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Identification and Comparative Studies of Different Volatile Fractions from *Monochaetia kansensis* by GCMS

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Abstract: Natural products from fungi are reported for various anticancer, antioxidant and other biological activities. So in search for novel bioactives from fungi isolated and identified from infected leaves of *Rhododendron* sp. Thus the isolated fungus was identified as *Monochaetia kansensis*, was extracted with variety of solvents of increasing polarity such as chloroform, Dichloromethane and ethylacetate for bioactive compounds extraction and identified by GCMS. In the chloroform extract, hexadecane (91.91%), E-15-Heptadecenal (3.98%) and phenol, 2, 4-bis (1,1- dimethyl ethyl) (1.57%) were identified. Hexadecane is reported for activities such as antibacterial, antioxidant activities. In dichloromethane extract, compounds such as phenol, 2, 4-bis (1, 1- dimethyl ethyl) (55.76%), 1- hexadecene (20.77%), 2-pentene, 3-ethyl, 4, 4- dimethyl (16.99%) and 1- octadecenol (6.48%) was noted. In ethyl acetate fraction, 2- tetradecene (22.00%), cyclodecane (19.39%), phenol, 2, 4-bis (1, 1- dimethyl ethyl) (9.78%), E-15-Heptadecenal (8.70%) and 1-octadene (12.29%) were noted. In all the three extracts, the presence of phenol, 2, 4-bis (1,1- dimethyl ethyl) was observed, where the compound is reported for antibacterial activity. E-15-Heptadecenal was present in both choloroform and ethylacetate extract at percentage of 3.98 and 8.70 respectively. In the chloroform and dichloromethane extracts, 1-hexadecene was observed at percentage of 0.93 and 20.77 respectively. Thus the basic study is focussed to study for the presence of volatile active compounds in the fungi isolated from the infected plant by GCMS.

Key words: Coelomycetous Fungi · GCMS · E-15-Heptadecenal · 1-Hexadecene · *Monochaetia Kansensis* · Phenol · 2, 4-Bis (1,1- Dimethyl Ethyl) · Volatile Compounds

INTRODUCTION

Cancer is of multistage nature and caused by environmental and genetic factors [1]. Thus there is a continuing search for novel anticancer compounds. Fungi have an excellent record in producing novel bioactive compounds and many of these presently have important medicinal and other uses [2]. Nowadays the researchers realizing the capability of microorganisms such as fungi to produce diverse bioactive molecules and the existence of an explored microbial diversity, research is underway to isolate and screen fungi from diverse habitats and unique environments for discovery of novel metabolites [3].

Fungi are an indispensable part of the life in the biosphere for they have a functional role in ecosystems. Most of the species reported are found to be saprobic or pathogenic on higher plants. One such group is Coelomycetes (mitosporic fungi) representing 7000 species recorded from widest range of ecological niches [4]. Coelomycetes are known to be imperfect fungi in which conidia are formed within a cavity lined by either fungal tissue, host tissue or a combinational of both [5-7]. Such organisms are the asexual reproducing states of ascomycetes [7]. They are commonly found and reported from cultivated and uncultivated soil of different types leaf litter and other organic debris from both natural [8, 9] and manufactured sources from saline and fresh water [10].

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The genus *Monochaetia* is classified under Coelomycetous fungi. The mycelium of *Monochaetia* is generally described as branched, septate, pale brown to hyaline; Conidia 4-euseptate, apical and basal cell hyaline. So far more than 120 taxa have been described in *Monochaetia* [7].

Species of the genus *Monochaetia* occur on different leaves of *Camellia japonica*, *C. caudate*, *C. sinensis*, *Camellia* sp., *Alium sativum*, Cassia *obtusifolia*, *Curcuma decepiens*, *Daucas carota*, *Rhododendron* sp., *Quercus* spp., [7, 11]. In this study *Monochaetia kansensis* was isolated and identified from *Rhododendron* sp. There are many works reported on various species of Coelomycetous fungi, a literature search revealed no references to previous work on *Monochaetia kansensis* fungal extract composition. Thus the present study was focused on extracting the active compounds from fungi, *Monochaetia kansensis* using three different solvents (chloroform, dichloromethane and ethylacetate), identifying and comparing the compounds present in three extracts by GC-MS analysis.

MATERIALS AND METHODS

Place of Collection: In the present study specimens of infected leaves from *Rhododendron* sp. with symptoms showing Coelomycetes were collected from Kodaikanal.

