## 4.1 Survey and sample collection:

The fresh sample (CBUS3) collected from Lachen Valley, North Sikkim, were freeze-dried and submitted to the Sikkim State Forest Herbarium (SSFH), Deorali, Sikkim Vide (SSFH SK005007). The samples obtained from Gassa (Lunana and Laha block) District of the Kingdom of Bhutan and Mechuka Valley of Shi-Yomi District of Arunachal Pradesh, India, were submitted to Bodoland University Botanical Herbarium, Department of Botany, Bodoland University, and accession numbers were obtained (CBUS1-BUBH0000881; CBUS2-BUBH0000882; CBUS4-BUBH0000883; CBUAP1-BUBH0000880; CBUB1-BUBH0000876; CBUB2-BUBH0000877; CBUB3-BUBH0000878; CBUB4-BUBH0000879)

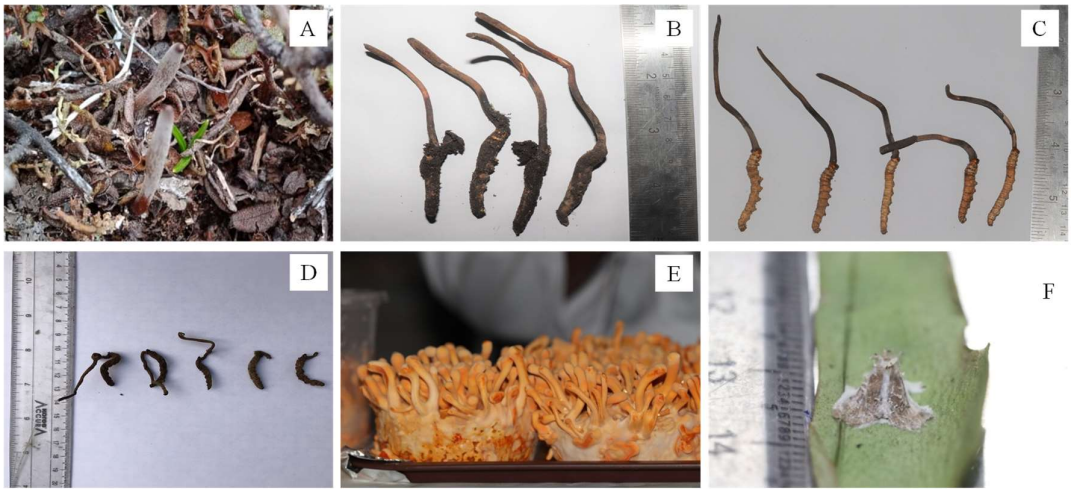



**Fig 4.1. A. *Ophiocordyceps sinensis* (CBUS3) growing in high altitude area of North Sikkim; B. *Ophiocordyceps sinensis* (CBUS3) collected from Sikkim; C. *Ophiocordyceps sinensis* (CBUS3) after cleaning; D. *Ophiocordyceps liangshensis* (CBUAP1) collected from Arunachal Pradesh; E. *Cordyceps miltaris* standard Strain; F. Wild *Cordyceps sp.* photographed at Ultapani forest Range, Assam**

## 4.2 Morphological Studies:

## Morphological Characteristics:

The morphological characteristics like local name and source of collection has been depicted in table 4.1

**Table 4.1. Description of collected sample**

| Sl. No. | Sample | Sample Description |
|---|---|---|
| 1 |  <br>SAMPLE 1: CBUS1 (Cordyceps Bodoland University Sikkim 1) | Sample Name – *Cordyceps* spp.<br>Local Name-Yarsagumba<br>Host Insect - Larva<br>Source of Collection- Lachung Valley,<br>District- North Sikkim<br>State- Sikkim<br>Average Length of Stalk- 19.6 mm<br>Average Breadth of Stalk- 1.4 mm<br>Average Length of Larva- 23.35 mm<br>Average Breadth of Larva- 2.77 mm |
| 2 |  <br>SAMPLE 2: CBUS2 (Cordyceps Bodoland University Sikkim 2) | Sample Name – *Cordyceps* spp.<br>Local Name-Yarsagumba<br>Host Insect - Larva<br>Source of Collection- Lachung Valley,<br>District- North Sikkim<br>State- Sikkim<br>Average Length of Stalk- 15.59 mm<br>Average Breadth of Stalk-2.62 mm<br>Average Length of Larva- 30.91 mm<br>Average Breadth of Larva- 3.76 mm |

| | | |
|---|---|---|
| 3 |  <br>SAMPLE 3: CBUS3 (Cordyceps Bodoland University Sikkim 3) | Sample Name – *Cordyceps* spp.<br>Local Name-Yarsagumba<br>Host Insect - Larva<br>Source of Collection- Lachen Valley,<br>District- North Sikkim<br>State- Sikkim<br>Average Length of Stalk- 54.59 mm<br>Average Breadth of Stalk-1.67 mm<br>Average Length of Larva- 28.42 mm<br>Average Breadth of Larva- 3.46 mm |
| 4 |  <br>SAMPLE 4: CBUS4 (Cordyceps Bodoland University Sikkim 4) | Sample Name – *Cordyceps* spp.<br>Local Name-Yarsagumba<br>Host Insect - Larva<br>Source of Collection- Lachung Valley,<br>District- North Sikkim<br>State- Sikkim<br>Average Length of Stalk- 15.20 mm<br>Average Breadth of Stalk-2.22 mm<br>Average Length of Larva- 36.19 mm<br>Average Breadth of Larva- 4.30 mm |
| | | Sample Name – *Cordyceps* spp.<br>Local Name-Yarsagumba |

| | | |
|---|---|---|
| 5 |  <br>SAMPLE 5: CBUAP1 (Cordyceps Bodoland University Arunachal Pradesh 1) | Host Insect - Larva<br>Sample Name – *Cordyceps* spp.<br>Host Insect – Larva<br>Local Name-Yarsagumba<br>Source of Collection- Mechuka Valley,<br>District- Shi-Yomi<br>State- Arunachal Pradesh<br>Average Length of Stalk- 48.66 mm<br>Average Breadth of Stalk- 2.56 mm<br>Average Length of Larva- 28.96 mm<br>Average Breadth of Larva- 6.05 mm |
| 6 |  <br>SAMPLE 6: CBUB1 (Cordyceps Bodoland University Bhutan 1) | Sample Name – Super A (Local Name)<br>Source of Collection- Bhutan<br>Local Name- yartsa guenboob<br>Block- Laya<br>District- Gassa<br>Average Length of Stalk- 32.06 mm<br>Average Breadth of Stalk- 3.91 mm<br>Average Length of Larva- 38.23 mm<br>Average Breadth of Larva- 4.65 mm |

| | | |
|---|---|---|
| 7 |  <br>SAMPLE 7: CBUB2 (Cordyceps Bodoland University Bhutan 2) | Sample Name – A+ (Local Name)<br>Source of Collection- Bhutan<br>Local Name- yartsa guenboob<br>Block- Lunana<br>District- Gassa<br>Average Length of Stalk- 24.76 mm<br>Average Breadth of Stalk- 2.50 mm<br>Average Length of Larva- 32.63 mm<br>Average Breadth of Larva- 4.65 mm |
| 8 |  <br>SAMPLE 8: CBUB3 (Cordyceps Bodoland University Bhutan 3) | Sample Name – B+ (Local Name)<br>Source of Collection- Bhutan<br>Local Name- yartsa guenboob<br>Block- Lunana<br>District- Gassa<br>Average Length of Stalk- 23.68 mm<br>Average Breadth of Stalk- 2.77 mm<br>Average Length of Larva- 28.07 mm<br>Average Breadth of Larva- 4.03 mm |
| 9 | | Sample Name – C+ (Local Name)<br>Source of Collection- Bhutan<br>Local Name- yartsa guenboob<br>Block- Laya |

| | | |
|---|---|---|
| |  <br>SAMPLE 9: CBUB4 (Cordyceps Bodoland University Bhutan 4) | District- Gassa<br>Average Length of Stalk- 30.55 mm<br>Average Breadth of Stalk- 2.18 mm<br>Average Length of Larva- 25.74 mm<br>Average Breadth of Larva- 3.77 mm |
| 10 |  <br>SAMPLE 10: CBUJ1 (Cordyceps Bodoland University Jharbari 1 | Sample Name – *Cordyceps* spp.<br>Host Insect - Moth<br>Source of Collection- Jharbari<br>Block- Dotma<br>District- Kokrajhar |

## 4.3 Isolation and Pure Culture:

Sample CBUS3 was the only sample collected freshly and was cultured in Petri plates containing potato dextrose agar media supplemented with 0.5 g/L magnesium sulphate and 50 mg thiamine hydrochloride. The mycelia started to grow after 5–7 days, followed by a subculture to obtain a pure culture of the sample. Similarly standard strain of *Cordyceps militaris* was also sub cultured in potato dextrose agar media supplemented with 0.5 g/L magnesium sulphate and 50 mg thiamine hydrochloride.

## 4.4 Cultivation of *Cordyceps militaris:*

The cultivation of *Cordyceps militaris* was carried out on different rice varieties to study the importance of rice as a substrate and also to compare its nutritional properties with the sample collected from wild.

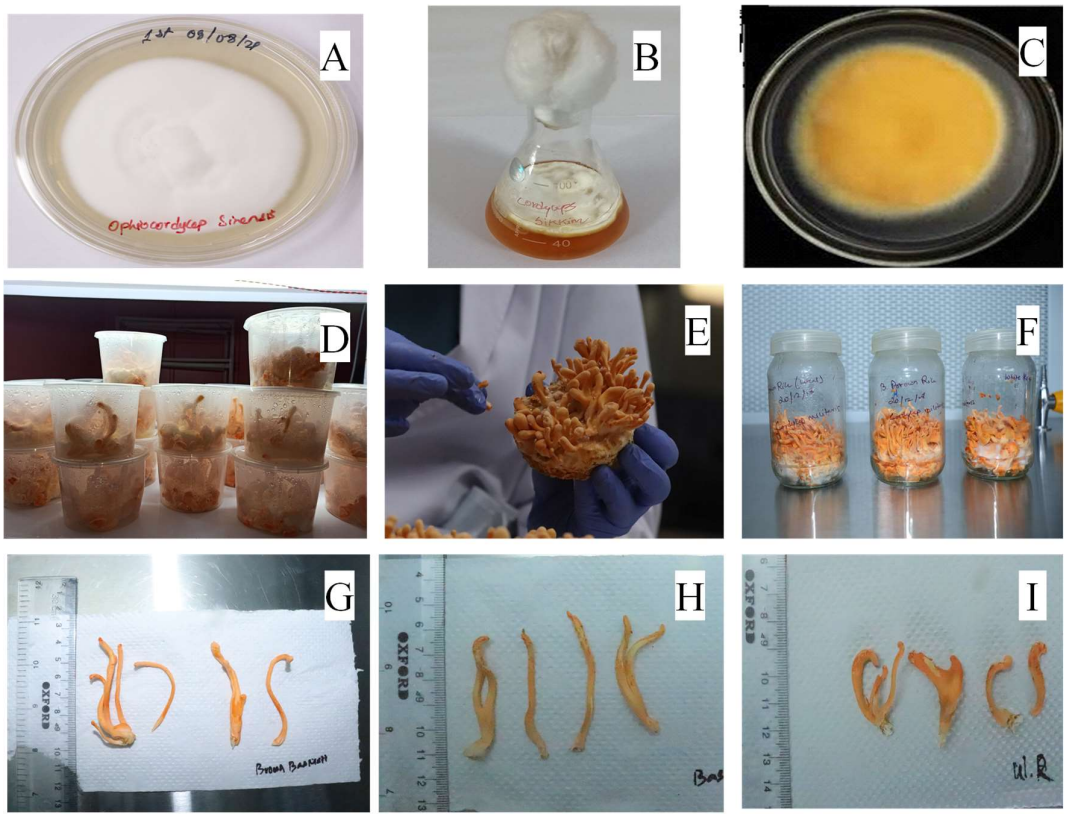



**Fig 4.2. A. Pure culture of *Ophiocordyceps sinensis* (CBUS3); B. Mycelia of *Ophiocordyceps sinensis* (CBUS3) grown on Potato dextrose broth; C. Pure culture of *Cordyceps militaris* (CBUCM); D. Fruiting bodies of *Cordyceps militaris* (CBUCM); E. Harvesting of fruiting bodies; F. *Cordyceps militaris* grown on different rice substrates; G. Fruiting bodies of *Cordyceps militaris* grown on Brown rice; H. Fruiting bodies of *Cordyceps militaris* grown on Basmati rice; I. Fruiting bodies of *Cordyceps militaris* grown on Basmati rice.**

## 4.5 Molecular Studies:

## 4.5.1 DNA isolation:

The DNA was isolated from the collected samples and subjected to electrophoresis for confirmation.

## 4.5.2 Electrophoresis:

The isolated DNA were visualized in 0.8% agarose gel electrophoresis.

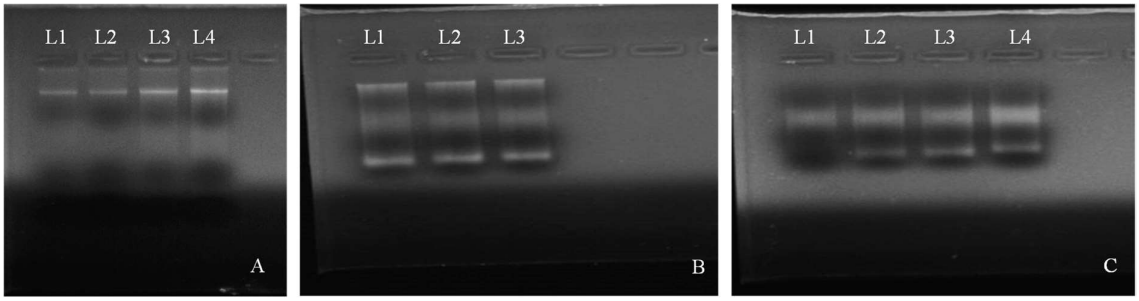



**Fig 4.3. Agarose gel (0.8%) run of Genomic DNA; A. Sample CBUS; L1- CBUS1: L2-CBUS2: L3- CBUS3: L4- CBUS4; B. Sample CBUAP1; L1- L3- CBUAP1; C. Sample CBUB; L1- CBUB1: L2-CBUB2: L3- CBUB3: L4- CBUB4**

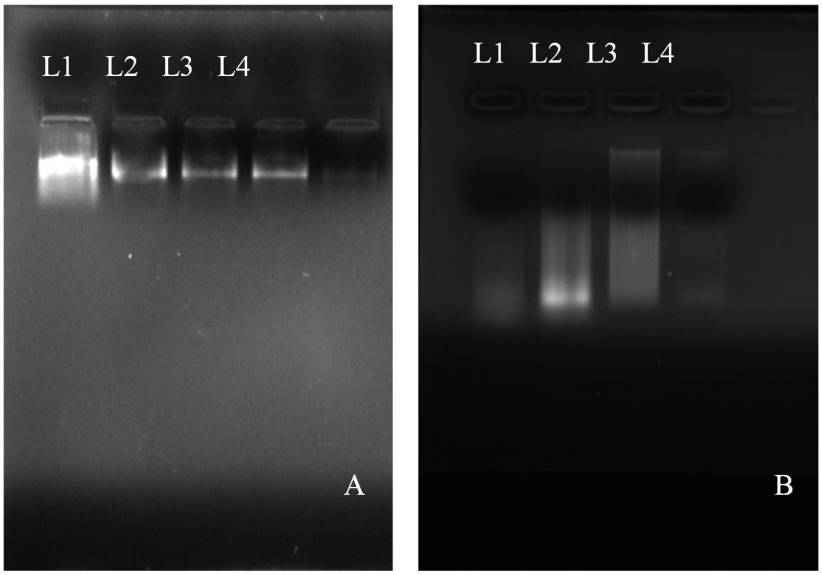



**Fig 4.4. Agarose gel (0.8%) run of Genomic DNA; A. Sample CBUCM; L1-L4-CBUCM; B. sample CBUS3 Mycelia L1-L4**

## 4.5.3 DNA Quantification:

DNA quantification was performed by Qubit 4 Fluorometer (Invitrogen) following the manufacturer's protocol. Concentration of DNA are depicted on Table 4.2

**Table 4.2. Concentration Genomic DNA (ng/µL).**

| Sl. No. | Sample Name | Concentration (ng/µL) |
|---|---|---|
| 1 | CBUS1 | 196 |

| | | |
|---|---|---|
| 2 | CBUS2 | 201 |
| 3 | CBUS3 | 186 |
| 4 | CBUS4 | 215 |
| 5 | CBUS3- Mycelia | 213 |
| 6 | CBUAP1 | 178 |
| 7 | CBUB1 | 258 |
| 8 | CBUB2 | 244 |
| 9 | CBUB3 | 215 |
| 10 | CBUB4 | 226 |
| 11 | CBUCM | 235 |

## 4.6 Sequencing:

The amplicon was visualized in 1.2 % agarose gel and photographs were taken on E-Gel imager System (Life Technologies, USA) (Figure 4.4) and concentration of DNA were evaluated using Qubit 4 fluorometer. The amplicon was then sequenced on an applied biosystem (Model No-AB13730XL) DNA Sequencer following sanger sequencing method. The sequenced raw data was processed in Bioedit version 7.2.5 and consensus sequence was made and submitted to Gene bank after identification (Table 4.3)

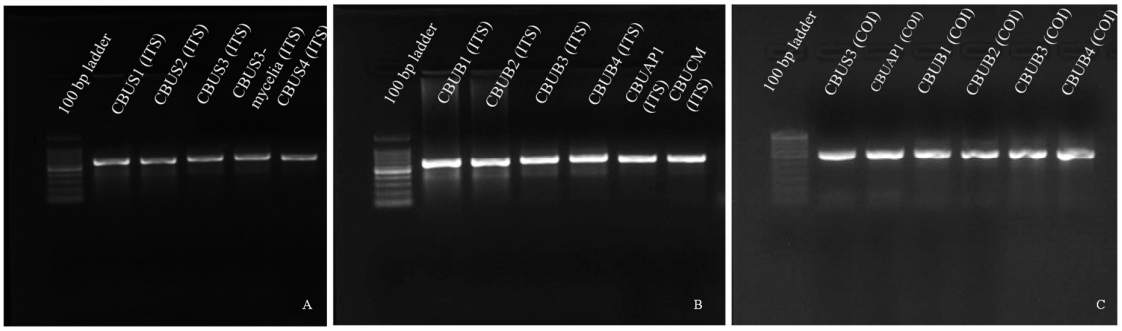



**Fig 4.5. Agarose gel (1.2 %) electrophoresis of amplified PCR product; A. L1- 100 bp ladder, L2-CBUS3(ITS): L3- CBUS3- Mycelia (ITS): L4- CBUB1(ITS): L5-CBUB2 (ITS): L6- CBUB3 (ITS): L7- CBUB4(ITS); B. L1- 100 bp ladder: L2 & L3-CBUAP1 (ITS): *:* L4- CBUCM (ITS); C. L1- 100 bp ladder, L2-CBUS3(COI): L3-CBUAP1 (COI): L4- CBUB1(COI): L5-CBUB2 (COI): L6- CBUB3(COI): L7-CBUB4(COI)**

