CHAPTER: IV

RESULTS

4.1. Taxonomic study:

4.1.1. *Volvariella volvacea:*

Volvariella volvacea (Bull.) Singer, *Lilloa* **22**:401 (1951) [1949]
=*Agaricus rhodomelas* Lasch, *Linnaea* **4**: 548(1829)
=*Agaricus volvaceus* Bull., *Herb. Fr*. (Paris) **6**: tab.262 (1786)
= *Agaricus volvaceus var. minor* Bull**.**, *Herb. Fr.* (Paris) **7**: tab. 330 (1788) [1787-88]
= *Agaricus volvaceus var. rhodomelas* (Lasch) Fr**.**, *Epicr. syst. mycol.* (Upsaliae): 138 (1838) [1836-1838]
= *Amanita virgata* Pers., *Tent. disp. meth. fung.* (Lipsiae): 18 (1797)
=*Vaginata virgata* (Pers.) Gray, *Nat. Arr. Brit. Pl.* (London) **1**: 601 (1821)
=*Volvaria rhodomelaena (*Lasch) P. Kumm., *Führ. Pilzk.* (Zerbst): 99 (1871)
=*Volvaria virgata var. fennica* P. Karst**.**, *Hedwigia* **30**: 246 (1891)
=*Volvaria volvacea* (Bull.) P. Kumm., *Führ. Pilzk.* (Zerbst): 99 (1871)
=*Volvaria volvacea var. edulis* Overeem, *De nuttige planten van Nederlandsch Indië* **1**: 72 (1927)
= *Volvaria volvacea var. fennica* (P. Karst.) Sacc., *Syll. fung.* (Abellini) **11**: 43 (1895)
=*Volvaria volvacea var. nigricans* Kawam., *Icones of Japanese fungi* **5**: 596 (1954)
=*Volvariella volvacea var. heimii* Singer
=*Volvariella volvacea var. masseei* Singer & Wasser [as '*massei*'], in Wasser, *Flora Gribov Ukrainy*, Bazidiomitsety. Amanital'nye Griby (Kiev): 101 (1992)
=*Volvariella volvacea var. nigricans* Kawam. ex Hongo, *J. Jap. Bot.* **38**: 233 (1963)
=*Volvariopsis volvacea* (Bull.) Murrill, *N. Amer. Fl.* (New York) **10**(2): 144 (1917) [Index Fungorum].

The pileus size ranged from (48)-89.7-(152.8) mm in diameter (orbicular in shape when looking down at the pileus), coloured pale grey (4-B1) in edge to grey earth to brownish grey (4-D1-5-F2) in central region narrowly parabolic with elongated volva during button stage which then becomes broadly parabolic in young and eventually from plano convex to hemispheric in mature stage and appears brown to dark brown in colour (paler towards edge) & broad, umbo, moist cuticle which peels easily up to the diameter and are smooth with wavy margin which are smooth. Lamellae free from close to crowded and are brittle with smooth gill edge and colour ranging from yellowish white (2-A2) in young to brownish orange to red haired (6-C3-C4) in matured stages. The stipe is terete to sub-cylindrical and sub-clavate thickening towards the base with length ranging from (53.8)-106.2-(180) mm and diameter ranging from (6.5)-10.96-(17.8) mm, fibrous, moist surface

with volva at the base coloured dark brown, the colour of flesh from pileus and stipe are white (1-A1), aroma and taste not distinctive.

Microscopic characters: basidiospores ellipsoid to ovoid in shape, smooth, inamyloid (5)-6.9- (8) μm × (4)-4.6-(6) μm, Q= (1.75)-1.48-(1.33), Qavg= (6.9)-1.5-(4.6) basidia clavate and tetrasterigmatic thin walled, hyaline measuring 18-23 × 6-9 μm. Pleurocystidia; common, fusoid-ventricose or lanceloid measuring 42-60 μm × 10- 16 μm. Cheilocystidia mucronate with elongated apices measuring 38-55 μm × 14-16 μm. Pileipellis have cylindrical septate hyphae without clamp connection. Stipitipellis: cylindrical, septate hyphae, Gills trama: cells of variable shapes. The collected sample matches well with *V. volvacea.*

Specimen examined: Department of Biotechnology, Bodoland University, Kokrajhar, Assam, India (26º46'93"N 90º29'52"E) on 26/09/17, 1/10/17, 3/10/2017, (BUR01).

4.1.2. *Termitomyces heimii:*

Termitomyces heimii Natarajan, Mycologia **71**(4): 853 (1979)
=*Sinotermitomyces cavus* M. Zang, *Mycotaxon* **13**(1):172(1981)
=*Termitomyces longiradicatus Sathe & J.T. Daniel [as 'longiradicata'], Maharashtra Association for the Cultivation of Science, Monograph No.1 Agaricales (Mushrooms) of South West India* (Pune): 102 (1981)] [Index Fungorum].

Pileus measuring up to 9 cm in diameter, bulbous with smooth surface with veil, then campanulate which turns convex and finally planoconvex with distinct white perforitorium that turns brownish grey at maturity, splitted margins with smooth and dry surface. Lamellae free, brittle, serete margin, close, initially white which later turn to pale pink at maturity. Annulus white on the upper part of stipe. Stipe white in colour, central, solid and cylindrical measuring up to 8 cm × 2.7 cm, fibrous, pseudorhiza hollow up to 45 cm long, tapering at the base.

Microscopic characters: Basidia measuring 12.5-18μm × 5-6 μm, clavate, with 2-4 sterigma, thin walled. Basidiospores measuring 6-7.5 × 4.5-6, Qavg 1.5-1.5-1.75 μm, ellipsoid, sub hyaline and non amyloid. Pleurocystidia 13-18 × 5-7 μm, clavate to pyriform, thin walled and hyaline. Cheilocystidia not observed. Stipitipellis with cylindrical and septate hyphae measuring 3.5-6 μm in diameter, without clamp connection. Gills trama measures 55-63 μm wide, septate, parallel and hyaline hyphae. Pileipellis radial and repent hyphae sometimes with branch measuring 4-5 μm in diameter, septate and cylindrical.

Sample examined: Department of Biotechnology, Bodoland University, Kokrajhar, Assam, India on 04/04/2019, 05/04/2019, 27/04/2019, BUR02.

4.1.3. *Lentinus sajor-caju:*

Lentinus sajor-caju (Fr.) Fr., *Epicr. Syst. Mycol.* (Upsaliae): 393(1838)
=*Agaricus sajor-caju Fr., Syst. mycol.* (Lundae) **1**: 175 (1821)
=*Pocillaria sajor-caju* (Fr.) Kuntze, *Revis. gen. pl.* (Leipzig) **2**: 866 (1891)
=*Pleurotus sajor-caju* (Fr.) Singer, *Lilloa* **22**: 271 (1951)
=*Lentinus tanghiniae* Lév., *Annls Sci. Nat*., Bot., ser.3 **5**: 119 (1846)
=*Pocillaria tanghiniae* (Lév.) Kuntze,*Revis. Gen*.pl. (Leipzig) **2**:866(1891)
=*Lentinus stenophyllus* Reichardt, *Verh.zool.-bot. Ges. Wien* **16**:375(1866)
=*Lentinus nicobarensis* Reichardt, *Reise der Osterr. Fregatte Novara* **1**(3): 141(1870)
=*Pocillaria nicobarensis* (Reichardt) Kuntze, Revis.gen.pl.(Leipzig) **2:**866(1891)
=*Lentinus glandulosus* Ces., *Atti Accad.Sci.fis.mat.Napoli* **8** (no.3):3(1879)
=*Pocillaria glandulosa* (Ces.) Kuntze, *Revis. Gen.pl.* (Leipzig) **2**:866(1891)
=*Lentinus murrayi* Kalchbr. & MacOwan, *in Kalchbrenner, Grevillea* **9**(no.52):136(1881)
=*Pocillaria murrayi* (Kalchbr. & MacOwan) Kuntze, *Revis. Gen.pl.* (Leipzig)**2**:866(1891)
=*Lentinus woodii* Kalchbr., *Grevillea* **9**(no.52):136(1881)
=*Pocillaria woodii* (Kalchbr.) Kuntze, , *Revis. Gen.pl.* (Leipzig) **2**:866(1891)
=*Lentinus tenuipes* Sacc. & Paol., *Atti Inst. Veneto Sci. Lett., ed Arti, Ser.6 **6**: 392(1888).*
=*Lentinus bonii Pat., Bull. Soc.mycol.Fr.* **8**(2):48(1892)
=*Lentinus bukobensis* Henn.,*Bot.Jb*.**17**:32(1893)
=*Lentinus tanghiniae var. annulatus* Henn**.**, *Bot.Jb*. **22**:94(1895)
=*Lentinus annulifer* De Seynes, *Recherches Fl.Champ.Congo franc*. **1:**25(1897)
=*Lentinus sajor-caju* f. laciniata Biers, *Bull. Trimmest.Soc.mycol.Fr*.**40**(3):235(1924)
=*Lentinus sajor-caju var. densifolius* Pilát, *Annls mycol.* **34**(1/2):128(1936)
=*Lentinus sajor-caju var. elegans* Pilát, *Annls mycol.* **34**(1/2):128(1936)
=*Lentinus sajor-caju var. medius* Pilát, *Annls mycol.* **34**(1/2):128(1936)
=*Lentinus sajor-caju var. obnubilus* Pilát, *Annls mycol.* **34**(1/2):128(1936)
=*Lentinus sajor-caju var. sparsifolius* Pilát, *Annls mycol.* **34**(1/2):128(1936)
=*Lentinus sajor-caju var. typicus* Pilát, *Annls mycol.* **34**(1/2):128(1936)
=*Lentinus sajor-caju var. vellereus* Pilát, *Annls mycol.* **34**(1/2):128(1936)
=*Lentinus sajor-caju var. velutinosquamulosus* Pilát, *Annls mycol.* **34**(1/2):128(1936)
=*Antromycopsis sajor-caju* L.N. Nair & V.P. Kaul, *Sydowia* **33**:223(1980)
=*Lentinus sajor-caju var. subdistans* Corner, *Beih. Nova Hedwigia* **69**:45(1981) [Index Fungorum].

Pileus ranging from 1.5- 4.8 cm, thin, convex, involute, umbilicate and infundibulbiform, ivory (4B3) during young turning light blonde (4C3) with maturity and white (5A1) in the centre, dry and leathery peeling easily to the diameter with smooth and matty surface. The edges are incurved with wavy lining, striated and smooth, veil absent, lamellae decurrent to subdecurrent with close fleshy gills coloured silver white (2B2) in young and mature. Stipe central to eccentric cylindrical and irregular measuring 4-6 mm in

thickness and 20-28 mm in length, leathery, solid and fleshy with dry surface bearing annulus, no particular taste but have woody smell.

Microscopic characters: Basidia 12.26-16.57 × 2.3-4.9 µm, clavate and tetra-sterigmate having basal clamp, Basidiospores measuring 5.1-7.09 × 1.65-2.21 µm, Q=3.2-3.25-3.5, narrowly ellipsoid to cylindrical, inamyloid containing oil droplets. Dimetic hyphal system bearing generative and skeletal hyphae measuring 3-4.6 µm and 3.32-6.1 µm in diameter. Generative hyphae with thick to thin walled hyaline bearing clamp and septate. Cystidia not seen.

Material Examined: Department of Biotechnology, Bodoland University, Kokrajhar, Assam, India. 17/10/2017, 21/08/2018, 26/08/2018. BUR03.

4.1.4. *Chlorophyllum hortense:*

Chlorophyllum hortense (Murrill) Vellinga, *Mycotaxon* **83**: 416 (2002)
=*Lepiota hortensis* Murrill, *N. Amer. Fl.* (New York) **10**(1): 59 (1917)
=*Leucoagaricus hortensis* (Murrill) Pegler, *Kew Bull.*, Addit. Ser. **9**: 414 (1983) [Index Fungorum].

Pileus 2.6-8 cm in diameter, oval at young turning conical then flattening with maturity bearing umbo scarcely fleshy, soft and brittle, partial veil and scarcely involute at young, moist, scaly, matt and ornamented, striated. Lamellae free, crowded 17/cm not interveined. Stipe central, long thin and cylindrical measuring 3-5.2 cm in length and 3.8-6.2 mm in diameter, centrally hollow, reddening on rubbing, cartilaginous with dry surface universal veil present at initial stage leftover remains at the stipe at maturity, flesh turn red on cut, no distinct smell or taste.

Microscopic characters: Basidiospores measuring 6.46-8.66 × 4.71-5.87 µm, Q=1.37-1.45-1.49 broadly ellipsoid, smooth thick walled bearing apicules without germ pore and hyaline. Basidia measuring 21-31 × 5.3-8.1 µm, clavate and tetrasterigmate. Cheilocystidia numerous measuring 30.53-62.42 × 5.92-12.53 µm, thick walled, clavate to sub cylindrical. Pleurocystidia absent, pileipellis parallel, septate, thick walled, cylindrical hyphae and terminal elements with obtuse and interwoven. Stipitipellis with longitudinal parallel hyphae, incomplete clamp connections (may be outgrowth of hyphae).

Material examined: Department of Biotechnology, Bodoland University, Kokrajhar, Assam, India. 12/10/2018, 13/10/2018, 28/12/2018, 17/01/2019. BUR04.

4.1.5. *Cantharellus subamethysteus:*

Cantharellus subamethysteus Eyssart. & D.Stubbe**,** Fungal Diversity **36**:62(2009) [Index Fungorum].

Macroscopic characters: *Cantharellus sp.* are found growing in the leaf of *Shorea robusta*, they appear in groups, pileus measuring 10-62 mm, infundibulbiform with moist and smooth surface, they have wavy, incurved and smooth margin, lamellae subdecurrent, close (11/cm). Stipe central, round solid in young and hollow in mature fruit body measuring 10-33 mm in length and 5- 9 mm in diameter. Fruity smell and Colour greyish yellow (1-B3) in the surface with paler hymenium. With distinct purplish lilac squamules in the centre of pileus in young fruiting body and in mature with brownish scales with purple tinge to some extent covering the pileus.

Hymenium continues to stipe with spores measuring (5.3)6-7.5(7.87) ×(4.27)4.5-5.5(5.63) µm shortly ellipsoid with Q=1.2-1.5, Qavg=1.4 from 40 individual spores. Basidia clavate sub hyaline measuring 52-75 × 7-8.5 µm, 4-6 spored, subhymenium not differentiated. Pileipellis bearing repent and thin walled cylindrical hyphae measuring 5-10 µm broad with numerous and obtuse free extremities. Stipitipellis bearing dense parallel septate hyphae sometimes branched measuring 4-11 µm width. Pleurocystidia and cheilocystidia absent. Clamp connections present.

Material Examined: Department of Biotechnology, Bodoland University, Kokrajhar, Assam, India. 5/11/2018, 7/11/2018, 14/01/2019, 15/01/2019. BUR05.

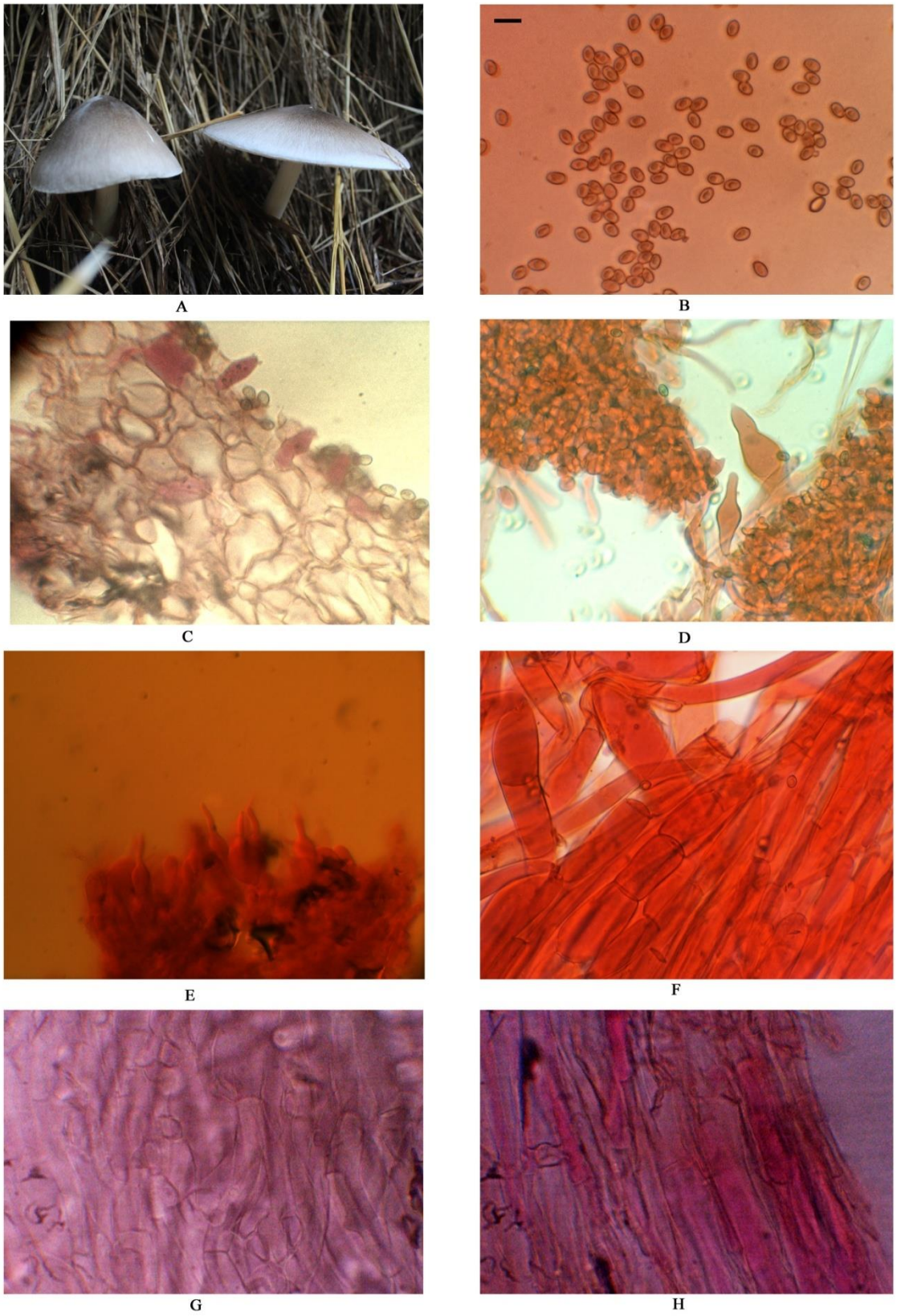


Figure 4.1A: *Volvariella volvacea* a. Fruit body, b. Basidiospores, c. Basidia, d. Pleurocystidia, e. Cheilocystidia, f. Hyphae at pileipellis, g. Hyphae at stipitipellis, h. Hyphae at volva.