Method of Collecting Coelomycetes: Coelomycetes are found on dead twigs, leaf litter, bark and infected leaves. By using a hand lens, the presence of conidiomata on the substrate should be confirmed in the field. As the specimen is collected, the data should be recorded on the envelope used to transport the material to the laboratory. It is essential that the identity of the substrate should be accurately known for the identification of many species is still based on a host basis. Unless the material is examined immediately it should be dried thoroughly to prevent the growth of moulds and unwanted saprophytes.

Examination of the Specimens: The following methods were used to study Coelomycetes on the specimen.

Direct Examination of the Specimen: Once the selected conidioma has been removed from the substrate it is transferred to a clean glass slide & mounted with water, for microscopic examinations.

Moist Chamber Incubation Method: This technique was used to induce sporulation. The specimen was incubated in 15 cm diameter sterilized petriplate lined with moist blotting paper. The plates were kept moist by adding sterile distilled water periodically but, the blotting paper was never flooded with water. The specimens were examined after a week under stereomicroscope and the conidiomata on them were studied. The fungi found in sporulating conditions were isolated, examined and identified down to species level.

Squash Preparation: Once the conidioma has been mounted, cleaned and examined, the preparation was tapped vigorously to break the conidioma and then reheated. In this way the conidia should be released and if one cell is diligent some conidiophores or conidiogenous cells will be evident with developing conidia attached.

Preparation of Permanent Slides: For the preparation of permanent slides lactophenol was used. The slides were sealed by DPX mountant.

Illustration and Photomicrographs: Photomicrographs of conidia were taken with the help of Carl Zeiss Axiostar Plus – Photomicroscope (Phase contrast) with Cannon Digital Camera.

Isolation of Fungi: The infected plant parts of approximately 4 mm^2 were sterilized by immersing for 1 min in 0.1% mercuric Chloride solution and washed by successive transfer through sterile distilled water thrice. These were then incubated in petriplate containing PDA medium with 5 mg/g chloramphenicol.

Culture Media: Potato Dextrose Agar (PDA)

Potato	-	200g
Dextrose	-	20g
Agar	-	20g
Distilled water	-	1000 ml

The pH of the media was adjusted to 6.8 – 7.0 prior to autoclaving.

Light Source: For light requiring species, (visible light) two Philips "Cool white" (6500K) fluorescent lamps providing energy of 50 $\mu E/m^2/S$ was used. A Philips 100w comptalax lamp served as the source of incandescent light.

The specimens were inoculated on PDA medium and they were incubated for 5 to 7 days with 12 h light and 12 h dark condition in a growth chamber. The culture was carefully removed with a fine needle directly from the

culture plate, sub-cultured and maintained in pure culture. They were also maintained in agar slants stored at 7°C and subcultured at monthly intervals.

Extraction of Crude Sample: The fungus was grown in 2 L conical flasks containing 1 lit of PDB medium. The culture was incubated for 21 days, at 20-22°C. After the incubation period the culture was filtered through four layers of cheese cloth to remove mycelia. *Monochaetia kansensis* was selected for studying the volatile compounds in their extract. Thus the culture filtrate of *Monochaetia kansensis* was extracted with two equal volumes of different solvent viz., ethylacetate, chloroform and dichloromethane. The organic phase was collected and the solvent was then removed by evaporation under reduced pressure at 65°c using rotary vacuum evaporator. The dry solid residue was re-dissolved in methanol for the subsequent separation and the crude extracts were analyzed by GC-MS analysis.

GC-MS Analysis

Preparation of Extract: 2 µl of the various fractions of the fungi *Monochaetia kansensis* was employed for GC/MS analysis.

Instruments and Chromatographic Conditions: GC-MS analysis was carried out on a GC clarus 500 Perkin Elmer system comprising a AOC-20i autosampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: column Elite-1 fused silica capillary column (30 × 0.25 mm ID ×1EM df, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1ml/min and an injection volume of 0.5 EI was employed (split ratio of 10:1) injector temperature 250°C; ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/min, to 200°C/min, then 5°C/min to 280°C/min, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 40 to 550 Da.

Identification of Components: 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 mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

RESULTS AND DISCUSSION

Identification of Test Organism: The characteristics of the fungal isolate were observed by making microslide preparations of the fruit bodies and conidia [Fig.1 a & b]. They were identified on the basis of the structure of fruit body (Conidiomata) and conidia [7]. The isolate was then identified as *Monochaetia kansensis,* was selected and screening for secondary metabolite study. The presence of appendages on the conidia which is now being recognized as an important taxonomic criterion was studied critically [7, 12].