**Table 4.3. Description of samples submitted to Gene Bank**

| Sl. No | Source | Sequence Length | Description | Gene Bank Accession Number |
|---|---|---|---|---|
| **1** | *Ophiocordyceps sinensis* (CBUS3- Fruiting Body) | 559 bp | Internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence. | MW990119 |
| **2** | *Thitarodes* sp. (CBUS3- Host) | 633 bp | cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial. | MZ956161 |
| **3** | *Ophiocordyceps sinensis* (CBUS3- *Mycelia*) | 549 bp | internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunitribosomal RNA gene, partial sequence. | OK041477 |
| **4** | *Ophiocordyceps liangshanensis* (CBUAP1) | 612 bp | internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunitribosomal RNA gene, partial sequence. | MZ318360.1 |
| **5** | *Thitarodes* sp. (CBUAP1- Host) | 601 bp | cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial. | PP715863 |
| **7** | *Cordyceps militaris* (CBUCM) | 535 bp | internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunitribosomal RNA gene, partial sequence. | MZ749691.1 |
| **8** | *Ophiocordyceps sinensis* (CBUB1) | 579 bp | internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunitribosomal RNA gene, partial sequence. | MN626441.1 |
| 9 | *Thitarodes* spp. (CBUB2- Host) | 361 bp | cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial. | PP583001 |
| **10** | *Ophiocordyceps sinensis* (CBUB2) | 571 bp | internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunitribosomal RNA gene, partial sequence. | MW774384.1 |

**Table 4.4. Nucleotide Blast results**

| Sl. No | Sample | Query Cover | Percent Identity | Closest Match with accession number |
|---|---|---|---|---|
| 1 | *Ophiocordyceps sinensis* (CBUS3- Fruiting Body) | 97 % | 99.58 | KJ175199.1 |
| 2 | *Thitarodes* sp. (CBUS3- Host) | 100 % | 95.56 | KC994917.1 |
| 3 | *Ophiocordyceps sinensis* (CBUS3- *Mycelia*) | 99 % | 100 | AB067720.1 |
| 4 | *Ophiocordyceps liangshanensis* (CBUAP1) | 97 % | 92.78 | KJ524691.1 |
| 5 | *Cordyceps militaris* (CBUCM) | 100% | 100% | ON553385.1 |
| 6 | *Ophiocordyceps sinensis* (CBUB1) | 98 % | 88.47 | KT232019.1 |
| 7 | *Ophiocordyceps sinensis* (CBUB2) | 98 % | 99.60 | KM197540.1 |

## 4.7 Phylogenetic analysis:

### 4.7.1 *Ophiocordyceps sinensis* collected from Sikkim (CBUS1-S4): Fruiting body and Host:

**Current Name: (Index Fungorum)**

***Ophiocordyceps sinensis*** (Berk.) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora, in Sung, Hywel-Jones, Sung, Luangsa-ard, Shrestha & Spatafora, Stud. Mycol. 57: 46 (2007) (Index Fungorum)

Synonymy:
*Cordyceps sinensis* (Berk.) Sacc., Michelia 1(no. 3): 320 (1878)

*Hirsutella sinensis* X.J. Liu, Y.L. Guo, Y.X. Yu & W. Zeng, Acta Mycol. Sin. 8(1): 37 (1989)
*Hirsutella sinensis* X. Fang, Mycological Society of China, Academic Annual Meeting Summary: 203 (2013)
*Sphaeria sinensis* Berk., London J. Bot. 2: 207 (1843)
*Torrubia sinensis* (Berk.) Tul. & C. Tul., Select. fung. carpol. (Paris) 3: 13 (1865)
(https://www.indexfungorum.org/names/names.asp)

The ITS amplicon of sample *Cordyceps* Bodoland University Sikkim was subjected to NCBI Nucleotide BLAST, subsequently, similar sequences were retrieved. The phylogenetic analysis of the *ITS* region of stroma and *COI gene* of the host was performed separately. A total of 22 sequences of *Cordyceps* spp. were retrieved from NCBI for the phylogenetic analysis of the *ITS* region of the stroma, and 19 sequences were retrieved for the phylogenetic analysis of the *COI* region of the host. The sequences were subject to multiple alignment using fast fourier transform (MAFFT) online tool. The alignment was optimized visually, and ambiguous regions were excluded from subsequent phylogenetic analyses. The best model was calculated by the model testing in Mega (version 11.0.13), and the Tamura-Nei model was chosen (Tamura and Nei 1993). Pairwise distance matrices were generated using Tamura-Nei model, and the phylogenetic analysis was performed in the Mega version 11.0.13. An NJ tree (Jukes and Cantor, 1969) with bootstrapping was constructed Initial tree(s) for the heuristic search were obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated using the Tamura-Nei model. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+*G*, parameter = 1.9527)) This analysis involved 26 nucleotide sequences. There was a total of 923 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar et al., 2018). *Volvariella volvacea* is used as an outgroup for phylogenetic analysis of the *ITS* region and *Anthera assama* as an outgroup for *COI* region of host larva. The comparative phylogenetics results using *ITS* sequence indicated that the presence of the sample collected from Sikkim clubbed with the sequences of *O. sinensis* sequences (China) retrieved from NCBI. The presence in the same clade diverging from the other related species confirmed that the sample belong to *O. Sinensis*, which is an entomo-parasitic fungus infecting larva of various genera (*Thitarodes* spp., *Endoclita* spp., *Napialus spp*.). The phylogenetic analysis of host was performed to confirm the larva. The sequence of *COI* of larva from the sample was compared to that of the common larva of *O. sinensis.* The sequences were retrieved from NCBI, and phylogenetic analysis was carried out.

Subsequently, the sample clubbed and grouped with the sequences with *Hepialidae* spp. which confirmed that the larva belongs to *Thitarodes* spp. The tree from Bayesian inference showed an identical tree topology.

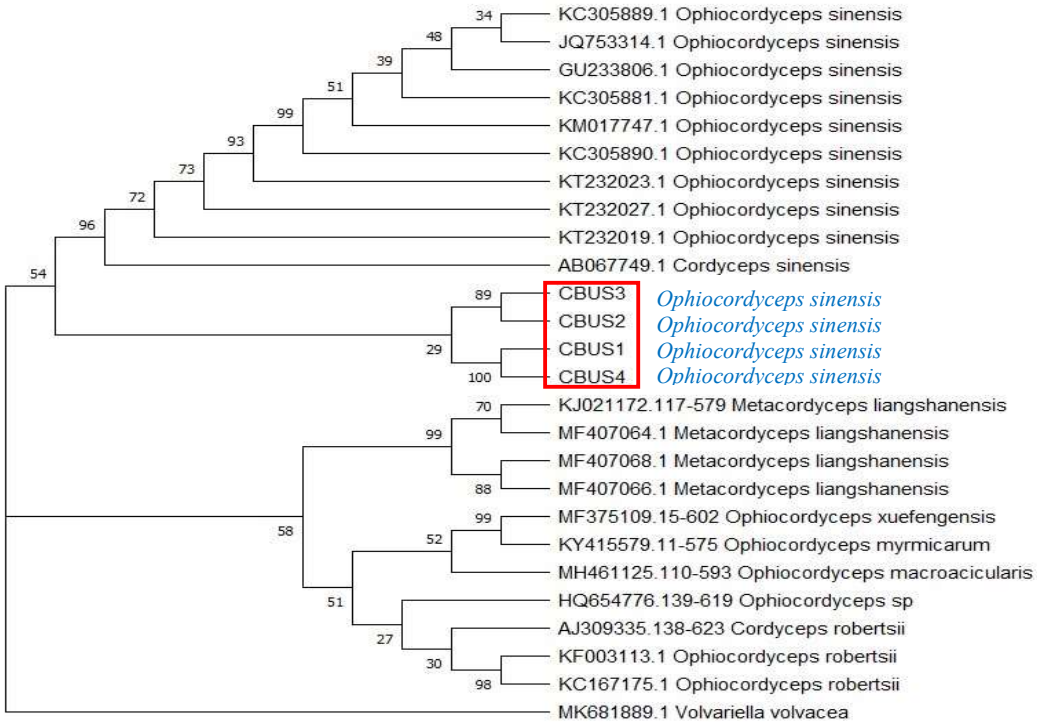



**Fig 4.6. Maximum Likelihood Tree of CBUS1-S4 (*ITS*) constructed using Mega Version 11.0.13**

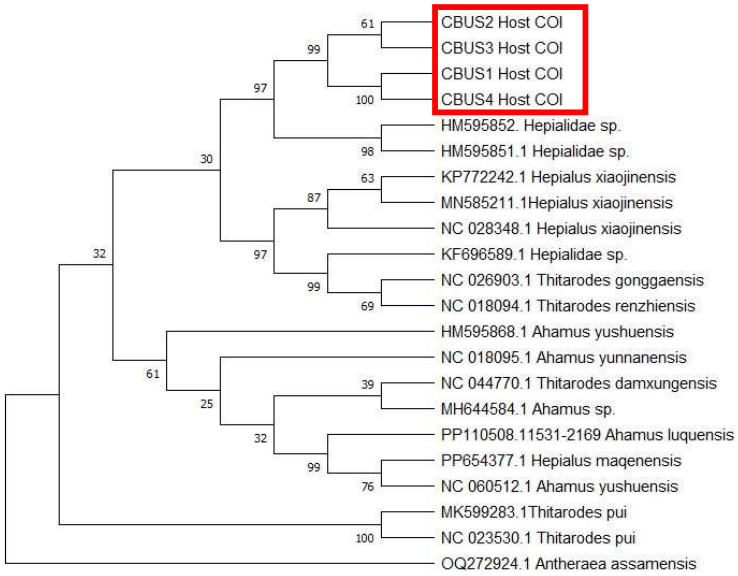



**Fig 4.7. Maximum Likelihood tree of CBUS1-S4 (*COI*) constructed using Mega Version 11.0.1**

### 4.7.2 *Ophiocordyceps liangshanensis* collected from Arunachal Pradesh (CBUAP1):

**Current Name: (Index Fungorum)**
***Ophiocordyceps liangshanensis*** (M. Zang, D.Q. Liu & R.Y. Hu) H. Yu, Y. Wang, Y.D. Dai, Zhu L. Yang & Y.B. Wang, in Wang, Dai, Yang, Guo, Wang, Yang, Ding & Yu, *Mycobiology* 49(4): 302 (2021)

**Synonymy:**
*Cordyceps liangshanensis* M. Zang, D.Q. Liu & R.Y. Hu, Acta bot. Yunn. 4(2): 174 (1982)
*Metacordyceps liangshanensis* (M. Zang, D.Q. Liu & R.Y. Hu) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora, in Sung, Hywel-Jones, Sung, Luangsa-ard, Shrestha & Spatafora, Stud. Mycol. 57: 35 (2007)
*Papiliomyces liangshanensis* (M. Zang, D.Q. Liu & R.Y. Hu) Luangsa-ard, Samson &Thanakitp., in Mongkolsamrit, Khonsanit, Thanakitpipattana, Tasanathai, Noisripoom, Lamlertthon, Himaman, Houbraken, Samson &Luangsa-ard, Stud. Mycol. 95: 240 (2020)

The PCR product of *ITS* and *COI* was 612 bp and 601 bp respectively. The amplicon was then sequenced on an applied biosystem (Model No-AB13730XL) DNA Sequencer following sanger sequencing method. The sequenced raw data was processed in Bioedit version 7.2.5 and consensus sequence was made. The *ITS* amplicon of CBUAP1 (fruiting body) covered a query of 94%, which is identical with *O. liangshanensis*. The sequencing data of *COI* gene had query cover of 94% identity to *Hepialidae* spp. A total of 28 nucleotide sequences were taken up for the phylogenetic analysis including an outgroup from NCBI database. The sequences were subject to multiple sequence alignment using fast fourier transform (MAFFT) online tool. The alignment was optimized visually, and ambiguous regions were excluded from subsequent phylogenetic analysis. The best model was calculated by the model testing in Mega (version 11.0.13), and the T92+G model was chosen (Kimura et al., 1980). Pairwise distance matrices were generated using Kimura models of nucleotide substitutions (Kimura et al., 1980, Kumar et al., 2018; Swofford et al., 1998), and the phylogenetic analysis was performed in the Mega version 11.0.13. An ML tree with bootstrapping was constructed with distance measured by the Jukes Cantor distance model and Kimura's two parameter distance model (Jukes and Cantor, 1969). To assess the confidence of phylogenetic relationships, the bootstrap test (Felsenstein, 1985) was conducted with 1000 resampling for ML analysis. The phylogenetic relationships of *O. liangshanensis*

were also analyzed using the Bayesian method (Ronquist et al., 2012). *Volvariella volvaceae* was used as an outgroup for phylogenetic analysis of the *ITS* region. The comparative phylogenetics results using ITS sequence indicated that the presence of the sample collected from Arunachal Pradesh clustered with the sequences of *Ophiocordyceps liangshanensis* sequences (China) retrieved from NCBI. The presence in the same clade diverging from the other related species confirmed that the sample belong to *O. liangshanensis* (*Metacordyceps lianshanensis*), which is an entomoparasitic fungus infecting larva of various genera (*Thitarodes*, *Endoclita*, *Napialus*). Similarly, the *COI* gene of host was subjected to phylogenetic analysis which clubbed with *Hapialidae* spp. confirming the host. The processed sequence of CBUAP1 was submitted to gene bank and accession number MZ318360.1 was obtained.

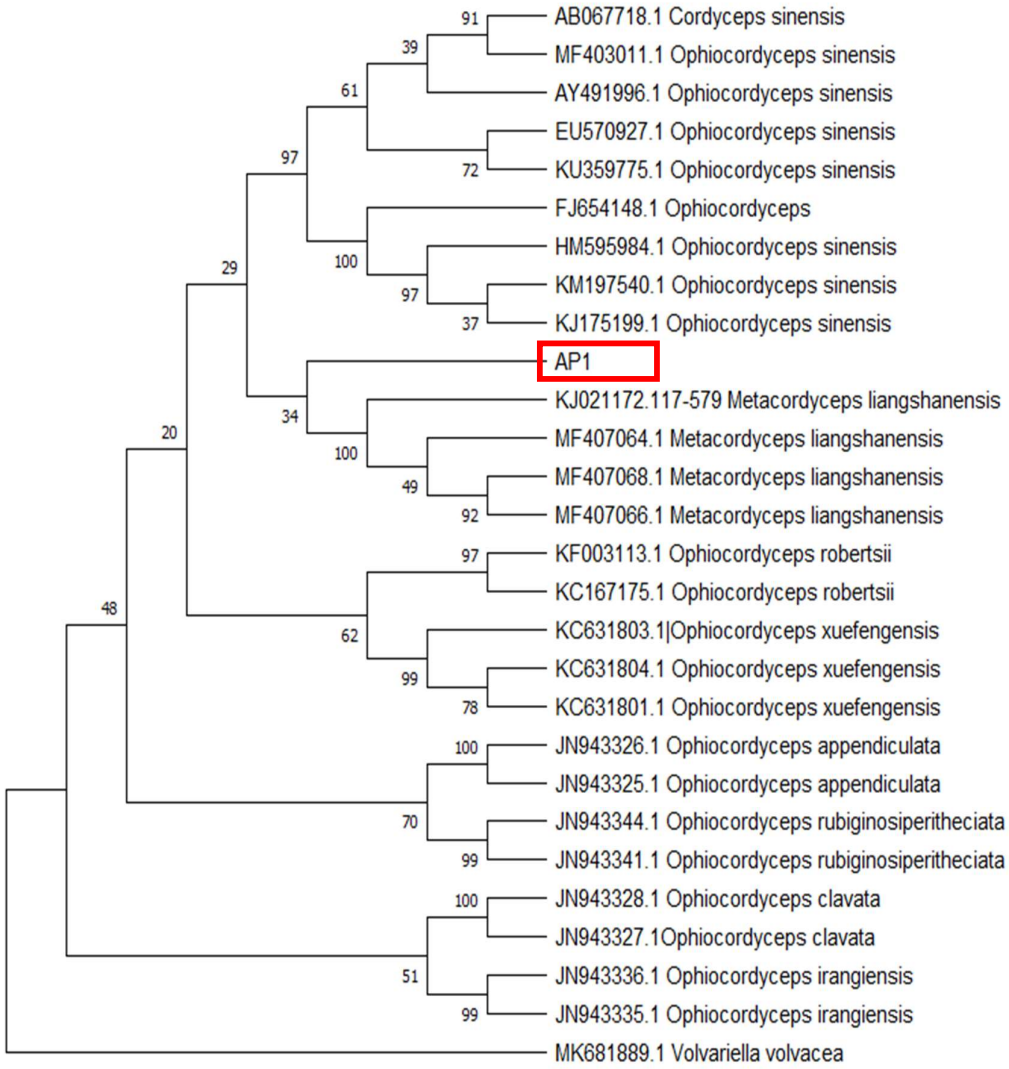



**Fig 4.8. Maximum Likelihood tree of CBUAP1(*ITS*) constructed using constructed using Mega Version 11.0.13**

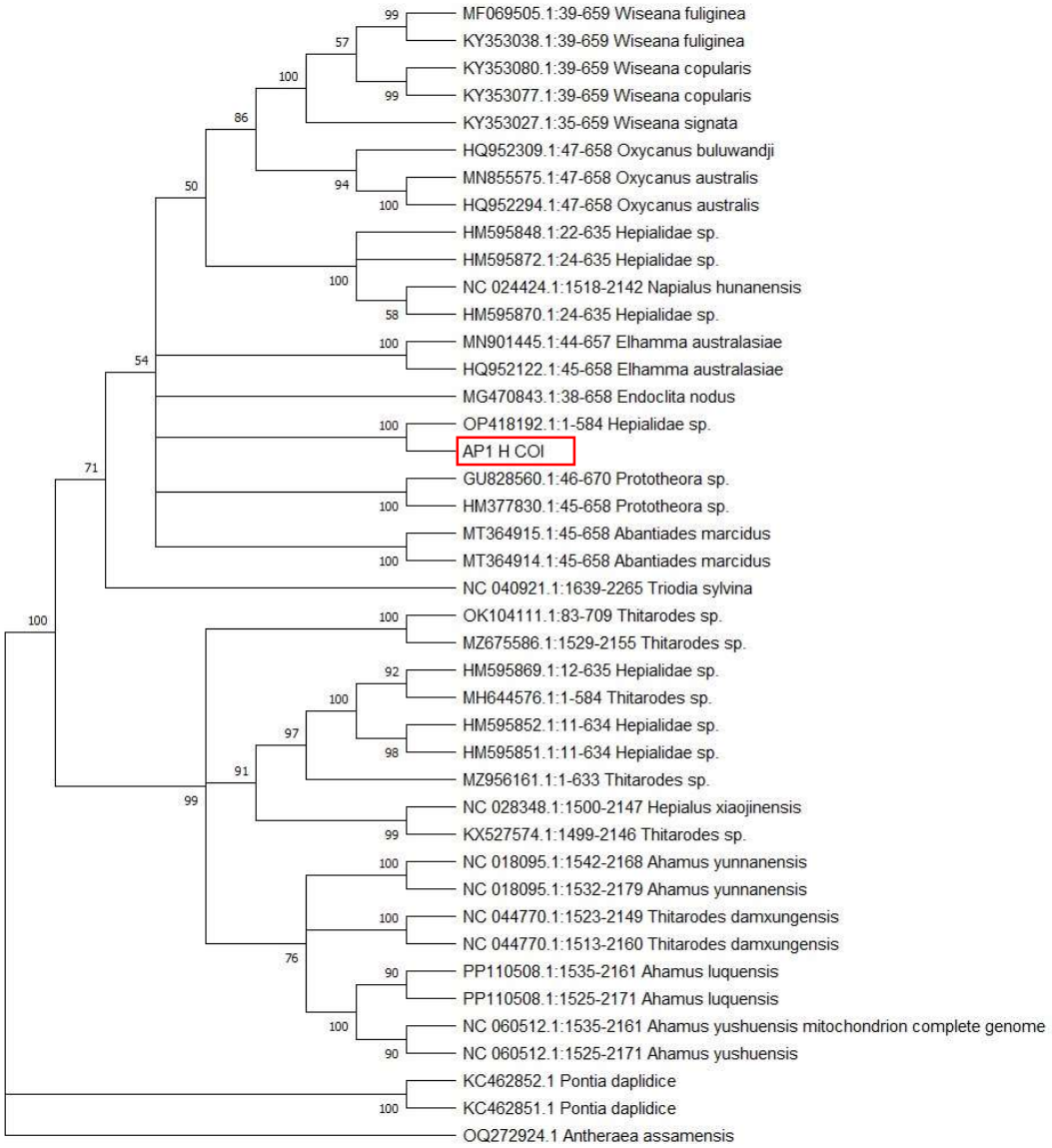



**Fig 4.9. Maximum Likelihood tree of CBUAP1(*COI*) constructed using constructed using Mega Version 11.0.13**

## 4.7.3 *Ophiocordyceps sinensis* (CBUB1-B4) collected from Bhutan:

**Current Name:** (Index Fungorum)

***Ophiocordyceps sinensis*** (Berk.) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora, in Sung, Hywel-Jones, Sung, Luangsa-ard, Shrestha & Spatafora, Stud. Mycol. 57: 46 (2007) (Index Fungorum)