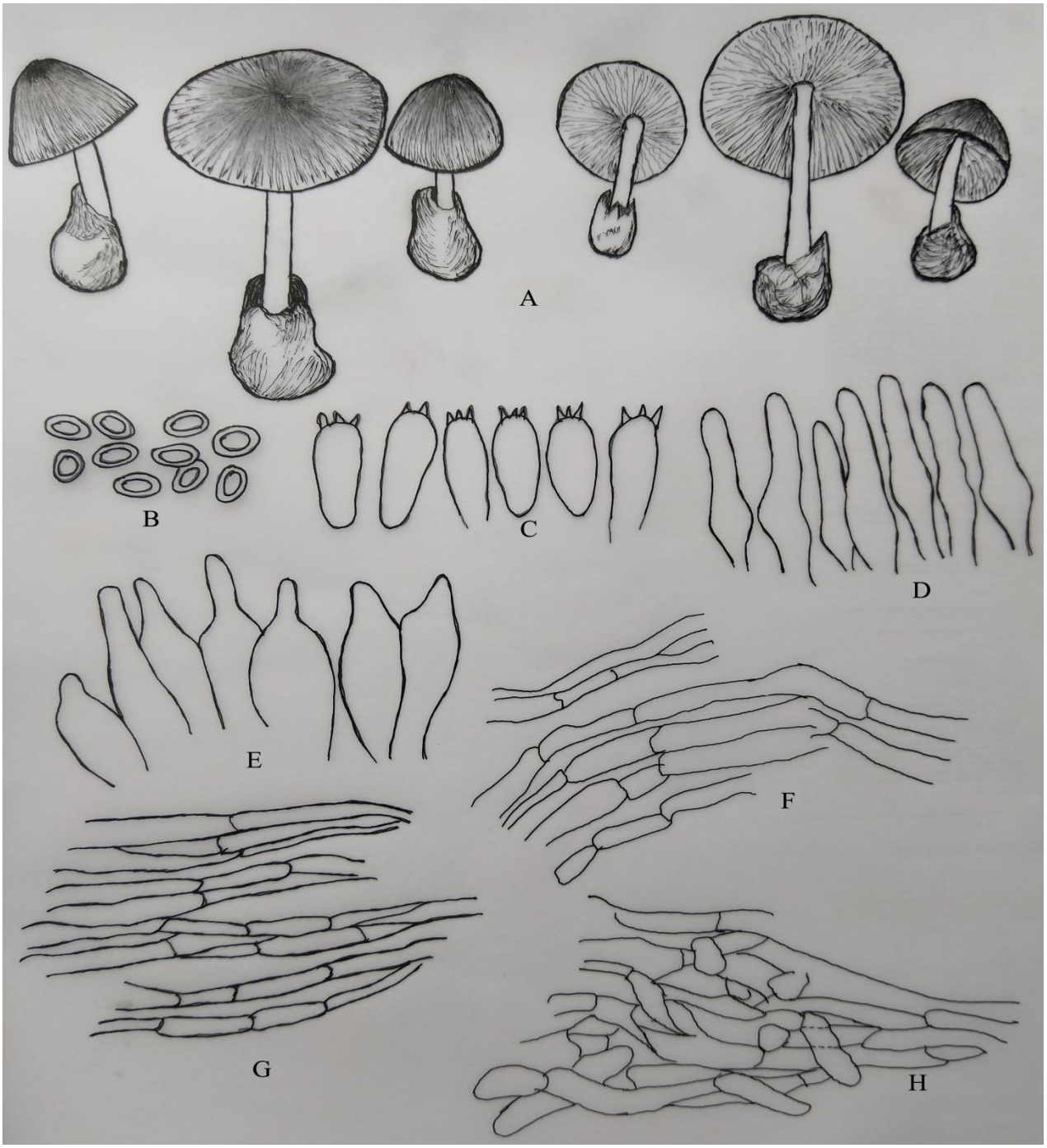


Figure: 4.1B. *Volvariella volvacea.* A- Fruit body, B- basidiospores, C- Basidia, D- Pleurocystidia, E- Cheilocystidia, F-Pileipellis, G- Hyphae at volva, H- Stipitipellis.

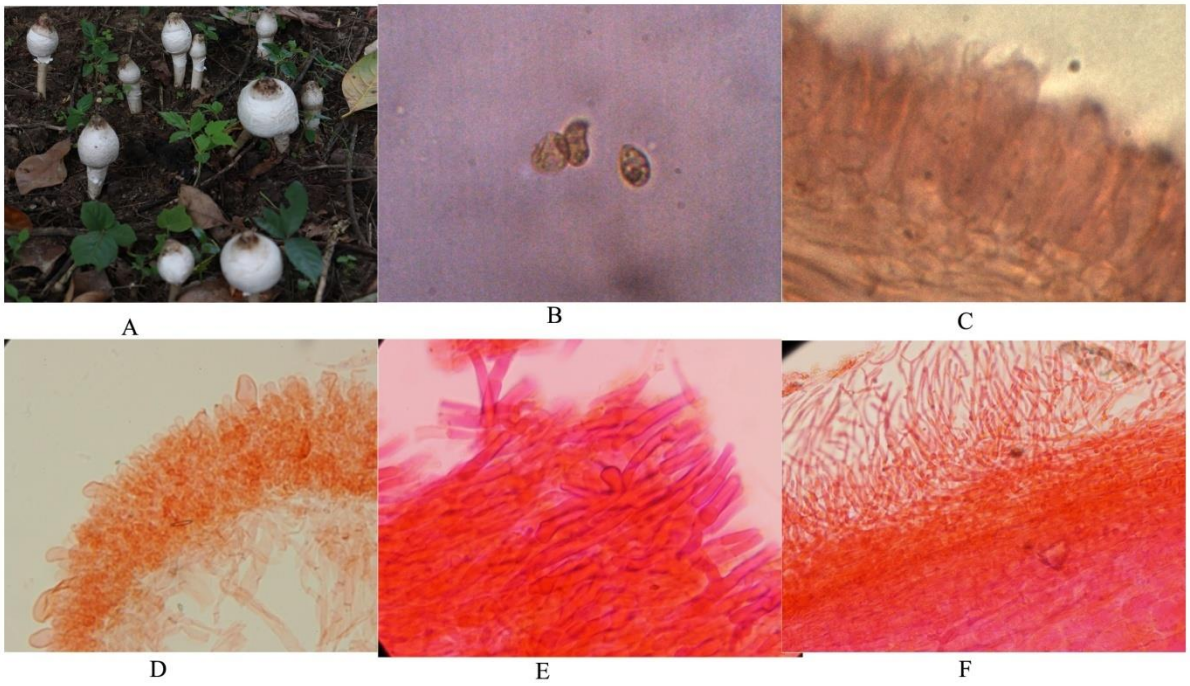


Figure 4.2A: *Termitomyces heimii;* a. Fruit body, b. Basidiospores, c. Basidia, d. Pleurocystidia, e. Hyphae at pileipellis, f. Hyphae at stipitipellis.

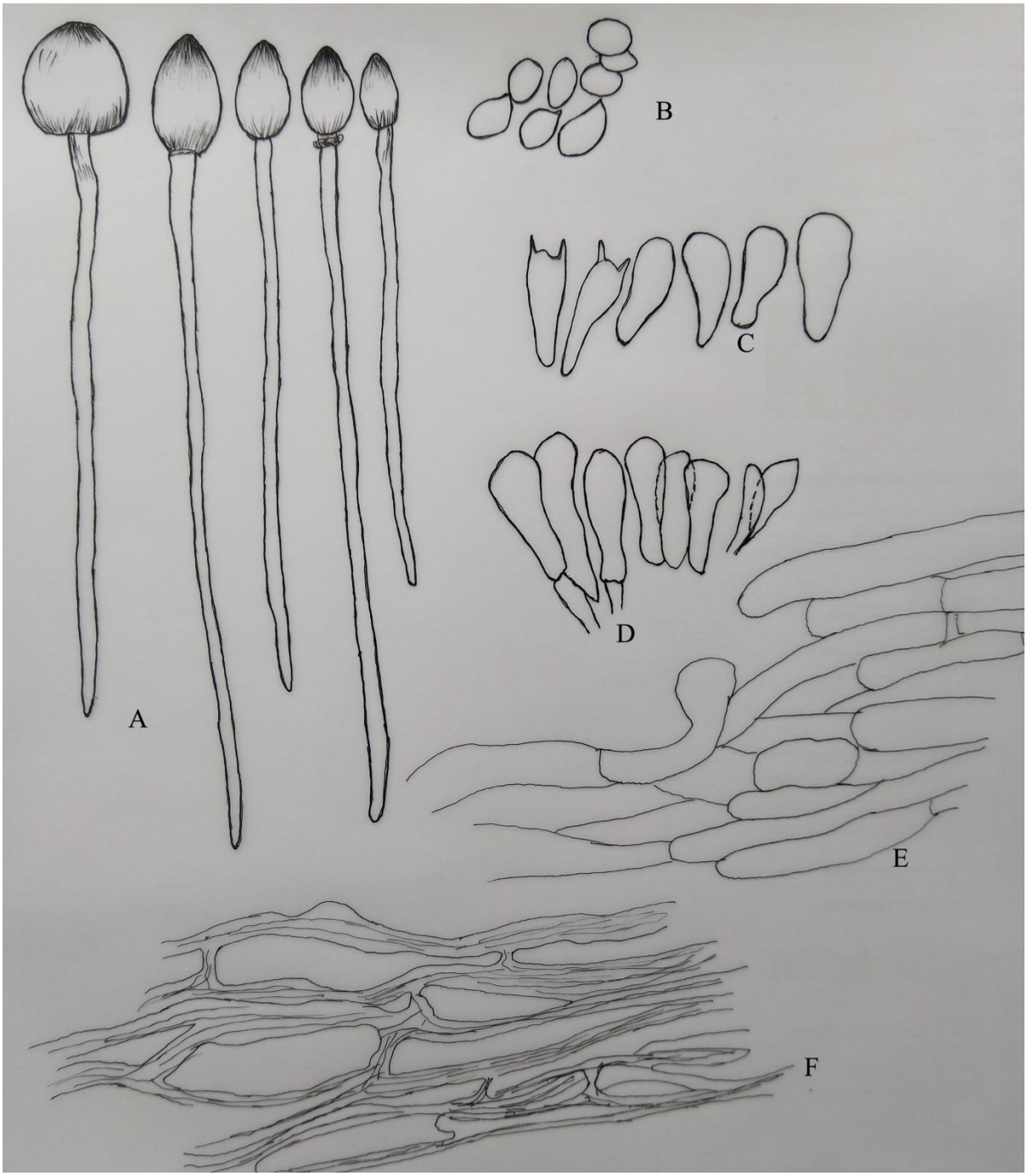


Figure 4.2B; *Termitomyces heimii*; A- Fruit body, B- Basidiospore, C- Basidia, D- Pleurocystidia, E- hyphae at stipitipellis, F- Pileipellis trama.

