

Analysis of the Bioactive Metabolites of the Endangered Mexican Lost Fungi *Campanophyllum* – A Report from India

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ABSTRACT

Meghalaya, (in India), in the region of the mega-biodiversity hotspots, is home to a plethora of wild mushrooms. The present study concerns the exploration of the order Agaricales, which includes rare gilled mushrooms considered endangered under IUCN A4c criteria, due to the declining habitat. Electron microscopy of the gill sections revealed an abundance of clamp connections, hyphal cell walls, cystidia, and basidia. This rare species which belongs to the family Cyphellaceae, exhibits morphological and molecular differences from the *Cyphella* spp. Phylogenetic analysis revealed that it formed a clade under the genus *Campanophyllum* of the order Agaricales, confirmed by both Neighbor Joining (NJ) and Bayesian phylogenetic analysis. Being nutritionally potent along with its efficient antioxidant value, the fungal extract shows significant rise of two-fold in the antimicrobial activity along with the commercial antibiotics. The compound, Phenol, 2, 4-bis (1, 1-Dimethylethyl) (2, 4-DTBP) showed in ample range in the fungal extract along with aliphatic hydrocarbons, terpene, alcohol and volatile organic compounds on further characterization in GCMS. The present study indicates the endangered *Campanophyllum proboscideum* could be a rich source of natural antioxidants and an effective pharmaceutical agent.

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1. Introduction

Meghalaya, extending across an area of approximately 22,549 km², is located between the coordinates of 25°47'–26°10'N latitude and 89°45'–92°47'E longitude. The region is noted for high rainfall during the summer receiving an annual average of above 12,000 mm and temperature touching a high of 28 °C in the summer to a low of 2 °C in the winter, with a periodic deviation to below freezing temperature. The high humidity prevailing in the area provides atmospheric conditions conducive for the growth of several saprophytes, including mushrooms growing in the forest tier or mycorrhiza associated with the plant life. Although the region is rich in specimens belonging to Boletaceae, Russulaceae, Clavariaceae, Gomphaceae, and Tricholmataceae, specific knowledge regarding the importance of these mushrooms is limited to the old and elderly villagers and the region continues to be scientifically explored for its mushroom flora [1]. The identification of the mushrooms in these regions is completely based on ethnic knowledge, which includes their colors and smells, with no information regarding their additional properties. Locals collect these

mushrooms for their livelihood, without any accurate knowledge in terms of their toxic essence, which sometimes leads to fatalities among them. This occurs because of the consumption of the toxic species which mimic their edible counterparts. Researchers have demonstrated that cryptic species are a very common occurrence among these fungi. Accuracy based on the morphological traits can prove nonreliable and hence the DNA barcode has become an authentic tool in identification [2,3].

The diversity of the gilled cyphelloid polyporous fungus, *Campanophyllum proboscideum* once reported is common among the “reduced series” members of *Campanella*, *Cyphella*, *Rimbachia*, and *Skepperiella*, which were earlier predominant in Mesoamerica [4]. The genus was limited in 2003 to include the species termed *Lentinus proboscoides*, the name applied to the mushroom, which had been collected by Örsted (more than 150 years ago) in Costa Rica, but later was restricted to genus *Campanophyllum* related to the “Gloeostereae” clade [4,5]. The study of the rDNA sequence has given valuable insight into its evolutionary relationships with the other basidiomycetes group. The species was also considered endangered under IUCN A4c

criteria due to its declining habitat and low population. As the genus features among the “reduced series”, no records regarding its polyphasic approach or bioactivity have been reported from the foothills of the Eastern Himalayas, in India.

At present, there are vast records of a variety of bioactive metabolites drawn from mushrooms which have fascinated many and kindled great scientific interest for their biological behavior as antioxidants, antimicrobials, anti-inflammatory, and anti-tumor agents [6,7]. High traces of phenolic compounds are present in mushrooms. Many *in vitro* and epidemiological studies have contributed toward the use of individual phenolic compounds possessing different antioxidant and antagonistic activities with the potential of being free radical polymerization inhibitors (chain breaker), peroxide decomposers, and oxygen scavengers [8].

In the present study, the specimen was collected from the East Khasi Hill district of Meghalaya, nestled in the foothills of the Eastern Himalayas in India and is illustrated and described. The total soluble phenol and its bioactive components in some commercial mushrooms have been researched by several authors, but no reports on detailed profiling of the chemical and nutritional compounds of the Mexican endangered mushroom, which can be utilized later as a potential pharmacological substance, are available in the database [9–11].

2. Materials and methods

2.1. Sample collection

Fresh mushroom was collected from the forest of Meghalaya (25°26.7'N and 91°44.92'E) at an altitude of 1788 m with 75% humidity. Collected sample was brought to the laboratory condition in a sterile bag. The sample was washed in luke warm water to remove the dirt, while maintaining safety using sterile gloves and mask. Macro-morphological traits were recorded at the site of collection for the fresh voucher. Field photograph of the fresh voucher was taken with a Canon camera. A unique laboratory identification number has been assigned against the collected sample, MBSRJ38.

2.2. Microscopic analysis

Transverse sections of the mushroom cap along with the gills were viewed in Leica Microsystems (CH-9435; Leica, Heerbrugg, Germany) using Melzer's solution [12].

Scanning electron microscope (SEM) analysis of the sample was carried as described earlier where the processed tissues were mounted on an aluminum stubs using double sided adhesive tape and

was layered with gold by sputtering in a vacuum sputter (JFC-1100; Jeol, Tokyo, Japan) at various magnification to obtain surface ornamentation at 20 kV of accelerating voltage [13].

2.3. Molecular analysis

The fungal tissue was considered for extraction of genomic nuclear DNA using HiPurA fungal DNA isolation kit (Himedia, Mumbai, India) and was amplified by two primers ITS1F (5'-TCCGTAGGTGAACCTG CCG-3') and ITS4R (5'-TCCTCCGCTTATTGATA TGC-3') [14]. Amplification was carried out in a GeneAmp 9700 Thermal Cycler (Applied Biosystems, Foster City, CA, USA) under the following conditions: initial denaturation at 95 °C for 5 min, 30 cycles of denaturation at 94 °C for 1 min, annealing at 52 °C for 30 s, extension at 72 °C for 1 min and final extension at 72 °C for 10 min. The amplified nucrDNAITS1-5.8S- ITS2 (ITS barcode) ribotypes were purified using QIA quick® gel extraction kit (Qiagen, Hilden, Germany) and was sequenced (Xcelris Lab, Gujarat, India).