The above mentioned test fungus was subcultured and maintained on Potato Dextrose Agar (PDA) medium. For screening the fungi for secondary metabolite production it was grown in Potato Dextrose Broth medium. The test fungi was deposited in Madras University Botany Laboratory (MUBL) culture collected of Centre for Advanced Studies in Botany, University of Madras, Guindy campus, Chennai – 25.

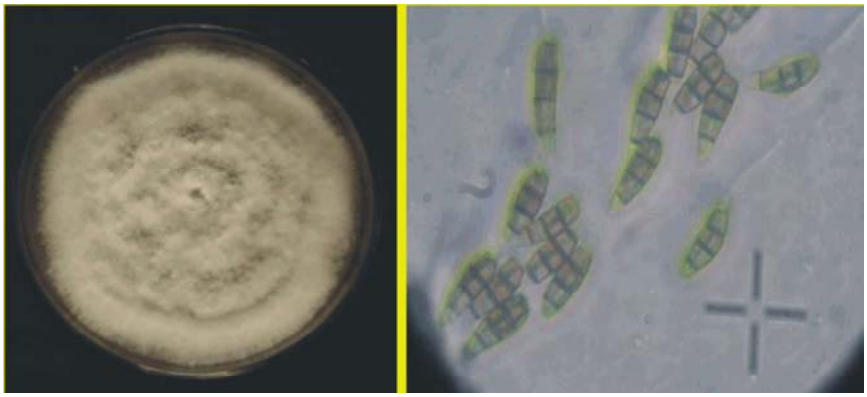

Fig. 1: Figure showing the *Monochaetia Kansensis* in culture plate and conidia (40X)

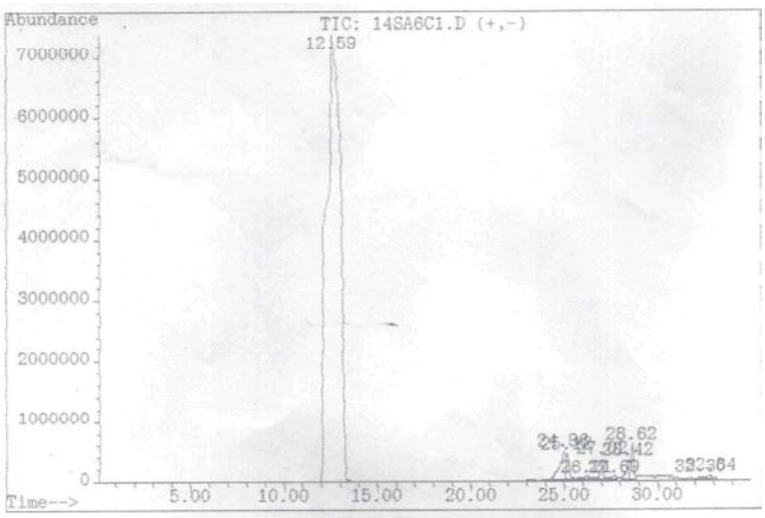

Fig. 2: GCMS chromatogram of chloroform fraction of *Monochaetia Kansensis*.

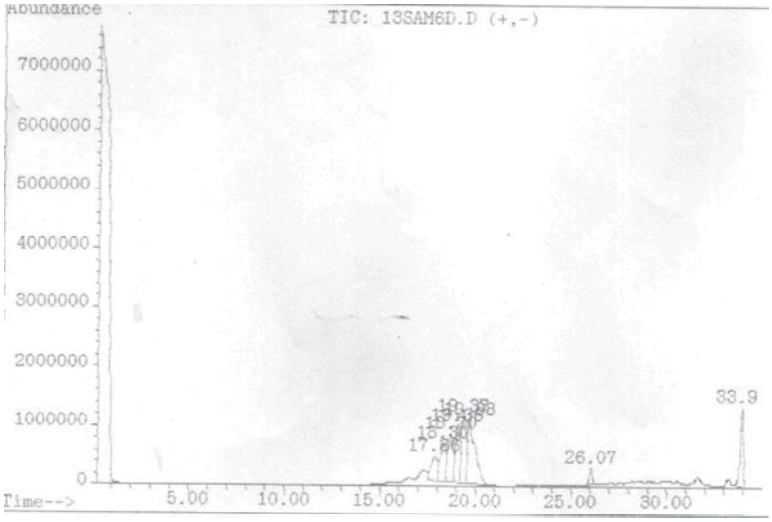

Fig. 3: GCMS chromatogram of dichloromethane fraction of *Monochaetia Kansensis*.