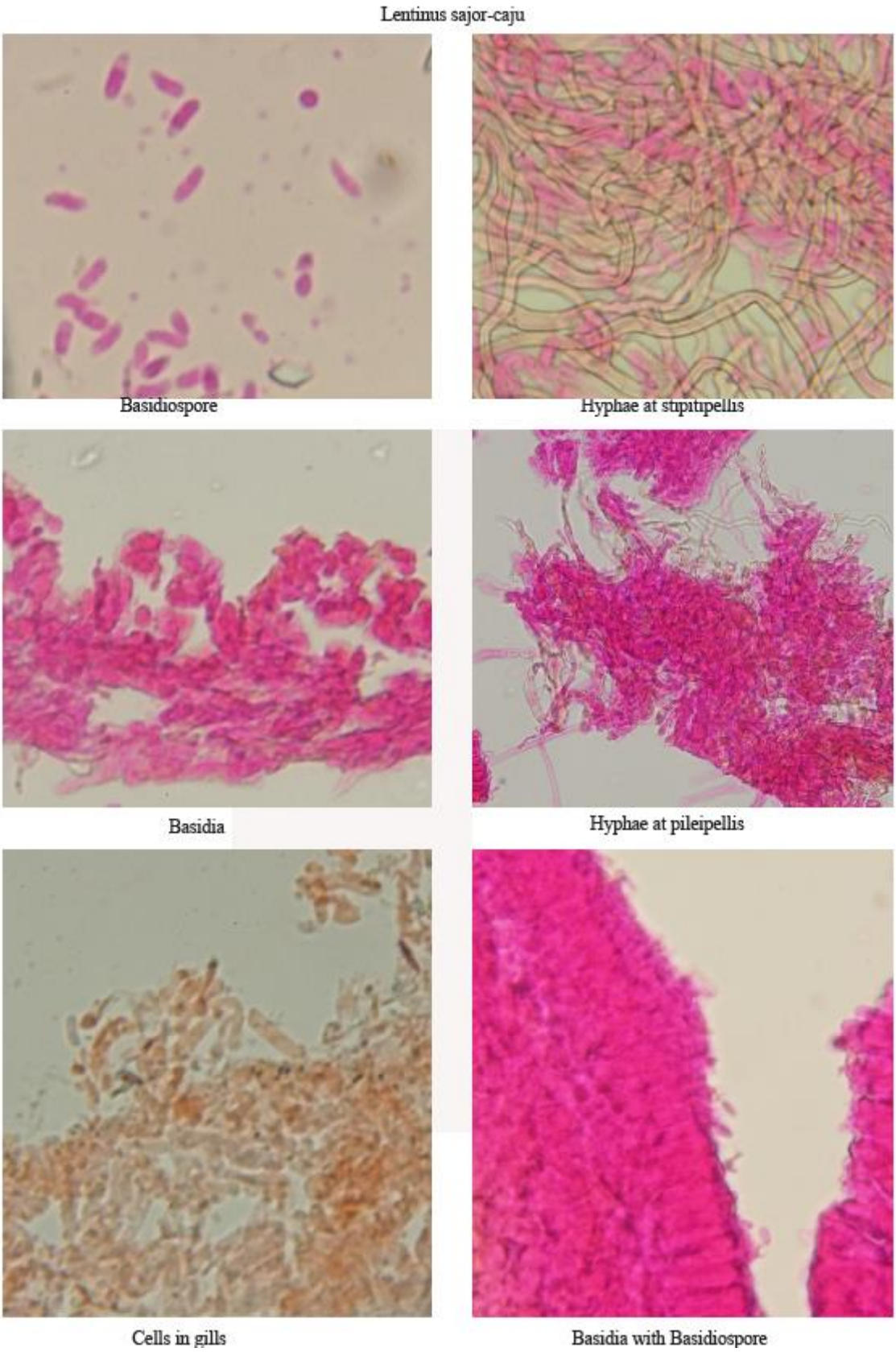


Figure 4.3A: Microscopical characters of *Lentinus sajor-caju.*

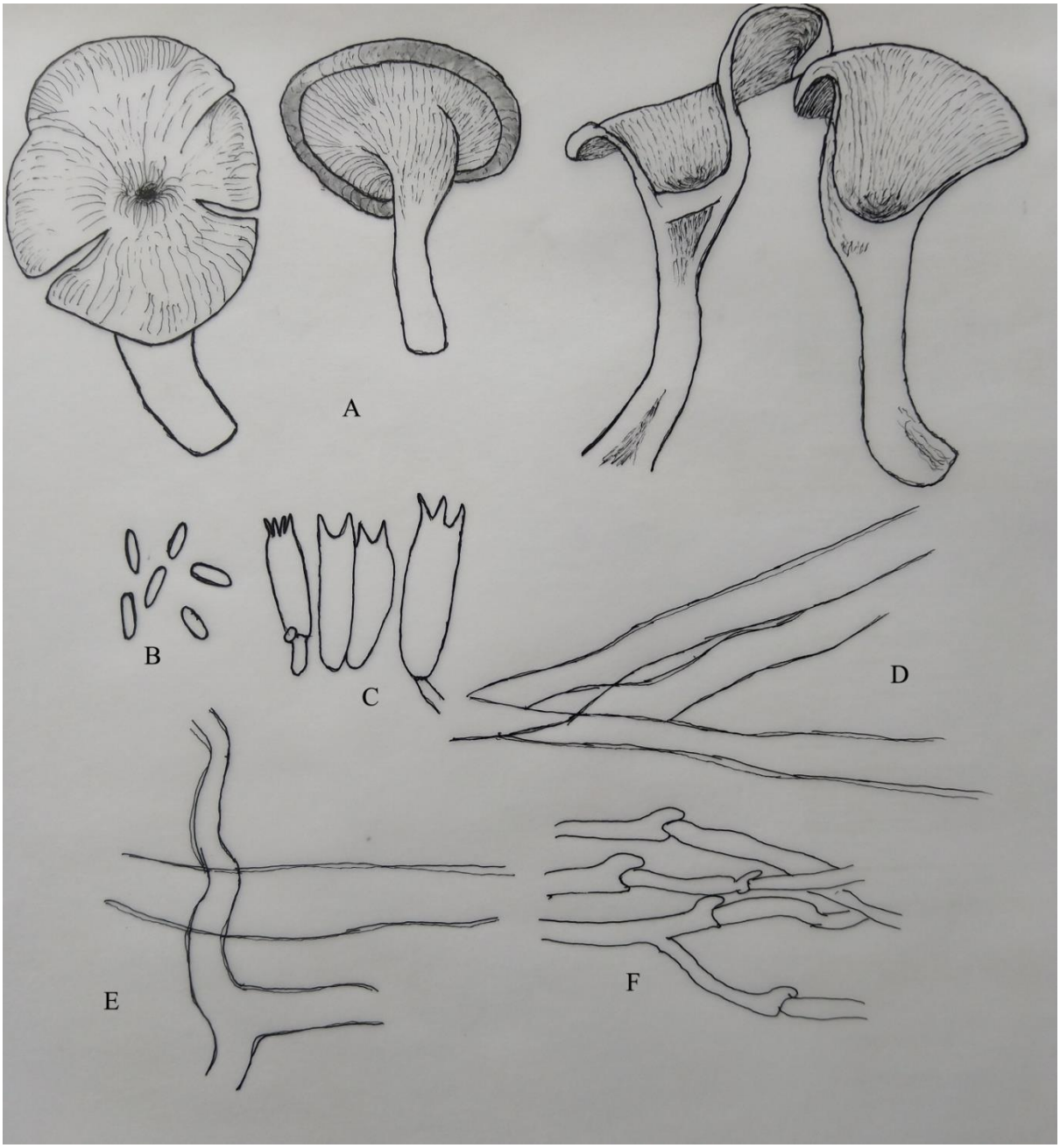


Figure 4.3B; *Lentinus sajor-caju.* A- Fruit body, B- Basidiospore, C- Basidia, D, E &F- Skeletal and generative hyphae.

Figure 4.4A: *Chlorophyllum hortense;* Microscopic characters.

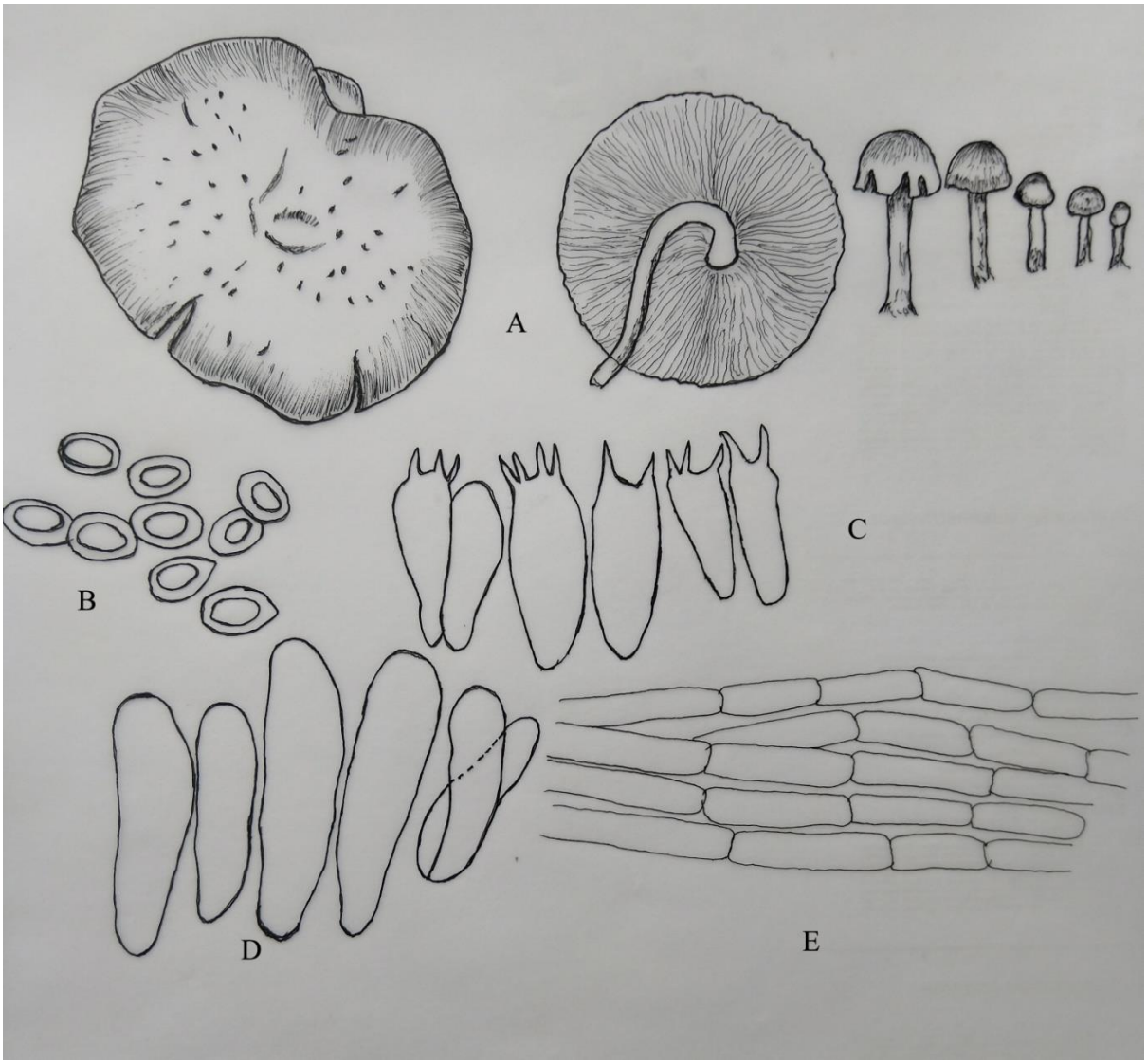


Figure: 4.4B; *Chlorophyllum hortense*. A- Fruiting body, B- Basidiospore, C- Basidia, D- Cheilocystidia, E-Stipitipellis.

Cantharellus subamethysteus

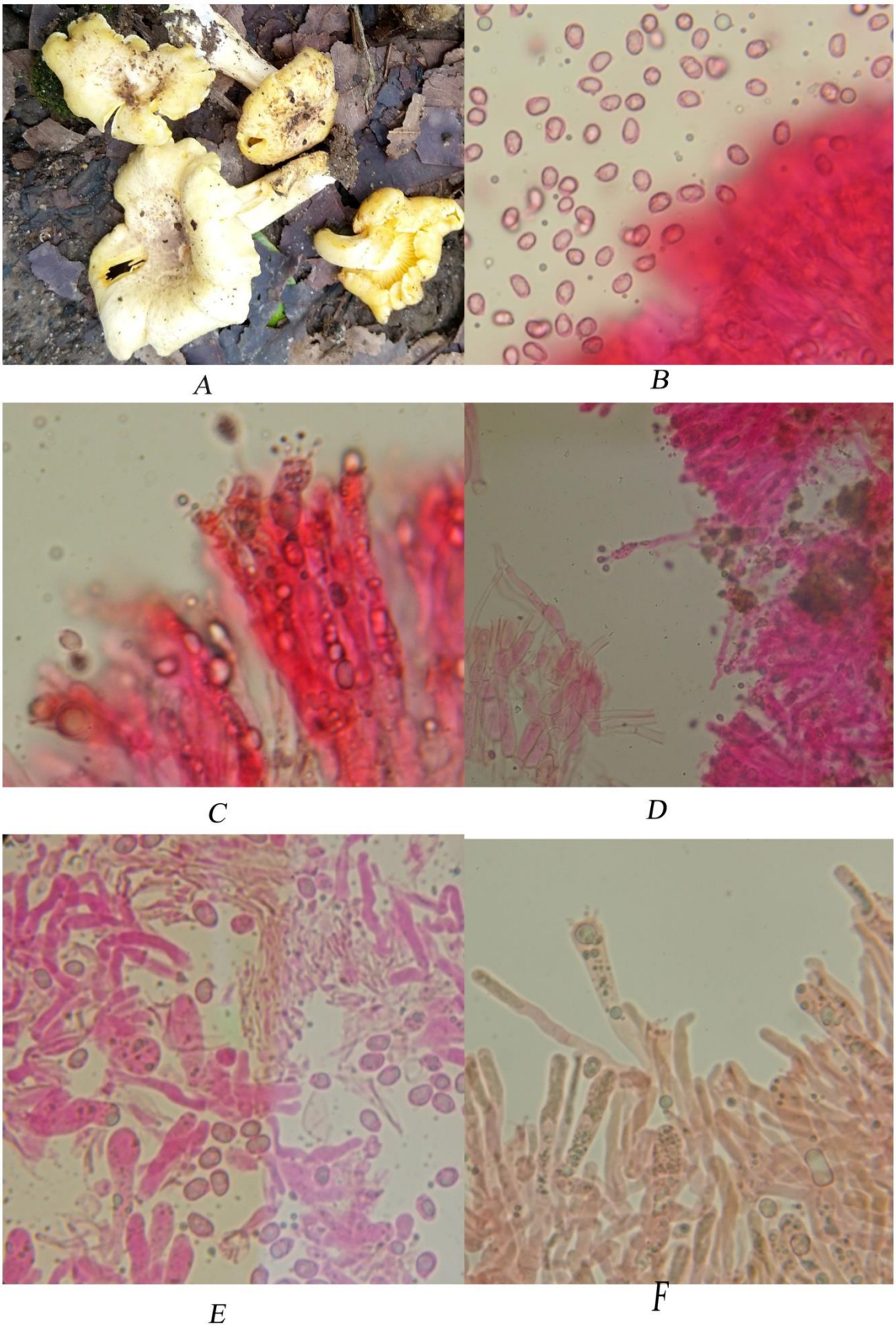


Figure 4.5A: *Cantharellus subamethysteus;* Microscopic characters**.** A. Fruiting body, B. Basidiospore, C. Basidia, D, E & F. Hyphae and cells at Pileipellis.

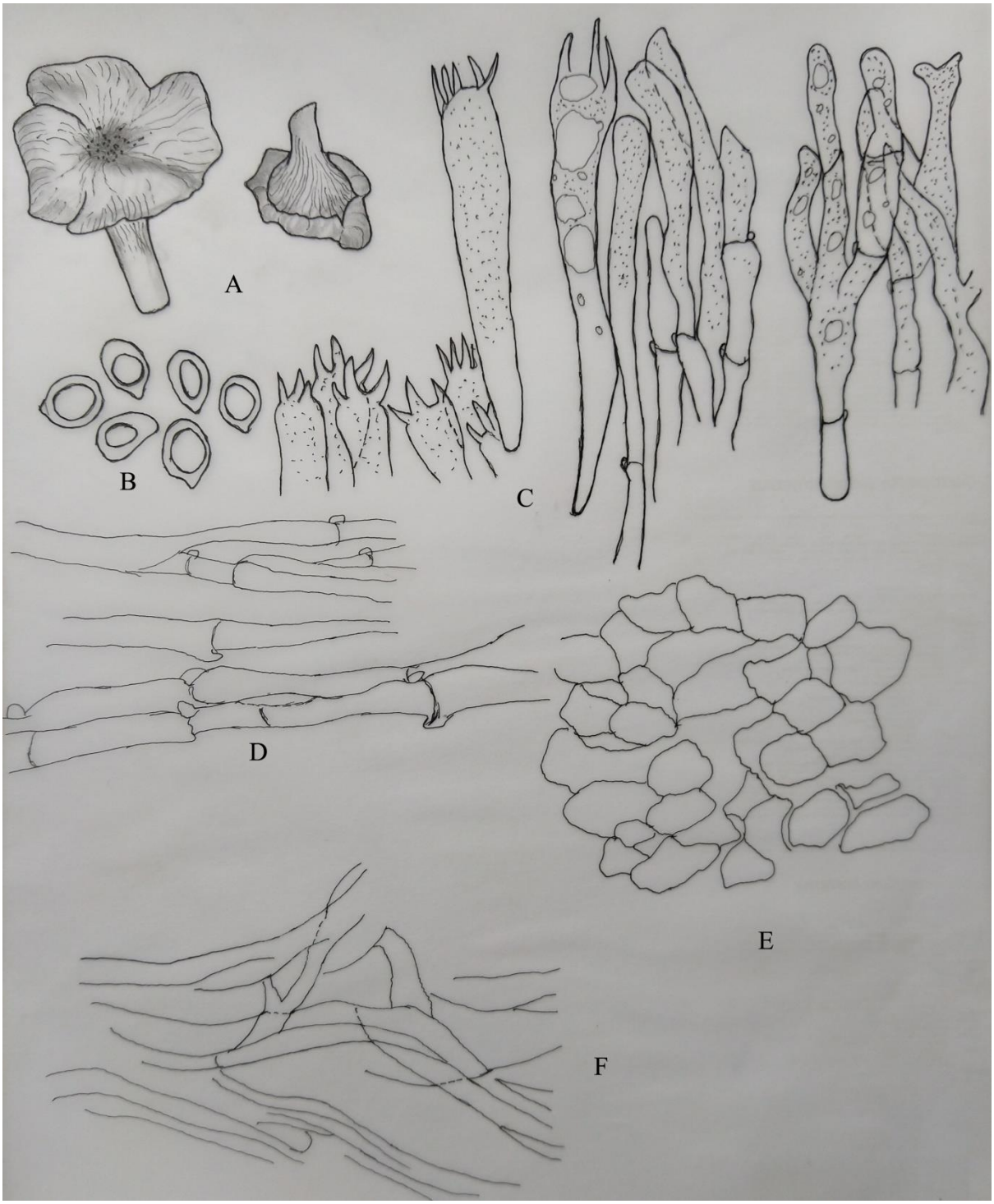


Figure: 4.5 B; *Cantharellus subamethysteus*. A. fruiting body, B. Basidiospore, C. Basidia, D. Stipitipellis, E &F. Gill's trama.

4.2. Molecular studies:

4.2.1. DNA isolation: The DNA was isolated and subjected to electrophoresis for confirmation.

4.2.2. **Electrophoresis**: The isolated DNA was visualized in 0.8 % agarose gel with Gel documentation.

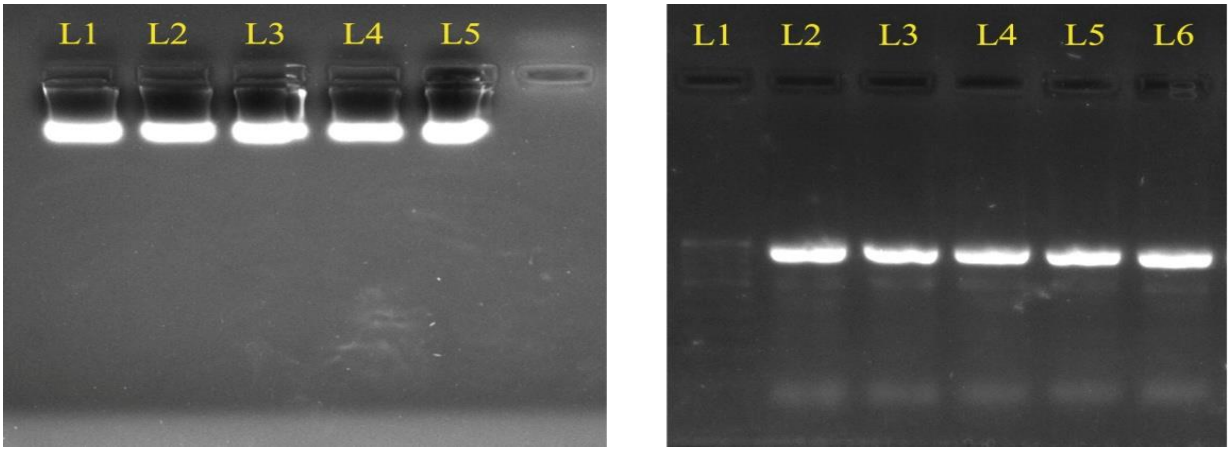


Figure 4.6: A. Genomic DNA L1- *V. volvacea*, L2- *T. heimii*, L3- *L. sajor-caju*, L4- *C. hortense*, L5- *C. subamethysteus*, B. PCR products. L1- marker 0.1 kb, L2- *V. volvacea*, L3- *T. heimii*, L4- *L. sajor-caju*, L5- *C. hortense*, L6- *C. subamethysteus*.