2.4. Sequence and phylogenetic analysis

The nucleotide sequence coding for the ITS gene was computed for their alignment along with its homology sequence using ClustalW (<https://www.ebi.ac.uk/Tools/msa/clustalo/>).

Electropherograms was manually edited to omit the gap using Chromas Lite (Technelysium, Queensland, Australia) and deposited to NCBI to obtain the accession number.

Neighbor joining (NJ) and Bayesian inference were used to analyze the evolutionary distance matrix between the query sequence and its homologous. NJ tree was computed using MEGA 6 software [15]. Robustness of NJ was calculated by analyzing the bootstrap of 1000 replicates (Kimura-2 parameter). Bayesian analysis was performed with MrBayes 3.2 and was run by specifying a general-time reversible (GTR) model with gamma-distributed variations across site [16]. Four Markov chains (3 heated and one cold chain) with incremental heating temperature of 0.05 were run for one million generations and sampled every 10 generations. The first 25% of the trees were discarded as burn-in. Clade posterior probabilities (PP) were determined for the combined sets of trees from two simultaneous runs.

2.5. Nutritional analysis

The mushroom sample was analyzed for its presence of nutritional composite using AOAC protocol for moisture, fat, fiber, and ash [17].

Total crude protein analysis of the examined sample was evaluated by Bradford test [18]. The tests were performed in three replicas to reduce the possibility of error.

2.6. Extraction of crude metabolite

The sample was dried and was extracted in methanolic solvent using the earlier protocol as described earlier [13].

2.7. Qualitative analysis for phytochemicals

The crude methanolic extract was further diluted at a concentration of 5 mg/mL for its various phytochemical assays. The presence of alkaloids, flavonoid, phenolic, carbohydrates, cardiac glycosides, terpenoids, and saponins were determined qualitatively [19]. Meixner test was performed to test the presence of deadly amanitin and other hallucinogenic alkaloids [20].

2.8. Determination of antioxidant assay

The free radical scavenging activity of the methanolic extract of the mushroom was performed [21]. Absorbance was measured at 517 nm in UV spectrophotometer. A low absorbance value indicated a high free-radical-scavenging activity. Butylated hydroxytoluene (BHT) was considered as a positive control for the test. The percentage free radical scavenging activity was analyzed using the following equation

$$\text{DPPH scavenging effect (\%)} = 100 \times (1 - A_C/A_D)$$

where A_C is the absorbance of the reaction with test sample and A_D is the absorbance of the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) reagent without the test sample.

Reducing power assay was performed using the protocol as described by Oyaizu [22]. To various concentrations of the methanolic extract (2.5 mL) were mixed the reagents and the absorbance was measured spectrophotometrically at 700 nm. Higher absorbance indicates higher reducing powers. BHT was considered as a standard.

2.9. Determination of bioactive compounds

For flavonoid content, the methanolic extract (5 mL) was treated with 10% of aluminum nitrate (1 mL) and 1 M aqueous potassium acetate (1 mL) and

absorbance was noted at 415 nm. Quercetin was used as a standard for the test [23].

Folin-Ciocalteu calorimetric method was used to determine the total soluble phenolic matter of the crude extract [24]. Results were expressed in terms of mg of gallic acid equivalents per gram (mg GAE g^{-1}) of the test mushroom.

β -carotene and lycopene in the metabolite was determined and was calculated with the following formula [25]

$$\beta\text{-carotene (mg/100mL)} = 0.216A_{663} - 1.22A_{645} - 0.304A_{505} + 0.452A_{453}$$

$$\text{Lycopene (mg/100mL)} = -0.0458A_{663} + 0.204A_{645} + 0.372A_{505} - 0.0806A_{453}$$

2.10. GCMS analysis

GC-MS analysis was performed using a GC-MS spectrometer (Clarus 680/600 EI; Perkin-Elmer, Norwalk, CT, USA) with a capillary column (30 m \times 0.25 mm \times 0.25 microm). The Clarus 680 GC used purified helium as the carrier gas, at a constant flow rate of 1 mL/min. One microliter of samples were injected and oven temperature was programmed from 60 °C to 300 °C for 2 min at the rate of 10 °C/min and then isothermally held for 6 min until the analysis was completed. Later the compounds were analyzed in mass spectrophotometer. Both the structure and molecular weight of the compounds were interpreted by comparing with the database of National Institute Standard and Technology (NIST) compound database.

2.11. Antimicrobial and synergistic assay

In vitro antimicrobial assay was carried out by well diffusion method against clinical pathogens [13]. The test bacterial strains, *Escherichia coli* (MTCC730), *Staphylococcus aureus* (MTCC96), *Streptococcus pyogenes* (MTCC1925), and *Klebsiella pneumonia* (MTCC109) were considered. The bacterial strains were grown on brain-heart infusion broth at temperature of 37 °C for 18–24 h until it reached a turbidity of 0.5 McFarland standards (10^6 colony forming units/mL). The antimicrobial assay was performed on Mueller Hinton Agar plates. The crude extract at a concentration of 1 mg/mL (40 μ L) was used for the assay along with the pure extraction solvent (DMSO) as a negative control. The plates were incubated at 37 °C for 24 h. Chloramphenicol (30 μ g) was used as a positive control to determine the sensitivity of the strains. Antimicrobial activity of the crude extract was determined by measuring the zone of inhibition around the well.

Synergistic activity of the metabolite was analyzed by calculating the mean surface area of zone of inhibition by antibiotic and to that of metabolite (1 mg/mL, 40 μ L) in combination with commercial antibiotic chloramphenicol. The percentage in fold increase area was determined by the following relation

$$(B^2 - A^2)/A^2 \times 100$$

where A is zone of inhibition for antibiotic and B is the metabolite in combination with antibiotic, respectively.

3. Results and discussion

3.1. Etymology

This label refers to the polyphasic similarity of the mushroom to *Campanophyllum proboscideum*.

3.2. Taxonomic analysis

Pileus: Pileus span from 2.5 to 3.9 cm diameter, asci with wavy and lobe-shaped margin to somewhat in-rolled, and curved. Slightly umbonated surface, sticky, moist, and fibrillose in texture. Lamellae sinuate, crowded, whitish in color when young, pale yellow at maturity, but becomes slightly darker brown during storage or drying. Stipe is absent. The asci-shaped pileus attaches on to the tree bark (Figure 1(A)).