**Synonymy:**
*Cordyceps sinensis* (Berk.) Sacc., Michelia 1(no. 3): 320 (1878)

*Hirsutella sinensis* X.J. Liu, Y.L. Guo, Y.X. Yu & W. Zeng, Acta Mycol. Sin. 8(1): 37 (1989)
*Hirsutella sinensis* X. Fang, Mycological Society of China, Academic Annual Meeting Summary: 203 (2013)
*Sphaeria sinensis* Berk., London J. Bot. 2: 207 (1843)
*Torrubia sinensis* (Berk.) Tul. & C. Tul., Select. fung. carpol. (Paris) 3: 13 (1865)
(https://www.indexfungorum.org/names/names.asp)

The sequenced raw data was processed in Bioedit version 7.2.5 and consensus sequence was generated. The phylogenetic analysis was performed by using the Maximum Likelihood method and Tamura-Nei model (Tamura and Nei, 1993). The bootstrap consensus tree inferred from 1000 replicates (Felsenstein, 1985) is taken to represent the evolutionary history of the taxa analyzed (Felsenstein, 1985). Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein, 1985). Initial tree(s) for the heuristic search were obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated using the Tamura-Nei model. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+*G*, parameter = 1.4200)). This analysis involved 23 nucleotide sequences. There was a total of 796 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar et al., 2018). *Volvariella volvacea* was used as an outgroup for phylogenetic analysis of the *ITS* region and *Anthera assama* as an outgroup for *COI* region of host larva. The comparative phylogenetics results using *ITS* sequence indicated that the presence of the sample collected from Bhutan clubbed with the sequences of *O. Sinensis* sequences (China) retrieved from NCBI. The presence in the same clade diverging from the other related species confirmed that the sample belong to *O. Sinensis*, which is an entomo-parasitic fungus infecting larva of various genera (*Thitarodes*spp., *Endoclita*spp., *Napialus spp*.). The phylogenetic analysis of host was performed to confirm the larva. The sequence of *COI* of larva from the sample was compared to that of the common larva of *O. Sinensis.* The sequences were retrieved from NCBI, and phylogenetic analysis was carried out. Subsequently, the sample clubbed and grouped with the sequences with *Hepialidae* spp. which confirmed that the larva belongs to *Thitarodes* spp. The tree from Bayesian inference showed an identical tree topology (Ronquist, et al., 2012).

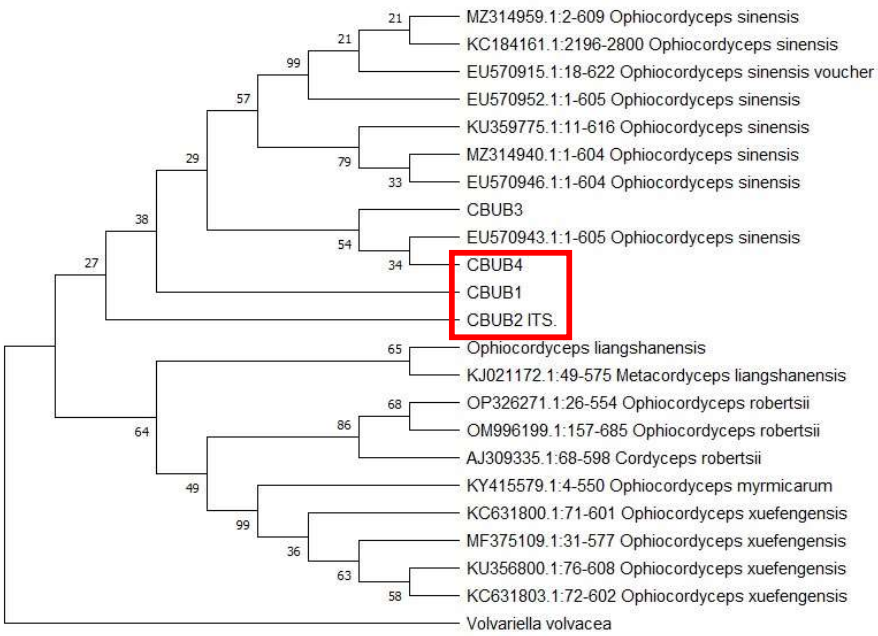



**Fig 4.10. Maximum Likelihood tree of samples collected from Bhutan (*ITS*) constructed using constructed using Mega Version 11.0.13**

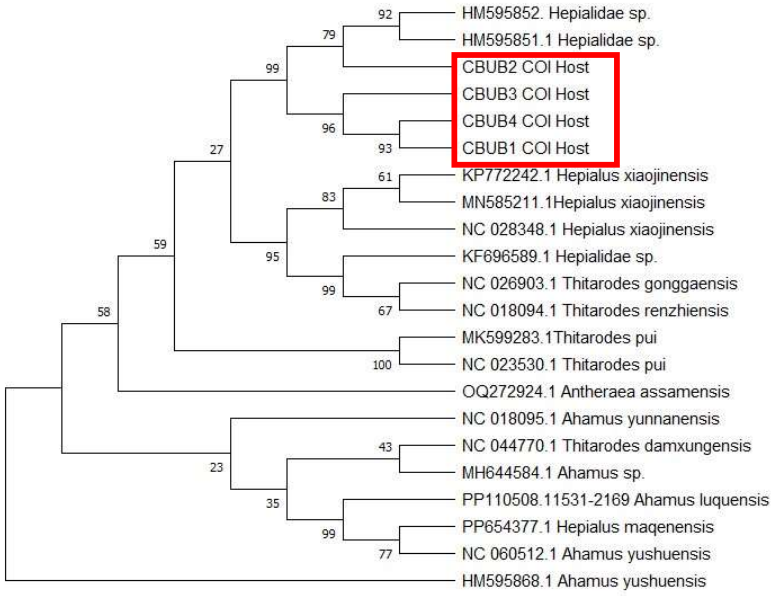



**Fig 4.11. Maximum Likelihood Tree of host larva collected from Bhutan (*COI*) constructed using constructed using Mega Version 11.0.13**

## 4.8 Microscopic Characteristics of CBUS3:

Details of macro-morphological characters of *O. sinensis* collected from Lachen Valley, North Sikkim

## Microscopic characteristics of stroma:

Stroma sparingly cylindrical, dark pinkish (fresh) and brown when dried, 38–40 mm in length, and about 2.5–3 mm in diameter. The total diameter of the transverse section is 298 µm, length and breadth of the asci were 84 µm and 33 µm, respectively. Perithecia oval to elliptical, elongated, and grouped at the fertile portion of stroma. Mycelia embedded with the asci. The outer layer of the stroma stained dark with Congo red. Larva body resembling a silkworm, 29–32 mm in length and 4–5 mm in breadth. Yellowish in color with 8 pairs of the leg. Light-creamish. Mycelia branch with 3-µm diameter. The fruiting body (stroma) is identified as *O. sinensis*, the host larva is identified as *Thitarodes* spp., and mycelia of fruiting body to be *O. sinensis*.

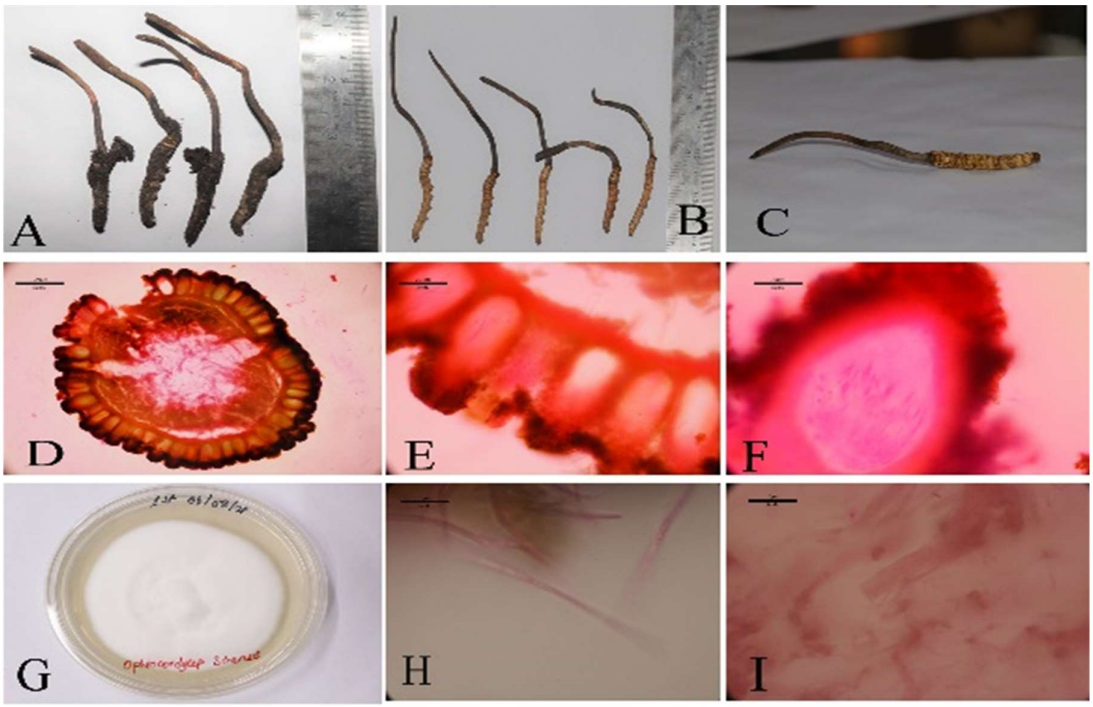



**Fig 4.12. A. *O. sinensis*; B. *O. sinensis* cleaned; C. *O. sinensis* with 8 pairs of legs on the abdomen and 4 pairs at the center; D. Transverse section of stroma; E. Perithecia at 20X; F. Perithecia at 40X G. Mycelial culture of *O. sinensis* grown on PDA media; H. Mycelia of *O. sinensis*; I. Central portion of stroma**

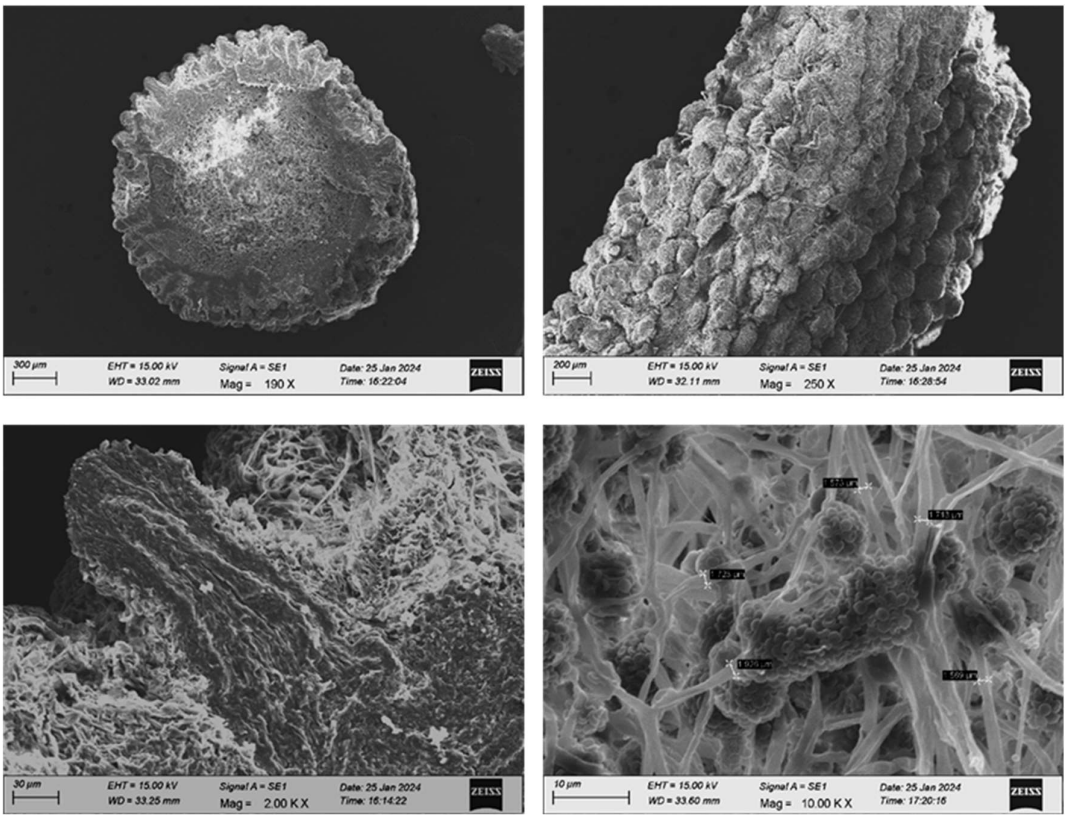



**Fig 4.13. SEM analysis of CBUS3 A. Transverse section of stroma: B. Fertile region of stroma at 250X: C. Transverse section of fertile region of stroma 2000X: D. Mycelia at 10000X**

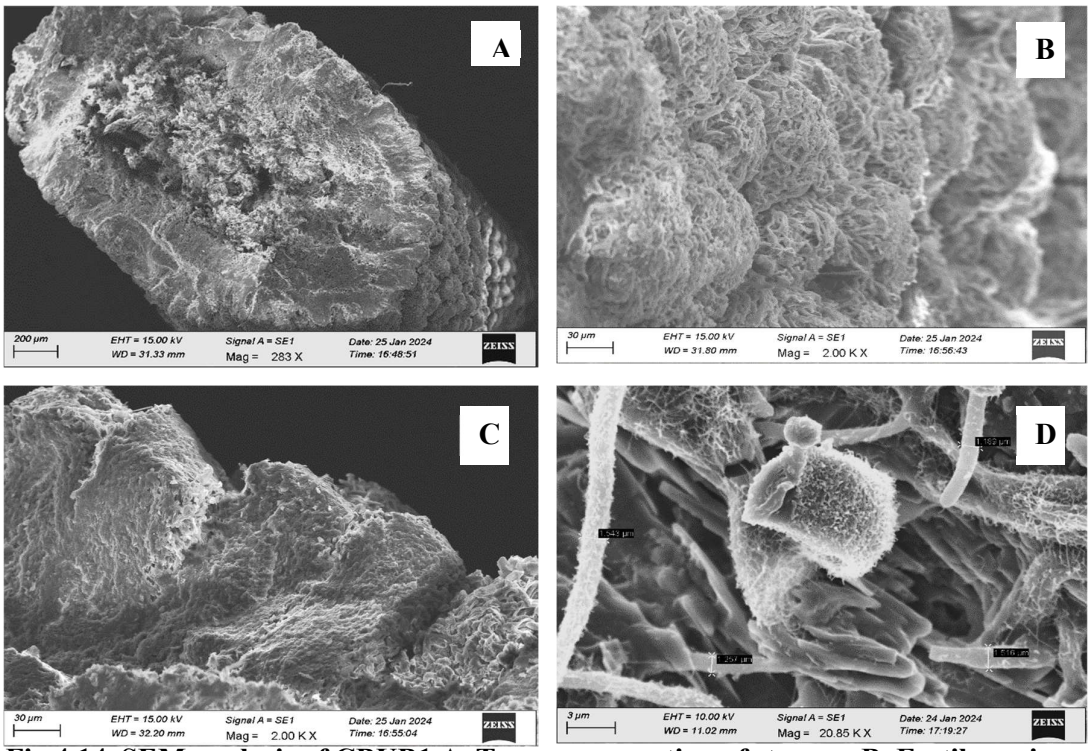



**Fig 4.14. SEM analysis of CBUB1 A. Transverse section of stroma: B. Fertile region of stroma at 300X: C. Transverse section of fertile region of stroma 2000X: D. Mycelia at 20850X**

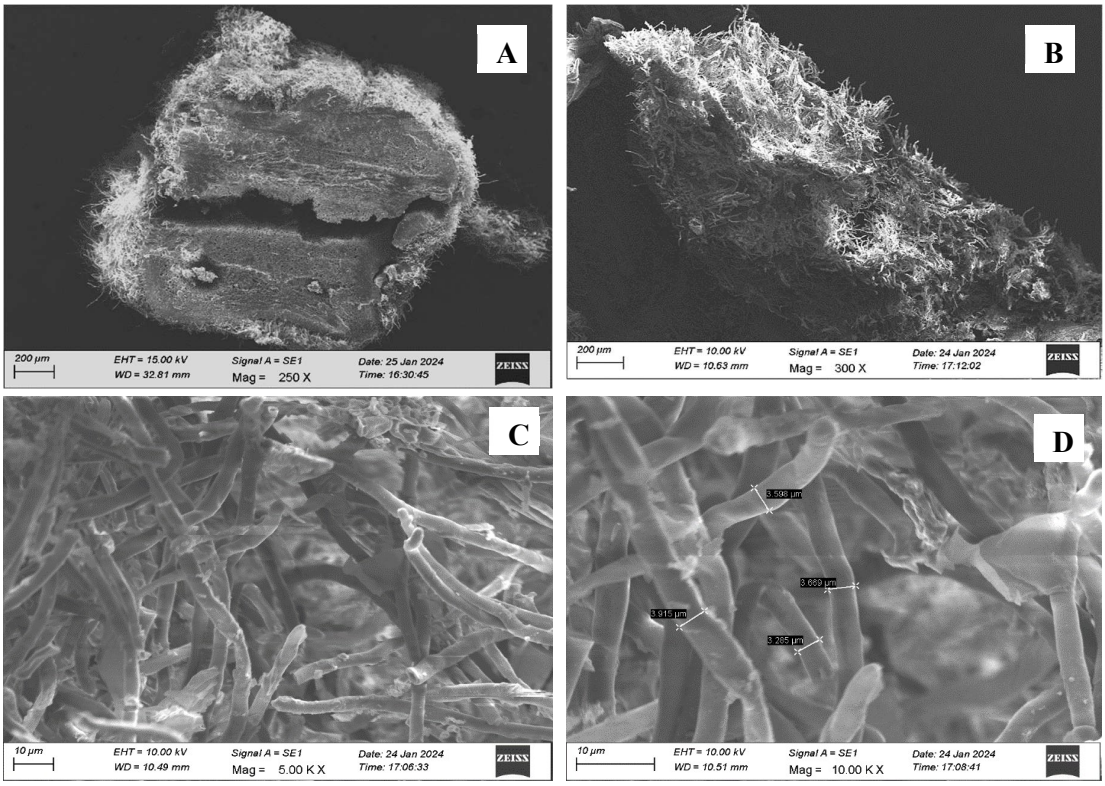



**Fig 4.15. SEM analysis of CBUAP1 A. Transverse section of stroma: B. Mycelia of stroma at 300X: C. Mycelia of stroma 5000X: D. Mycelia at 10000**

## 4.9. Protein Estimation:

The protein content of all the samples were investigated. CBUS4 (*Ophiocordyceps sinensis*) exhibited the highest protein content of 14.1% (±0.01), followed by CBUCM (*Cordyceps militaris*) cultivated on Joha Rice of 13.96% (±0.02), CBUCM (*Cordyceps militaris*) grown on Brown Rice exhibited the lowest protein content of 5.25% (±0.02). The detailed protein content of samples is depicted on Table 4.5.

