Bioprospecting of Bioactive Metabolites from 
*Monochaetia karstenii*

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**Abstract**

In the present study, to optimize the media for the production of bioactive compounds from *Monochaetia karstenii* was carried out and compounds were identified by GC-MS. *M. karstenii* was identified from infected *Camellia japonica* leaves by classical and molecular taxonomy. It was cultured in different media and determined their mycelial biomass and antibacterial activity. Further Maltose Maltose tartrate broth (MTB) was altered for its media components such as carbon, nitrogen, minerals, amino acids and vitamins sources and physical parameters like temperature, pH and incubation periods for growth and production of secondary metabolites from *M. karstenii*. The antimicrobial and antioxidant compounds were performed from three different solvent extracts (Chloroform, Dichloromethane and Ethyl acetate) of *M. karstenii* from optimized medium. *M. karstenii* had optimum growth in MTB showing mycelial growth of 13.16 g/L. The ethyl acetate extract observed significant antibacterial activity against *Escherichia coli* (21 mm), *Staphylococcus aureus* (20 mm) and *Vibrio cholerae* (18 mm). *In-vitro* antioxidant activity revealed that, the IC<sub>50</sub> values for 2, 2’-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS), 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and total antioxidant radical scavenging assay of 100.58 μg/ml, 140 μg/ml and 141.91 μg/ml from ethyl acetate extract respectively. Thus the antimicrobial and antioxidant activity of the fungal extract has been due to the presence of biocompounds such as cyclohexenone derivatives, cinnamic acid, isoaxazoline 3-phenyl- benzodiazepine, 2-propenoic acid 3-phenyl-(E)-dodecene and 3-undecen -1-yne (E) were characterized by gas chromatography-mass spectrometry (GC–MS) and reported first time in *M. karstenii*. We conclude that *M. karstenii* possess excellent antimicrobial and antioxidant potential and can be exploited for the discovery of new drug molecules.

**Keywords:** *Monochaetia karstenii*, anti-bacterial, antioxidant, GC-MS analysis, media optimization, cinnamic acid

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INTRODUCTION

Fungi has been the source of most profitable industrial products used for medicine such as two anti-cholesterol strains, antibiotic penicillin and immunosuppressant cyclosporine A. Media and growth conditions such as pH, temperature optimization is most necessary for high production of bioactive compounds. Secondary metabolites are excreted out of cell protect the fungi from extreme conditions, competitors and also help in survival. There is always a search for novel drug molecules from different sources. There are limited studies on plant pathogenic fungi as source of secondary metabolites which are unexplored. Various studies have showed that altering the growth environment has major effect on the production of fungal secondary metabolites. Moreover, the carbon and nitrogen sources leads for fungal growth and secondary metabolites production. A report showed that nitrogen sources had a biggest impact in studies conducted on three genera of Entomophthorales. Whereas the environmental factors such as growth factors, pH, moisture, temperature, pressure, incubation period, are most deciding factors for antibiotic biosynthesis. The requirements of nutritional conditions vary from fungus to fungus depending upon the genera to which they belong.

The genera Monochaetia, Pestalotia and Pestalotiopsis have similar conidia provided with apical appendage. Monochaeta differs from the latter two genera with conidia, septate and a single apical appendage. Species in the genus are typically plant parasites, saprophytes and cause leaf spot diseases on various hosts. However most of the Monochaeta species lack molecular data and it may be a rare occurrence and distribution. Monochaeta species are observed to produce bioactive compounds like taxol, ambuic acid and chaetiacandin. In our previous study, we reported diverse volatile fractions from Monochaetia kansensis showing presence of bioactive compound phenol, 2, 4-bis (1,1- dimethyl ethyl). The comparative study among different extraction, ethyl acetate extract possessed good yield of bioactive compounds. So far there is no report in M. karstenii for optimization of culture conditions. Therefore, the current study aimed for determination of optimum growth of M. karstenii for their production of bioactive secondary metabolites and characterization of bioactive compounds.