GC-MS Analysis: In the chloroform extract of fungi *Monochaetia kansensis* (Figure 2), the main compounds are hexadecane (91.91%), E-15-Heptadecenal (3.98%) and phenol, 2, 4-bis (1,1- dimethyl ethyl) (1.57%) (Table 1). Hexadecane is also reported in hexane fraction of facultative marine fungi *Aspergillus ustus* [13]. 1-hexadecane and hexadecene were observed to be present in crude extracts of *Spirulina platensis*, showing antibacterial activity [14]. Hexadecane was reported in the hexane extract of Malaysian red algae, *Acanthaphora spicifera.* The extract had significant inhibitory effect on *P.aeruginosa* exhibiting antimicrobial as well as antioxidant activity [15]. Hexadecane present in the essential oil of *Cestrum noctrunum* had inhibitory effect on bacteria. 1-Docosene is identified in the hexane extract facultative marine fungi *Aspergillus ustus* [13]. Thus GCMS have been used for identification of active constituents from bark extracts of *Litsea polyantha* [16]. Cyclohexane, 1-ethyl, 4-methyl have been

Table 1: Volatile compounds in the chloroform fraction of *Monochaetia kansensis*

No	RT	Name of the compound	Peak Area(%)
1.	12.50, 26.21	1-Hexadecane	91.91
2.	24.96, 25.12	E-15-Heptadecenal	3.98
3.	27.02	Cyclohexane, 1, 1-dimethyl	0.99
4.	27.70	Chloromethyl, 4- chloro undeconate	0.33
5.	28.43	1- Hexadecene	0.93
6.	28.61	Phenol, 2, 4-bis (1, 1-dimethyl ethyl)-	1.57
7.	32.30	17- Pentatriacontene	0.11
8.	32.84	1- Docosene	0.18

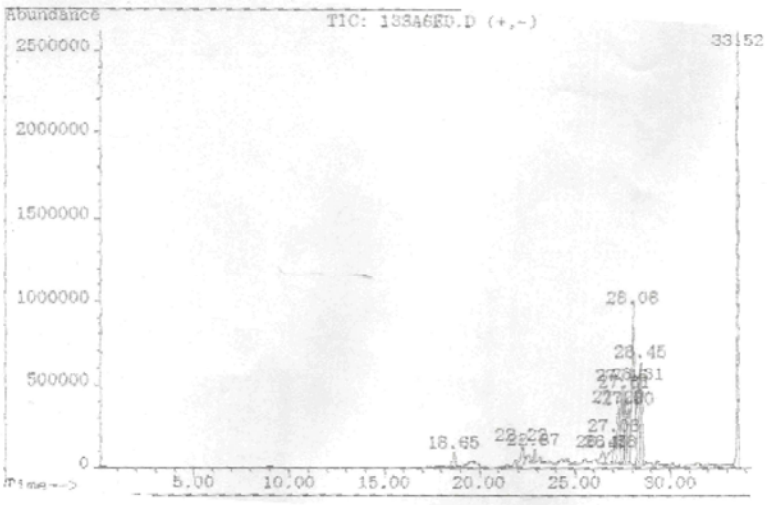

Fig. 4: GCMS chromatogram of ethylacetate fraction of *Monochaetia Kansensis.*

reported as one of the constituents present in the ethanolic leaf extract of *Tabebuia rosea* as analysed by GCMS [17].

In dichloromethane extract of fungi *Monochaetia kansensis* (Figure 3), the presence of compounds such as phenol, 2, 4-bis (1, 1- dimethyl ethyl) (55.76%), 1- hexadecene (20.77%), 2-pentene, 3-ethyl, 4, 4- dimethyl (16.99%) and 1- octadecenol (6.48%) was noted (Table 2). 1-Octadecanol, aldehyde has molecular weight, 266; molecular formula, $C_{18}H_{34}O$ [18]. Octadecanol is one of the main compounds identified by GCMS in the extracts of *Camellia sinensis* [14]. 1,6- Octadedien-3-ol, 3,7-dimethyl (11.55%) is one of major compound present in essential oil of *Cinnamomum pubescens* contributing to the antioxidant and antibacterial properties of extract [19]. 9-Octadenal is reported as one of the constituents in the active fraction of *Laurencia bradenii* as analysed by GCMS [20].