4.2.3. **Quantification**: The isolated DNA was quantified and the results are given in Table 4.1.

Table 4.1: Quantification and purity of Genomic DNA.

Sl no	Sample	Quantity (ng/µL)	Purity (260/280)
1	*Volvariella volvacea*	212	1.81
2	*Termitomyces heimii*	187	1.83
3	*Lentinus sajor-caju*	231	1.94
4	*Chlorophyllum hortense*	259	1.87
5	*Cantharellus subamethysteus*	255	1.97

4.2.4. Sequencing: The amplified PCR products were visualized in 2% agarose gel (Figure 4.6b) and sequenced. Sequences obtained were edited and submitted to GenBank. Description with accession numbers are given in the table 4.2.

Table 4.2: Sequence length, description and accession no of submitted sequences.

Sl no	Sample	Sequence Length	Description	GenBank accession no
1	*Volvariella volvacea*	826 bp	SSU partial, ITS 1, 5.8S, ITS 2 complete, LSU partial	MK681889

2	*Termitomyces heimii*	703 bp	SSU partial, ITS 1, 5.8S, ITS 2 complete, LSU partial	MK724034
3	*Lentinus sajor-caju*	576 bp	ITS 1 partial, 5.8S rRNA, ITS 2 complete, LSU partial	MK660091
4	*Chlorophyllum hortense*	488 bp	5.8s rRNA partial, ITS 2 complete, LSU partial	MK660092
5	*Cantharellus subamethysteus*	906 bp	28S rRNA partial sequence	MK660093

Table: 4. 3: BLAST results.

Sl no	Sample	Query cover	Percent Identical	Closest match with Accession no
1	*Volvariella volvacea*	64%	76.42%	KC142117 (*V. volvacea*)
2	*Termitomyces heimii*	98%	99.14%	GU001667 (*Termitomyces sp.*)
3	*Lentinus sajor-caju*	98%	97.89%	KP012899 (*L. sajor-caju*)
4	*Chlorophyllum hortense*	99%	98.98%	MK554576 (*C. hortense*)
5	*Cantharellus subamethysteus*	90%	92.90%	NG060404 (*C. subamethysteus*)

4.3. Nutritional Analysis:

4.3.1. Proximate analysis: Mushrooms are admired by different groups of people for their nutritional content and flavour. A fresh fruit body of mushroom comprises mainly water ranging from 80-95 %, which is also relevant in current study. The moisture content of five edible mushrooms falls in the range of 88-92 % without much variation. The moisture content was found to be maximum in *Cantharellus subamethysteus* and minimum in *Termitomyces heimii*. Dry matter content of mushrooms ranged from 40-55 mg/g. Mushrooms are preferred as a good diet due to its supplement of rich protein source with low fat, the crude fat content ranged from 1-3.2% on dry weight basis which is comparably a good range for the health conscious people. On the basis of fat content *Chlorophyllum hortense* dominated the list with 3.21% and *Lentinus sajor-caju* had the least fat content among the studied mushrooms. The total soluble sugar was found to be highest in *Lentinus sajor-caju* and least in *Termitomyces heimii*. The protein and total sugar content was higher in *Lentinus sajor-caju* with 52.08 and 46.6% respectively. The protein content was lower in *V. volvacea* with 29.7%, and sugar content was lower in *Termitomyces heimii*. (Table 4.4).

Table 4.4. Proximate analysis.

Samples	Moisture (%)	Ash (mg/g)	Fat (%)	Protein (%)	Sugar (%)
Volvariella volvacea	90.27±0.74	50.8	2.14	29.76	33.2
Termitomyces heimii	88.32±1.06	40.7	1.54	35.37	20.4
Lentinus sajor-caju	89.51±0.6	45.7	1.02	52.08	46.6
Chlorophyllum hortense	90.11±1.21	50.22	3.21	37.34	34.16
Cantharellus subamethysteus	91.72±1.13	53.32	3.18	36.6	22.4

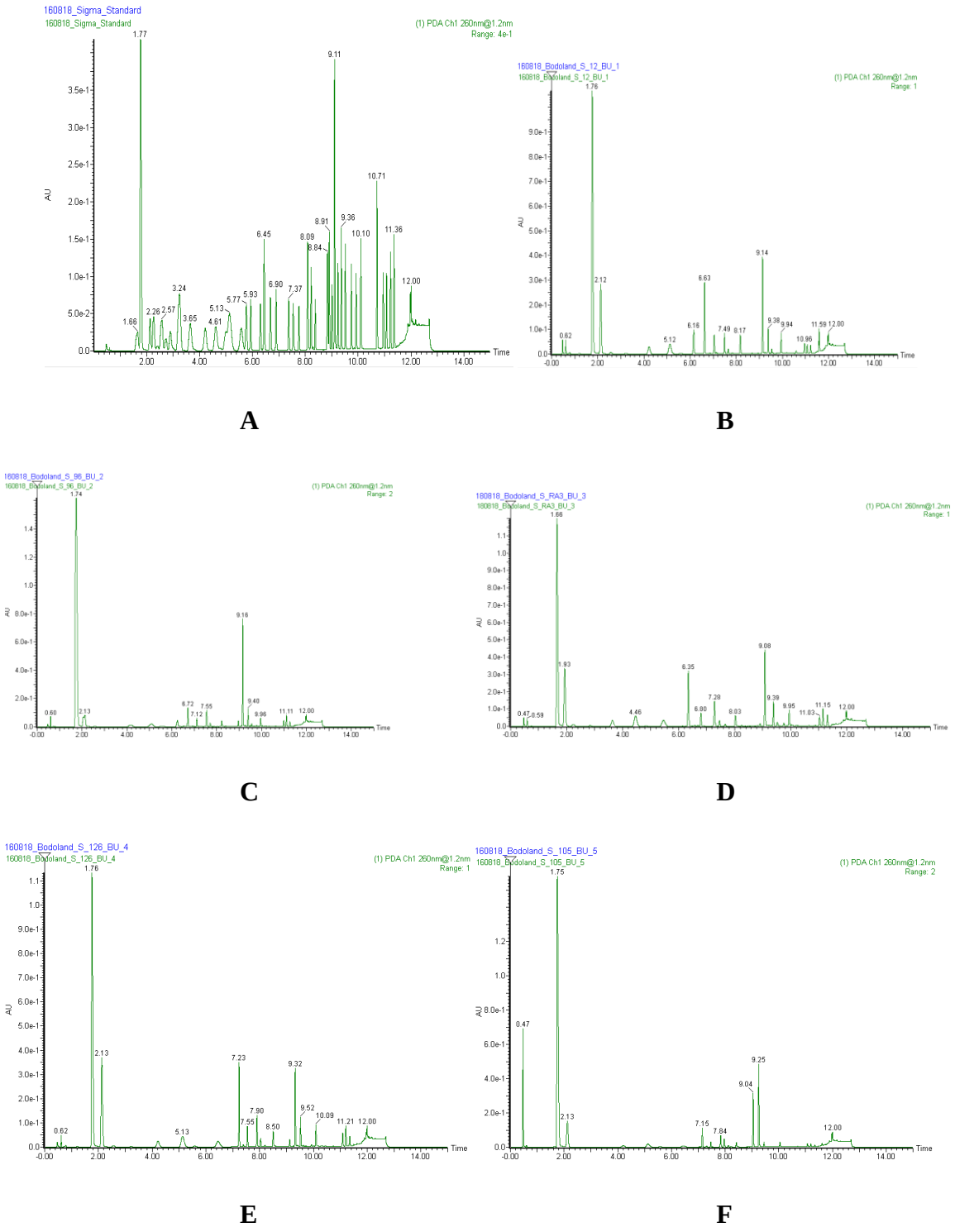
4.3.2. Amino Acid content:

The amino acid content of the wild edible samples were analysed and results are tabulated in Table 4.5.

The studied mushrooms were found to be rich in most of the essential amino acids. Lysine and valine were not detected in *V. volvacea*, Valine was not detected in *Termitomyces heimii*, isoleucine & leucine were not detected in *Chlorophyllum hortense* and *Cantharellus subamethysteus* respectively. Among the semi essential amino acids histidine & arginine were not detected in any of the five samples. Serine and glutamic acid were not detected among the non essential amino acids in any of the samples. Glycine was absent except in *Lentinus sajor-caju* and aspergine was absent except in *Termitomyces heimii.* They were not detected in other samples in the present study.

Cantharellus subamethysteus had the highest content of lysine. Leucine, valine, tryptophan, phenylalanine, tyrosine. Methionine was found to be higher in *Chlorophyllum hortense*. *V. volvacea* had higher content of isoleucine, threonine, alanine and glutamine. Amino acids like cystine and proline was higher in *Termitomyces heimii*. *Lentinus sajor-caju* had higher content of aspartic acid.

The percentages of essential amino acids were higher in *Cantharellus subamethysteus* followed by *V. volvacea*, *Chlorophyllum hortense*, *Termitomyces heimii* and *Lentinus sajor-caju*. The percentage of semi essential and non essential amino acids was higher in *Termitomyces heimii*. The content of non standard amino acid was found to be higher in *Lentinus sajor-caju* followed by *Chlorophyllum hortense*, *Termitomyces heimii*, *V. volvacea* and *Cantharellus subamethysteus*.



A **B**

C **D**

E **F**

Figure: 4.7. Chromatogram for amino acids, A- Standard, B- *Volvariella volvacea*, C- *Termitomyces heimii*, D- *Lentinus sajor-caju*, E- *Chlorophyllum hortense*, F- *Cantharellus subamethysteus*.

Table 4.5: Amino acid content (Standard amino acids).

Sl no	Amino acids	*Volvariella volvacea* µg/g	*Termitomyces heimii* µg/g	*Lentinus sajor-caju* µg/g	*Chlorophyllum hortense* µg/g	*Cantharellus subamethysteus* µg/g
1	Lysine	ND	7068.58	4171.06	4726.32	68376.33
2	Leucine	5448.17	5934	3798.86	7672.5	ND
3	Isoleucine	1133.72	593.74	284.724	ND	513.49
4	Methionine	2317.18	955.96	1371.51	10520.7	475.87
5	Valine	ND	ND	1005.38	1190.69	286.63
6	Tryptophan	6193.84	6190.89	5321.53	7072.04	3098.08
7	Threonine	22624.8	717.01	3509.08	8452.91	2540.96
8	Phenylalanine	6924.46	14111.3	8850.33	14606.2	3738.68
	%	16.40433	9.916993	3.156251	12.251503	40.850701
9	Cystine	11922.3	23530.73	7375.4	885.106	8458.07
10	Tyrosine	11231.3	13698.01	8041.97	38691.7	3455.62
11	Histidine	ND	ND	ND	ND	ND
12	Arginine	ND	ND	ND	ND	ND
	%	8.508084	10.379021	1.718715	8.9392183	6.1581974
13	Alanine	31783.1	16147.65	4722.16	ND	ND
14	Aspergine	ND	907	ND	ND	ND
15	Aspartic acid	1431.3	3366.83	8510.35	1870.47	1343.48
16	Glutamic acid	ND	ND	ND	ND	ND
17	Glutamine	15036.9	5862.35	ND	12201	5004.51
18	Glycine	ND	ND	3460.7	ND	ND
19	Proline	8047.39	48269.02	ND	27997.3	24127
20	Serine	ND	ND	ND	ND	ND
	%	20.68766	20.78463	1.860945	9.5020786	15.752550
	% standard amino acid	45.60008	41.08064	6.735915	30.69280	62.76144

ND- Not detected.

Table 4.6: Amino acid content. (Non standard amino acids).

Sl no	Amino acids	*Volvariella volvacea* µg/g	*Termitomyces heimii* µg/g	*Lentinus sajor-caju* µg/g	*Chlorophyllum hortense* µg/g	*Cantharellus subamethysteus* µg/g
1	Phosphaserine	1642.96	ND	232697	326.61	2220.51
2	Hydroxyl proline	86604.3	20882.59	49160.3	112822	48803.6
3	PEA	2266.48	1157.59	ND	2090.44	361.28
4	1-Methyl-Histidine	600.92	ND	ND	415.641	161.34
5	Ethanolamine	3138.42	2335.35	ND	3624.66	1615.24
6	Sarcosine	15857.5	11961.11	21983.4	ND	ND
7	GABA	9277.07	999.16	ND	ND	9742.84
8	aAAA	19224.9	ND	17625.8	56314	ND
9	Norvaline	9429.44	7239.82	5659.92	10156	ND
10	3-methyl Histidine	ND	1864.61	ND	ND	ND
11	Cystathionine-2	ND	149155.6	491505	ND	ND
12	bAIBA	ND	ND	6420.55	ND	9137.14
13	Ornithine	ND	3214.07	ND	ND	ND
14	aABA	ND	14393.82	ND	121096	ND
15	Cystathionine-1	ND	ND	9688.77	ND	ND
	% of non standard amino acids	54.39	58.91	93.26	69.30	37.23

ND- Not Detected.

Among the standard amino acids *V. volvacea* was dominated by alanine. Proline was highest in *Termitomyces heimii* & phenylalanine was highest in *Lentinus sajor-caju*. *Chlorophyllum hortense* have higher content of tyrosine and amino acid content of *Cantharellus subamethysteus* was dominated by proline.

4.3.3. Fatty Acid content:

The fatty acid analysis of wild mushrooms revealed the presence of many beneficial fatty acids, mushroom samples were dominated by unsaturated fatty acid except

Termitomyces heimii which was higher in saturated fatty acids. Among the saturated fatty acids palmitic acid was found to be dominating except in *Chlorophyllum hortense* which had stearic acid in higher concentration. Mono unsaturated fatty acids like oleic acid and palmitoleic acid were found to be abundant in all the studied samples except in *Cantharellus subamethysteus* where oleic acid was not detected. Linoleic acid was found to be present in higher content among the fatty acid except in *Chlorophyllum hortense* where oleic acid was in higher content.

Volvariella volvacea was found to have considerable amount of different fatty acid. Palmitic acid was plentiful with 19.44% and stearic acid with 9.95%. These two fatty acids contributed maximum with lignoceric acid and toluic acids etc which in total contributed towards saturated fatty acid with 35.7%. *Termitomyces heimii* was found to contain higher percentage of palmitic acid with 39.37% compared to other mushrooms. Stearic acid content was 5.16% and other fatty acids were in much lower percentage. In *Lentinus sajor-caju* the share of palmitic acid was 28.67% with lower stearic acid content compared to other mushrooms at 3.86%. *Chlorophyllum hortense* had much lower content of saturated fatty acid as compared to other studied mushrooms, it have palmitic, stearic and lignoceric acid at 1.66, 7.53 and 3.47% respectively. The sum total of saturated fatty acid along with other minor fatty acid was 23.73%. *Cantharellus subamethysteus* was found to have higher percentage of stearic acid comparably than other studied mushrooms with further contribution of palmitic acid, myristic acid, pentadecanoic acid and margaric acid with sum total of 33.07% of saturated fatty acid.

The highest content of mono unsaturated fatty acid was recorded from *Chlorophyllum hortense* having 45.71% and 12.72% of oleic acid and palmitoleic acid respectively. Other studied mushrooms had mono unsaturated fatty acid at the range of 12- 27%. Among the polyunsaturated fatty acids, linoleic acid contributed the maximum ranging from 33-46% except in *Chlorophyllum hortense* which had low content at 10.16%.

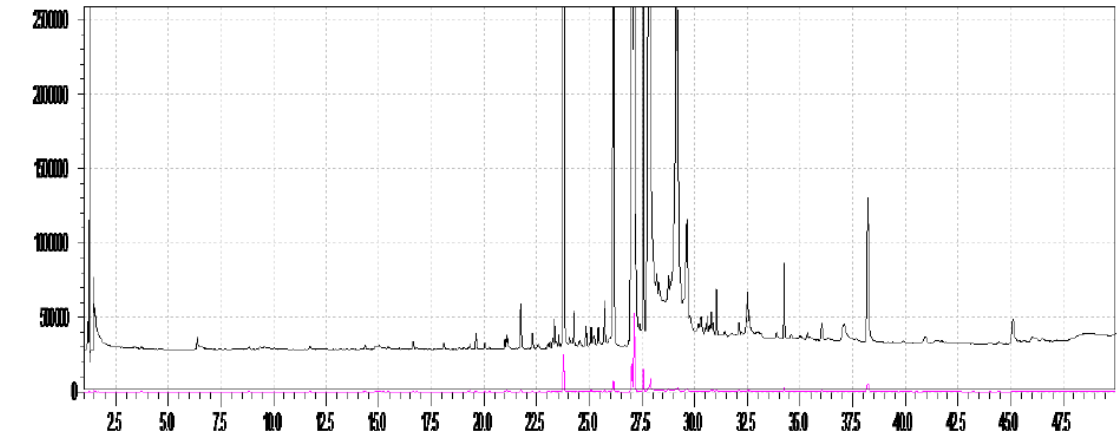


Figure 4.8. *Volvariella volvacea*: Chromatogram for fatty acid and bioactive compounds.