MATERIALS AND METHODS

Isolation, molecular identification of M. karstenii

M. karstenii was isolated from infected leaves of Camellia japonica L. from Kodaikanal, Tamil Nadu, India. Classical identification was followed by Sutton and molecular identification was carried out from the isolated genomic DNA using standard method. The genomic DNA was amplified by thermal PCR condition with help of ITS1 and ITS4 rDNA gene primer. The PCR product was sequenced, compared by nBLAST analysis and identified by species level. Further rDNA sequence of M. karstenii was aligned by Clustal-W and analyzed for phylogenetic tree using by MEGA6 software with maximum parsimony method.

M. karstenii growth and antibacterial activity in different liquid media

About 100 ml of Cornmeal Peptone yeast extract (CPYEB), Czapexdox broth-I, II (CDB-I), CDB-II, Malt extract broth (MEB), Oat Meal broth (OMB), Maltose tartrate broth (MTB), Potato carrot broth (PCB), Modified 1 medium broth (M1DB), Potato Dextrose Broth (PDB), Crabill’s medium, Potato Dextrose Yeast Extract Broth (PDYEB) and Sabouraud Dextrose Broth (SDB) were separately prepared. Three mycelial discs (10mm) of M. karstenii was inoculated in to the respective media containing flask individually under the influence of 12 h light followed by 12 h dark condition (per day). After 21 days of incubation cultures were harvested. In order to measure the fungal biomass dry weight, mycelial mat was filtered through filter paper and dried for 12 hrs at 50°C. Initial screening such as antibacterial studies were carried out using twelve different culture filtrates against S. aureus and Klebsiella pneumoniae by agar well diffusion method. Briefly, bacterial cultures speared on Nutrient Agar medium separately and well (0.5 cm) was created using sterile corkborer. To each wells, 100 μl of each different culture filtrates were added and incubated at 37°C for 24 hrs. The development of inhibition zone was measured.

Growth of M. karstenii in different chemical and physical conditions

The different media components of carbon sources (20g/L): sucrose, glucose, dextrose,
maltose, fructose, galactose, lactose, xylose, sorbitol and mannitol; nitrogen sources (2.8g/L): ammonium nitrate (NH₄NO₃), yeast extract, beef extract, peptone, potassium nitrate (KNO₃), urea (CH₄N₂O), ammonium tartrate (C₄H₆N₂O₄), ammonium sulphate ([NH₄]₂SO₄), and sodium nitrate (NaNO₃); minerals (0.5g/L): manganese chloride (MnCl₂), calcium nitrate (Ca(NO₃)₂), sodium chloride (NaCl), magnesium sulphate (MgSO₄), copper sulphate (CuSO₄), potassium chloride (KCl), zinc sulphate (ZnSO₄), sodium sulphate (Na₂SO₄), ferrous sulphate (FeSO₄), and potassium iodide (KI); amino acid (50mg/L): histidine, glycine, alanine, cysteine, methionine, tryptophan, aspartic acid, phenylalanine and threonine; vitamin sources (50mg/L): Vitamin B1, B2, B6, B8 and B9; Physical parameters such as pH 5.0, 5.5, 6.0-7.0, 7.5 and 8.0; incubation period 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25 and 27 days were selected for optimization processes. These conditions were used for optimization process in terms of mycelial dry weight.

**Mass cultivation and preparation of different fungal extracts**

A 15 days old culture of ten mycelial discs (10mm) were inoculated in 5 litre Hoffkin flask containing two litre of optimized medium (magnesium sulphate - 0.5 g/l, yeast extract - 2.8 g/l, glucose - 20 g/l, potassium dihydrogen phosphate -1 g/l, trace elements (Zinc - 0.2 mg/l, Iron - 0.2 mg/l, manganese - 0.1 mg/l) and histidine - 50 mg/l) with pH 6.8, under the influence of 12 h light followed by 12 h dark condition (per day) for incubation. The fungal culture filtrate was filtered after 21 days and extracted with double the volume of chloroform, dichloromethane and ethyl acetate separately. Each organic solvent extracts were condensed by vacuum rotary evaporator at 40°C, all organic extracts were dissolved in 0.4% Dimethyl sulfoxide (DMSO) and it used for bio-assays separately.