3.3. Microscopic analysis

The asci-shaped *C. proboscideum* differs from the sac silhouette of *Cyphella digitalis* in its morphological trait, despite being a member of the same family, Cyphellaceae. Among the subicular hymenium observed, the cystidium is the biggest cell, with an average length of 6–8.83 μ m and is cylindrical or globular to subclavate (Figure 1(B,C)). The

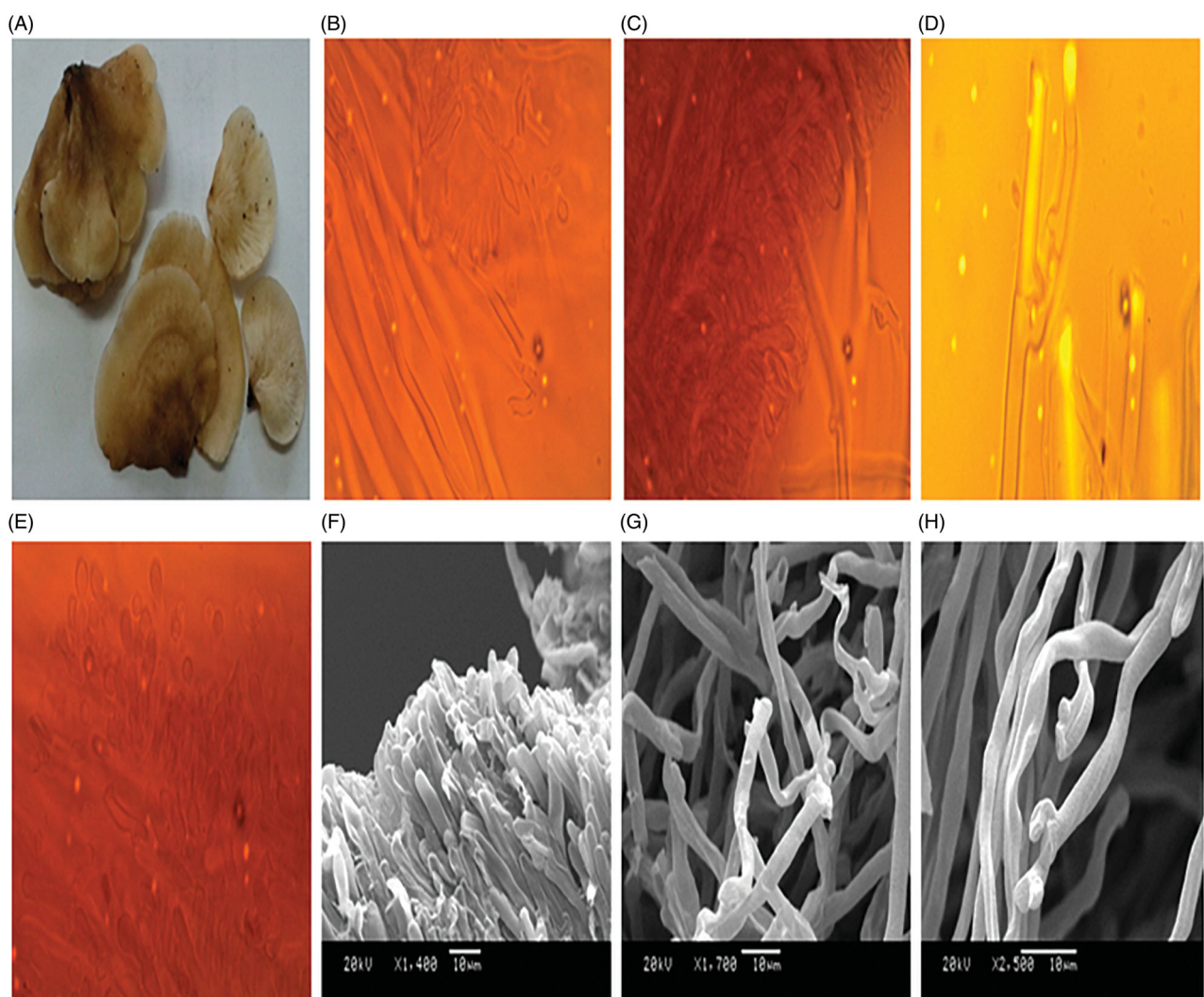


Figure 1. (A) Fresh basidiomycota of *Campanophyllum proboscideum*; (B) mycelia morphology showing cystidia with tapering end; (C) cystidia with globular end; (D) clamp connection; (E) Globular basidia formation observed in the transverse section of gills; (F) Scanning electron micrograph of gills showing globular end; (G) Scanning electron micrograph of gills with hymenium; (H) Scanning electron micrograph of gills showing clamp connection.

subhymenia are mainly generative with a slightly double-thickened wall, leaving the cytoplasmic components in some parts of the hymenia. The hyphal system appears monomitic in form, having a width of 2.77 μm . The hymenium is narrow and clavate and frequently shows clamp connections, with the clamp ranging in diameter from 2.94 μm to 4.95 μm (Figure 1(D)). The hyphae have been observed to be swallowed up in some regions. The cheilocystidia are noted to have short necks present at the lamellar edge, which are similar to pleuramacrocystidia (Figure 1(D)) [26].

The cheilocystidia are crystalline in the center. The lamellae are observed to become mature with the basidia formation having a diameter width of 2.42 μm to 2.65 μm at the apex (Figure 1(E)). Some basidia appear to have a clavate structure. The lower parts of the basidia are noted to have swallow cells. Sterigmata and basidiospores are absent. The lamellar trama, which is seen to acquire a yellowish color with Melzer's reagent, possess a hollow tube like structure at its apex. The pileipellis is somewhat thick. Marginal cells appear cylindrical to short-ellipsoidal in shape.

Most of the hyphal trama appear to be having a clear club-shaped apex with globular basidia in the juvenile stage; they lack sterigmata and basidiospores, as well as the basidioles in the SEM image. The hymenial cells were observed to be short in length (Figure 1(F)). The hyphae were thick-walled, loosely interwoven, and distantly aerial. The apex was somewhat enveloped. Clamp connections were observed and were often branched at the junctions, with wide angles (Figure 1(G)). Microscopically, abundant fusiform to cystidia cylindrical in structure were present, often bulbous and tapering at the apex (Figure 1(H)). The unique feature of *C. proboscideum* was the long thick hyphal cell wall showing the presence of clamp connections, together with the cheilocystidia along the lamellar edge, which were evident in the section of the gills under the optical microscope.

