose lichen which mainly grows on bark of trees, rocks, etc. Triterpenoids along with many important compounds have been extracted from *Cryptothecia* sp. which showed strong antimicrobial activity (Tuan et al., 2021, Devi et al. 2022). It has been hypothesized that the medicinal properties of lichens might be due to the associated endolichenic fungi. In the present study *Penicillium* sp. isolated from *Cryptothecia striata* was characterized with the help of GC-MS and FTIR analysis to identify the bioactive compounds present in it which might be responsible for the antimicrobial properties of the lichen.

## Materials and Methods

### Collection of lichen

Healthy lichen thallus was collected from Tipi region (93.61°N and 27.14° E) of Western Arunachal Pradesh which is a part of Indo-Burma belt. The identification of the lichen species was done considering anatomy and its morphology features. External morphology was studied under Leica EZ4 and Leica S9 stereozoom microscopes. Anatomical details were examined under Leica DM2500 compound microscope. Lichen substances were identified by colour spot tests following Orange et al. (2001).

### Isolation and identification of endolichenic fungi

The lichen thallus of *Cryptothecia striata* was firstly surface sterilized following standard protocol (Guo et al., 2003). The surface sterilized lichen thallus was cut into smaller fractions and were air dried. The dried surface sterilized lichen fragments were placed onto three media namely PDA (Potato Dextrose Agar), MEA (Malt extract Agar) and WA (water Agar) which were supplemented with 0.01% Streptomycin sulphate. The plates were incubated at 28°C in BOD incubator until the growth of endolichenic fungi appeared. The identified endolichenic fungi were inoculated in PDA slants and stored at 4°C.

### GC MS and FTIR analysis

GC-MS chromatography of the crude metabolite was analysed with the help of Claurus 680 Gas chromatograph and Claurus 600C Mass spectrometer with GC having Liquid Autosampler model. The phase reference was 35% diphenyl 65% dimethyl polysiloxane used as Stationary phase and Helium (He) gas was used as a mobile phase. The details of the bioactive compounds were provided by NIST (2008). Similarly the FTIR analysis for the determination of functional groups

was done with the help of Nicolet is10 model. KBr pellet was made by mixing KBr with the crude metabolite sample in 10:1 ratio and IR spectra were obtained. The possible presence of functional groups was determined (Coates et al., 2006).

## Result and Discussion

### Identification of lichen

Identification of the lichen was done by following the literature (Awasthi 2007). Based on morphological and microscopic observations the selected lichen species was identified as *Cryptothecia straita*.

### Identification of *Penicillium* sp. (CRP 3a)

Colonies are light greenish in colour with black side yellowish. Colonies are highly spreading and grows tremendously in PDA. The morphological characterization shows the presence of many branched structures bearing many spores.

### FTIR and GC-MS analysis

FTIR analysis of crude ethyl acetate extract of *Penicillium* sp.(CRP 3a) shows the presence of many functional groups( Table 1 and Figure 1).GC-MS analysis of methanolic acid of *Penicillium* sp.(CRP 3a) shows the presence of eight compounds which were identified. GC-MS analysis of the crude extract of *Penicillium* sp.(CRP 3a) shows the presence of Phenol 3,5-Bis(1-1 Dimethyl ether), Pentanoic acid,5-hydroxy-2,4-di-t-butyl phenyl esters, Pentanedioic acid,(2,4-Di-T-Butylphenyl ester) ,ethyl chrysanthemate , Carissanol Dimethyl ether, Vanillin and Diethyl,2-6 – Pyridine- dicarboxylate(Table 2 and Figure 2)

The presence of Phenol-3-5-Bis (1-1, Dimethyl ether) was also observed in Neem extract that shows antimicrobial activity against *E. coli*, *S. aureus* and *C. albicans* and also possess antioxidant activity (Chandrasekar et al.,2015). Pentanoic acid, 5-hydroxy-2, 4-di-t-butyl phenyl ester exhibits anticancerous activity in *Cadenula officinalis*(Abduljalill , 2014).Our study also shows the presence of this compound.The presence of Pentanedioic acid, (2, 4-Di-T-Butylphenyl ester) in our result shows similarity with that of the result shown found in some plants such as *Prunus africana* and *Harrisonia abyssinica* which exhibits antimicrobial activity against *S. aureus*, *B. subtilis*, *E. coli*, *P. aeruginosa* ,*C.albicans* (Madivoli et al., 2018).Two compounds particularly Ethyl chrysanthemate and Vanillin are new compounds isolated from the EtOAc extract of *Penicillium* sp. These two compounds are reported to be the new compounds isolated from endolichenic fungi. No records have been found denoting the presence of these compounds from endolichenic fungi.