**Biological activity of M. karstenii extracts**

**Antibacterial activity**

Different solvent extracts (Chloroform, Dichloromethane and Ethylacetate) of *M. karstenii* and 0.4% DMSO served as a control were analysed their antibacterial activity by agar well diffusion method against *S. aureus*, *E. coli* and *V. cholerae*. The reaction mixture containing 1.0 ml of 0.1 mM DPPH methanol solution and 50 µl of different concentrations of fungal ethyl acetate extract at concentration of 10-150 µg/ml, incubated for 30 minutes, absorbance was recorded at 517 nm and DPPH radical scavenging activity was calculated.

**Bioactive compounds from M. karstenii extract**

Ethyl acetate extract (2 µl) of *M. karstenii* was subjected to GC/MS analysis in GC Clarus 500 Perkin Elmer system with AOC-20i autosampler, gas chromatograph-mass spectrometer. The reaction mixture containing 1.0 ml of 0.1 mM DPPH methanol solution and 50 µl of different concentrations of fungal ethyl acetate extract at concentration of 10-150 µg/ml, incubated for 30 minutes, absorbance was recorded at 517 nm and DPPH radical scavenging activity was calculated.

**Total antioxidant radical scavenging assay**

Total antioxidant radical scavenging assay carried out using standard method. Fungal ethyl acetate extract with varying concentrations (75-200 µg/ml) of 0.2 ml and standard (Ascorbic acid) separately, distilled water (1.8 ml), 1 ml of Phosphomolybdenum reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) were added, incubated at 95°C for 90 min and cooled to 37°C. The absorbance of each solution was recorded at 695 nm. The result was calculated as percentage of inhibition which is ratio of (absorbance of control solution minus sample solution) to absorbance of control multiplied by 100 and same as was followed for other assays.

**ABTS radical scavenging assay**

Fresh ABTS solution (1.8 mM) containing 5.0 ml of 4.9 mM potassium persulphate solution to 5.0 ml of 14 mM ABTS solution, diluted to have absorbance of 0.650 ± 0.20 at 734 nm. About 50 µl of fungal test sample at concentrations of 10-110 µg/ml and 950 µl of ABTS radical solution, standard (Ascorbic acid) were tested individually and absorbance read at 734 nm and calculated.

**DPPH assay**

Ethyl acetate fungal extract and standard (Quercetin) were measured the DPPH assay by standard method. The reaction mixture containing 1.0 ml of 0.1 mM DPPH methanol solution and 50 µl of different concentrations of fungal ethyl acetate extract at concentration of 10-150 µg/ml, incubated for 30 minutes, absorbance was recorded at 517 nm and DPPH radical scavenging activity was calculated.
Statistical analysis

Data are given as mean ± S.E.M with three triplicate values. Statistical comparisons were made using one way ANOVA followed by Tukey’s family error test. P-value < 0.05 was considered as significant.

RESULTS AND DISCUSSION

Fungi have been always the potential source of novel metabolites for curing various diseases. In this study, fungus was isolated from C. japonica and identified as a M. karstenii. The genus belonging to the order of Melanconiales in imperfect fungi of Coelomycetes, based on conidial structure by classical taxonomy with following descriptions. Mycelia on media was yellow (mature) and white (young) colour (Fig. 1a); Conidiomata acervular; Conidia with thick walls, 4-celled or 3 celled, 13 x 6.5 µm, 10-5 µm long, hyaline, 1-2 apical appendage, basal cell thin-walled (Fig. 1b). Molecular taxonomy of M. karstenii was identified by nBLAST analysis, ITS sequence was submitted in GenBank, NCBI, USA with an accession number of JN222973. Phylogenetic tree of M. karstenii was constructed by MEGA6 using with maximum parsimony method with additional of 100 randomly sequences. The monophyletic group of M. karstenii showing 100% similarity with other Monochaetia species. Colletotrichum gloeosporioides from coelomycetes and Amanita muscaria from basidiomycetes were used for an out group (Fig. 1c).