**Table 4.5. Protein content**

| Sl. No. | Sample | Protein Content (%) |
|---|---|---|
| 1 | *CBUS1 (Ophiocordyceps sinensis)* | $13.5^{d}$ (±0.01) |
| 2 | *CBUS2 (Ophiocordyceps sinensis)* | $13.80^{c}$ (±0.03) |
| 3 | *CBUS3 (Ophiocordyceps sinensis)* | $13.00^{e}$ (±0.01) |
| 4 | *CBUS3- Mycelia (Ophiocordyceps sinensis)* | $13.9^{bc}$ (±0.04) |

| | | |
|---|---|---|
| 5 | *CBUS4 (Ophiocordyceps sinensis)* | 14.1 $^{a}$ (±0.01) |
| 6 | CBUAP1 (*Ophiocordyceps liangshanensis*) | 12.70$^{f}$ (±0.04) |
| 7 | *CBUCM* (*Cordyceps militaris*) grown on Brown Rice | 5.25$^{i}$ (±0.02) |
| 8 | *CBUCM (Cordyceps militaris)* grown on Black *Rice* | 8.75$^{g}$ (±0.08) |
| 9 | *CBUCM (Cordyceps militaris)* grown on Joha *Rice* | 13.96$^{b}$ (±0.02) |
| 10 | *CBUCM (Cordyceps militaris)* grown on Rozana *Rice* | 8.75$^{g}$ (±0.05) |
| 11 | *CBUCM (Cordyceps militaris)* grown on Barni Rice | 7.52$^{h}$ (±0.05) |

## 4.10. Total Dietary Fiber:

Total Dietary fiber of the samples was evaluated, CBUS3 (*Ophiocordyceps sinensis*) demonstrated the highest total dietary fiber content of 42.72% (±0.05), followed by CBUS4 (*Ophiocordyceps sinensis*) of 41.33% (±0.04) and CBUS1 (*Ophiocordyceps sinensis*) of 40.01% (±0.01). CBUS3- *Mycelia* (*Ophiocordyceps sinensis*) exhibited the lowest total dietary fiber content of 21.33(±0.08). Among the *Cordyceps militaris* samples cultivated on different rice varieties, brown rice exhibited the highest fiber content of 34.25% (±0.03). Conversely, *Cordyceps militaris* grown on Black Rice and Joha Rice exhibited lower fiber percentages of 23.24% (±0.08) and 23.26% (±0.09) respectively. The detailed total dietary fiber content of samples is depicted on Table 4.6.

**Table 4.6. Total Dietary Fiber**

| **Sl. No.** | **Sample** | **Total Dietary Fiber (%)** |
|---|---|---|
| 1 | *CBUS1 (Ophiocordyceps sinensis)* | 40.01$^{c}$ (±0.01) |
| 2 | *CBUS2 (Ophiocordyceps sinensis)* | 39.56$^{d}$ (±0.08) |
| 3 | *CBUS3 (Ophiocordyceps sinensis)* | 42.72$^{a}$ (±0.05) |
| 4 | *CBUS3- Mycelia (Ophiocordyceps sinensis)* | 21.33$^{j}$ (±0.08) |
| 5 | *CBUS4 (Ophiocordyceps sinensis)* | 41.33$^{b}$ (±0.04) |
| 6 | CBUAP1 (*Ophiocordyceps liangshensis*) | 38.55$^{e}$ (±0.04) |
| 7 | CBUCM (*Cordyceps militaris*) grown on Brown Rice | 34.25$^{f}$ (±0.03) |
| 8 | CBUCM *(Cordyceps militaris)* grown on Black Rice | 23.24$^{i}$ (±0.08) |

| | | |
|---|---|---|
| 9 | CBUCM *(Cordyceps militaris)* grown on Joha Rice | $23.26^{i}$ (±0.09) |
| 10 | CBUCM *(Cordyceps militaris)* grown on Rozana Rice | $27.28^{g}$ (±0.04) |
| 11 | CBUCM *(Cordyceps militaris)* grown on Barni Rice | $26.89^{h}$ (±0.03) |

## 4.11. DPPH Scavenging Activity:

The study evaluated the radical scavenging activity of different samples of *Cordyceps* mushrooms. The findings indicated a favorable outcome that *Cordyceps* mushrooms exhibit substantial radical scavenging activity. The DPPH radical scavenging activity was found to be highest on CBUS3 *(Ophiocordyceps sinensis)*62.2 (± 0.07), followed by CBUS3- Mycelia *(Ophiocordyceps sinensis)* and lowest on *Cordyceps militaris* grown on brown rice 44.55 (± 0.01). The detailed inhibition of samples is depicted on Table 4.7.

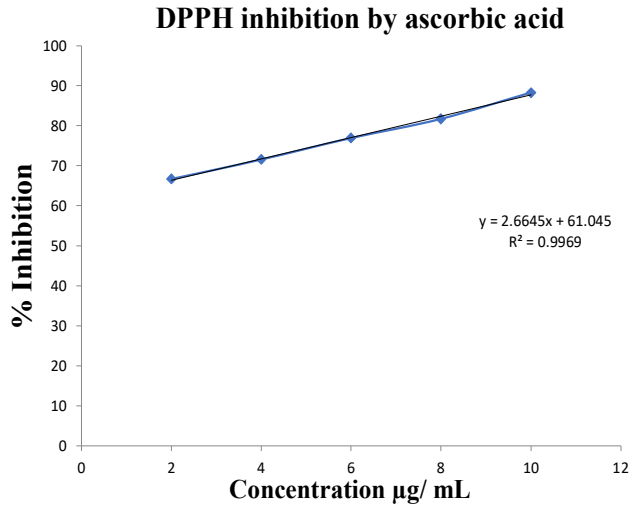



**Fig 4.16. DPPH Standard calibration curve**

**Table 4.7. DPPH Scavenging Activity:**

| Sl. No. | Sample | $IC_{50}$ Value |
|---|---|---|
| 1 | CBUS1 *(Ophiocordyceps sinensis)* | $60.5^{c}$ (± 0.04) |
| 2 | CBUS2 *(Ophiocordyceps sinensis)* | $60.33^{c}$ (± 0.04) |
| 3 | CBUS3 *(Ophiocordyceps sinensis)* | $62.2^{a}$ (± 0.07) |
| 4 | CBUS3- Mycelia *(Ophiocordyceps sinensis)* | $61.52^{b}$ (± 0.05) |
| 5 | CBUS4 *(Ophiocordyceps sinensis)* | $58.97^{d}$ (± 0.02) |
| 6 | CBUAP1 (*Ophiocordyceps liangshanensis*) | $56.28^{e}$ (± 0.08) |

| | | |
|---|---|---|
| 7 | CBUCM (*Cordyceps militaris*) grown on Brown Rice | $44.55^{j}$ (± 0.01) |
| 8 | CBUCM (*Cordyceps militaris*) grown on Black Rice | $47.28^{i}$ (± 0.04) |
| 9 | CBUCM (*Cordyceps militaris*) grown on Joha Rice | $49.35^{g}$ (± 0.04) |
| 10 | CBUCM (*Cordyceps militaris*) grown on Rozana Rice | $50.11^{f}$ (± 0.08) |
| 11 | CBUCM (*Cordyceps militaris*) grown on Barni Rice | $48.05^{h}$ (± 0.08) |

## 4.12 FRAP (Ferric Reducing Antioxidant Power):

The Ferric Reducing Antioxidant Potential of the collected samples were analyzed and it was found that, CBUS3 *(Ophiocordyceps sinensis)* exhibited highest ferric reducing ions with FRAP value of 58.89(± 0.04), followed by CBUS3- *Mycelia (Ophiocordyceps sinensis).* Among the *Cordyceps militaris* samples grown on Joha rice exhibited the highest ferric reducing ions with FRAP value of 51.33(± 0.04) and lowest was observed *Cordyceps militaris* grown on Brown rice with 44.55 (± 0.05) frap value. The results were expressed as µmol $Fe^{2+}$ equivalent, 1 mg aqueous extract was found to be equivalent to 100 µmol of $Fe^{2+}$ against the standard curve prepared using ferrous sulphate.

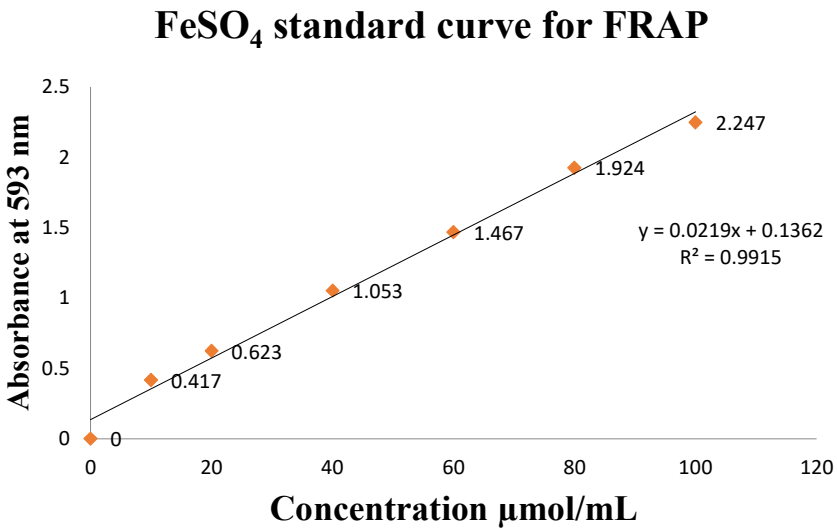



**Fig 4.17. FRAP Standard calibration curve**

**Table 4.8. FRAP value**

| Sl. No. | Sample | FRAP Value |
|---|---|---|
| 1 | CBUS1 *(Ophiocordyceps sinensis)* | $52.89^{e}$ (± 0.02) |
| 2 | CBUS2 *(Ophiocordyceps sinensis)* | $53.45^{d}$ (± 0.03) |
| 3 | CBUS3 *(Ophiocordyceps sinensis)* | $58.89^{a}$ (± 0.04) |
| 4 | CBUS3- Mycelia *(Ophiocordyceps sinensis)* | $54.54^{c}$ (± 0.04) |
| 5 | CBUS4 *(Ophiocordyceps sinensis)* | $56.97^{b}$ (± 0.05) |
| 6 | CBUAP1 (*Ophiocordyceps liangshanensis*) | $48.67^{g}$ (± 0.05) |
| 7 | CBUCM (*Cordyceps militaris*) grown on Brown Rice | $44.55^{j}$ (± 0.05) |
| 8 | CBUCM (*Cordyceps militaris*) grown on Black Rice | $46.38^{i}$ (± 0.07) |
| 9 | CBUCM (*Cordyceps militaris*) grown on Joha Rice | $51.33^{f}$ (± 0.04) |
| 10 | CBUCM (*Cordyceps militaris*) grown on Rozana Rice | $47.25^{h}$ (± 0.03) |
| **11** | CBUCM (*Cordyceps militaris*) grown on Barni Rice | $48.78^{g}$ (± 0.04) |

## 4.13 ABTS radical scavenging activity:

The ABTS radical scavenging activity were studied from the extracts of the collected samples and found that CBUS3 *(Ophiocordyceps sinensis)* had the highest potential to scavenge ABTS free radicals with $IC_{50}$ value of 389.95µg (±0.05) followed by CBUS3-Mycelia *(Ophiocordyceps sinensis)* with $IC_{50}$ value of 385.28 µg (±0.02), while lowest potential to scavenge ABTS was recorded in *Cordyceps militaris* grown on Barni rice with $IC_{50}$ value 345.21µg (±0.06) respectively as given in table 4.10.

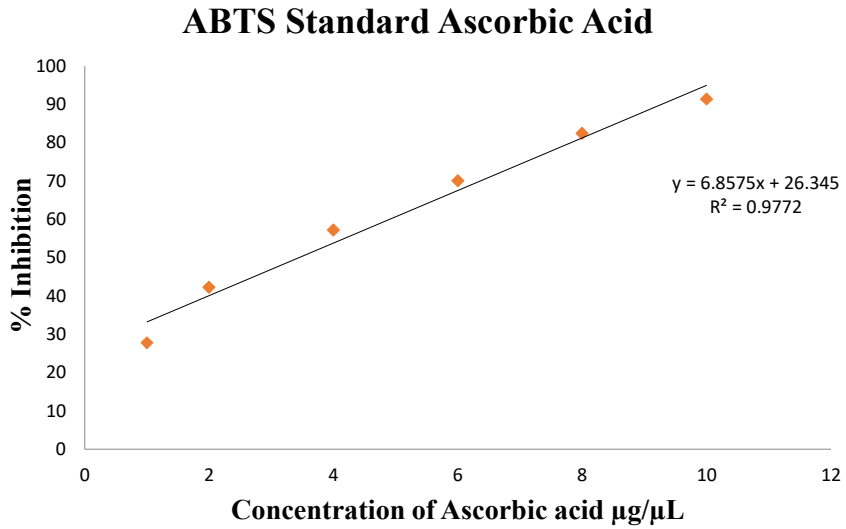



**Fig 4.18.** ABTS Standard calibration curve

**Table 4.9. ABTS radical scavenging activity**

| Sl. No. | Sample | $IC_{50}$ Value |
|---|---|---|
| 1 | CBUS1 *(Ophiocordyceps sinensis)* | $370.51^{d}$ µg (±0.01) |
| 2 | CBUS2 *(Ophiocordyceps sinensis)* | $362.88^{f}$ µg (±0.08) |
| 3 | CBUS3 *(Ophiocordyceps sinensis)* | $389.95^{a}$ µg (±0.05) |
| 4 | CBUS3 Mycelia *(Ophiocordyceps sinensis)* | $385.28^{b}$ µg (±0.02) |
| 5 | CBUS4 *(Ophiocordyceps sinensis)* | $380.66^{c}$ µg (±0.04) |
| 6 | CBUAP1 (*Ophiocordyceps liangshanensis*) | $366.85^{e}$ µg (±0.03) |
| 7 | CBUCM (*Cordyceps militaris*) grown on Brown Rice | $356.56^{g}$ µg (±0.04) |
| 8 | CBUCM (*Cordyceps militaris*) grown on Black Rice | $349.25^{i}$ µg (±0.05) |
| 9 | CBUCM (*Cordyceps militaris*) grown on Joha Rice | $347.37^{j}$ µg (±0.06) |
| 10 | CBUCM (*Cordyceps militaris*) grown on Rozana Rice | $351.75^{h}$ µg (±0.04) |
| 11 | CBUCM (*Cordyceps militaris*) grown on Barni Rice | $345.21^{k}$ µg (±0.06) |

## 4.14 Antimicrobial activity:

The methanolic aqueous (70:30) of the samples was evaluated for its antimicrobial properties against six bacterial strains, the samples exhibited with variable intensity. CBUB2 and *Cordyceps militaris* grown on Joha rice exhibited the highest inhibition against *Staphylococcus aureus* with 27 mm zone of inhibition while other samples also exhibited potential inhibition against the bacteria in the concentration of 200 µg/ mL extract concentration. Similarly, CBUS3 exhibited the highest inhibition against *Salmonella typhi* and *Mycobacterium smegmatis* with 20 mm and 19 mm zone of inhibition respectively, while *Cordyceps militaris* grown on Basmati rice exhibited lowest inhibition of 2 mm against *Salmonella typhi. Cordyceps militaris* grown on Basmati rice recorded the highest inhibition of *Pseudomonas aeruginosa i*with 18 mm zone of inhibition, while CBUS3 and *Cordyceps militaris* grown on Black rice exhibited lowest inhibition zone of 9 mm. CBUS3 exhibited highest inhibition of 14 mm against *Escherrichia coli*, while *Cordyceps militaris* grown on Joha rice exhibited lowest inhibition zone of 7 mm. Similarly, CBUS3 exhibited highest inhibition of 14 mm against *Bacillus cereus*, while CBUB2 exhibited lowest inhibition zone of 8 mm. The detailed zone of inhibition of all tested the samples, standard antibiotic (Ampicillin 10 µg) and DMSO (Positive control).