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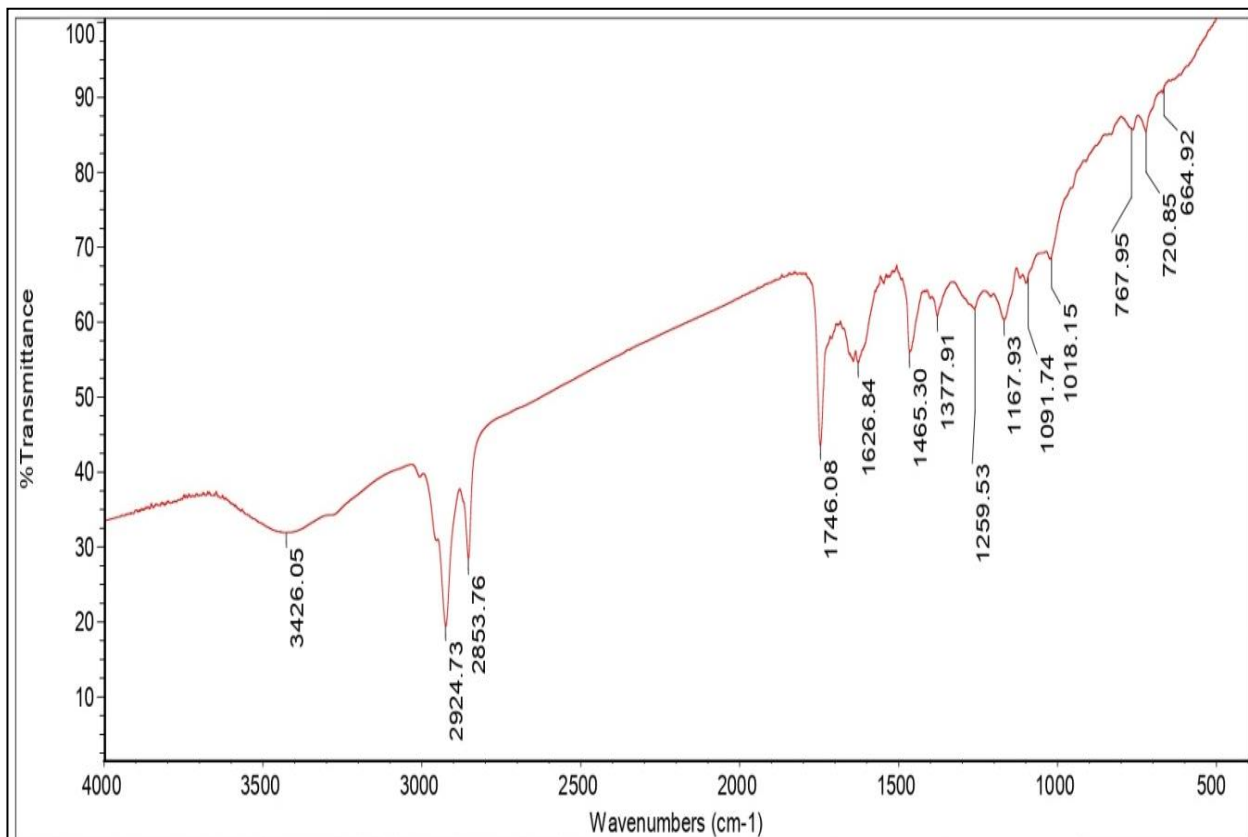
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**Table 1- FTIR analysis of the crude ethyl acetate of *Penicillium* sp. (CRP 3a )**