3.4. Molecular analysis

The macrofungi rRNA-ITS region was considered the confirmatory identification, in which the sequence data were aligned using BLAST and deposited on to the NCBI to obtain the accession number (KP843881). Both the Neighbor Joining (NJ) and Bayesian analysis were performed to confirm the relationship clade. The tree based on the ITS rRNA revealed the sequence to be included in the clade with *C. proboscideum*, having 93% sequence homology with a strong supported bootstrap of 1000 replicates while computing Neighbor joining which

was later confirmed with the Bayesian topology. The Bayesian topology was analyzed to verify the second hypothetical interference of the concatenated file. The topology test specified that the sequence data (MBSRJ38) needed to be in same clade with *C. proboscideum* with one posterior probability, which supports the best-scoring NJ analysis (Figure 2). The species is identified as *Campanophyllum* based on its morphological and microscopic structure, including basidia, cystidia, and hyphal cell wall, and is authenticated by molecular characterization using ITS. The fungal sequence showed hits of 83% identity to *C. proboscideum* with a query cover of 94% in the NCBI BLAST. Being monotypic, the sequence supports 93% bootstrap and forms a clade with *C. proboscideum* identified in Costa Rica in 2003 (NCBI Accession: AY230867) [4].

3.5. Nutritional analysis

The nutritional analysis of the fruiting body varies significantly in its content of protein, fiber, moisture, ash, and fat. The protein content was found to be significantly higher. The protein content was found to be significantly higher. The sample was analyzed for total moisture, fiber content in its dried mass, fat content, and crude ash (Table 1). The crude protein content of the mushroom in this experiment was found to be appreciably good. According to a few reports, the crude protein present in mushrooms ranges from 19 to 39 g in 100 g of dry weight [27–29]. The low fat content and higher protein level reveal it to be a source of treasured dietary compounds, indicating that the mushroom in this study is essentially highly beneficial in terms of human health.

3.6. Phytochemical analysis

The experimental mushroom was analyzed for the presence of phyto-constituents which was found to be positive for alkaloids, flavonoids, terpenes, saponins, cardiac glycosides, phenolics, and carbohydrates. From the Meixner test, the metabolite was analyzed to be negative for both deadly amanitin and hallucinogenic alkaloid psilocybin, as the extract did not show any color change when treated with high-lignin content newspaper and hydrochloric acid, making it safe for human consumption.

3.7. Antioxidant assay

The DPPH forms a free radical, which when it reacts with the active antioxidant can donate its hydrogen or electron, and get reduced from dark violet in color to pale yellow. The reduction or

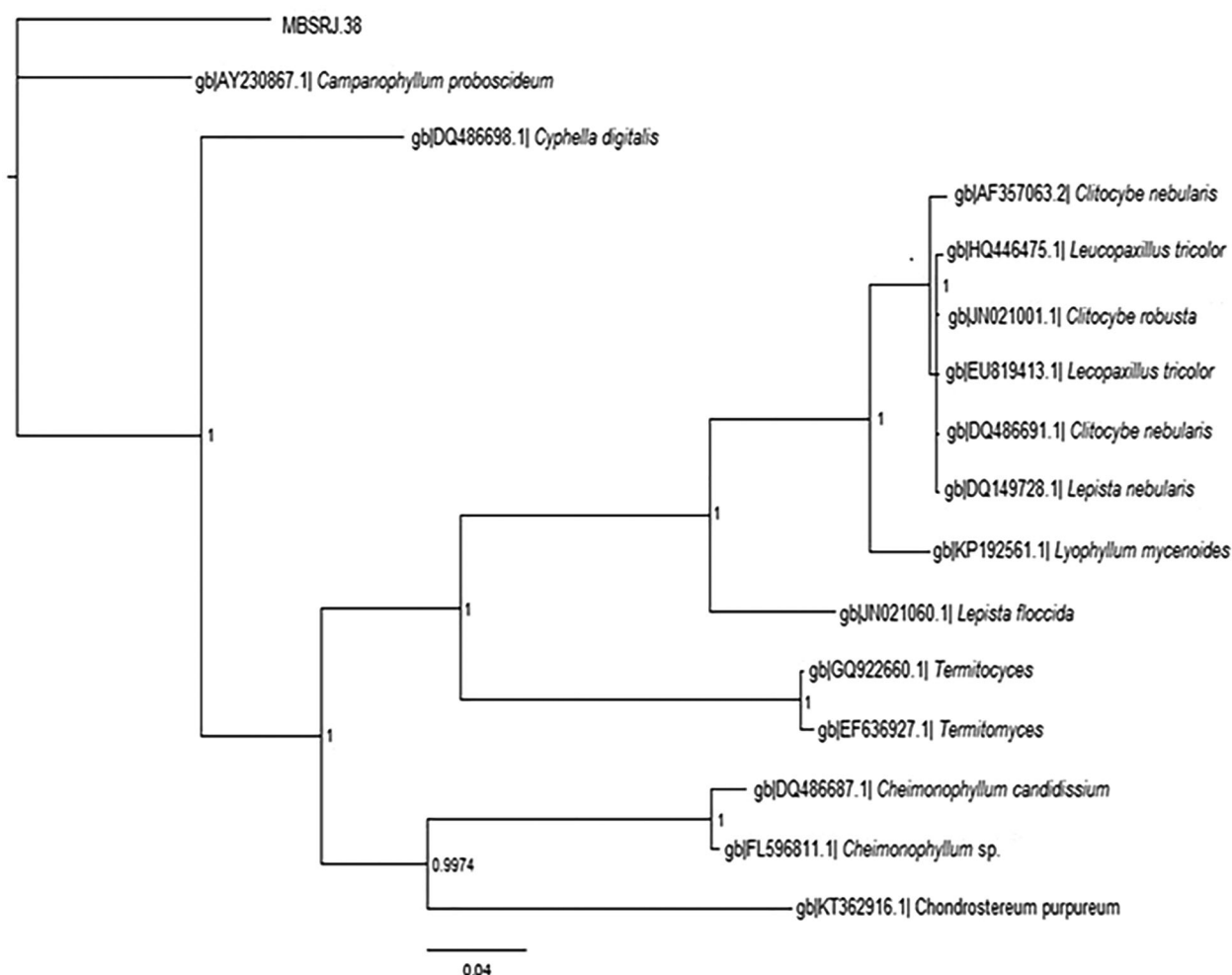


Figure 2. Bayesian tree inferred from ITS nucleotide sequence with 1 posterior probability.

Table 1. Nutritional profiling of *Campanophyllum proboscideum* (g/100g).