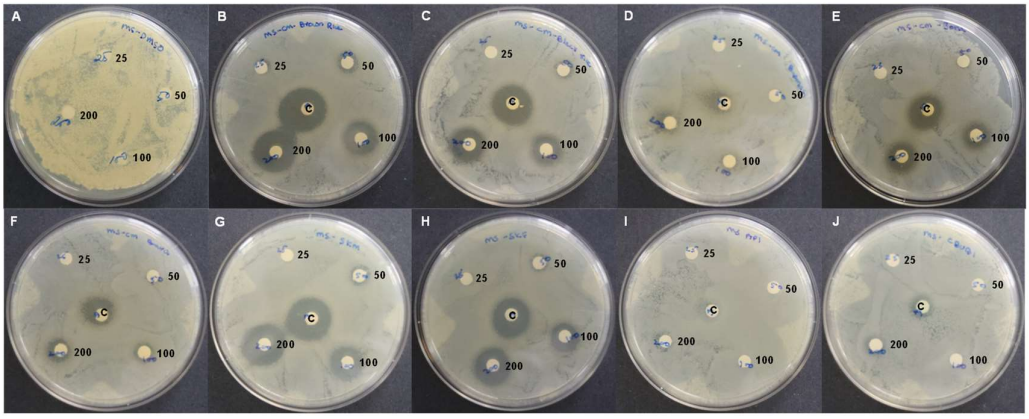


**Fig 4.19. Assessment of antimicrobial efficacy on *Mycobacterium smegmatis;* A. DMSO; B. CBUCM (cultivated on Brown rice); C. CBUCM (Cultivated on Black rice); D. CBUCM (cultivated on Basmati rice); E. CBUCM (cultivated on Joha rice); F. CBUCM (cultivated on Barni rice); G. CBUS3(Mycelia); H. CBUS3(fruiting); I. CBUAP1; and J. CBUB1**

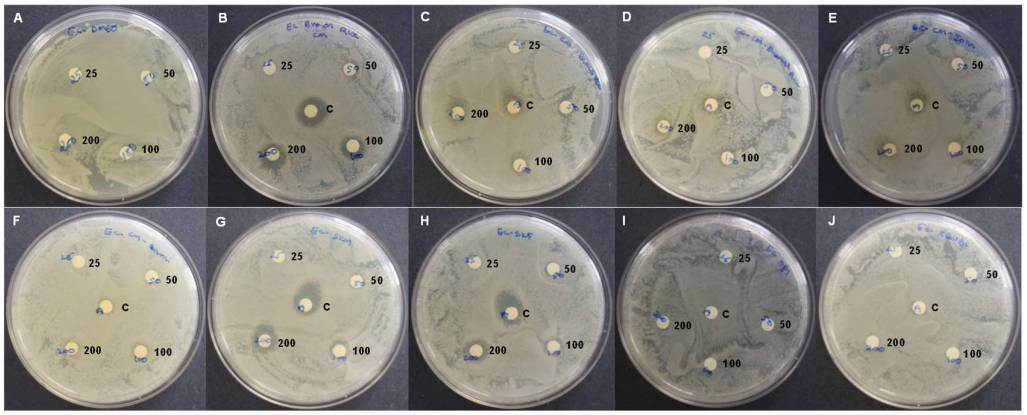


**Fig 4.20. Assessment of antimicrobial efficacy on *Escherichia coli*; A. DMSO; B. CBUCM (cultivated on Brown rice); C. CBUCM (Cultivated on Black rice); D. CBUCM (cultivated on Basmati rice); E. CBUCM (cultivated on Joha rice); F. CBUCM (cultivated on Barni rice); G. CBUS3(mycelia); H. CBUS3(fruiting); I. CBUAP1; and J. CBUB1**

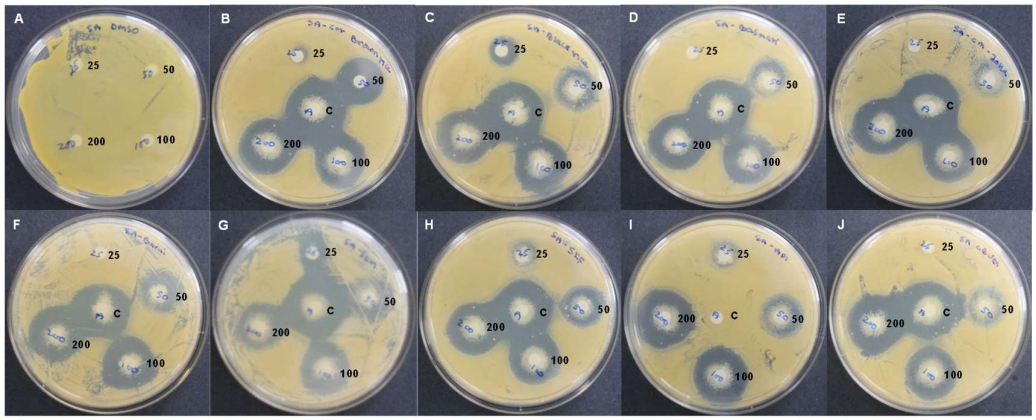


**Fig 4.21. Assessment of antimicrobial efficacy on *Staphylococcus aureus;* A. DMSO; B. CBUCM (cultivated on Brown rice); C. CBUCM (Cultivated on Black rice); D. CBUCM (cultivated on Basmati rice); E. CBUCM (cultivated on Joha rice); F. CBUCM (cultivated on Barni rice); G. CBUS3(mycelia); H. CBUCBUS3(fruiting); I. CBUAP1; and J. CBUB1**

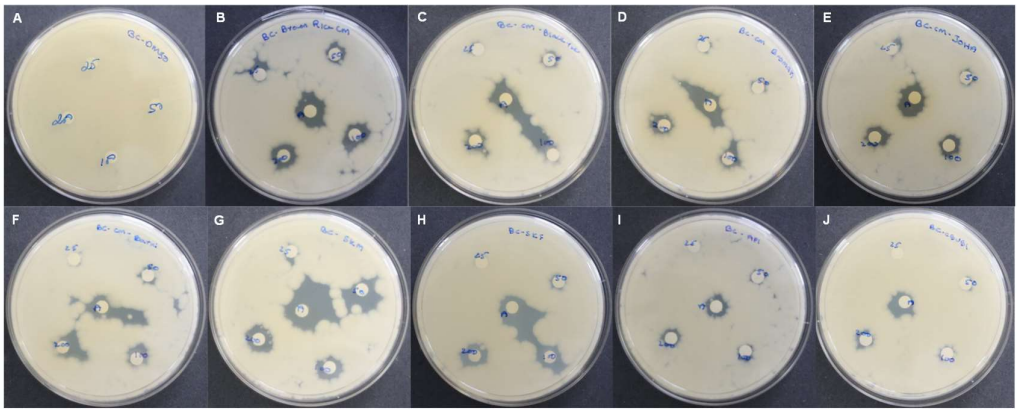


**Fig 4.22. Assessment of antimicrobial efficacy on *Bacillus cereus;* A. DMSO; B. CBUCM (cultivated on Brown rice); C. CBUCM (Cultivated on Black rice); D. CBUCM (cultivated on Basmati rice); E. CBUCM (cultivated on Joha rice); F. CBUCM (cultivated on Barni rice); G. CBUS3(mycelia); H. CBUS3(fruiting); I. CBUAP1; and J. CBUB1**

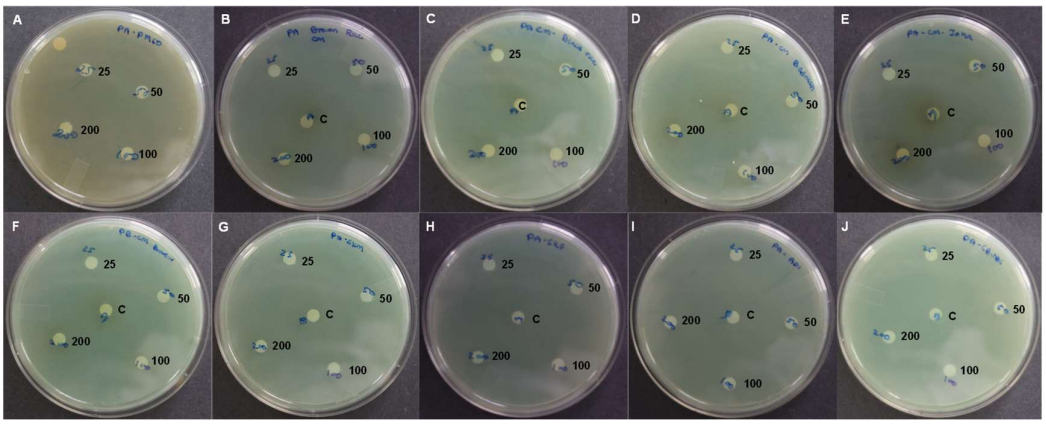


**Fig 4.23. Assessment of antimicrobial efficacy on *Psudomonas aeruginosa*; A. DMSO; B. CBUCM (cultivated on Brown rice); C. CBUCM (Cultivated on Black rice); D. CBUCM (cultivated on Basmati rice); E. CBUCM (cultivated on Joha rice); F. CBUCM (cultivated on Barni rice); G. CBUS3(mycelia); H. CBUS3(fruiting); I. CBUAP1; and J. CBUB1**

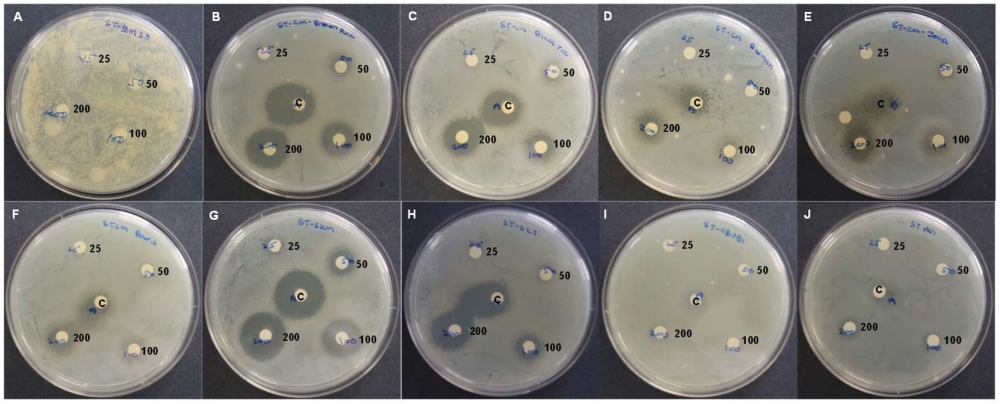


**Fig 4.24. Assessment of antimicrobial efficacy on *Salmonella typhimurium*; A. DMSO; B. CBUCM (cultivated on Brown rice); C. CBUCM (Cultivated on Black rice); D. CBUCM (cultivated on Basmati rice); E. CBUCM (cultivated on Joha rice); F. CBUCM (cultivated on Barni rice); G. CBUS3(mycelia); H. CBUS3(fruiting); I. CBUAP1; and J. CBUB1**

**Table: 4.10. Detailed antimicrobial activity in Zone of Inhibition (in mm)**

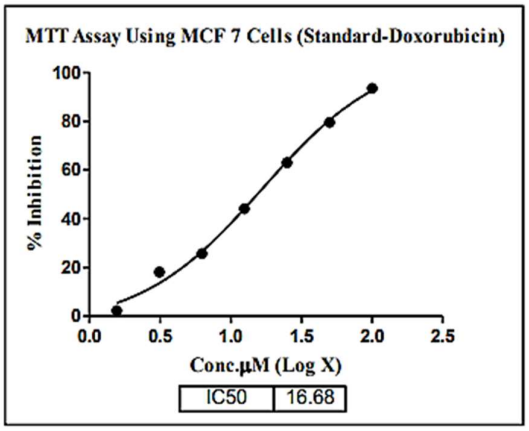
| Bacteria Name | Sample Concentration | CBU BrR | CB UBlkR | CBU BasmatiR | CBUJohaR | CBU barniR | CB US KM | CB US KF | CB UA P1 | CBUB 2 |
|---|---|---|---|---|---|---|---|---|---|---|
| ***Escherrichia coli*** | 25 | NI | NI | NI | NI | NI | NI | NI | NI | NI |
| | 50 | NI | 8 | NI | NI | NI | 7 | 7 | NI | NI |
| | 100 | 9 | 8 | 7 | NI | NI | 9 | 8 | NI | NI |
| | 200 | 12 | 8 | 8 | 7 | NI | 14 | 10 | NI | NI |
| | Antibiotics | 15 | 15 | 11 | 13 | 11 | 15 | 14 | NI | NI |
| | **MIC** | **100** | **50** | **100** | **200** | **NI** | **50** | **50** | **NI** | **NI** |
| ***Mycobacterium smegmatis*** | 25 | NI | NI | NI | NI | NI | NI | NI | NI | NI |
| | 50 | 11 | 8 | NI | 7 | NI | 8 | 7 | NI | NI |
| | 100 | 14 | 12 | 7 | 9 | 11 | 12 | 11 | NI | 7 |
| | 200 | 13 | 14 | 9 | 14 | 13 | 19 | 11 | NI | 9 |
| | Antibiotics | 19 | 18 | 11 | 18 | 16 | 22 | 11 | NI | 13 |
| | **MIC** | **50** | **50** | **100** | **50** | **100** | **50** | **50** | **NI** | **100** |
| ***Staphylococcus aureus*** | 25 | NI | 8 | NI | NI | NI | 13 | 7 | NI | NI |
| | 50 | 19 | 17 | 11 | 18 | 8 | 23 | 13 | 4 | 17 |
| | 100 | 22 | 23 | 22 | 24 | 22 | 24 | 23 | 14 | 23 |
| | 200 | 24 | 26 | 26 | 27 | 24 | 25 | 24 | 23 | 27 |
| | Antibiotics | 31 | 29 | 28 | NI | 31 | 28 | 25 | 29 | 22.9 |
| | **MIC** | **50** | **25** | **50** | **50** | **50** | **25** | **25** | **50** | **50** |
| ***Bacillus cereus*** | 25 | 0.7 | NI | NI | NI | NI | NI | NI | NI | NI |
| | 50 | 9 | 7 | NI | NI | 8 | 13 | 7 | 7 | NI |
| | 100 | 12 | 8 | 8 | 8 | 11 | 13 | 0 | 9 | 7 |
| | 200 | 13 | 9 | 9 | 11 | 13 | 14 | 11 | 11 | 8 |
| | Antibiotics | 19 | 14 | 13 | 15 | 18 | 22 | 14 | 16 | 12 |
| | **MIC** | **25** | **50** | **100** | **100** | **50** | **50** | **50** | **50** | **100** |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| ***Salmonella typhi*** | 25 | NI | NI | NI | NI | NI | NI | NI | NI | NI |
| | 50 | 7 | 8 | NI | 7 | NI | 12 | 7 | NI | NI |
| | 100 | 13 | 12 | 8 | 9 | 9 | 18 | 8 | 8 | 7 |
| | 200 | 2 | 16 | 11 | 13 | 11 | 20 | 11 | 9 | 9 |
| | Antibiotics | 25 | 18 | 13 | 17 | 14 | 24 | 14 | 11 | 11 |
| | **MIC** | **50** | **50** | **100** | **50** | **100** | **50** | **50** | **100** | **100** |
| ***Pseudomonas aeruginosa*** | 25 | NI | NI | NI | NI | NI | NI | NI | NI | NI |
| | 50 | NI | NI | NI | NI | NI | NI | NI | NI | NI |
| | 100 | NI | 8 | 7 | NI | NI | NI | 7 | NI | NI |
| | 200 | NI | 9 | 18 | NI | NI | NI | 9 | NI | NI |
| | Antibiotics | NI | 12 | 11 | 13 | NI | 13 | 13 | NI | NI |
| | **MIC** | **NI** | **100** | **100** | **NI** | **NI** | **NI** | **100** | **NI** | **NI** |
| **DMSO** | 25 | NI | NI | NI | NI | NI | NI | NI | NI | NI |
| | 50 | NI | NI | NI | NI | NI | NI | NI | NI | NI |
| | 100 | NI | NI | NI | NI | NI | NI | NI | NI | NI |
| | 200 | NI | NI | NI | NI | NI | NI | NI | NI | NI |

## 4.15 Anticancer Activity:

### 4.15.1 MTT Assay:

We studied the cytotoxicity of extracts prepared from the fruiting bodies and mycelia of CBUS3 (*Ophiocordyceps sinensis*) and CBUAP1 (*Ophiocordyceps liangshanensis*) collected from Sikkim and Arunachal Pradesh in the MCF-7, HeLa, and SKOV3 cell lines. CBUAP1 exerted moderate cytotoxicity against MCF-7 with an $IC_{50}$ of 292.8; CBUS3 (fruiting) did not exert active cytotoxicity ($IC_{50}$> 100 µg/mL); however, extract CBUS3 (mycelia) exhibited potent activity with an $IC_{50}$value of 77.48. Extracts CBUAP1 and CBUS3 (fruiting) were not active ($IC_{50}$> 100 µg/mL). However, CBUS3 (Mycelia) exerted moderate activity against MCF-7, with IC50 of 294.3 against the HeLa cell line. The extracts CBUAP1 and CBUS3 (fruiting) exerted low cytotoxic activity with no active activity ($IC_{50}$> 100 µg/ml); however, extract CBUS3 (mycelia) exhibited an $IC_{50}$ of 185.6 against the SKOV3 cell line.



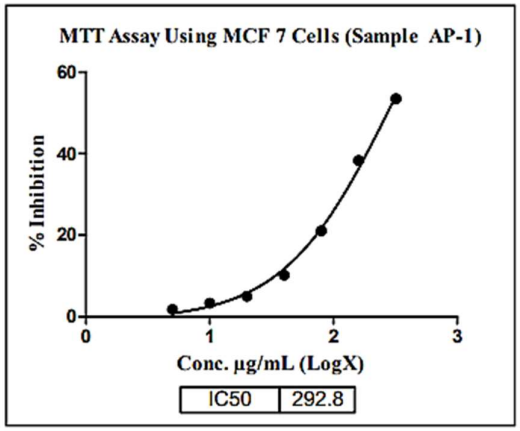




**Fig: 4.25. MTT Assay of Standard Doxorubicin Fig: 4.26. MTT Assay of CBUAP1**

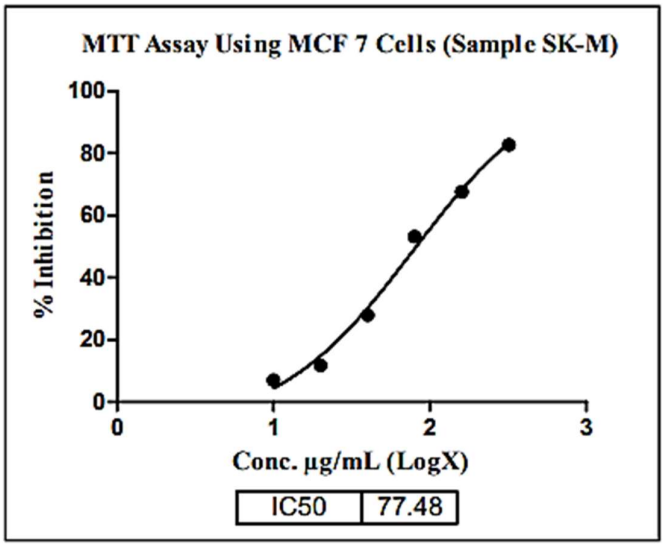


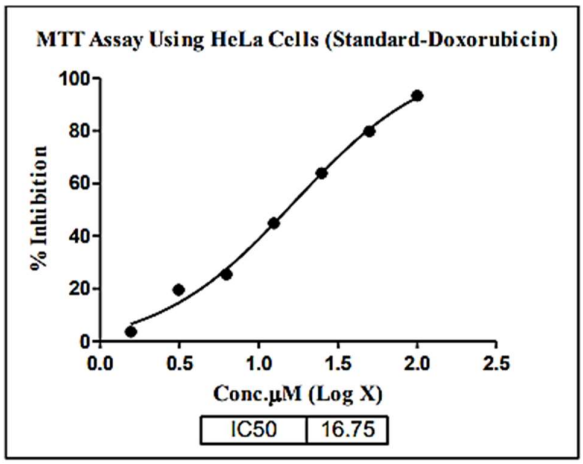

**Fig: 4.27. MTT Assay of CBUS3- Mycelia**

**Table 4.11. $IC_{50}$ value for MTT assay of MCF 7 (Breast Cancer) Cell line**

| MCF 7 | | | | |
|---|---|---|---|---|
| **Test Sample** | **Conc. in µM** | **OD @ 590nm** | **% Inhibition** | **$IC_{50}$ in µM** |
| **Vehicle Control** | **0** | 0.626 | 0.00 | |
| | 1.560 | 0.612 | 2.29 | |
| | 3.125 | 0.513 | 18.11 | |

| | | | | |
|---|---|---|---|---|
| **Doxorubicin** | 6.25 | 0.465 | 25.72 | **16.68** |
| | 12.5 | 0.350 | 44.16 | |
| | 25 | 0.231 | 63.06 | |
| | 50 | 0.128 | 79.53 | |
| | 100 | 0.040 | 93.56 | |
| | | | | |
| **Test Sample** | **Conc. in µg/mL** | **OD @ 590nm** | **% Inhibition** | **$IC_{50}$ in µM** |
| **Vehicle Control** | 0 | 0.626 | 0.00 | **292.8** |
| **CBUAP1 (*Ophiocordyceps liangshanensis*)** | 5 | 0.615 | 1.75 | |
| | 10 | 0.605 | 3.35 | |
| | 20 | 0.595 | 5.01 | |
| | 40 | 0.562 | 10.22 | |
| | 80 | 0.494 | 21.09 | |
| | 160 | 0.386 | 38.34 | |
| | 320 | 0.291 | 53.45 | |
| | | | | |
| **Test Sample** | **Conc. in µg/mL** | **OD @ 590nm** | **% Inhibition** | **$IC_{50}$ in µM** |
| **Vehicle Control** | 5 | 0.621 | 0.80 | |
| **CBUS3 (Fruiting) *Ophiocordyceps sinensis*** | 10 | 0.611 | 2.40 | |
| | 20 | 0.598 | 4.47 | |
| | 40 | 0.524 | 16.29 | |
| | 80 | 0.507 | 19.01 | |
| | 160 | 0.496 | 20.77 | |
| | 320 | 0.437 | 30.19 | |
| | | | | |

| Test Sample | Conc. in µg/mL | OD @ 590nm | % Inhibition | $IC_{50}$ in µM |
|---|---|---|---|---|
| **Vehicle Control** | 5 | 0.601 | 3.99 | |
| **CBUS3 (Mycelia)- *Ophiocordyceps sinensis*** | 10 | 0.582 | 7.03 | |
| | 20 | 0.557 | 11.01 | |
| | 40 | 0.451 | 27.96 | **77.48** |
| | 80 | 0.293 | 53.19 | |
| | 160 | 0.205 | 67.25 | |
| | 320 | 0.102 | 83.77 | |