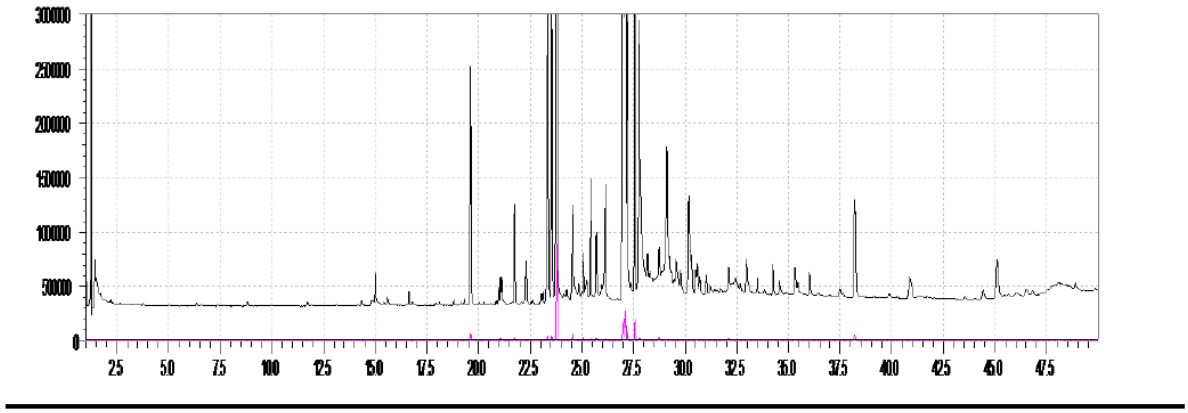


Figure:4.9. *Termitomyces heimii*: Chromatogram for fatty acid and bioactive compounds.

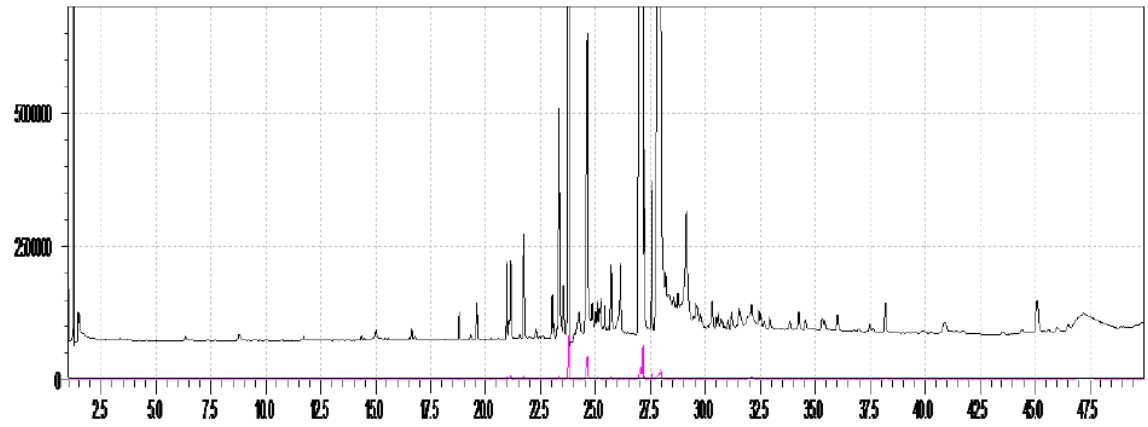


Figure: 4.10. *Lentinus sajor-caju*: Chromatogram for fatty acid and bioactive compounds.

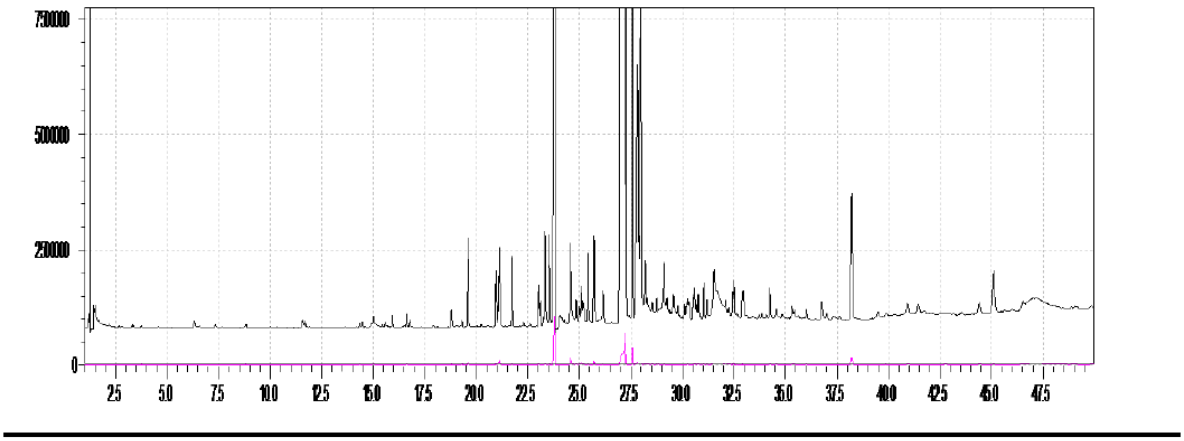


Figure: 4.11. *Chlorophyllum hortense*: Chromatogram for fatty acid and bioactive compounds.

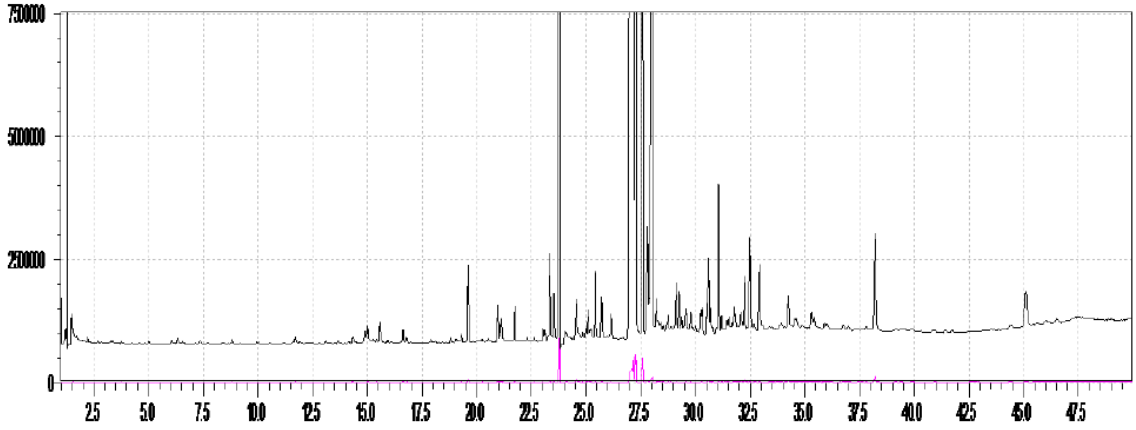


Figure: 4.12. *Cantharellus subamethysteus*: Chromatogram for fatty acid and bioactive compounds.

Table 4.7: Fatty acid profile.

Sl no	Fatty acid	*Volvariella volvacea*	*Termitomyces heimii*	*Lentinus sajor-caju*	*Chlorophyllum hortense*	*Cantharellus subamethysteus*
1	Cyclopropaneoctanoic acid	0.26	ND	ND	1.27	ND
2	Lauric acid	0.17	0.601	0.218	0.31	0.84
3	Myristic acid	0.19	3.9	1.553	2.075	3.74
4	Palmitic acid	19.44	39.376	28.67	1.66	4.89

5	Arachidic acid	0.68	0.178	ND	0.5	ND
6	Heneicosanoic acid	0.23	ND	ND	0.36	ND
7	Caprylic acid	ND	ND	ND	ND	0.218
8	Tricosanoic acid	0.21	0.25	0.213	0.17	ND
9	Lignoceric acid	2.9	ND	0.436	3.47	0.088
10	Hyenic acid	0.16	ND	ND	ND	ND
11	Ethyl iso-allochate	0.05	ND	0.04	0.3	0.82
12	Capric acid	ND	ND	ND	ND	0.33
13	Hydrocinnamic acid	ND	0.053	0.209	ND	ND
14	Azelaic acid	ND	0.058	0.01	0.07	0.42
15	Pentadecenoic acid	ND	1.373	2.032	2.15	1.49
16	Methyl undecyl ether	0.02	ND	ND	ND	ND
17	Triacontanoic acid	0.103	0.076	ND	ND	ND
18	Stearic acid	9.95	5.162	3.86	7.53	18.44
19	Behenic acid	ND	0.266	ND	ND	ND
20	Margaric acid	ND	0.685	0.216	2.39	1.2
21	A-toluic acid	1.29	0.151	0.32	0.581	0.599
22	Tridecanoic acid	ND	ND	ND	0.41	ND
23	Dotriacontane	0.05	ND	ND	ND	ND
24	Cyclopropanebutanoic acid	ND	0.045	0.703	ND	ND
25	Pentacosanoic acid	ND	ND	0.497	0.49	ND
	% of SFA	35.70	52.01	38.97	23.73	33.075
26	9(11)-dehydroergosteryl benzoate	1.17	0.7	0.801	1.86	1.91
27	1-nonadecene	0.07	0.106	0.41	0.622	0.38
28	Oleic acid	8.16	1.725	6.704	45.71	ND
29	palmitoleic acid	4.86	9.755	8.896	12.729	11.78
30	1-heptadecene	0.095	0.09	0.091	0.159	ND
31	9-hexadecanal	12.72	0.05	ND	ND	ND
	Other		0.145	0.202	0.06	0.207

	% of MUFA	27.07	12.571	17.104	61.48	14.277
	Other PUFA	3.46	0.266	3.16	3.15	2.79
32	linoleic acid	32.2	33.718	41.63	10.16	43.73
	% of PUFA	35.66	33.984	44.79	13.75	46.52

ND-Not Detected.

4.4. Mineral content:

The wild edible mushrooms were analysed for six minerals viz: cobalt, manganese, magnesium, nickel, zinc and iron. Mg was found to be maximum among the minerals with *V. volvacea* and *Chlorophyllum hortense* having 93.87 and 93.49 mg respectively. The duo had higher content of Zn than other mushrooms. Co and Mn content was higher in *Termitomyces heimii* with 0.053 and 2.21 mg respectively. *Lentinus sajor-caju* had higher content of Ni with 0.51 mg and *Cantharellus subamethysteus* had higher content of iron with19.65 mg.

Table 4.8: Mineral content.

Sl no	Sample	Co mg/100g	Mn mg/100g	Mg mg/100g	Ni mg/100g	Zn mg/100g	Fe mg/100g
1	*Volvariella volvacea*	0.036±0.003	1.28±0.15	93.87±2.9	0.38±0.07	5.22±0.63	8.08±0.92
2	*Termitomyces heimii*	0.053±0.011	2.21±0.43	67.06±2.4	0.44±0.05	2.97±0.24	7.60±1.3
3	*Lentinus sajor-caju*	0.006±0.002	1.00±0.12	79.57±1.9	0.51±0.03	4.95±0.81	10.81±1.1
4	*Chlorophyllum hortense*	0.032±0.006	0.89±0.13	93.49±2.7	0.49±0.09	5.04±1.03	4.28±0.9
5	*Cantharellus subamethysteus*	0.022±0.004	1.66±0.26	67.84±2.3	0.40±0.06	0.30±0.09	19.65±1.64

4.5. Antioxidant properties:

4.5.1. Phenolic content:

The phenolic content of five wild edible mushrooms were studied with aqueous, ethanolic and methanolic extracts with reference to standard caliberation curve prepared using gallic acid. The mushroom species were found to have good phenolic content ranging

from 12.47 mg/g in methanolic extract of *Termitomyces heimii* to 59.13 mg/g (Fig 9) in aqueous extract of *Lentinus sajor-caju*. The study revealed the phenolic content was better in aqueous extract than in alcoholic extracts. The highest phenolic content was present in *Lentinus sajor-caju* in all the three extracts and *Termitomyces heimii* had the lowest content when compared in all the three extracts.

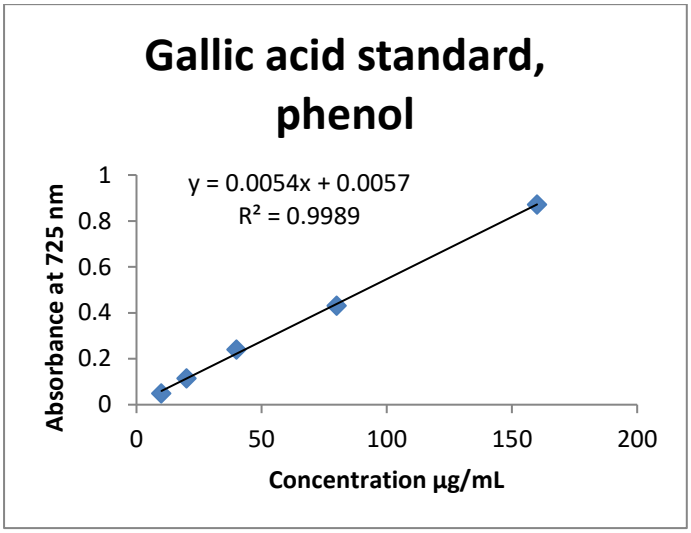


Figure 4.13. Phenol standard.

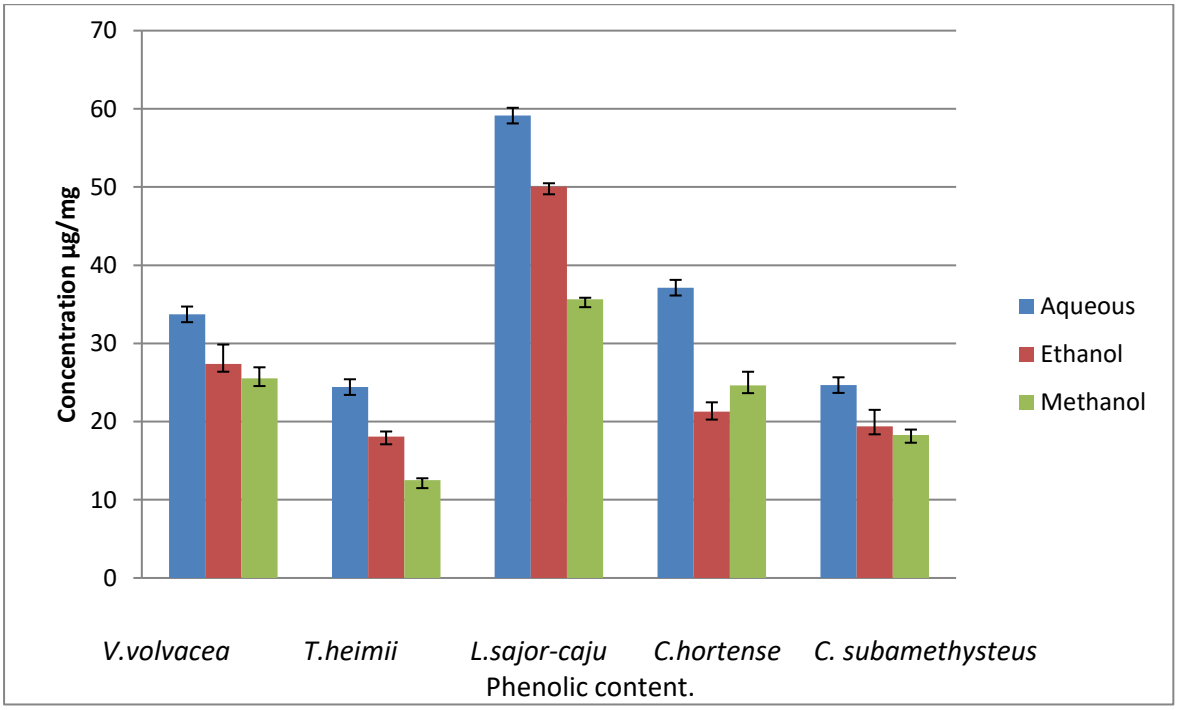


Figure 4.14: Phenolic content of three extraxts of *V. volvacea, T. heimii, L. sajor-caju, C. hortense* and *C. subamethysteus.* The results presented are mean of 3 readings ± standard deviation.