Fungal biomass was observed in twelve different liquid media for enhancing biomass production and antibacterial activity were recorded against S. aureus and K. pneumoniae.

Fig. 1. (a) Culture plate showing growth of M. karstenii on MTA medium; (b) Spore morphology showing mature conidia of M. karstenii in 40X; (c) Phylogenetic relationship of M. karstenii by Maximum parsimony analysis
Among the media used, MTB (1.07 g) showed maximum biomass production followed by PDB (0.92 g), PDYEB (0.65 g) and M1D (0.77 g). The ethyl acetate fungal extract of MTB showed best inhibition against *S. aureus* and *K. pneumoniae* (Fig. 2). Relatively, Timnick et al. reported that MTB medium supported the growth of *Melanconium* belonging to the order of Melanconiales.

There has been substantial challenge to supply the essential nutrients favouring production of secondary metabolites. So, the present study deals with optimization of *M. karstenii* for highest biomass production using different media components of carbon, nitrogen, mineral, aminoacid and vitamin sources. The effect of different carbon sources, the highest biomass (1.25 g) was observed in glucose followed by fructose, sucrose and maltose. There is no significant biomass observed in xylose, sorbitol, mannitol, galactose and lactose (Fig.3a). Similar observation

![Fig. 2.](image1.png)

**Fig. 2.** *M. karstenii* growth comparison in various media and antibacterial activity.

![Fig. 3.](image2.png)

**Fig. 3.** Growth of *M. karstenii* in different chemical parameters: (a) carbon; (b) nitrogen; (c) mineral; (d) amino acid; (e) Vitamin; without respective nutrition containing medium refers to the control.
was confirmed by Ranzoni\textsuperscript{28} stating that \textit{Anguillo sporalongissima} and \textit{A. gigantea} growth were supported with glucose as best carbon source. Our study shows, minimum biomass was observed in lactose. Supporting this, Sati and Bisht\textsuperscript{29} observed that very least growth using lactose as carbon source for \textit{Tetraechatun elegans} and \textit{Tetracladium marchalianum}.

Among the nine different nitrogen sources, yeast extract supported highest biomass (1.28 g) followed by sodium nitrate, ammonium tartarate, beef extract and peptone. No growth was observed in urea and control (Fig. 3b). Similar to our study results, \textit{Fusarium} sp. (SS2) has elevated level of antibacterial compounds and biomass produced in medium supplemented with yeast extract\textsuperscript{30}. Different mineral sources, the constant biomass production (1.28 g) was observed in magnesium sulphate followed by calcium nitrate, manganese chloride and sodium chloride. Growth was not exhibited in copper sulphate, ferrous sulphate and zinc sulphate (Fig. 3c). Notably, constant result was observed when added magnesium sulphate in basal and optimized medium. This results seem to be similar to Jonathan and Fasidi\textsuperscript{31} reported \textit{Psathyrella atrioimbonata} best mycelial growth when supplemented with magnesium and calcium. Similarly, Sehgal and Anand\textsuperscript{32} also observed that magnesium sulphate supported the growth of \textit{Cordyseps militaris}.

![Fig. 4. Growth of \textit{M. karstenii} in different physical parameters: (a) pH; (b) incubation periods](image)

![Fig. 5. Antibacterial activity of different solvent extracts of \textit{M. karstenii}; control – 0.4% DMSO; CDCl\textsubscript{3} extract - chloroform; EtoAc extract - ethyl acetate; DCM extract -dichloromethane.](image)
From amino acid sources, the highest biomass (1.29 g) was observed in histidine followed by glycine, alanine, methionine, tryptophan, aspartic acid (Fig.3d). Related result was observed in the growth of Colletotrichum gloeosporioides. Vitamin supplement did not support the growth of M. karstenii in our studies (Fig.3e) which is connected to reports of Painter with studies on Geotrichum sp. and F. aqueductum, but Trichosporon cutaneum showed maximum growth in medium containing thiamine, whereas Sepedonium sp. requires both thiamine and biotin.