Moisture (%)	Fiber	Fat	Ash	Protein
56.78 ± 0.13	5.78 ± 0.43	0.7 ± 0.03	2.87 ± 0.18	21.67 ± 0.67

Result considered was the mean ± SEM of 3 trials (one-way ANOVA).

removal of the hydrogen ion from DPPH is determined by its lowered absorption when determined at 517 nm. Figure 3(A) shows the scavenging activity (%) on the DPPH radical by the experimental mushroom metabolite in terms of the commercially synthesized BHT as the positive control. The % inhibition by the methanolic extract of *C. proboscideum* ranges between 33.52 ± 0.27% and 82.94 ± 0.22%. The free radical scavenging effect was found to be in an exponential phase with the increase in the concentration, whereas the IC₅₀ value obtained from the regression graph was around 4.6537 mg/mL. The data obtained from this study are much higher than a report on a few wild mushrooms as recorded earlier, when the scavenging effect by the methanolic extract of the wild mushroom was noted to be 84.4–85.8% at a concentration of 30 mg/mL for both the stipe and pileus, individually [29].

The reducing power assay is based on the ability of the metabolite to reduce the yellow ferric iron to the blue ferrous form by donating an electron. The reducing power of the experimental mushroom was found to be clearly manifested ($p < 0.05$), where the BHT was accepted as the standard. The higher the absorbance the higher the reducing power (Figure 3(B)), and this might be due to the high phenolic or flavonoid content which participates in electron donation and stabilizes the free radical reaction [22]. The reducing power of the methanolic extract of *C. proboscideum* ranges from 0.85 ± 0.13 to 1.648 ± 0.14. The commercially synthesized antioxidant BHT reveals a higher range of reducing activity, with mean absorbance ranging from 1.79 ± 0.1 to 2.76 ± 0.14 from 2 mg/mL to 10 mg/mL concentration. However, a good reducing power was reported at a concentration of 1–5 mg/mL for ranges from 0.73 to 0.84 [30].

3.8. Determination of the bioactive compounds

Flavonoids are known to possess strong antioxidant activities to scavenge the free radicals. The total flavonoid content in the experimental mushrooms was

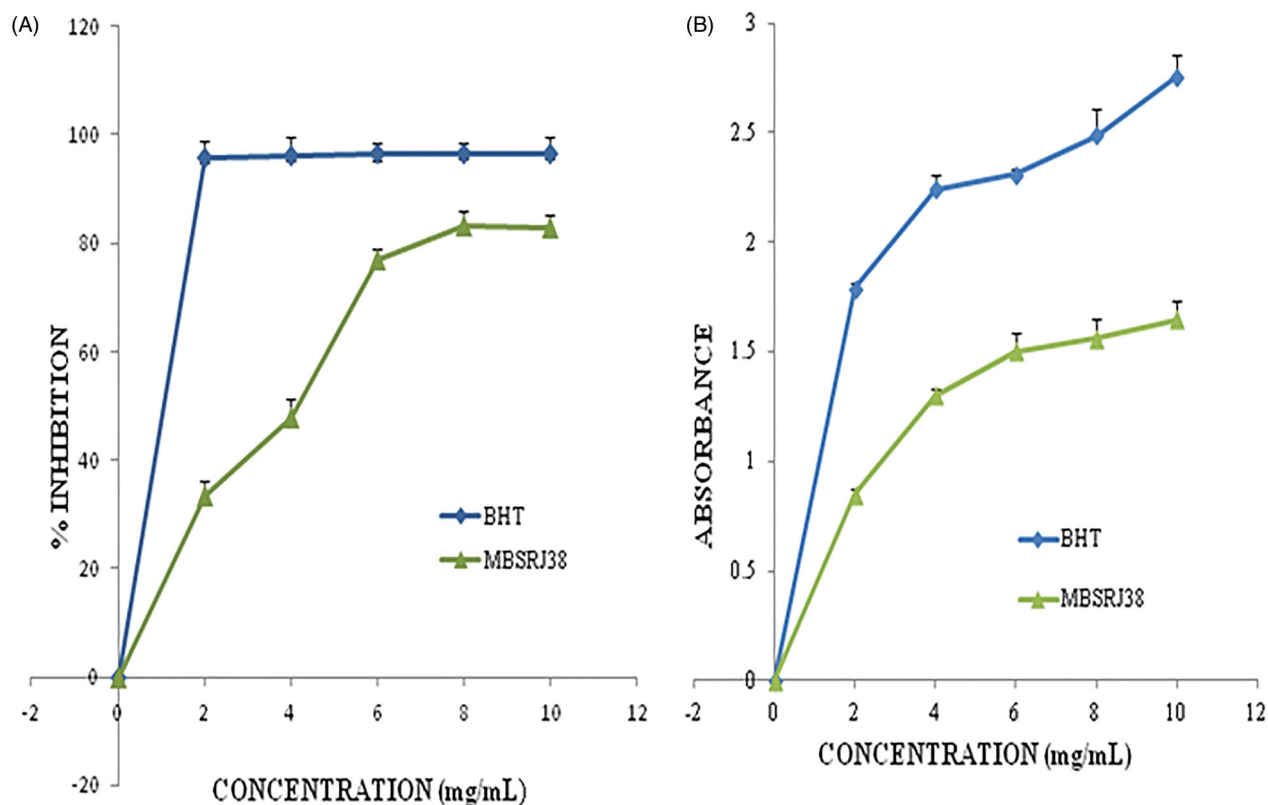


Figure 3. (A) Free radical scavenging activity of the methanolic extract at various concentrations; (B) Reducing power activity of the mushroom methanolic extract at various concentrations.

Table 2. Bioactive compound profiling of *Campanophyllum proboscidium*.

Total flavonoids (mg QE/g dry weight)	Total phenolics (mg GAE/g dry weight)	β -carotene (μ g/g)	Lycopene (μ g/g)
0.94 ± 0.037	0.5127 ± 0.020	7.42 ± 0.04	3.84 ± 0.02

Result considered was the mean \pm SEM of 3 trials (one way ANOVA).

estimated in terms of Quercetin (Table 2). The flavonoid level is a little lower than that of the other wild mushrooms, which ranges from 115.16 mg/100 g–134.31 mg/100 g in the literature and which may be the result of several epigenetic factors [31].