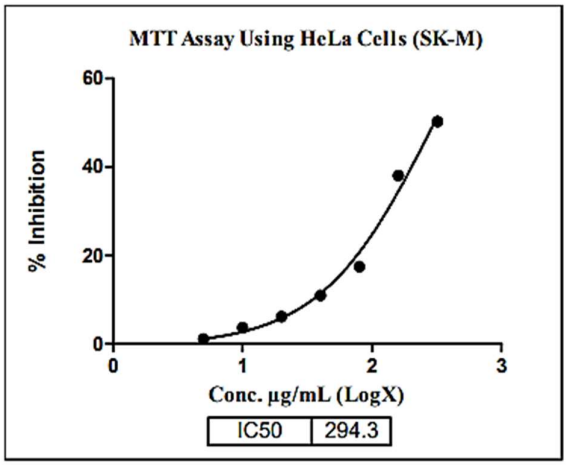



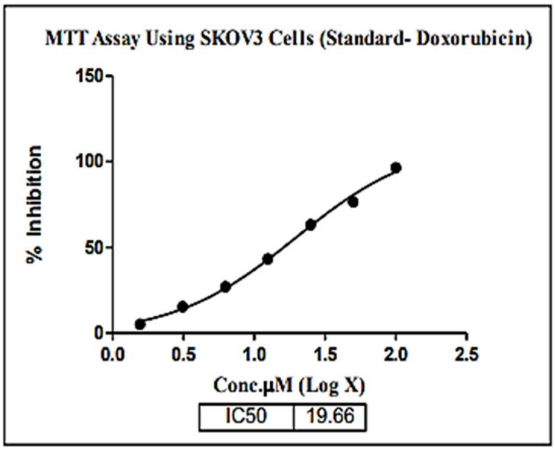

**Fig: 4.28. MTT Assay of Standard Doxorubicin** **Fig: 4.29. MTT Assay of CBUS3-Fruiting**

**Table 4.12. $IC_{50}$ value for MTT assay of HELA (Cervical Cancer) Cell line**

| HELA | | | | |
|---|---|---|---|---|
| **Test Sample** | **Conc. in µM** | **OD @ 590nm** | **% Inhibition** | **$IC_{50}$ in µM** |
| **Vehicle Control** | **0** | **0.636** | **0** | |
| | 1.560 | 0.613 | 3.59 | |
| | 3.125 | 0.512 | 19.55 | |
| | 6.25 | 0.474 | 25.47 | |

| | | | | |
|---|---|---|---|---|
| **Doxorubicin** | 12.5 | 0.351 | 44.88 | **16.75** |
| | 25 | 0.229 | 63.96 | |
| | 50 | 0.128 | 79.85 | |
| | 100 | 0.042 | 93.35 | |
| | | | | |
| **Test Sample** | **Conc. in µg/mL** | **OD @ 590nm** | **% Inhibition** | **$IC_{50}$ in µM** |
| **Vehicle Control** | 0 | 0.636 | 0.00 | |
| **CBUAP1 (*Ophiocordyceps liangshanensis*)** | 5 | 0.630 | 0.94 | |
| | 10 | 0.623 | 2.03 | |
| | 20 | 0.609 | 4.30 | |
| | 40 | 0.595 | 6.42 | |
| | 80 | 0542 | 14.85 | |
| | 160 | 0.520 | 18.29 | |
| | 320 | 0.434 | 31.73 | |
| | | | | |
| **Test Sample** | **Conc. in µg/mL** | **OD @ 590nm** | **% Inhibition** | **$IC_{50}$ in µM** |
| **Vehicle Control** | 5 | 0.634 | 0.31 | |
| **CBUS3 (Fruiting)- *Ophiocordyceps sinensis*** | 10 | 0.623 | 2.12 | |
| | 20 | 0.611 | 3.97 | |
| | 40 | 0.586 | 7.82 | |
| | 80 | 0.542 | 14.74 | |
| | 160 | 0.504 | 20.74 | |
| | 320 | 0.476 | 25.16 | |
| | | | | |
| **Test Sample** | **Conc. in µg/mL** | **OD @ 590nm** | **% Inhibition** | **$IC_{50}$ in µM** |

| | | | | |
|---|---|---|---|---|
| **Vehicle Control** | 5 | 0.629 | 1.10 | |
| **CBUS3 (Mycelia) *Ophiocordyceps sinensis*** | 10 | 0.613 | 3.69 | **294.3** |
| | 20 | 0.597 | 6.18 | |
| | 40 | 0.566 | 10.97 | |
| | 80 | 0.525 | 17.49 | |
| | 160 | 0.413 | 35.05 | |
| | 320 | 0.291 | 54.29 | |



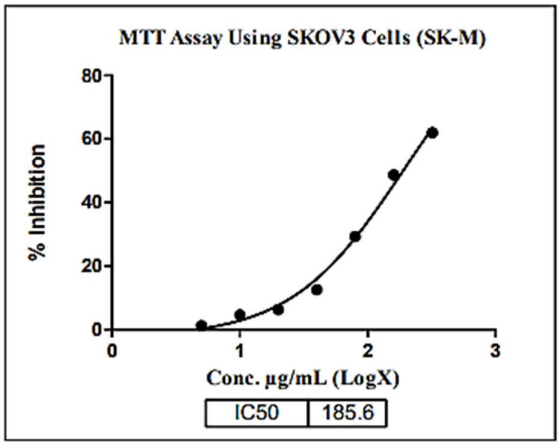




**Fig: 4.30. MTT Assay of Standard Doxorubicin** **Fig: 4.31. MTT Assay of CBUS3-Fruiting**

**Table 4.13. $IC_{50}$ value for MTT assay of SKOV3 (Ovarian Cancer) Cell line**

| **SKOV3** | | | | |
|---|---|---|---|---|
| **Test Sample** | **Conc. in µM** | **OD @ 590nm** | **% Inhibition** | **$IC_{50}$ in µM** |
| **Vehicle Control** | **0** | **0.558** | **0** | |
| **Doxorubicin** | 1.560 | 0.530 | 5.09 | |
| | 3.125 | 0.473 | 15.26 | |
| | 6.25 | 0.408 | 26.95 | **19.66** |
| | 12.5 | 0.317 | 43.22 | |
| | 25 | 0.205 | 63.22 | |

| | | | | |
|---|---|---|---|---|
| | 50 | 0.131 | 76.61 | |
| | 100 | 0.020 | 96.44 | |
| | | | | |
| **Test Sample** | **Conc. in µg/mL** | **OD @ 590nm** | **% Inhibition** | **$IC_{50}$ in µM** |
| **Vehicle Control** | 0 | 0.558 | 0.00 | |
| **CBUAP1 (*Ophiocordyceps liangshanensis*)** | 5 | 0.547 | 1.97 | |
| | 10 | 0.542 | 2.85 | |
| | 20 | 0.504 | 9.68 | |
| | 40 | 0.475 | 14.87 | |
| | 80 | 0.428 | 23.30 | |
| | 160 | 0.399 | 28.49 | |
| | 320 | 0.376 | 32.62 | |
| | | | | |
| **Test Sample** | **Conc. in µg/mL** | **OD @ 590nm** | **% Inhibition** | **$IC_{50}$ in µM** |
| **Vehicle Control** | 5 | 0.554 | 0.72 | |
| **CBUS3 (Fruiting) *Ophiocordyceps sinensis*** | 10 | 0.542 | 2.87 | |
| | 20 | 0.521 | 6.63 | |
| | 40 | 0.495 | 11.29 | |
| | 80 | 0.461 | 17.38 | |
| | 160 | 0.449 | 19.53 | |
| | 320 | 0.418 | 25.09 | |
| | | | | |
| **Test Sample** | **Conc. in µg/mL** | **OD @ 590nm** | **% Inhibition** | **$IC_{50}$ in µM** |
| **Vehicle Control** | 5 | 0.551 | 1.25 | |
| | 10 | 0.532 | 4.66 | |

| | | | | |
|---|---|---|---|---|
| **CBUS3 (Mycelia) *Ophiocordyceps sinensis*** | 20 | 0.523 | 6.32 | **185.6** |
| | 40 | 0.488 | 12.59 | |
| | 80 | 0.395 | 29.28 | |
| | 160 | 0.286 | 48.69 | |
| | 320 | 0.212 | 61.95 | |

## 4.15.2 LDH Assay:

LDH assays of sample CBUAP1, CBUS3 (fruiting), and CBUS3 (mycelia) showed increased levels of LDH at higher concentrations, i.e., 1891.54 U/L, 1554.91 U/L, and 1944.97 U/L at 320 µg/ml respectively, when compared to control (untreated), which was 390.06 U/L in MCF 7 cells. At 100µM treatment, standard doxorubicin released 2324.35 U/L of LDH in MCF 7 cells. Comparing Sample CBUAP1, CBUS3 (fruiting), and CBUS3 (mycelia) to Control (untreated), which had 368.69 U/L in HeLa cells, the three samples had greater amounts of LDH (1656.43 U/L, 1496.13 U/L, and 1854.14 U/L at 320µg/ml respectively). At 100 µM treatment, standard doxorubicin released 2527.40 U/L of LDH in HeLa cells. The LDH levels in CBUAP1, CBUS3 (fruiting), and CBUS3 (mycelia) were greater at higher doses (1683.15 U/L, 1474.76 U/L, and 1907.57 U/L at 320 µg/ml respectively) than in the control (untreated) group, which had 384.72 U/L in SKOV3 cells. At 100µM treatment, standard doxorubicin released 2703.73 U/L of LDH in SKOV3 cells.

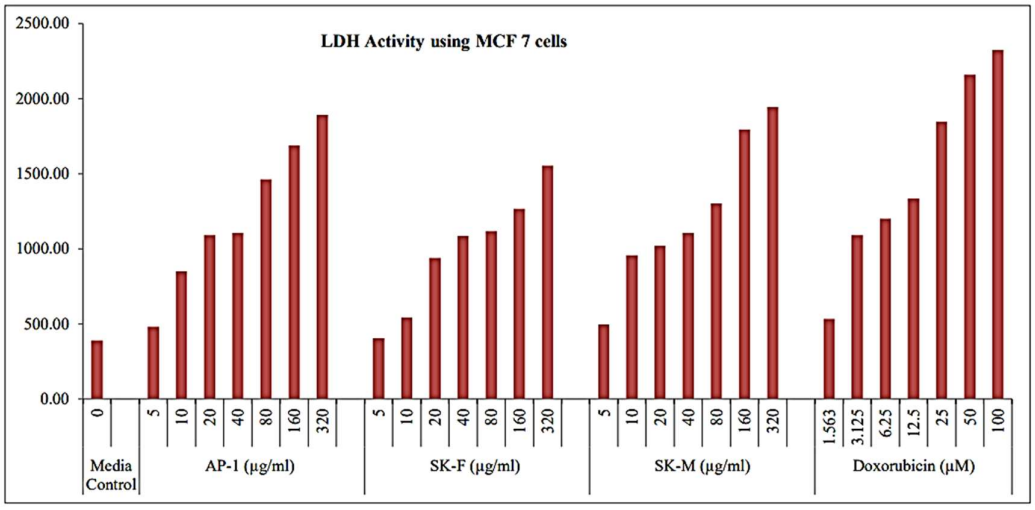



**Fig: 4.32. LDH Assay of CBUAP1, CBUS3- Fruiting, CBUS3- Mycelia and standard Doxorubicin on MCF-7 cell line**

**Table 4.14. Results of LDH Assay on MC-7 Cell line**

| MCF-7 | | | | | |
|---|---|---|---|---|---|
| **Sample** | **Concentration** | **Absorbance at 490 nm** | | **Absorbance** | **LDH activity (U/L)** |
| | | **1 min** | **3 min** | | |
| **Media Control** | 0 | 0.853 | 0.926 | 0.073 | 390.06 |
| | | | | | |
| **Doxorubicin (µM)** | 1.563 | 0.862 | 0.962 | 0.100 | 534.33 |
| | 3.125 | 0.897 | 1.101 | 0.204 | 1090.04 |
| | 6.25 | 0.912 | 1.137 | 0.225 | 1202.25 |
| | 12.5 | 0.926 | 1.176 | 0.250 | 1335.83 |
| | 25 | 0.942 | 1.288 | 0.346 | 1848.79 |
| | 50 | 0.978 | 1.382 | 0.404 | 2158.71 |
| | 100 | 0.994 | 1.429 | 0.435 | 2324.35 |
| | | | | | |
| **CBUAP1 (*Ophiocordyceps liangshanensis*) (µg/mL)** | 5 | 0.832 | 0.922 | 0.090 | 480.90 |
| | 10 | 0.896 | 1.055 | 0.159 | 849.59 |
| | 20 | 0.902 | 1.106 | 0.204 | 1090.04 |
| | 40 | 0.914 | 1.121 | 0.207 | 1106.07 |
| | 80 | 0.923 | 1.197 | 0.274 | 1464.07 |
| | 160 | 0.930 | 1.246 | 0.316 | 1688.49 |
| | 320 | 0.941 | 1.295 | 0.354 | 1891.54 |
| | | | | | |
| | 5 | 0.884 | 0.960 | 0.076 | 406.09 |
| | 10 | 0.884 | 0.968 | 0.102 | 545.02 |
| | 20 | 0.896 | 1.072 | 0.176 | 940.43 |
| | 40 | 0.925 | 1.128 | 0.203 | 1084.70 |

| | | | | | |
|---|---|---|---|---|---|
| **CBUS3 (Fruiting) *Ophiocordyceps sinensis* (µg/mL)** | 80 | 0.937 | 1.146 | 0.209 | 1116.76 |
| | 160 | 0.942 | 1.179 | 0.237 | 1266.37 |
| | 320 | 0.954 | 1.245 | 0.291 | 1554.91 |
| | | | | | |
| **CBUS3 (Mycelia) *Ophiocordyceps sinensis* (µg/mL)** | 5 | 0.844 | 0.937 | 0.093 | 496.93 |
| | 10 | 0.884 | 1.063 | 0.179 | 956.46 |
| | 20 | 0.910 | 1.101 | 0.191 | 1020.58 |
| | 40 | 0.921 | 1.128 | 0.207 | 1106.07 |
| | 80 | 0.938 | 1.182 | 0.244 | 1303.77 |
| | 160 | 0.962 | 1.298 | 0.336 | 1795.36 |
| | 320 | 0.987 | 1.351 | 0.364 | 1944.97 |

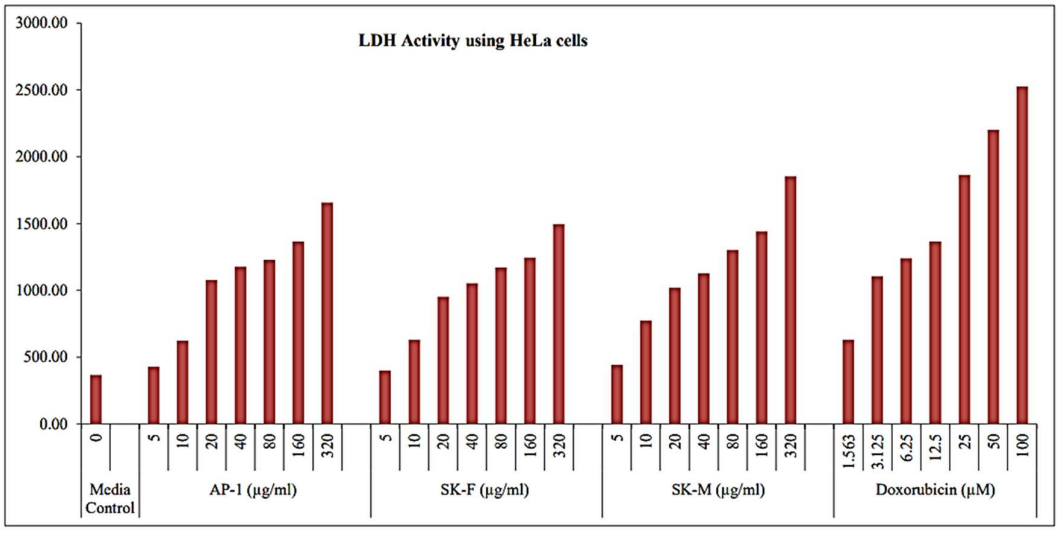



**Fig: 4.33. LDH Assay of CBUAP1, CBUS3- Fruiting, CBUS3- Mycelia and standard Doxorubicin on HeLa cell line**

**Table 4.15. Results of LDH Assay on HeLa Cell line**

| HeLa | | | | | |
|---|---|---|---|---|---|
| **Sample** | **Concentration** | **Absorbance at 490 nm** | | **Absorbance** | **LDH activity (U/L)** |
| | | **1 min** | **3 min** | | |
| **Media Control** | 0 | 0.868 | 0.937 | 0.069 | 368.69 |
| | | | | | |
| **Doxorubicin(µM)** | 1.563 | 0.875 | 0.993 | 0.118 | 630.51 |
| | 3.125 | 0.905 | 1.112 | 0.207 | 1106.07 |
| | 6.25 | 0.919 | 1.151 | 0.232 | 1239.65 |
| | 12.5 | 0.931 | 1.187 | 0.256 | 1367.89 |
| | 25 | 0.945 | 1.294 | 0.349 | 1864.82 |
| | 50 | 0.971 | 1.383 | 0.412 | 2201.45 |
| | 100 | 0.998 | 1.471 | 0.473 | 2527.40 |
| | | | | | |
| **CBUAP1 (*Ophiocordyceps liangshanensis*) (µg/mL)** | 5 | 0.868 | 0.948 | 0.080 | 427.47 |
| | 10 | 0.886 | 1.003 | 0.117 | 625.17 |
| | 20 | 0.905 | 1.107 | 0.202 | 1079.35 |
| | 40 | 0.919 | 1.139 | 0.220 | 1175.53 |
| | 80 | 0.925 | 1.115 | 0.230 | 1228.97 |
| | 160 | 0.937 | 1.193 | 0.256 | 1367.89 |
| | 320 | 0.943 | 1.253 | 0.310 | 1656.43 |
| | | | | | |
| | 5 | 0.863 | 0.938 | 0.075 | 400.75 |
| | 10 | 0.876 | 0.994 | 0.118 | 630.51 |
| | 20 | 0.891 | 1.069 | 0.178 | 951.11 |
| | 40 | 0.918 | 1.115 | 0.197 | 1052.64 |

| | | | | | |
|---|---|---|---|---|---|
| **CBUS3 (Fruiting)- *Ophiocordyceps sinensis* (µg/mL)** | 80 | 0.927 | 1.146 | 0.219 | 1170.19 |
| | 160 | 0.935 | 1.168 | 0.233 | 1245.00 |
| | 320 | 0.941 | 1.221 | 0.280 | 1496.13 |
| | | | | | |
| **CBUS3 (Mycelia)- *Ophiocordyceps sinensis* (µg/mL)** | 5 | 0.844 | 0.927 | 0.083 | 433.50 |
| | 10 | 0.852 | 0.997 | 0.145 | 774.78 |
| | 20 | 0.910 | 1.101 | 0.191 | 1020.58 |
| | 40 | 0.921 | 1.132 | 0.211 | 1127.44 |
| | 80 | 0.938 | 1.182 | 0.244 | 1303.77 |
| | 160 | 0.942 | 1.212 | 0.270 | 1442.70 |
| | 320 | 0.945 | 1.292 | 0.347 | 1854.14 |