The M. karstenii growth effects at different pH were studied. The stable biomass (1.29 g) was observed in pH 6.8 followed by pH 6.5, 6.6 and 6.7. Poor growth was observed in pH 5.0, 5.5, 7.5 and 8. This result suggested that optimum pH of the medium was 6.8 for M. karstenii (Fig.4a) in altered optimized MTB medium. P. theae showed good growth at pH 6.7. M. karstenii was harvested at different incubation periods showed fluctuations in growth. The growth increased gradually from 5th day to 21st day and declined later. These results indicated that the constant growth (1.29 g) was achieved on 21st day (Fig. 4b).

Present study indicates that, the optimized MTB medium per litre for M. karstenii with composition of potassium dihydrogen phosphate – 1 g, glucose – 20 g, magnesium sulphate - 0.5 g, yeast extract - 2.8 g, trace elements (Zinc - 200 µg, Iron - 200 µg, manganese - 100 µg) and histidine – 50 mg with pH 6.8 for 21 days showed best yield and compounds. The biomass of 13.16 g from per litre of optimized medium was supported

### Table 1. Antioxidant activities of M. karstenii

| Fungal ethyl acetate extract (µg/ml) | % of Inhibitions |
|-------------------------------------|------------------|
|                                     | DPPH | ABTS | Total antioxidant |
| 50                                  | 8.99 ± 0.17  | 18.46 ± 0.36 | 22.50 ± 0.45 |
| 100                                 | 40.79 ± 0.81 | 49.71 ± 0.99 | 44.28 ± 0.88 |
| 150                                 | 53.34 ± 1.06 | 74.60 ± 1.49 | 52.85 ± 1.05 |
| 200                                 | 71.40 ± 1.42 | 98.50 ± 1.99 | 60.00 ± 1.20 |
| 250                                 | 89.30 ± 1.78 | 99.60 ± 2.49 | 75.41 ± 1.50 |
| Ascorbic acid (100 µg)              | -    | 79.72 ± 1.59 | 95.65 ± 1.91 |
| Quercetin (10 µg)                   | 92.78 ± 1.85 | -    | -                  |
| IC_{50} value                       | 140.60 ± 2.81 | 100.58 ± 2.01 | 141.91 ± 2.83 |