4.5.2. Flavonoid content:

Unlike phenolic content which showed higher content in aqueous extract of all the mushrooms, the flavonoid content was higher in ethanolic extracts in three mushrooms (Fig 11). The flavonoid content was also found to be higher than the rest of the mushrooms considerably in *Lentinus sajor-caju* with 44.87 mg/g of extract followed by *Chlorophyllum hortense,V. volvacea, Termitomyces heimii*. *Cantharellus subamethysteus* had the lowest content in the methanolic extracts with 6.56 mg/g extract calculated according to caliberation curve of quercetin (Fig 10).

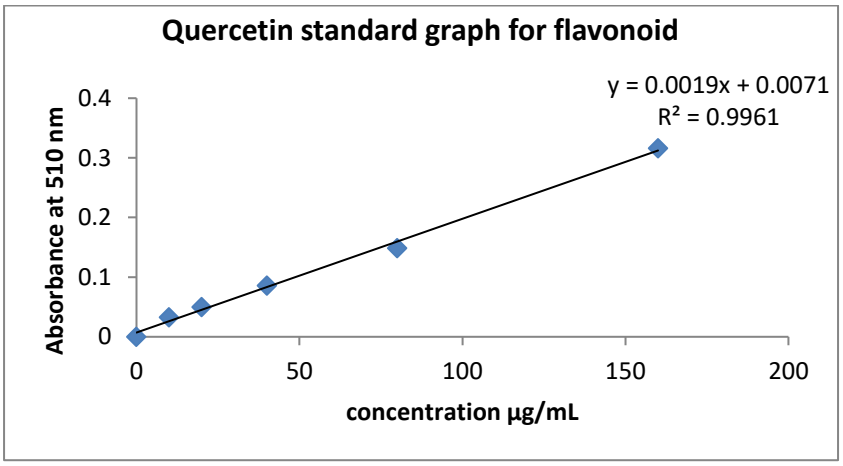


Figure 4.15: Flavonoid standard.

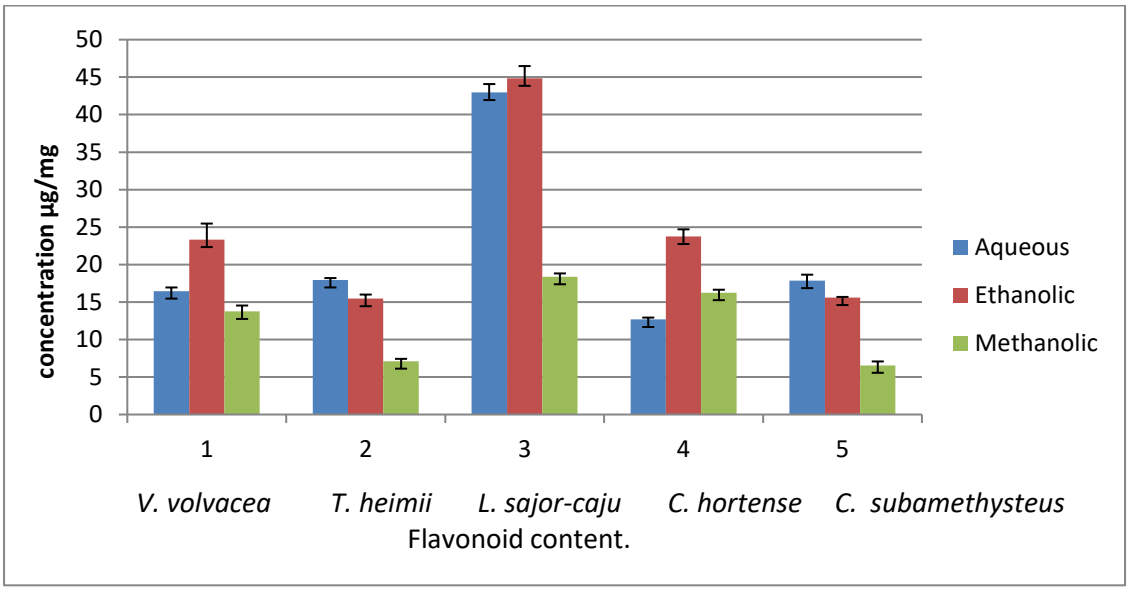


Figure: 4.16: Flavonoid content of aqueous, ethanolic and methanolic extracts of *V. volvacea, T. heimii, L. sajor-caju, C. hortense, C. subamethysteus.*The results are mean of 3 readings ± standard deviation.

4.5.3. Ferric Reducing Antioxidant Assay:

The Ferric Reducing Antioxidant Potential of wild mushroom extracts were analysed and it was found to be better in aqueous extracts. Among the five edible mushrooms *Lentinus sajor-caju* showed better result compared to others with FRAP value of 1.699 and *Cantharellus subamethysteus* have been least effective in reducing ferric ions with FRAP value of 0.77. When the results were expressed as µmol Fe^{2+} equivalent, 1 mg of *Lentinus sajor-caju* aqueous extract was found to be equivalent to 100 µmol of Fe^{2+} against the standard curve prepared using ferrous sulphate.

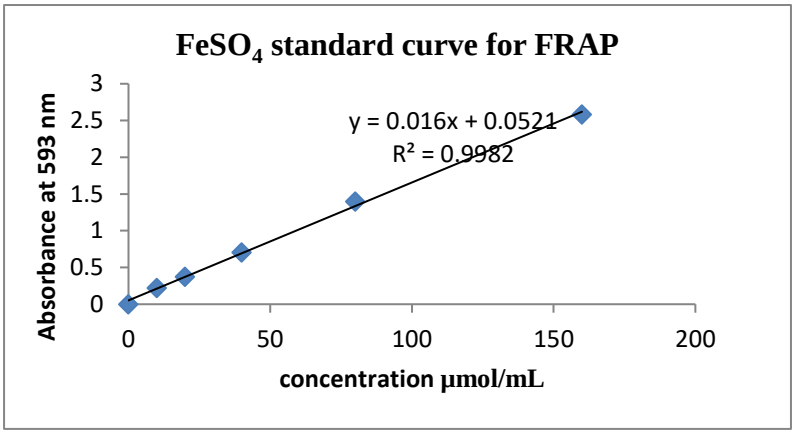


Figure 4.17: FRAP standard $FeSO_4$ µmol/mL concentration, Absorbance at 593 nm.

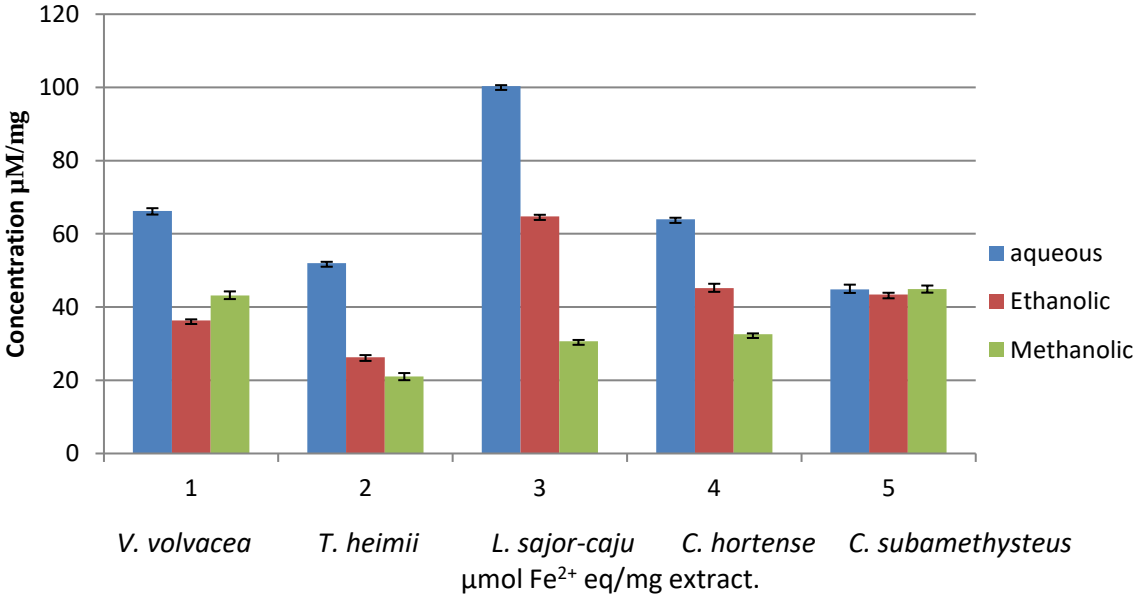


Figure: 4.18. FRAP, µmol Fe^{2+} eq/mg extract. Aqueous, ethanolic and methanolic extracts of *V. volvacea*, *T. heimii*, *L. sajor-caju*, *C. hortense* and *C. subamethysteus.* The results are mean of 3 readings ± standard deviation.

4.5.4. ABTS radical scavenging activity:

The ABTS free radical scavenging activity were studied from the methanolic extracts of the collected wild mushroom samples and found that *L. sajor-caju* had the highest potential to scavenge ABTS free radicals with IC_{50} value of 0.08 µg (y=100.3x+58.50, R^2 of 0.800) followed by *V. volvacea, C. hortense, T. heimii and C. subamethysteus* with IC_{50} values of 0.226, 0.228, 0.283 and 0.416 respectively as given in table 9.

4.5.5. DPPH radical scavenging activity:

The radical scavenging activity of different extracts of wild edible mushrooms was studied and the result had been favourable to infer that mushrooms have good radical scavenging activity. The DPPH radical scavenging activity was better in aqueous extract of mushrooms and the best among the studied sample was found in *L. sajor-caju* with ability to reduce 50 % of 100 µM DPPH at the concentration of 0.79 µg extract followed by *V. volvacea, C. subamethysteus, T. heimii* and *C. hortense*. The methanolic extract showed better result than ethanolic when compared. The *L. sajor-caju* was also better in reducing ABTS free radicals than the other studied mushrooms with IC_{50} value of 0.08 µg and *C. subamethysteus* had least activity in reducing free radicals.

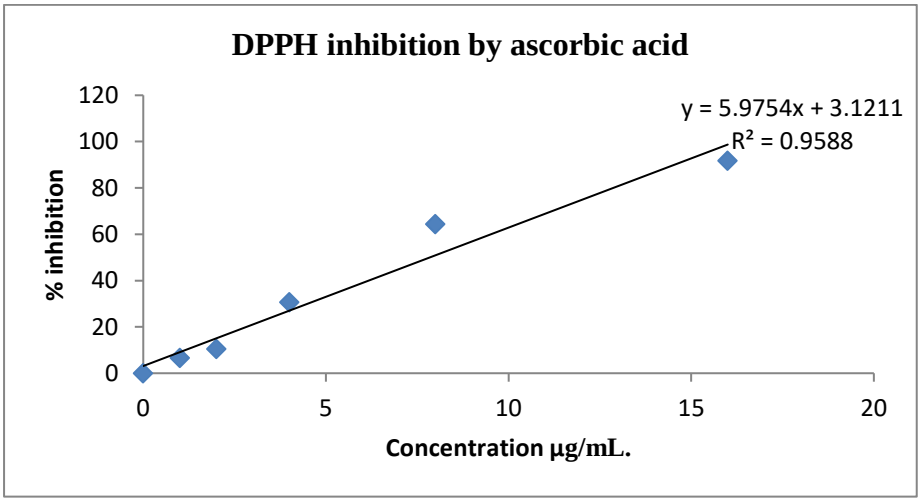


Figure 4.19: DPPH standard calibration curve.

4.5.6. Super oxide scavenging assay:

The super oxide radical scavenging activity was studied with the five wild edible mushrooms and found to have variable range of activity. *Volvariella volvacea* have good superoxide scavenging activity with IC_{50} value of 182 µg followed by *Chlorophyllum*

hortense, *Lentinus sajor-caju*, *Cantharellus subamethysteus* and *Termitomyces heimii* with IC_{50} values of 185 µg, 206 µg, 330 µg and 435 µg respectively. (Table 9).

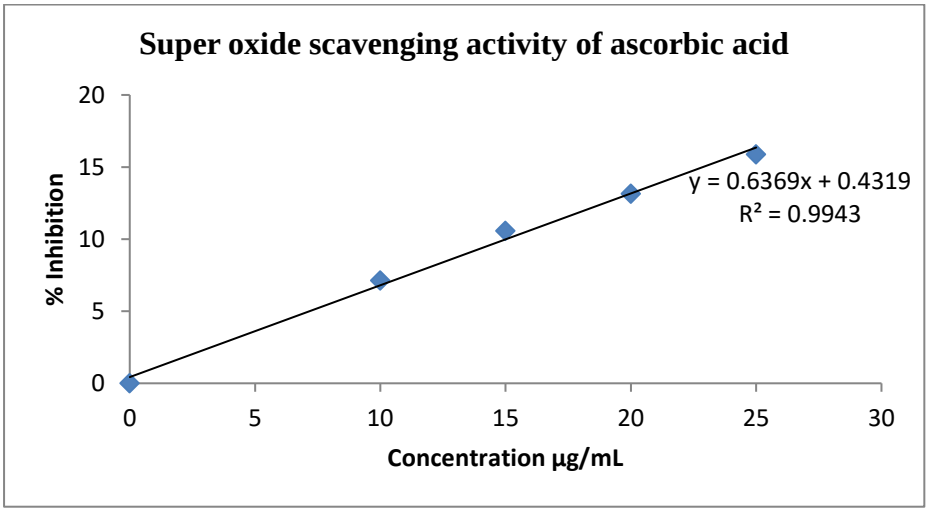


Figure 4.20: Calibration curve for Superoxide scavenging activity of ascorbic acid.

4.5.7. Nitric oxide scavenging assay:

The collected wild mushrooms was analysed for nitric oxide scavenging activity. The free radicals generated were incubated with extracts of wild mushrooms to study their activity and found to have scavenging activity at different concentrations. The graph was prepared against the scavenging activity and the regression equation was followed to calculate the IC_{50} value. The scavenging activity was found to be directly proportional to its concentration and the IC_{50} value was best in *V. volvacea* with 272 µg followed by *L. sajor-caju* with IC_{50} of 285 µg, *C. subamethysteus* with IC_{50} of 301 µg, *C. hortense* with IC_{50} of 315 µg and *T. heimii* with IC_{50} value of 569 µg. (Table 9)

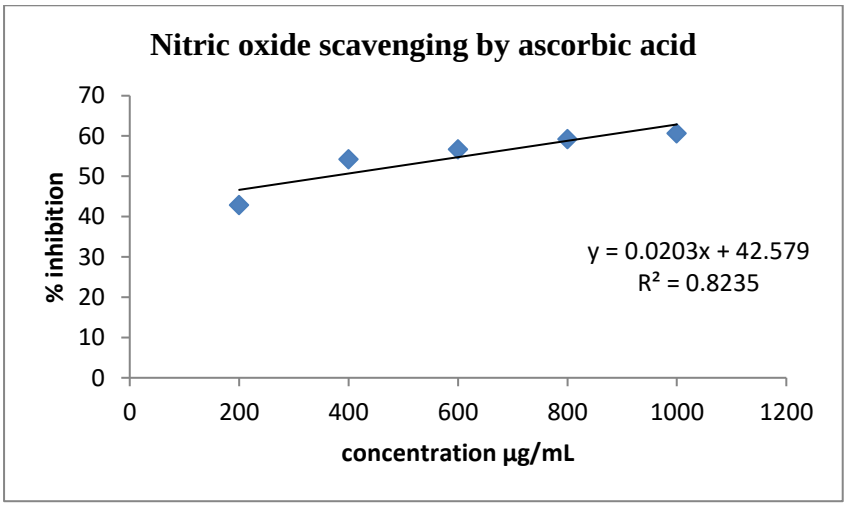


Figure 4.21: Calibration curve for nitric oxide scavenging activity of ascorbic acid.

Table 4.9: Radical scavenging activity of *V. volvacea, T. heimii, L. sajor-caju, C. hortense* and *C. subamethysteus.*

Sample	DPPH radical scavenging activity IC_{50} in µg			ABTS radical scavenging activity IC_{50} in µg	Superoxide radical scavenging activity IC_{50} in µg	Nitric oxide scavenging activity IC_{50} in µg
	Aqueous	Ethanolic	Methanolic			
Volvariella volvacea	1.37±0.21	2.03±0.21	1.51±0.16	0.226±0.02	182± 12	272± 13
Termitomyces heimii	1.55±0.13	3.05±0.37	2.36±0.21	0.283±0.03	435± 23	569± 29
Lentinus sajor-caju	0.79±0.11	2.24±0.29	1.78±0.19	0.08±0.01	206±21	285± 13
Chlorophyllum hortense	2.15±0.22	2.96±0.17	1.59±0.14	0.22±0.03	185 ±19	315±17
Cantharellus subamethysteus	1.57±0.12	4.62± 0.39	2.69±0.23	0.41±0.04	330 ±22	301± 21

4.6. Antimicrobial Assay:

4.6.1. *Volvariella volvacea:*

The extracts of the wild mushrooms were tested against five different microbes. Aqueous and petroleum ether extracts of *V. volvacea* were not effective against *E. coli*, but the alcoholic extracts showed inhibition. Methanolic extracts showed better capability to inhibit the growth of *E. coli* with inhibition zone of 12.33 mm at 100 µg concentration. Other microbes were inhibited to various extents by different extracts. Methanolic extract was more efficient against *Bacillus cereus* with zone of inhibition of 10.74 mm, *Proteus vulgaris* was better inhibited by ethanolic extract with 12.29 mm inhibition zone. Aqueous extract had greater ability to inhibit *Klebsiella pneumonae* with 12.70 mm. Petroleum ether extract had higher inhibition zone for *Staphylococcus aureus* with 12.44 mm.