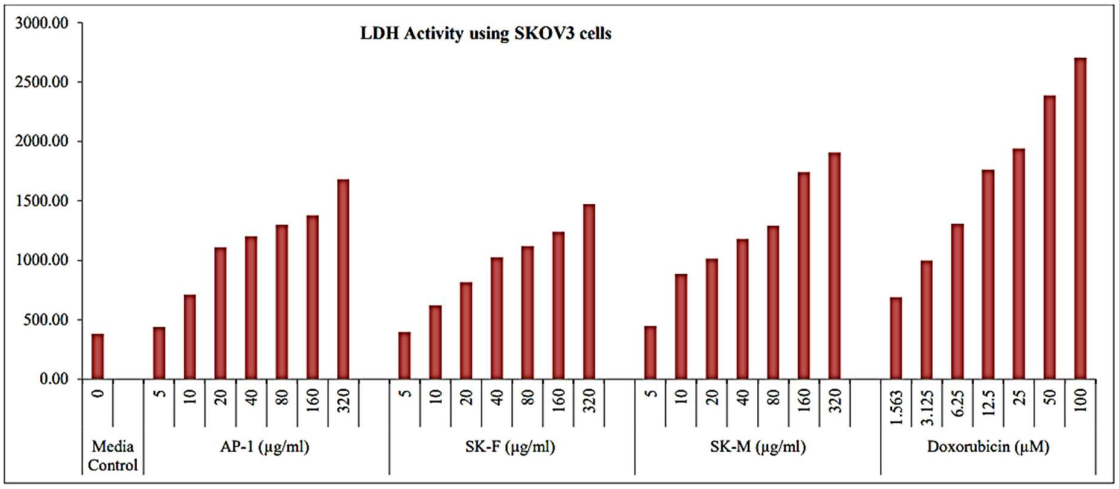



**Fig: 4.34. LDH Assay of CBUAP1, CBUS3- Fruiting, CBUS3- Mycelia and standard Doxorubicin on SKOV3 cell line**

**Table 4.16. Results of LDH Assay on SKOV3 cell line**

| SKOV3 | | | | | |
|---|---|---|---|---|---|
| **Sample** | **Concentration** | **Absorbance at 490 nm** | | **Absorbance** | **LDH activity (U/L)** |
| | | **1 min** | **3 min** | | |
| **Media Control** | 0 | 0.862 | 0.934 | 0.072 | 384.72 |
| | | | | | |
| **Doxorubicin(µM)** | 1.563 | 0.865 | 0.994 | 0.129 | 689.29 |
| | 3.125 | 0.915 | 1.102 | 0.187 | 999.20 |
| | 6.25 | 0.927 | 1.172 | 0.245 | 1309.12 |
| | 12.5 | 0.943 | 1.273 | 0.330 | 1763.30 |
| | 25 | 0.965 | 1.328 | 0.363 | 1939.63 |
| | 50 | 0.976 | 1.423 | 0.447 | 2388.47 |
| | 100 | 0.987 | 1.493 | 0.506 | 2703.73 |
| | | | | | |
| **CBUAP1 (*Ophiocordyceps liangshanensis*) (µg/mL)** | 5 | 0.872 | 0.945 | 0.082 | 438.15 |
| | 10 | 0.909 | 1.042 | 0.133 | 710.66 |
| | 20 | 0.917 | 1.125 | 0.208 | 1111.41 |
| | 40 | 0.926 | 1.151 | 0.225 | 1202.25 |
| | 80 | 0.931 | 1.174 | 0.243 | 1298.43 |
| | 160 | 0.946 | 1.204 | 0.258 | 1378.58 |
| | 320 | 0.955 | 1.270 | 0.315 | 1683.15 |
| | | | | | |
| | 5 | 0.858 | 0.932 | 0.074 | 395.41 |
| | 10 | 0.866 | 0.982 | 0.116 | 619.83 |
| | 20 | 0.873 | 1.026 | 0.153 | 817.53 |
| | 40 | 0.912 | 1.104 | 0.192 | 1025.92 |

| | | | | | |
|---|---|---|---|---|---|
| **CBUS3 (Fruiting)- *Ophiocordyceps sinensis* (µg/mL)** | 80 | 0.925 | 1.135 | 0.210 | 1122.10 |
| | 160 | 0.930 | 1.162 | 0.232 | 1239.65 |
| | 320 | 0.935 | 1.211 | 0.276 | 1474.76 |
| | | | | | |
| **CBUS3 (Mycelia)- *Ophiocordyceps sinensis* (µg/mL)** | 5 | 0.784 | 0.868 | 0.084 | 448.84 |
| | 10 | 0.832 | 0.998 | 0.166 | 886.99 |
| | 20 | 0.905 | 1.095 | 0.190 | 1015.23 |
| | 40 | 0.917 | 1.138 | 0.211 | 1180.88 |
| | 80 | 0.925 | 1.167 | 0.242 | 1293.09 |
| | 160 | 0.939 | 1.265 | 0.326 | 1741.93 |
| | 320 | 0.954 | 1.311 | 0.357 | 1907.57 |

## 4.16 HPLC Analysis:

High Performance Liquid Chromatography was used for the quantification of adenosine and cordycepin because of its boasted medicinal attributes. The results demonstrated that the wild collected samples (CBUS3, CBUAP1, CBUB1 and CBUB2), CBUS3 had the highest adenosine content of 119 µg/ mg of extract and lowest concentration of adenosine was recorded from CBUAP1 of 30 µg/ mg. The bioactive compound Cordycepin was found only from CBUAP1 at a concentration of 10 µg/ mg among the wild collected samples. Similarly, among the standard *Cordyceps militaris* samples, *Cordyceps militaris* grown on barni rice had the highest concentration of adenosine content of 260µg/mg extract, followed by *Cordyceps militaris* grown on brown rice 250 µg/mg extract and the lowest concentration of adenosine was found to be on *Cordyceps militaris* grown on basmati rice 145 µg/mg. The concentration of cordycepin was found to be highest in *Cordyceps militaris* grown on brown rice of 300µg/ mg, followed by *Cordyceps militaris* grown on basmati rice 295 µg/mg and the lowest concentration of cordycepin was recorded from *Cordyceps militaris* grown on black rice 100µg/mg of extract.

**Table: 4.17. HPLC analysis**

| Sl. No. | Sample | Peak Name | RT | Area | Area % | Height | Amount |
|---|---|---|---|---|---|---|---|
| 1 | CBUS3 | Adenosine | 10.249 | 1233921 | 9.91 | 41328 | 119 µg/ mg |
| | | Cordycepin | 13.422 | | | | ND |
| 2 | CBUAP1 | Adenosine | 15.377 | 88245 | 1.22 | 2868 | 30 µg/ mg |
| | | Cordycepin | 18.418 | 28916 | 0.40 | 1119 | 10 µg/ mg |
| 3 | CBUB1 | Adenosine | 11.058 | 543572 | 3.62 | 16263 | 91µg/ mg |
| | | Cordycepin | 15.400 | | | | ND |
| 4 | CBUB2 | Adenosine | 10.866 | 1070119 | 10.23 | 37666 | 105 µg/ mg |
| | | Cordycepin | 15.400 | | | | ND |
| 5 | CBUCM (*Cordyceps militaris*)-Brown Rice | Adenosine | 11.132 | 1419840 | 6.33 | 77990 | 250µg/ mg |
| | | Cordycepin | 15.469 | 3094275 | 13.80 | 96719 | 300µg/ mg |
| 6 | CBUCM (*Cordyceps militaris*)-Basmati Rice | Adenosine | 11.487 | 816254 | 4.32 | 43371 | 145 µg/mg |
| | | Cordycepin | 15.967 | 3090564 | 16.37 | 101362 | 295 µg/mg |
| 7 | CBUCM (*Cordyceps militaris*)-Joha Rice | Adenosine | 11.249 | 969488 | 5.42 | 54327 | 170 µg/ mg |
| | | Cordycepin | 15.736 | 1858818 | 10.4 | 59277 | 180 µg/mg |
| 8 | CBUCM (*Cordyceps militaris*)-Barni Rice | Adenosine | 11.01 | 1471508 | 6.34 | 82799 | 260 µg/mg |
| | | Cordycepin | 15.308 | 1935084 | 8.34 | 63377 | 185 µg/mg |
| 9 | CBUCM (*Cordyceps militaris*)-Black Rice | Adenosine | 11.214 | 1047256 | 6.43 | 56292 | 185 µg/mg |
| | | Cordycepin | 15.901 | 1036739 | 6.37 | 34305 | 100 µg/mg |