S.NO	FREQUENCY	GROUP
01	1167.93	Aromatic amine
02	1377.91	Methyl C-H asym./sym. bend
03	1465.30	Methylene C-H bend
04	1626.84	Alkenyl C=C stretch
05	1746.08	Ester
06	2853.76	Methylene C-H asym./sym. stretch
07	2924.73	Methylene C-H asym./sym. stretch
08	3426.05	Aromatic primary amine ,NH stretch

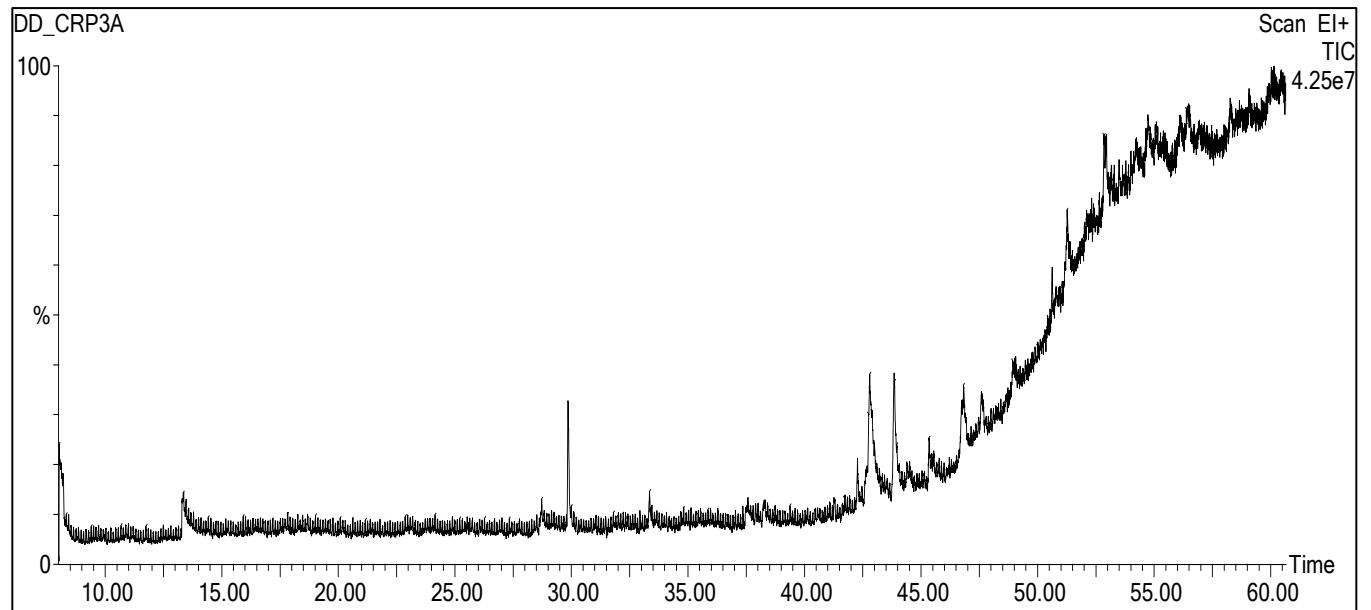


**Fig 1-FTIR chromatogram of Ethyl acetate extract of Endolichenic fungus *Penicillium* sp.(CRP 3a)**

**Table 2- GC-MS chromatography result of the crude ethyl acetate extract of *Penicillium* sp. (CRP 3a)**

S.NO	COMPOUND NAME	MOLECULAR WEIGHT	PEAK AREA
01	PHENOL, 3,5-BIS(1,1-DIMETHYLETHYL)-	206	29.847
02	PENTANOIC ACID, 5-HYDROXY-, 2,4-DI-T-BUTYLPHENYL ESTE	306	29.847
03	PENTANEDIOIC ACID, (2,4-DI-T-BUTYLPHENYL) MONO-ESTER	320	29.847
04	ETHYL 4-T-BUTYLBENZOATE	206	29.847
05	ETHYL CHRYSANTHEMATE	196	49.792
06	DIETHYL 2,6-PYRIDINEDICARBOXYLATE	223	43.838
07	CARISSANOL DIMETHYL ETHER	404	43.838
08	VANILLIN	152	43.838





**Fig 2- GC-MS chromatogram of the ethyl acetate organic crude extract obtained from endolichenic fungus *Penicillium* sp. (CRP 3a)**