Table: 4.10: CFU and Zone of Inhibition with standard antibiotics.

Microbial culture	**CFU/mL**	**Antibiotic**	**Zone of inhibition**
Escherichia coli (MTCC 40)	6.58×10^3	Penicillin (10 µg)	9.32 mm
Bacillus cereus (MTCC 430)	4.09×10^3	Oxytetracyclin (30 µg)	23.78 mm
Proteus vulgaris (MTCC 7299)	2.04×10^3	Ciprofloxcin (30 µg)	36.90 mm
Klebsiella pneumonae (MTCC 9751)	3.28×10^3	Ampicillin (10 µg)	8.46 mm
Staphylococcus aureus (MTCC 7443)	6.95×10^3	Penicillin (10µg)	14.74 mm

Table 4.11: Antimicrobial activity of *Volvariella volvacea.*

Microbial culture	Aqueous	Ethanolic	Methanolic	Petrolium Ether
Escherichia coli (MTCC 40)	NI	MIC 10 µg 100 µg 7.45 mm±0.3	MIC 10 µg 100 µg 12.33 mm±0.92	NI
Bacillus cereus (MTCC 430)	MIC 40 µg 100 µg 8.75 mm±0.29	MIC 10 µg 100 µg 8.75mm±0.7	MIC 10 µg 100 µg 10.74 mm±0.65	MIC 10 µg 100 µg 8.80 mm±0.58
Proteus vulgaris (MTCC 7299)	MIC 40 µg 100 µg 9.66 mm±0.75	MIC 10 µg 100 µg 12.29 mm±0.84	MIC 10 µg 100 µg 10.57 mm±0.36	MIC 40 µg 100 µg 11.37 mm±0.51
Klebsiella pneumonae (MTCC 9751)	MIC 40 µg 100 µg 12.70 mm±1.12	MIC 20 µg 100 µg 10.22 mm±0.78	MIC 20 µg 100 µg 9.73 mm±0.54	MIC 10 µg 100 µg 11.82 mm±0.96
Staphylococcus aureus (MTCC 7443)	MIC 20 µg 100 µg 10.43 mm±0.58	MIC 10 µg 100 µg 10.43 mm±0.63	MIC 10 µg 100 µg 10.43 mm±0.54	MIC 20 µg 100 µg 12.44 mm±0.93

Table 4.12: Antimicrobial activity of *Termitomyces heimii.*				
Microbial culture	Aqueous	Ethanolic	Methanolic	Petrolium Ether
Escherichia coli (MTCC 40)	NI	MIC 10 µg 100 µg 16.15mm±2.1	MIC 10 µg 100 µg 11.37mm±0.6	NI
Bacillus cereus (MTCC 430)	MIC 40 µg 100 µg 7.54 mm±0.4	MIC 10 µg 100 µg 8.02mm±0.5	MIC 10 µg 100 µg 9.32mm±0.7	MIC 10 µg 100 µg 7.83mm±0.4
Proteus vulgaris (MTCC 7299)	MIC 40 µg 100 µg 7.84 mm±0.3	MIC 10 µg 100 µg 9.75 mm±0.6	MIC 10 µg 100 µg 11.02mm±0.8	MIC 40 µg 100 µg 8.42mm±0.6
Klebsiella pneumonae (MTCC 9751)	MIC 40 µg 100 µg 6.56 mm±0.3	MIC 20 µg 100 µg 7.99mm±0.9	MIC 10 µg 100 µg 7.97 mm±0.4	MIC 10 µg 100 µg 7.78mm±0.7
Staphylococcus aureus (MTCC 7443)	MIC 20 µg 100 µg 7.25mm±0.3	MIC 10 µg 100 µg 7.99mm±0.4	NI	MIC 20 µg 100 µg 6.94mm±0.2

Table 4.13: Antimicrobial activity of *Lentinus sajor-caju.*				
Microbial culture	Aqueous	Ethanolic	Methanolic	Petroleum Ether
Escherichia coli (MTCC 40)	MIC 100 µg 9.03mm±0.6	MIC 10 µg 100 µg 12.9mm±0.9	MIC 40 µg 100 µg 11.0mm±0.8	MIC 10 µg 100 µg 12mm±0.7
Bacillus cereus (MTCC 430)	MIC 40 µg 100 µg 8.4mm±0.4	MIC 20 µg 100 µg 12.2mm±0.6	MIC 20 µg 100 µg 10.13mm±0.9	MIC 10 µg 100 µg 13.3mm±1.2
Proteus vulgaris (MTCC 7299)	MIC 20 µg 100 µg 11.2mm±0.5	MIC 40 µg 100 µg 10.4mm±0.4	MIC 10 µg 100 µg 13.3mm±0.7	MIC 10 µg 100 µg 13.04mm±0.7

Klebsiella pneumonae (MTCC 9751)	NI	MIC 20 µg 100 µg 14.04mm±1.1	MIC 10 µg 100 µg 12.3mm±0.4	MIC 40 µg 100 µg 10mm±0.6
Staphylococcus aureus (MTCC 7443)	NI	MIC 20 µg 100 µg 11.0mm±0.7	MIC 40 µg 100 µg 9.2mm±0.5	MIC 40 µg 100 µg 11.5mm±0.6

Table 4.14: Antimicrobial activity of *Chlorophyllum hortense.*

Microbial culture	Aqueous	Ethanolic	Methanolic	Petroleum Ether
Escherichia coli (MTCC 40)	NI	MIC 40 µg 100 µg 14.01±1.0mm	MIC 100 µg 100 µg 11.2mm±0.8	MIC 20 µg 100 µg 13.1±0.6
Bacillus cereus (MTCC 430)	MIC 100 µg 100 µg 8.02mm±0.2	MIC 10 µg 100 µg 12.12mm±0.6	MIC 40 µg 100 µg 9.1mm±0.4	MIC 20 µg 100 µg 12.10mm±0.4
Proteus vulgaris (MTCC 7299)	NI	MIC 40 µg 100 µg 10.5mm±0.3	MIC 100 µg 100 µg 10.2mm±0.5	MIC 20 µg 100 µg 13.06mm±0.7
Klebsiella pneumonae (MTCC 9751)	MIC 100 µg 100 µg 9.4mm±0.4	MIC 100 µg 100 µg 9.54mm±0.3	MIC 100 µg 100 µg 8.01mm±0.3	MIC 20 µg 100 µg 9.1mm±0.4
Staphylococcus aureus (MTCC 7443)	MIC 20 µg 100 µg 9.4mm±0.4	MIC 40 µg 100 µg 10.99mm±0.7	MIC 100 µg 100 µg 10.7mm±0.5	MIC 10 µg 100 µg 12.99mm±0.8

Table 4.15: Antimicrobial activity of *Cantharellus subamethysteus.*

Microbial culture	Aqueous	Ethanolic	Methanolic	Petroleum Ether

Escherichia coli (MTCC 40)	NI	NI	MIC 20 µg 100 µg 9.16mm±0.3	NI
Bacillus cereus (MTCC 430)	MIC 10 µg 100 µg 9.55mm±0.4	MIC 40 µg 100 µg 11.09mm±0.6	MIC 20 µg 100 µg 11.32mm±0.3	MIC 10 µg 100 µg 12.35mm±0.4
Proteus vulgaris (MTCC 7299)	MIC 20 µg 100 µg 10.37mm±0.6	MIC 10 µg 100 µg 10.99mm±0.9	MIC 10 µg 100 µg 10.31mm±0.4	MIC 10 µg 100 µg 10.38mm±0.6
Klebsiella pneumonae (MTCC 9751)	MIC 40 µg 100 µg 12.11mm±0.7	MIC 10 µg 100 µg 9.50mm±0.3	MIC 10 µg 100 µg 13.79mm±0.9	MIC 10 µg 100 µg 13.14mm±0.9
Staphylococcus aureus (MTCC 7443)	MIC 20 µg 100 µg 10.49±0.6	MIC 10 µg 100 µg 11.56mm±0.5	MIC 40 µg 100 µg 12.63mm±0.3	MIC 10 µg 100 µg 9.44mm±0.6

4.6.2. *Termitomyces heimii:*

Termitomyces heimii ethanolic extract was better against *E. coli* compared to methanolic extract with zone of inhibition of 16.15 mm. Petroleum ether extract and aqueous extract were ineffective. *Bacillus cereus* and *Proteus vulgaris* was inhibited to greater extent by methanolic extracts with zone of inhibition of 9.32 mm and 11.02 mm respectively. Ethanolic extract was better against *Klebsiella pneumonae* and *Staphylococcus aureus* with inhibition zone of 7.99 mm each (Table 12).

4.6.3. *Lentinus sajor-caju*:

In *Lentinus sajor-caju* ethanolic, methanolic and petroleum ether extracts were capable of inhibiting all the tested microorganisms except the inability of aqueous extract to stop the growth of *Klebsiella pneumonae* and *Staphylococcus aureus*. *Escherichia coli* was inhibited best by ethanolic extract to the extent of 12.9 mm while petroleum ether extracts was effective against *Bacillus cereus*, *Proteus vulgaris* and *Staphylococcus aureus* with inhibition zone of 13.3 mm, 13.04 mm and 11.5 mm respectively. Ethanolic extract was more effective against *Klebsiella pneumonae* (Table 13).

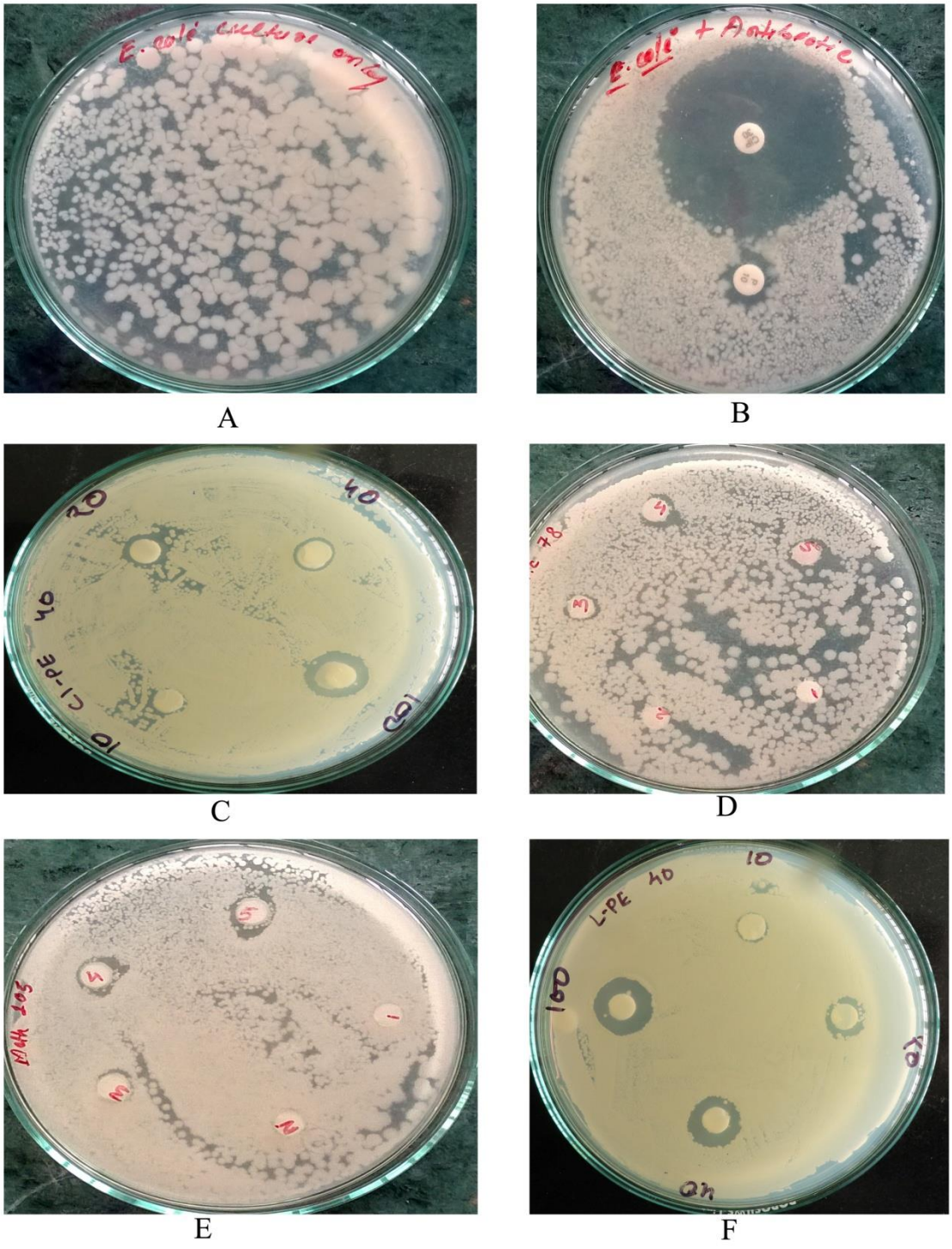


Figure: 4.22: A. *E. coli* culture, B. Inhibition by standard antibiotics, C. Inhibition by *C. hortense*, D. Inhibition by *T. heimii*, E. Inhibition by *C. subamethysteus*, F. Inhibition by *L. sajor-caju*.

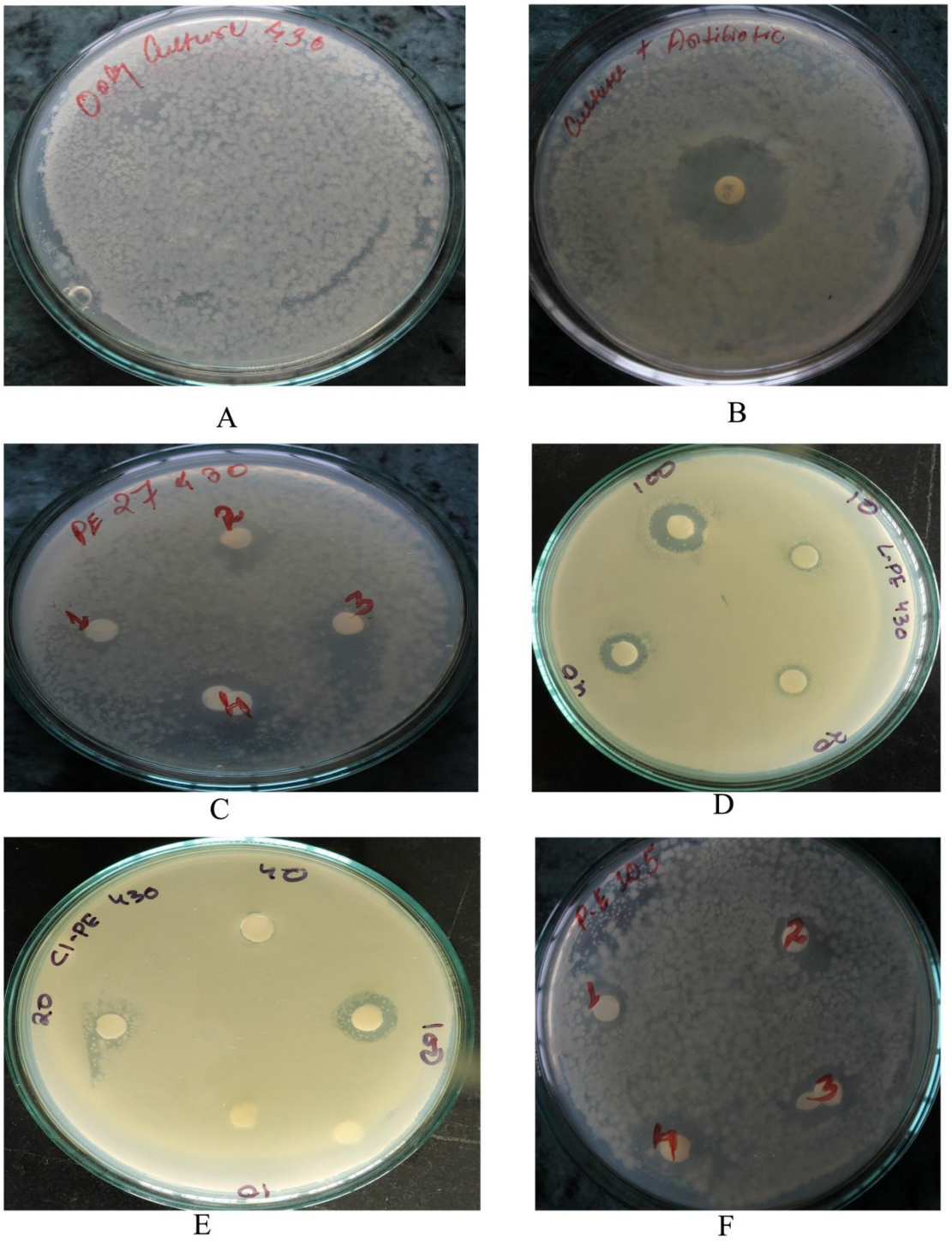


Figure 4.23; A. *B. cereus* culture, B. Inhibition by standard antibiotics, C. Inhibition by *V. volvacea*, D. Inhibition by *L. sajor-caju*, E. Inhibition by *C. hortense*, F. Inhibition by *C. subamethysteus*.