Values are expressed as mean ± S.E.M of 3 replicates; - indicates not tested

### Table 2. Bioactive compounds from M. karstenii

| No | Name of the compounds                          | Retention Time | Peak Area (%) | Mass (g/mol) | Molecular formula |
|----|-----------------------------------------------|----------------|---------------|--------------|-------------------|
| 1  | Styrene                                       | 21.77          | 0.44          | 104.15       | C_{8}H_{8}       |
| 2  | 2-Propenoic acid, 3-Phenyl/cinnamic acid      | 24.03          | 11.19         | 148.161      | C_{9}H_{8}O       |
| 3  | 2 (1H)-Pyridinone, 1-ethyl                    | 26.02          | 1.43          | 123.152      | C_{2}H_{5}NO     |
| 4  | 1-[α-(1- adamantyl) benzylidenyl] thiosemicarbazide | 26.67          | 1.59          | 313.463      | C_{18}H_{23}N_{3}S |
| 5  | 2-Isoxazoline, 3-phenyl-                      | 26.98          | 4.62          | 147.174      | C_{5}H_{10}NO    |
| 6  | Cis 1- chloro-1, 3- dimethyl silacyclohexane   | 27.65          | 3.14          | 162.73       | C_{9}H_{13}ClSi  |
| 7  | 1H, 1, 5- Benzodiazepine, 2, 3, 4, 5- tetrahydro | 28.27          | 5.58          | 148.15       | C_{10}H_{14}N_{4} |
| 8  | Oximino acid/Nicotinic acid N-oxide           | 28.47          | 10.55         | 139.109      | C_{9}H_{13}NO_{3} |
| 9  | 2, 2- dimethyl- 1- oxo- spiro (2, 3) hexane   | 29.46          | 9.54          | 112.172      | C_{8}H_{10}O     |
| 10 | Cis, 3, 5- dimethyl cyclohexanone              | 29.92          | 3.18          | 126.196      | C_{9}H_{14}O     |
| 11 | 1- Dodecane                                   | 30.25          | 14.38         | 168.319      | C_{12}H_{26}      |
| 12 | 3- undecen-1- yne                             | 31.17          | 10.38         | 150.26       | C_{11}H_{18}      |
| 13 | Neopentylidenecyclohexane                     | 32.23          | 4.86          | 152.276      | C_{12}H_{20}      |
to produce secondary metabolites from culture filtrate. Thus providing media with necessary chemical constituents leads to accumulation of metabolites. This study revealed that optimization of these culture growth conditions were first time reported in MTB medium as well in *M. karstenii*. The extraction of chemical metabolites was carried out using, chloroform, dichloromethane and ethyl acetate separately. The antibacterial study results had inhibition zones of 20 and 21 mm found in ethyl acetate extract against *S. aureus* and *E. coli* respectively. Whereas for dichloromethane and chloroform extract inhibition zones found to be 19 and 18 mm; 14 and 13 mm respectively against *E. coli* and *V. cholera* respectively (Fig.5). Study results shows that ethyl acetate extract has prominent potential active principle to control (0.4% DMSO) growth of microbes. Xu *et al.* reported most of the bioactive compounds were obtained from ethyl acetate extract of *Pestalotiopsis* sp. In this study, the fungal ethyl acetate extract was taken for in-vitro antioxidant activities. The IC₅₀ value was obtained at 141.91, 140.60 and 100.58 µg/ml for total antioxidant, DPPH and ABTS radical scavenging assay compared with standard respectively (Table 1). Similarly, Sharma and Vijaya proved that ethyl acetate extract of *Aspergillus terreus* had highest antioxidant activity. *M. karstenii* exhibited potent biological activity in ethyl acetate extract by antibacterial and antioxidant assays.

In order to find out the bioactive compounds, fungal ethyl acetate extract proceeded further for GC-MS analysis. The GC-MS spectral data revealed thirteen bioactive peaks corresponding to compounds such as cyclohexenone derivatives, cinnamic acid, isooxazoline 3-phenyl-, benzodiazepine, 2-propenoic acid 3-phenyl-(E)-, dodecene and 3-undecen -1-yne (E), etc. were identified and reported first time from *M. karstenii*. Molecular formula, mass value, retention time and area % of chemical composition are listed in Table 2. Ambuc acid, cyclohexenone moiety, reported for its antifungal activity has been isolated from rain forest plant endophytic fungi *Pestalotiopsis* spp. and *Monochaetia* sp. The main components such as 1-Dodecene, neopentylidenecyclohexane, cinnamic acid and oxiniacic acid were observed and reported for antimicrobial, antitumor, antioxidant and anti-hyperlipoproteinemic agent respectively.

**CONCLUSION**

Overall from this study, carbon and nitrogen sources are both important for growth of *M. karstenii*. Nitrogen is essential for the growth of fungus and carbon has an even greater importance. It is necessary for energy production and synthesis of various cell wall lipids. Studies confirmed that the ethyl acetate extract of *M. karstenii* have potent antibacterial and antioxidant activity. GC-MS studies have revealed the presence of interesting biocompounds for curing bacterial, fungal, cancer and several diseases. Thus further research is in progress to isolate the novel bioactive compounds by chromatographic and spectroscopic methods.

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**CONFLICT OF INTEREST**

The authors declare that there is no conflict of interest.

**AUTHORS’ CONTRIBUTION**

YS- Methodology, visualization, investigation and writing-original draft preparation; RS- supported for GC-MS analysis, Editing and reviewing of the manuscript, LB- contributed for anti-oxidant assay; KS - Editing and reviewing of the manuscript.

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DATA AVAILABILITY
The datasets analysed during the current study are available in this manuscript and NCBI database repository, Accession No: JN222973.

ETHICS STATEMENT
This article does not contain any studies with human participants or animals performed by any of the authors.

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