The total phenolic content of the mushroom sample examined was estimated with standard curve equation: $y = 0.9138x + 0.0064$, $R^2 = 0.9801$. Soluble phenolic compounds have been studied to facilitate the consumption of edible mushroom by the tribal communities, and which may also be the source of effective anti-inflammatory, anti-tumor, antibacterial, antioxidant, and antiviral agents [9]. The total phenol found in our experimental species was much less than that of some wild mushrooms, which could be due to certain genetic factors (species-to-species variations), epigenetic factors, solvent concentration, solvent-to-solid ratio, solvent polarity, or moisture content of the mushroom [32–34]. A similar study on tea, catechin, and caffeine has also reported much higher total phenol content in the ethanolic extract than that of methanol or water extract, which may be due to the inability of the solvent to permeate into the tissue [33].

β -carotenes are known as the precursors of Vitamin A and the presence of lycopene is considered the most efficient supply of an oxygen quencher [31]. In this study, the presence of carotenoid in the mushroom species makes them significant and on par with vegetables. The β -carotene and lycopene content was estimated from the studied mushroom (Table 2). Various reports have suggested the presence of high quantities of β -carotene and lycopene in the wild mushroom, including *Russula delica*, *Lentinus squarrosulus*, and *L. sajor-caju* [35,36].

3.9. Chromatographic analysis of the active components

The total ionic count from the chromatographic analysis is shown in Figure 4. The alcoholic, long-chain aliphatic hydrocarbon, phenolics and benzoquinone, as well as the terpenes, esters, ethyl group, carboxylic group, and volatile organic compounds were detected. At a retention time of 11.6, a terpene, 2H-pyran, tetrahydro-4-methyl-2-(2-methyl-1-propenyl)- was present, a well-recognized flavor-

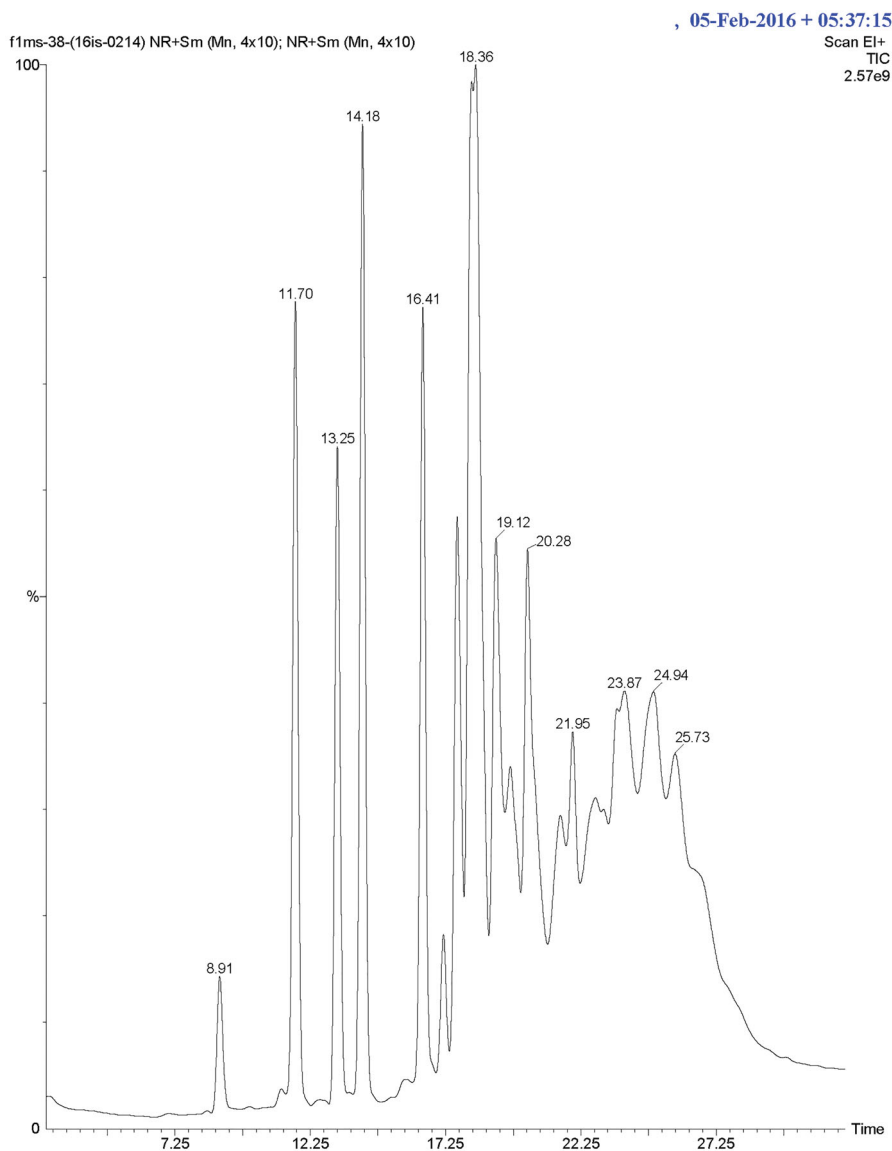


Figure 4. Total ionic count of the mushroom metabolic by GCMS.

Table 3. GCMS profiling of *Campanophyllum proboscidium*.

RT (min)	Compounds (Identified)	Mol. formula	<i>m/z</i>	Peak abundance (%)	Activity
11.6	2H-Pyran, Tetrahydro-4-methyl-2-(2-methyl-1-propenyl)-	C ₁₀ H ₁₈ O	154	1.5	Flavoring agent
13.25	2-methylsulfanyl-1-(thiomorpholin-4-yl) Ethanone	C ₇ H ₁₃ NOS ₂	191.30	100	–
13.25	Phenol, 2, 6bis (1, 1-Dimethylethyl) -	C ₁₄ H ₂₂ O	206	12.5	Anti-inflammatory
13.27	Phosphetane, 1, 1-bis (1, 1-dimethylethyl) 1-fluoro-1, 1-dihydro	C ₁₁ H ₂₄ FP	206	7.5	–
14.18	Cyclohexadecane	C ₁₆ H ₃₂	224	2.0	–
16.4	2, 6-dimethoxybenzoquinone	C ₈ H ₈ O ₄	168	1.5	Toxic (mutagenic, cytotoxic, hepatotoxic)
17.04	3-methyl-2-(2-oxopropyl) furan	C ₈ H ₁₀ O ₂	138	1.02	Anti-inflammatory, antipyretic, hepatoprotective
17.6	2-hydroxy-4-methylthiobutanoate	C ₅ H ₉ O ₃ S	149.18 100	–	–
18.3	1-Eicosene	C ₂₀ H ₄₀	280	2.5	Anti-diabetic, anti-cancer
19.12	S-propyl N-butyl-N-ethylcarbamothioate	C ₁₀ H ₂₁ NOS	203.13	0.5	–
19.12	1-Methyl-1-histidine, trimethylsilyl ester	C ₁₀ H ₁₉ N ₃ O ₂ Si	241	0.8	–
19.12	Sarcosine, n-pentafluoropropionyl-, propyl ester	C ₉ H ₁₂ F ₅ NO ₃	277	0.3	–
20.29	- (-) Epicatechin	C ₅ H ₁₄ O ₆	207.1043	3.25	Antioxidant