In ethyl acetate fraction of the fungi *Monochaetia kansensis* (Figure 4), main compounds identified by GCMS was 2- tetradecene (22.00%), cyclodecane (19.39%), phenol, 2, 4-bis (1, 1- dimethyl ethyl) (9.78%), E-15-Heptadecenal (8.70%), 5-eicosene (7.64%), 1-octadene (12.29%), cycloeicosane (9.26%), 11- tricosene (2.53%) and phytol (2.13%) (Table 3). Phytol, a diterpene has molecular weight, 296; molecular formula $C_{20}H_{40}O$. Octadecene and Eicosene are said to be found in higher percentages in the hexane fraction of facultative marine fungi *Aspergillus ustus* [13]. Octodene is also reported to be present in the ethanolic root extract of *Plumbago zeylanica* [21]. Octadene is said to possess various activities such as anticancer, antioxidant and antimicrobial activities [14, 22, 23]. Phytol is one of the main compounds present in extracts of Mentha spicata and Camella sinensis [18]. Phytol is reported for various biological activities such as antimicrobial, anticancer, anti-inflammatory and diuretic activity [24].

Table 2: Volatile compounds in the dichloromethane fraction of *Monochaetia kansensis*

No	RT	Name of the compound	Peak Area(%)
1.	17.83	1- Hexadecene	20.77
2.	18.29	Phenol, 2, 4-bis (1, 1-dimethyl ethyl)-	55.76
	18.70		
3.	26.07	2- Pentene-3-ethyl-4,4 dimethyl	16.99
4.	33.92	1- Octadecenol	6.48

Table 3: Volatile compounds in the Ethyl acetate fraction of *Monochaetia kansensis*

No	RT	Name of the compound	Peak Area(%)
1.	18.55	Trans-3-decene	1.84
2.	22.22	Benzaldehyde	2.84
3.	22.87	1- Hexanol, 2-ethyl	0.91
4.	26.43	Phytol	2.13
5.	26.88	11-tricosene	2.53
6.	27.08	Cycloeicosane	9.26
7.	27.29		
	27.62	1- Octadecene	12.29
8.	27.48	5- Eicosene	7.64
11.	28.06	Cyclododecane	19.39
12.	28.31	E- 15- Heptadecenal	8.70
13.	28.46	Phenol, 2, 4-bis (1, 1-dimethyl ethyl)-	9.78
14.	33.51	2-tetradecene	22.00

Eicosane, a fatty acid is found in essential oil and organic extracts of *Cestrum nocturnum*, is found to have activity on food borne pathogens [25]. Eicosane is also present ion the flower of *Allium atroviolaceum*, contributing to the antimicrobial activity of extract [26]. Eicosane and phytol are reported to be the main

constituents in the *Aloe vera* extract responsible for high antimicrobial activity against clinical pathogens (27).

In all the three extracts, the presence of phenol, 2, 4-bis (1, 1- dimethyl ethyl) was observed, with a percentage of 1.57, 55.76 and 9.78 respectively for chloroform, diuchloromethane and ethylacetate extracts of *Monochaetia kansensis*. Phenol, 2, 4-Bis (1, 1-Dimethyl ethyl) has molecular weight, 206; molecular formula, $C_{14}H_{22}O$ has good antioxidant activity [21]. Phenol, 2, 4-Bis (1, 1-Dimethyl ethyl) was identified in the Malaysian mango kernel by GCMS and reported to be responsible for antibacterial activity of the extract [28].

E-15-Heptadecenal was present in both choloroform and ethylacetate extract at percentage of 3.98 and 8.70 respectively. E-15-Heptacenal, an aldehyde is present in the crude extract of *Spirulina platensis*, reported for antibacterial activity [14].

In the chloroform and dichloromethane extracts, 1-hexadecene was observed at percentage of 0.93 and 20.77 respectively. Hexadecene was found in higher proportions in the hexane fraction of facultative marine fungi *Aspergillus ustus* [13]. Heptadecane and hexadecane are common hydrocarbons reported in seaweeds [15].

Thus further research is in progress to identify the active compounds in various extracts. Thus the study offers further research interests in study of these active fungal metabolites as antimicrobials and anticancer agents against various diseases.

CONCLUSION

Our systematic investigation reveals the potential of *Monochaetia kansensis* can be a good source of bioactive compounds such as alkanes, alkenes and hydrocarbons. The study also suggests that the different solvent extracts produced different ratios of bioactive compounds but the extraction by ethyl acetate was found to yield a variety of compounds. This study also gives a better understanding for identification and comparison of volatile compounds in fungal extracts by GCMS and further interest's researchers to study for bioactivity and isolation of pure compounds.

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