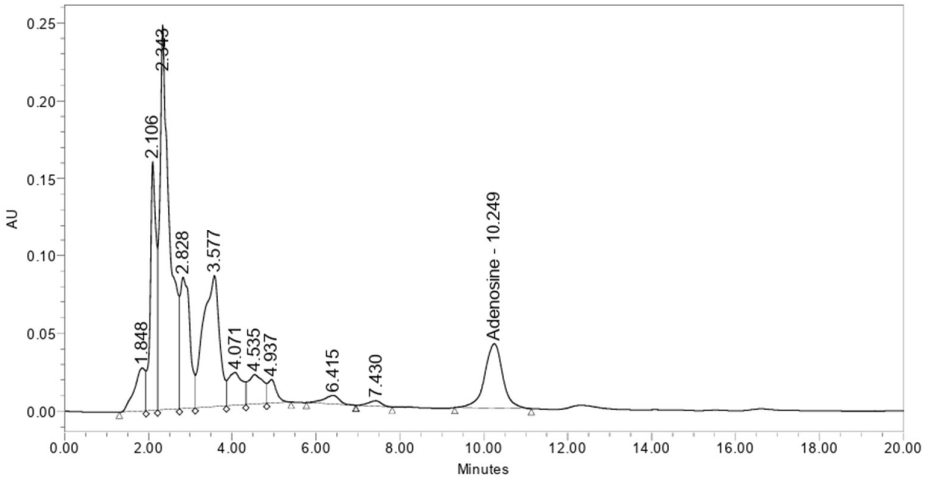



**Fig 4.35. HPLC Chromatogram of CBUS3 (Fruiting)**

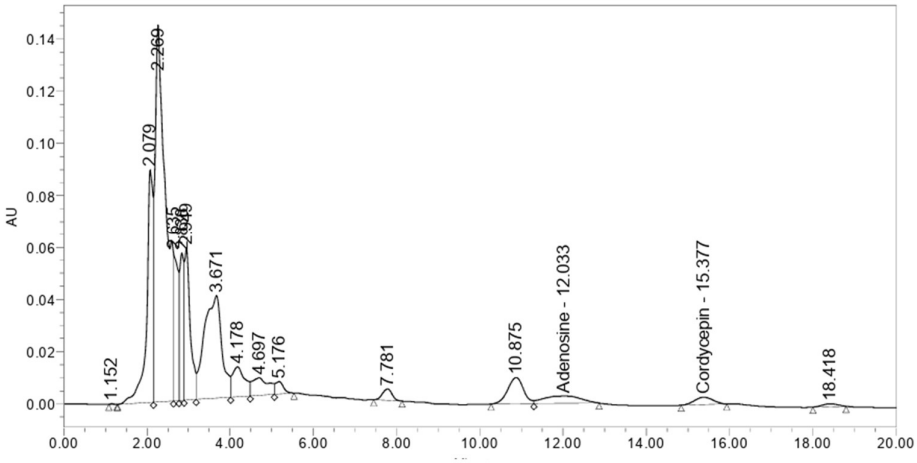



**Fig 4.36. HPLC Chromatogram of CBAP1**

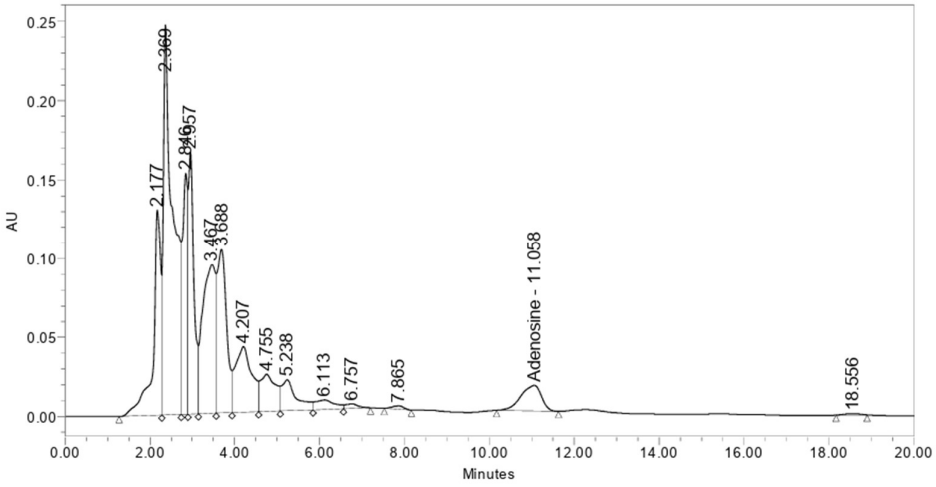



**Fig 4.37. HPLC Chromatogram of CBUB1**

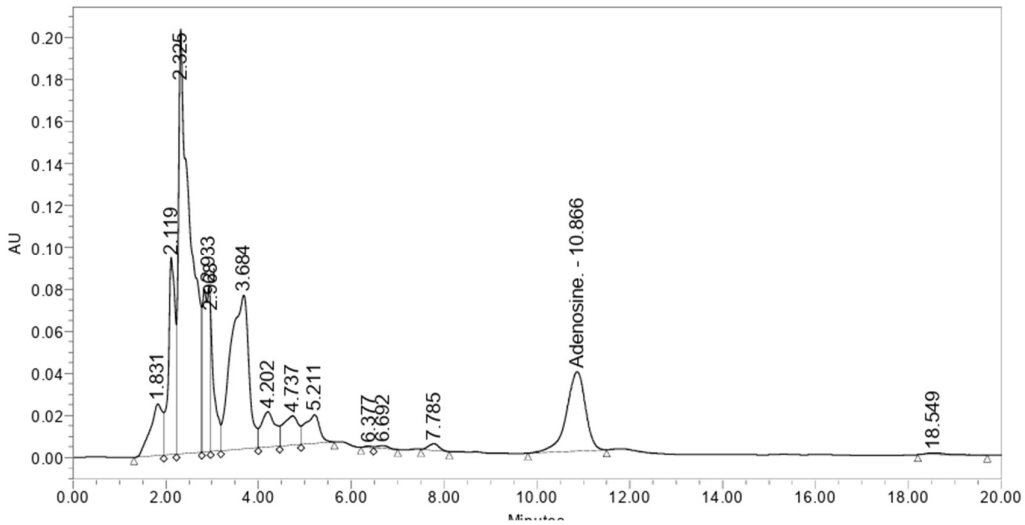



**Fig 4.38. HPLC Chromatogram of CBUB2**

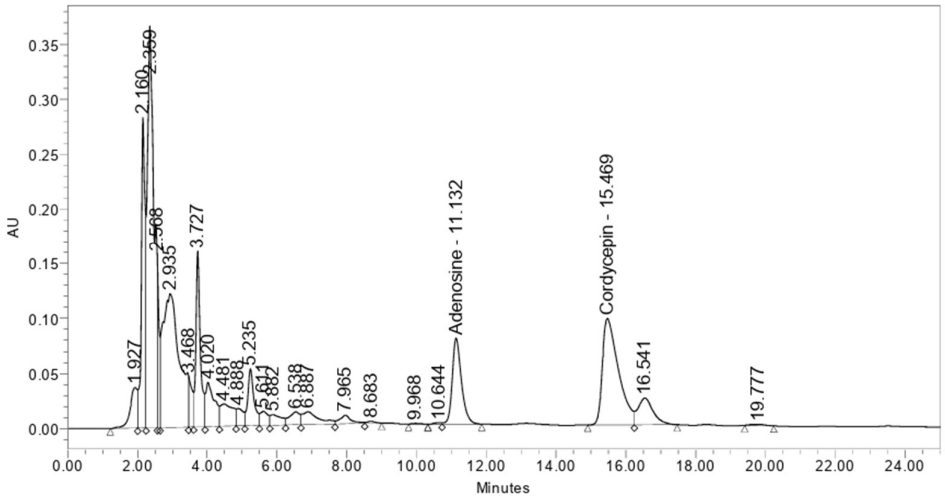



**Fig 4.39. HPLC Chromatogram of CBUCM (*Cordyceps militaris*) grown on Brown Rice**

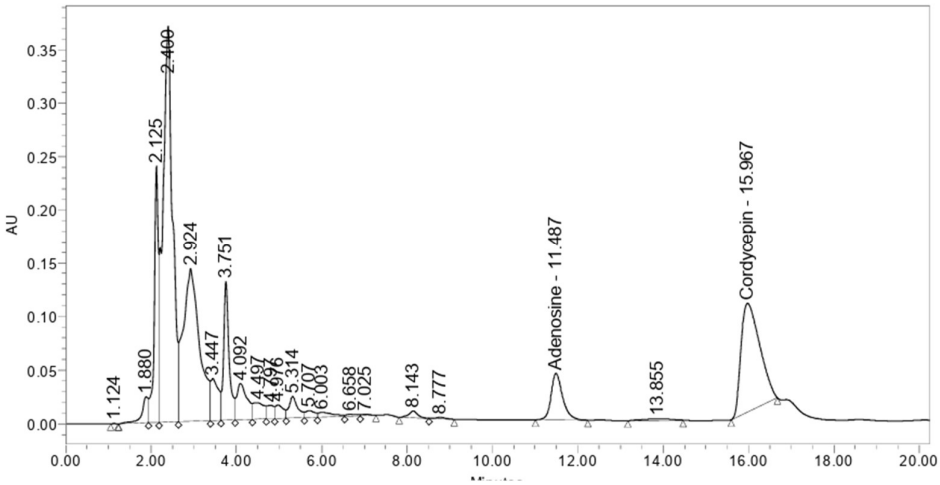



**Fig 4.40. HPLC Chromatogram of CBUCM (*Cordyceps militaris*) grown on Basmati Rice**

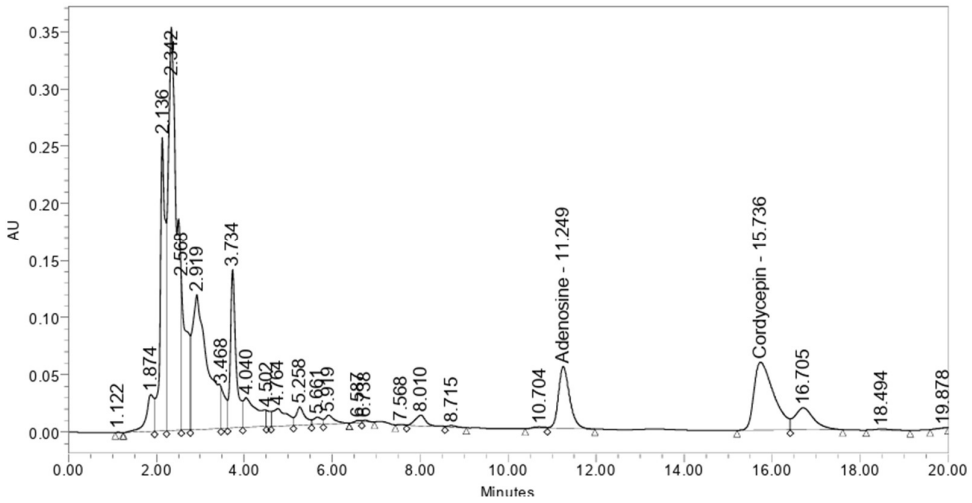



**Fig 4.41. HPLC Chromatogram of CBUCM (*Cordyceps militaris*) grown on Joha Rice**

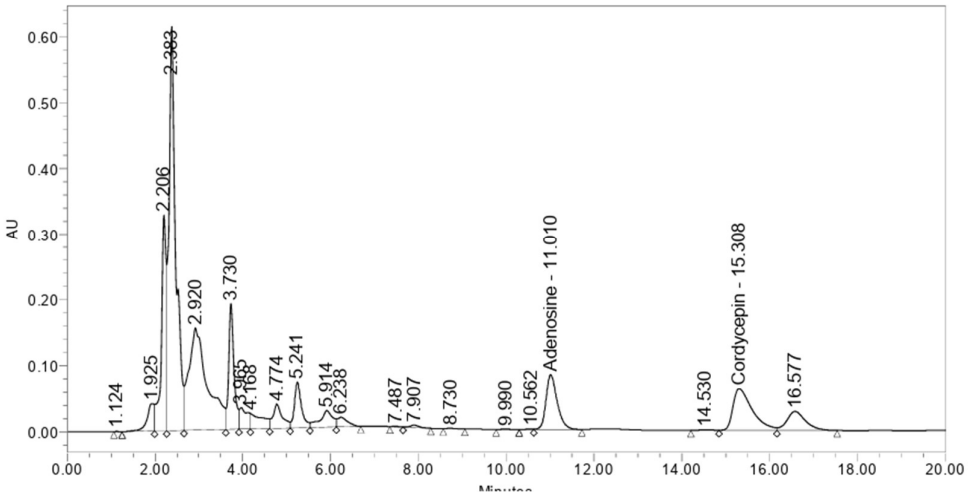



**Fig 4.42. HPLC Chromatogram of CBUCM (*Cordyceps militaris*) grown on Barni Rice**

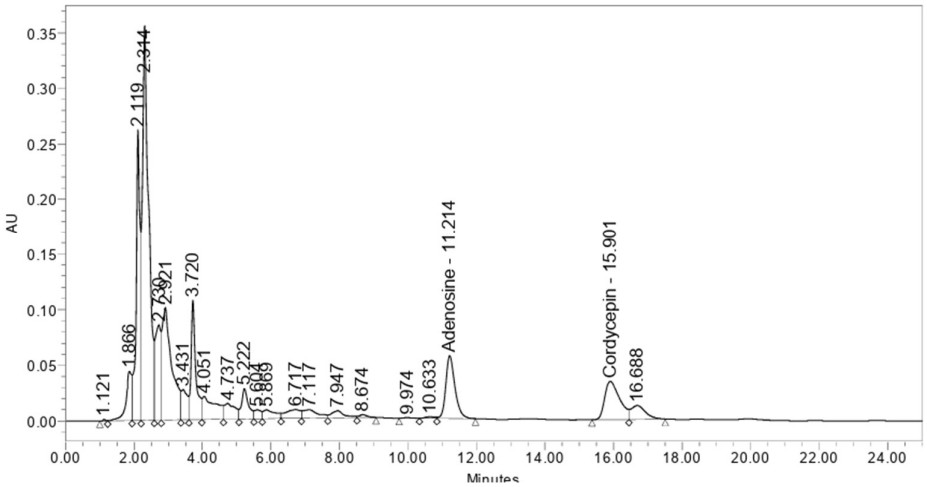
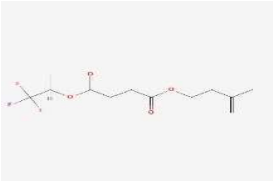



**Fig 4.43. HPLC Chromatogram of CBUCM (*Cordyceps militaris*) grown on Black Rice**

## 4.17 GCMS Analysis:

The samples were analysed using GCMS, which allowed for the identification of several bioactive chemicals based on their peaks observed on a chromatogram. The peaks were subsequently compared to the peaks of recognised chemicals recorded in NIST libraries. The fragmentation of molecules was compared based on their mass-to-charge ratio (m/z), and the compounds were identified. The bioactive chemicals found exhibited diverse actions and characteristics, including antibacterial, antioxidant, and antiviral effects. The chemicals that have been identified are given in Table (4.18-4.21). The majority of the chemicals exhibit similarity across the whole sample that was studied.

**Table 4.18. Bioactive compounds identified from CBUAP1**

| Sl. No. | Retention Time (Min) | Compound Name | Composition (%) | Molecular Weight (g/mol) | Molecular Formula | Structure | Activity |
|---|---|---|---|---|---|---|---|
| 1 | 14.616 | SUCCINIC ACID, 1,1,1-TRIFLUOROPROP-2-YL 3-METHYLBUT-3-EN-1-YL ESTER | 0.505 | 282.26 | $C_{12}H_{17}F_3O_4$ |  | Antibacterial activity (Huang et al., 2022) |

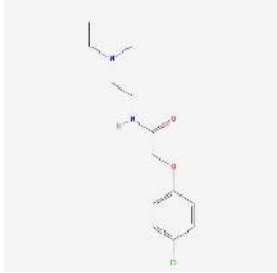
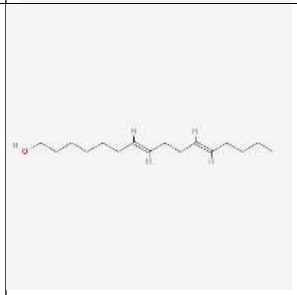
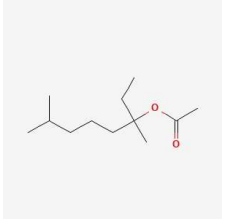
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 2 | 21.814 | CLOFEXAMIDE | 9.453 | 284.78 | $C_{14}H_{21}ClN_2O_2$ |  | Antidepressant (Tareq et al., 2023) |
| 3 | 23.365 | Z,Z-7,11-HEXADECADIEN-1-OL | 0.374 | 238.41 | $C_{16}H_{30}O$ |  | Pheromone used as Insect attractants-mating disrupter (Vick et al., 1974) |
| 4 | 24.840 | 3-OCTANOL, 3,7-DIMETHYL | 0.237 | 200.32 | $C_{12}H_{24}O_2$ |  | Cytotoxic activity (anticancer), andioxidant activity (Coêlho et al., 2022) |

**Table 4.19. Bioactive compounds identified from CBUB1**

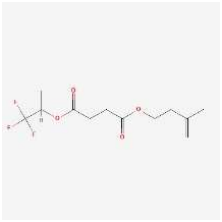
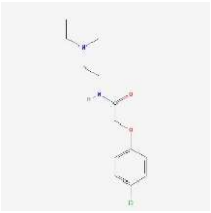
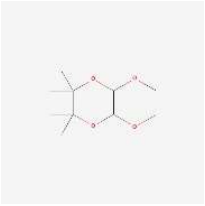
| Sl. No. | Retention Time (Min) | Compound Name | Composition (%) | Molecular Weight (g/mol) | Molecular Formula | Structure | Activity |
|---|---|---|---|---|---|---|---|
| 1 | 14.591 | SUCCINIC ACID, 1,1,1-TRIFLUOROPROP-2-YL 3-METHYLBUT-3-EN-1-YL ESTER | 0.493 | 282.26 | $C_{12}H_{17}F_3O_4$ |  | Antibacterial activity (Huang et al., 2022) |
| 2 | 21.789 | CLOFEXAMIDE | 10.790 | 284.78 | $C_{14}H_{21}ClN_2O_2$ |  | Antidepressant (Tareq et al., 2023) |
| 3 | 23.375 | [1,4]DIOXINO[2,3-B]-1,4-DIOXIN, HEXAHYDRO-2,2,3,3-TETRAMETHYL | 0.438 | 202.25 | $C_{10}H_{18}O_4$ |  | phytotoxic, cytotoxic, or antimicrobial activity (Sebald et al., 2019) |

**Table 4.20. Bioactive compounds identified from CBUS3 (fruiting)**

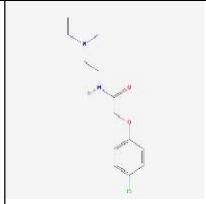
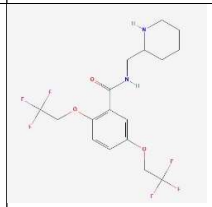
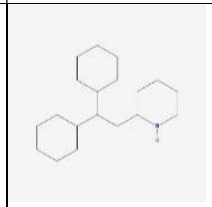
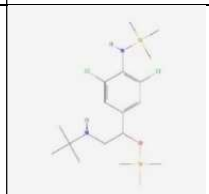
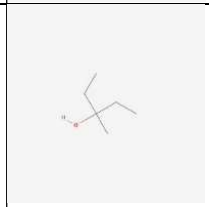
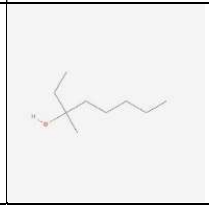
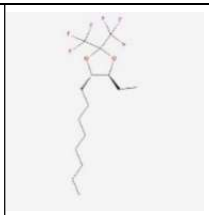
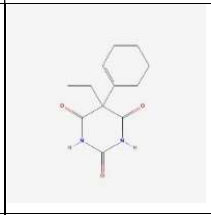
| Sl. No. | Retention Time (Min) | Compound Name | Composition (%) | Molecular Weight (g/mol) | Molecular Formula | Structure | Activity |
|---|---|---|---|---|---|---|---|
| 1 | 21.804 | CLOFEXAMIDE | 10.790 | 284.78 | $C_{14}H_{21}C_lN_2O_2$ |  | Antidepressant (Tareq et al., 2023) |
| 2 | 25.315 | Flecainide | 0.271 | 414.34 | $C_{17}H_{20}F_6N_2O_3$ |  | Antiarrhythmic activity, used for treatment of irregular heart beat (Arunachalam & Alzahrani, 2019.) |
| 3 | 25.330 | PIPERIDINE, 2-(2,2-DICYCLOHEXYLETHYL)- | 0.271 | 277.5 | $C_{19}H_{35}N$ |  | anticancer, antiviral, antimalarial, antimicrobial, antifungal, antihypertension, analgesic, anti-inflammatory, anti-Alzheimer, antipsychotic and/or anticoagulant agents. (Abdelshaheed et al., 2017) |

**Table 4.21. Bioactive compounds identified from CBUS3 (Mycelia)**

| Sl. No. | Retention Time (Min) | Compound Name | Composition (%) | Molecular Weight (g/mol) | Molecular Formula | Structure | Activity |
|---|---|---|---|---|---|---|---|
| **1** | 21.699 | CLENBUTEROL, 2TMS DERIVATIVE | NA | 421.5 | $C_{18}H_{34}Cl_2N_2OSi_2$ |  | Used to treat asthma, muscle attropy (Jiang et al., 2011) |
| **2** | 26.516 | 3-PENTANOL, 3-METHYL | NA | 102.17 | $C_6H_{14}O$ |  | Antidepressant and anticonvulsant (Pharmaceutical Manufacturing Encyclopedia (Vol. 1) 2007) |
| **3** | 29.782 | 3-OCTANOL, 3-METHYL | 0.411 | 144.25 | $C_9H_{20}O$ |  | Flavouring agent in meat industry (Hsu et al., 1982) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **4** | 34.194 | 1,3-DIOXOLANE, 4-ETHYL-5-OCTYL-2,2-BIS(TRIFLUOROMETHYL)-, TRANS | 3.786 | 350.34 | $C_{15}H_{24}F_6O_2$ |  | Antioxidant activity (Khan et al., 2016) |
| **5** | 37.175 | CYCLOBARBITAL | 7.326 | 236 | $C_{12}H_{16}N_2O_3$ |  | Antidepressant and used for treatment of insomnia (Breyer-Pfaff, U., Jerg, H., & Petruch, F. 1979) |

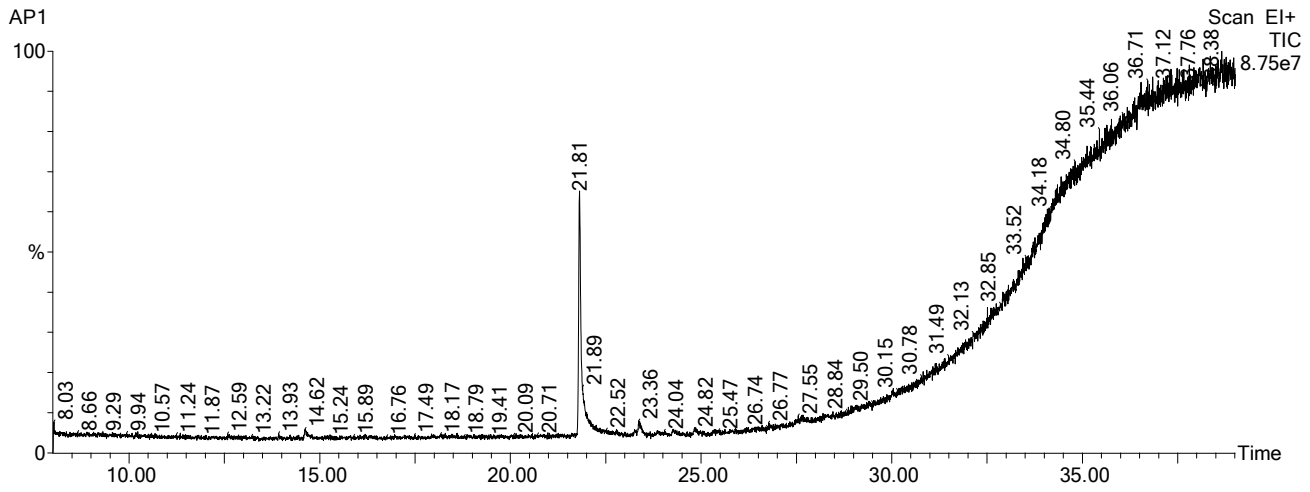



**Fig 4.44.** GCMS chromatogram of CBAP1

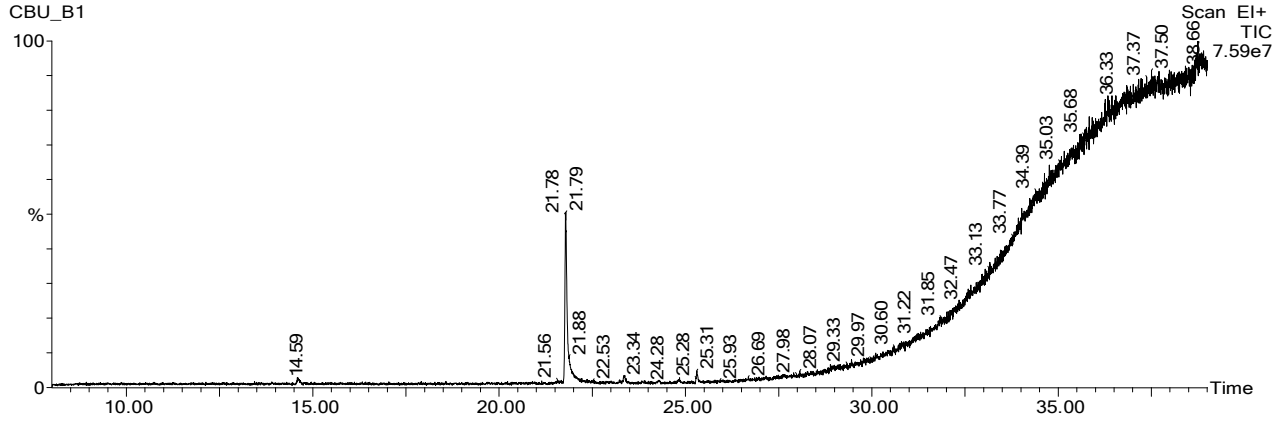



**Fig 4.45.** GCMS chromatogram of CBUB1

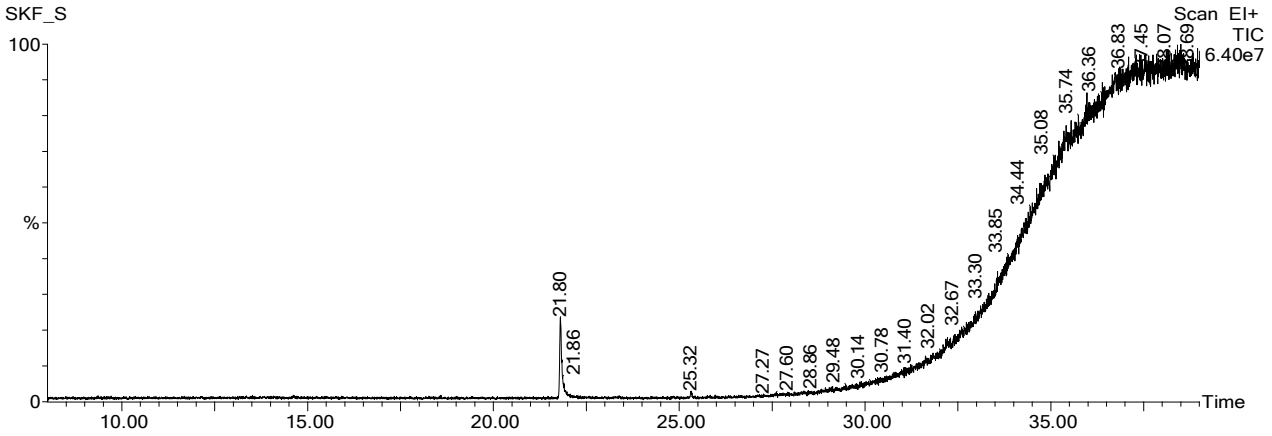



**Fig 4.46. GCMS chromatogram of CBUS3(fruiting)**

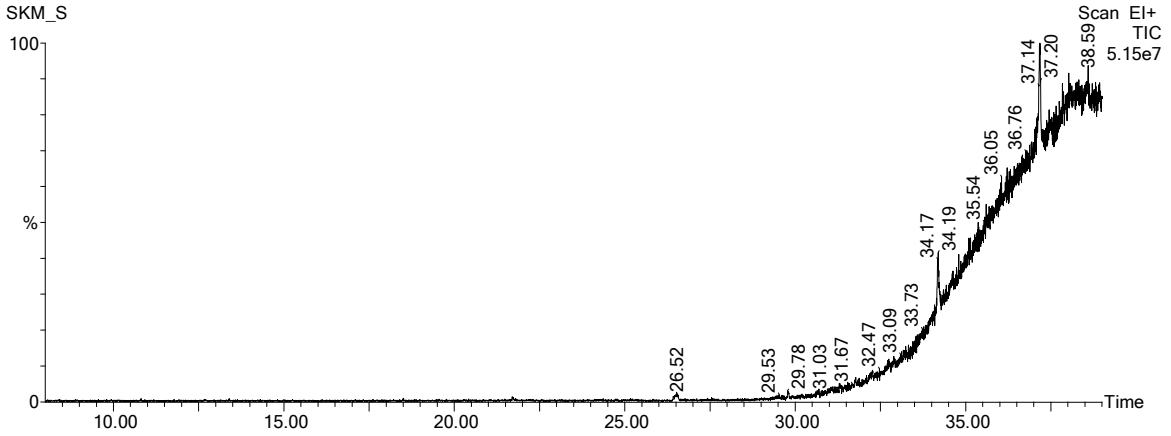



**Fig 4.47. GCMS chromatogram of CBUS3(Mycelia)**