enhancer. Terpenoids like ergosta-5, 7, 22-trien-3-ol, 22-tetraen-3.β.-ol, *D*-xenialactol, gosterol, ergothione, dimethylhydrazine were earlier reported in various higher fungi [37,38]. Terpenoids are known to possess antioxidant, anticancer, anti-

inflammatory, anticholesterol properties [38]. The phenolic compound, 2, 6-bis (1, 1-dimethylethyl) was present at 13.25 retention time with peak abundance touching as high as 12.5. This compound is commonly known as a precursor of the complex

compound utilized as an antioxidant. At 13.27 retention time, with peak abundance of 7.5%, Phosphetane, 1, 1-bis (1, 1-dimethylethyl)-1-fluoro-1, 1-dihydro was observed. Toxic benzoquinone, 2, 6-dimethoxybenzoquinone (2, 6-DMBQ) was present at a retention time of 16.4. The aliphatic hydrocarbon, 1-Eicosene (m/z of 280) was observed at a retention time of 18.3, with peak abundance of 2.5% (Table 3). Aliphatic hydrocarbons are known to

indirectly express metabolic activity by stimulating alcohol and ester formation, which further facilitates the growth of the cellular biomass by metabolizing the Krebs cycle. A few of these compounds have been reported for their potential in pharmaco-activities. Some compounds like alcoholic, volatile organic compounds, and ester components have been known to play a substantial roles either as a flavoring component in the pharmaceutical

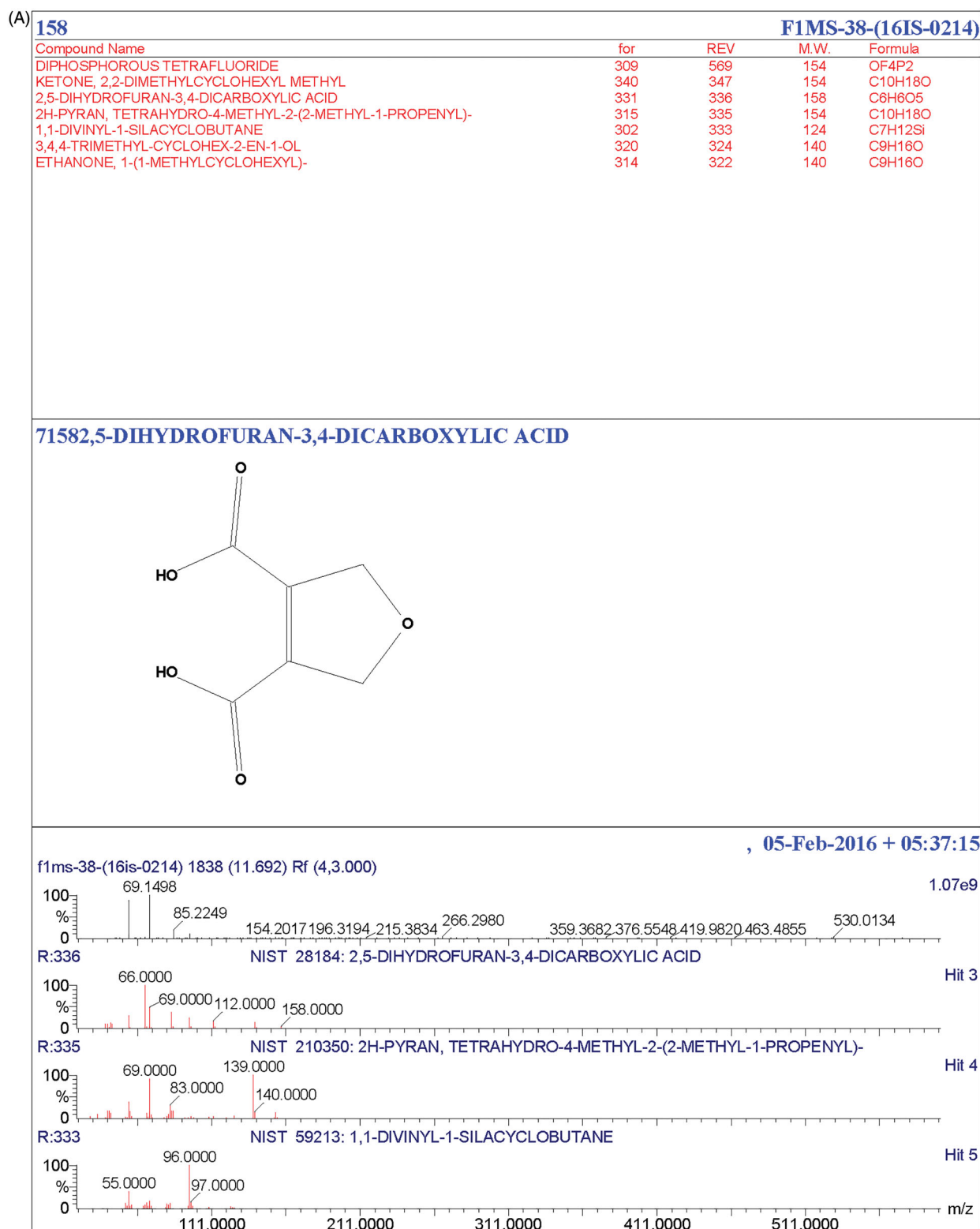


Figure 5. (A) NIST library analysis of active compound 71582. (B) NIST library analysis of an active compound 41682.

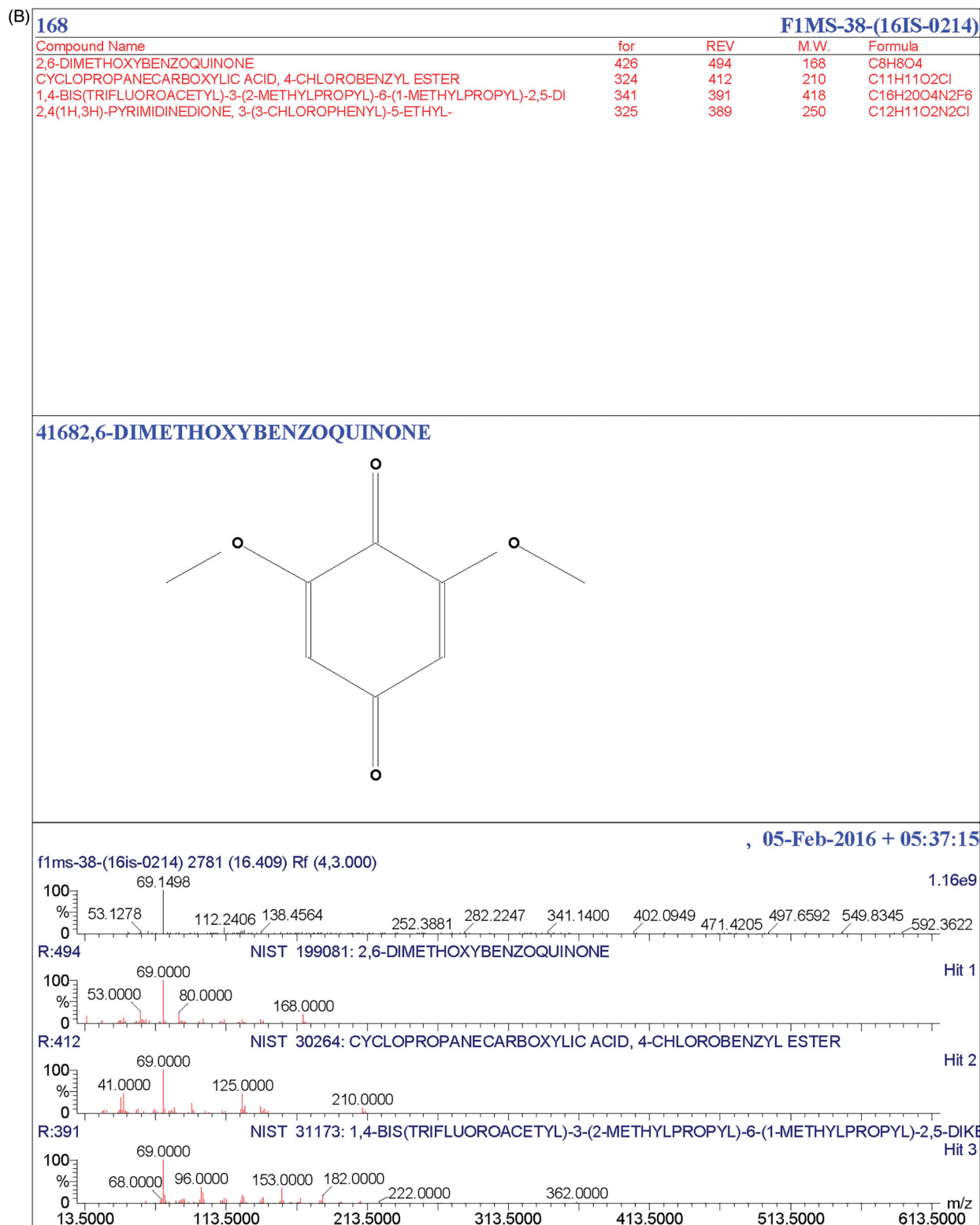


Figure 5. Continued.

Table 4. Antimicrobial and synergistic activity of the metabolite against pathogens *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes* and *Klebsiella pneumoniae*.

Clinical pathogens	Zone of inhibition (in mm)	Antibiotic [Chloramphenicol] A	Metabolite + Antibiotic Zone of inhibition (in mm) B	Fold area increase (%) (B2 - A2)/A2 × 100 A-Antibiotic (ZOI) B-Antibiotic + metabolite
<i>Escherichia coli</i>	10 mm	32	33	6.34
<i>Staphylococcus aureus</i>	12 mm	31	32	6.55
<i>Streptococcus pyogenes</i>	>10	32	36	26.56
<i>Klebsiellapneumoniae</i>	>10	31	32	6.55

industries or as a defoaming agent and many more. In addition, esters are recognized as a major fraction in various edible mushrooms, in several fruits (apple, apricot, pineapple) as reported, and are known to enhance the fragrance [39–42]. The presence of cyclohexadecane, a heterocyclic metabolite, and a few pyrrole derivatives are known to protect the mushroom from grazing animals. The peaks obtained in the chromatogram spectra were compared with the database from the NIST libraries (Figure 5(A,B)).

3.10. Antimicrobial and synergistic efficiency

The mushroom metabolite showed a strong antimicrobial assay in terms of the commercial antibiotic chloramphenicol (Table 4). The observed zone of inhibition, together with its synergistic effect implies its bactericidal properties. The increased fold area of the mushroom metabolite was significantly higher (with 26.56% in *Streptococcus pyogenes*) than that of the other test pathogens, which may differ in their cell wall compositions.

4. Conclusion

Ecologically, *C. proboscideum* grows in the areas of highest rainfall. It was initially reported from Costa Rica, where the climatic variations are dependent upon the quantity of rainfall received. It is well known that the region receives the highest rainfall between May and November, with the annual rainfall exceeding 5000 mm in some areas. Due to a few climatic similarities, the sample studied was collected twice, from one of the preserved forests of Meghalaya (Mawphlang), which receives the highest rainfall (as high as 12,000 mm) throughout the year, with the humidity higher than 85%. The findings suggest that the biogeographic features have played a unique role in determining the evolutionary traits of this fungus. The specimen was given due attention, and its ultrastructure and molecular characterization were analyzed using the Internal Transcribed Spacer (ITS) markers. However, the morphological evidence of *Campanophyllum* suggests an evolutionary clade in the basidiomycetes, based on the presence of the basidia. Besides, much attention was paid to the molecular structure of the specimen and its bioactive metabolites. Terpenes, esters, and heterocyclic metabolites were very frequently found in the dried extract. The presence of flavonoids was rather scarce when compared with the wild mushroom reported, which may show variations due to different genetic and epigenetic factors. However, it has the capacity to scavenge the free radicals and can be a powerful antioxidant source. It has shown

dynamic synergistic activity and its various bactericidal properties can be used in the pharmaceutical industry. Further investigations will uncover greater details regarding their distribution, availability and toxicity perspective.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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