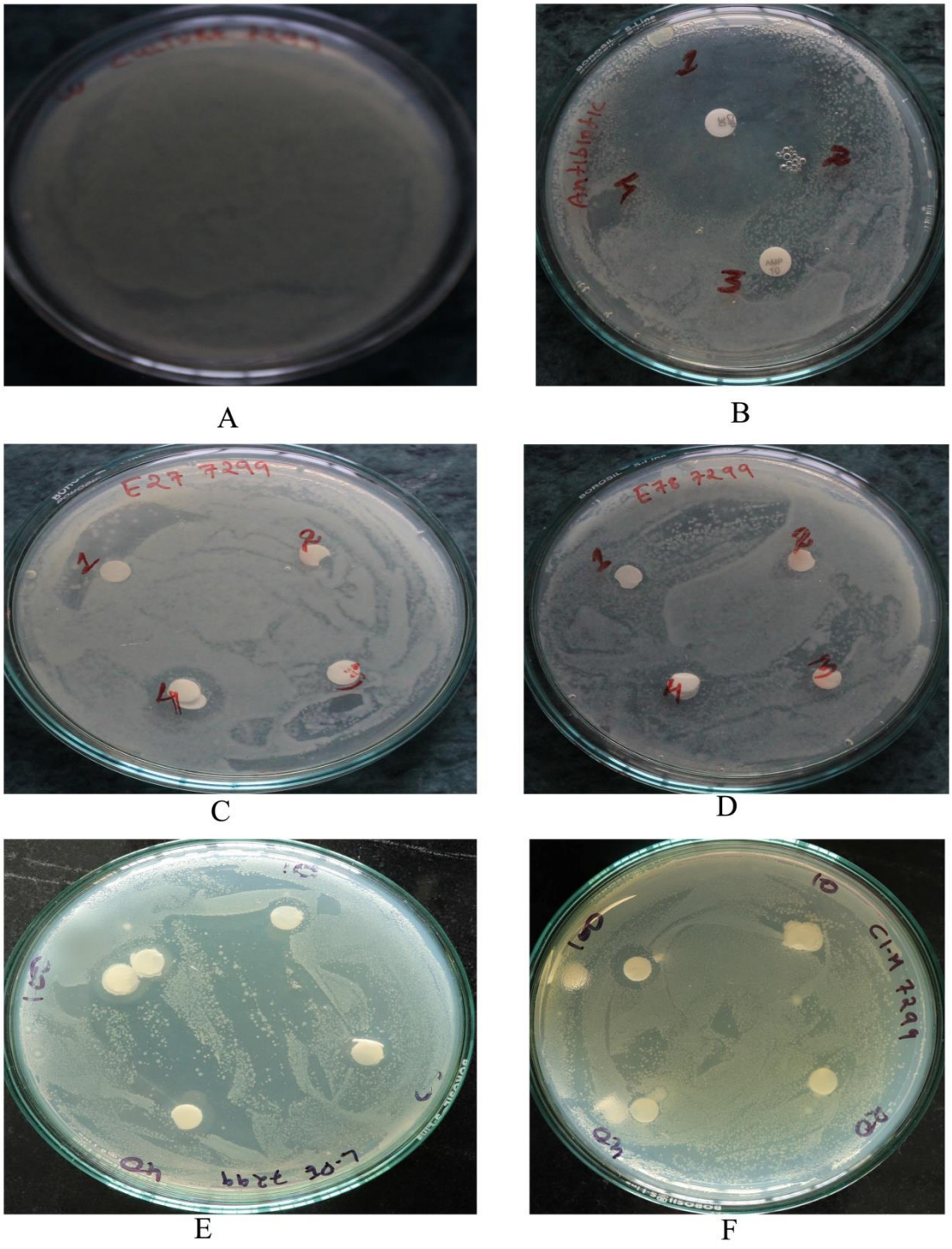


Figure 4.24; A. *P. vulgaris* culture, B. Inhibition by standard antibiotics, C. Inhibition by *V. volvacea*, D. Inhibition by *T. heimii*, E. Inhibition by *L. sajor-caju*, F. Inhibition by *C. hortense*.

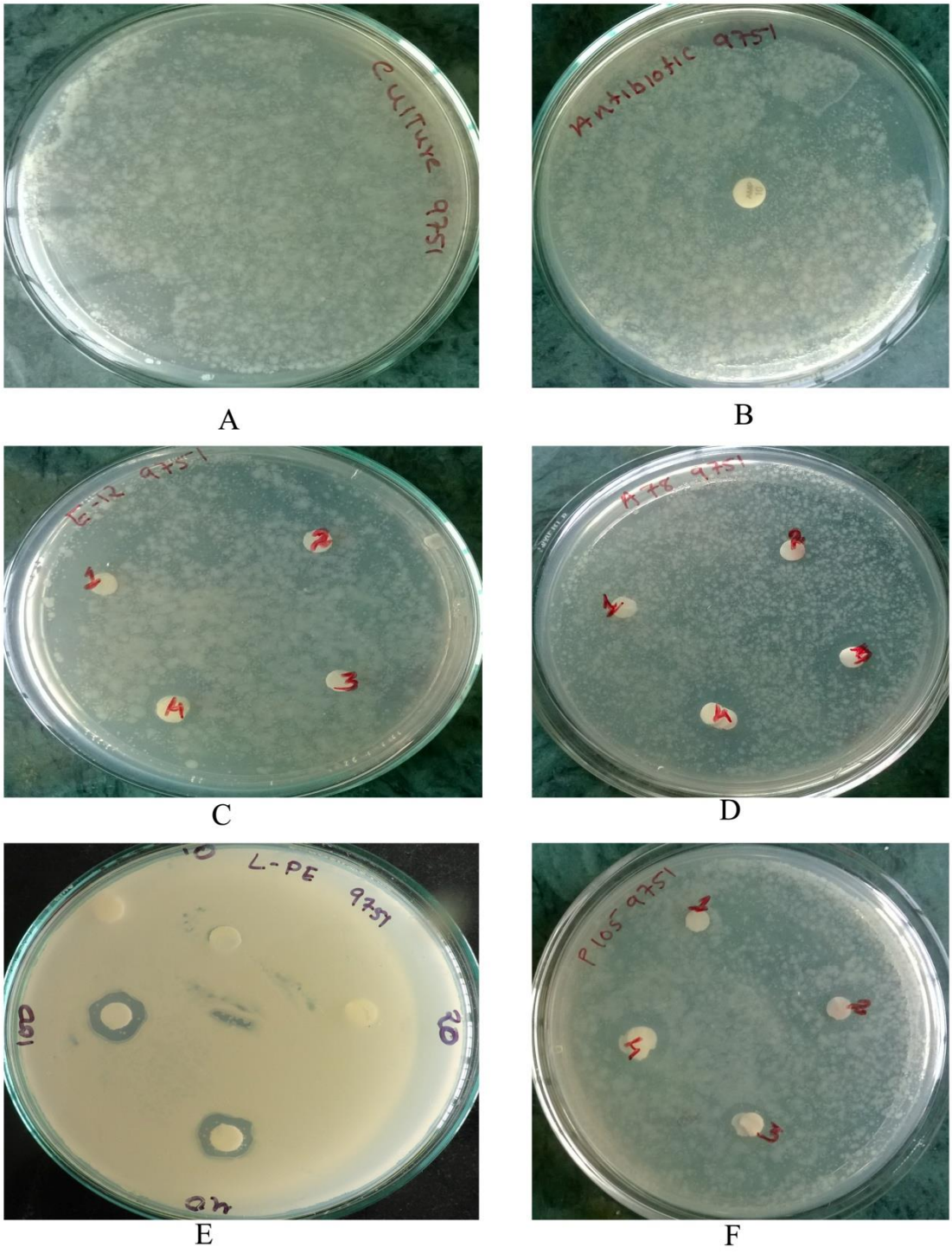


Figure: 4. 25; *K. pneumonae* culture, B. Inhibition by standard antibiotics, C. Inhibition by *V. volvacea*, D. Inhibition by *T. heimii*, E. Inhibition by *L. sajor-caju*, F. Inhibition by *C. subamethysteus*.

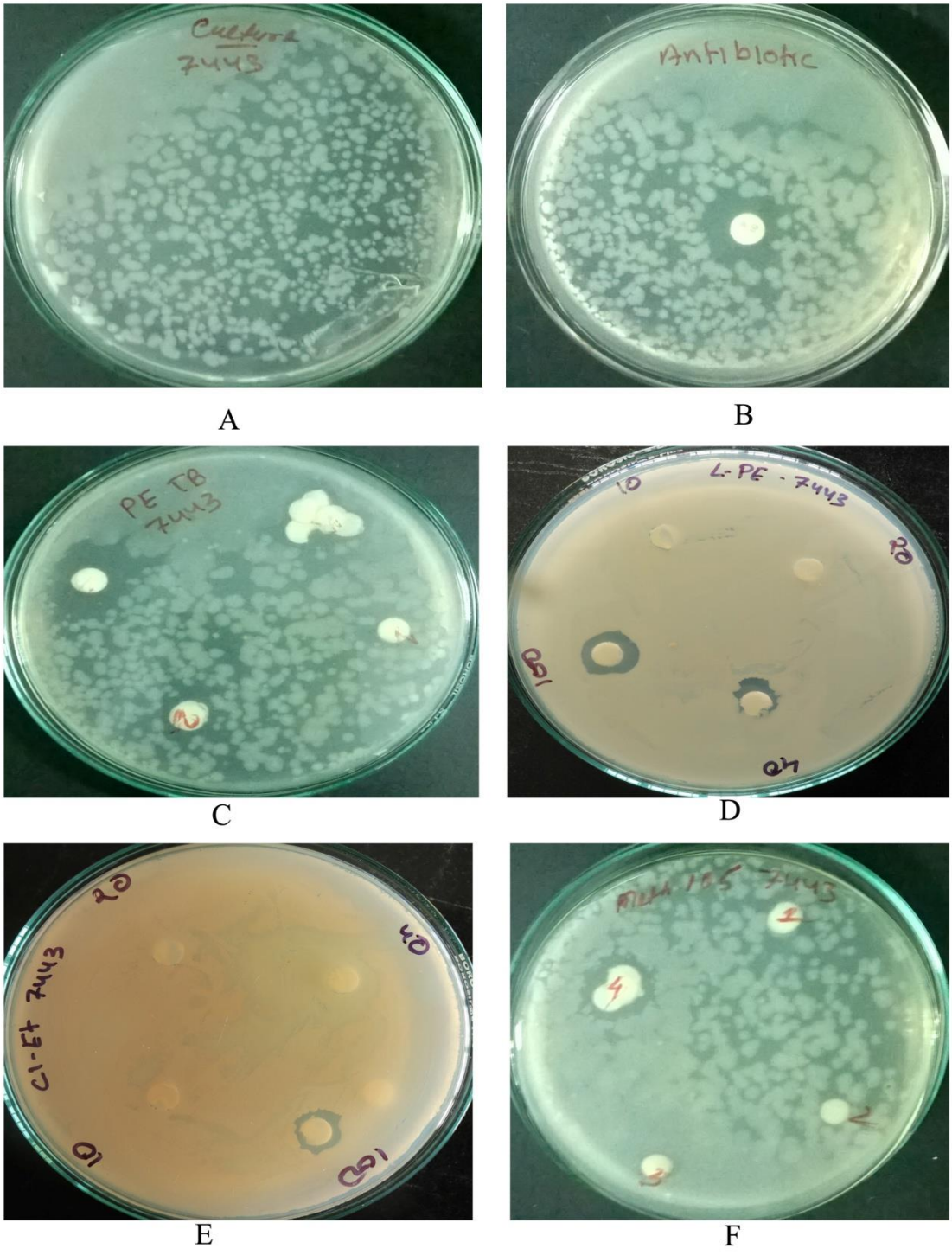


Figure 4.26: A. *S. aureus* culture, B. Inhibition by standard antibiotics, C. Inhibition by *T. heimii*, D. Inhibition by *L. sajor-caju*, E. Inhibition by *C. hortense*, F. Inhibition by *C. subamethysteus*.

4.6.4. *Chlorophyllum hortense:*

Chlorophyllum hortense ethanolic extract was effective in inhibiting *Escherichia coli, Bacillus cereus* and *Klebsiella pneumonae* with inhibition zone of 14.01 mm, 12.12 mm and 9.54 mm respectively. *Proteus vulgaris* and *Staphylococcus aureus* was inhibited to greater diameter by petroleum ether extract with inhibition zone of 13.06 mm and 12.99 mm respectively. The aqueous extract was unable to inhibit the growth of *E. coli* and *Proteus vulgaris* (Table 4.14).

4.6.5. *Cantharellus subamethysteus:*

Except for methanolic extract of *Cantharellus subamethysteus* other extracts did not show any effect on the growth of *Escherichia coli.* Petroleum ether extract was effective against *Bacillus cereus* with inhibition zone of 12.35 mm and methanolic extract was better in inhibiting the growth of *Klebsiella pneumonae* and *Staphylococcus aureus* with zone of inhibition measuring 13.79 mm and 12.63 mm respectively, ethanolic extract showed better activity against the growth of *Proteus vulgaris* with inhibition zone of 10.99 mm (Table 15).

4.7. GC-MS Analysis:

The GCMS analysis of the samples resulted in identification of many bioactive compounds based on their peaks on chromatogram. The peaks were then compared with the peaks of known compounds stored in NIST libraries. The fragmentation of molecules with their mass to charge ratio (m/z) was compared and the compounds were ascertained. The bioactive compounds identified were having various activities and properties like antibacterial, antioxidant, and antiviral. Some of the compounds like penicillamine are already been used as drugs. The identified compounds are listed in (Table 16-table 20). Most of the compounds are similar in the entire studied sample. The compounds include mainly fatty acid methyl ester, terpenoids, alcohols and phenolic acids.

Table 4.16: Bioactive compounds from *Volvariella volvacea.*

Sl.no	NAME OF COMPOUND	RT	M/Z	AREA
1	3-Pentanethiol	1.203	55	64954
2	Benzeneacetic acid, methyl ester	6.372	91	256420
3	1-Heptadecene	16.635	55	18906
4	Dodecanoic acid, trimethylsilyl ester	18.091	73	19578
5	7-Hexadecenoic acid, methyl ester, (Z)-	20.03	82	13878
6	Dotriacontane (CAS) n-Dotriacontane	20.252	57	10332

7	1-Nonadecene	21.065	55	19267
8	Heneicosanoic acid, methyl ester	20.967	74	44013
9	Methyl undecyl ether	21.74	70	5623
10	Dodecanoic acid, 2,3-bis(acetyloxy)propyl ester	22.291	73	47756
11	Cyclopropaneoctanoic acid, 2-[[2-[(2-ethylcyclopropyl)methyl]cyclopropyl]methyl]-, methyl ester	23.343	67	12321
12	7-Hexadecenoic acid, methyl ester, (Z)-	23.345	55	42999
13	5,10-Diethoxy-2,3,7,8-tetrahydro-1H,6H-dipyrrolo[1,2-a;1',2'-d]pyrazine	23.771	70	100781
14	Oleic acid, trimethylsilyl ester	24.251	73	89627
15	6-Hexadecenoic acid, 7-methyl,methyl ester (E)	25.078	55	27973
16	Heneicosanoic acid, methyl ester	25.427	74	15996
17	Cyclopropaneoctanoic acid, 2-hexyl-, methyl ester	25.7	55	52069
18	Hexadecanoic acid, 14-methyl-, methyl ester	25.699	74	159202
19	Oleic acid, trimethylsilyl ester	26.137	73	1659848
20	9,12-Octadecenoic acid (Z,Z)-, methyl ester	27.017	55	7442170
21	cis-9-Hexadecenal	27.559	55	576558
22	9,12-Octadecadienoic acid (Z,Z)-	27.821	67	563191
23	Oleic acid, trimethylsilyl ester	29.119	73	358713
24	9,12-Octadecanoic acid, trimethylsilyl ester	29.202	117	137157
25	Z,Z-8,10-Hexadecadien-1-ol	30.781	67	31352
26	Eicosanoic acid, methyl ester	31.029	74	169702
27	Z,Z-8,10-Hexadecadien-1-ol	32.508	71	36257
28	7-Hexadecenal, (Z)-	32.583	55	7697
29	cis-1-Chloro-9-octadecene	35.348	55	9458
30	Tricosanoic acid, methyl ester	36.029	74	52234
31	Tetracosanoic acid, methyl ester	38.216	74	719614
32	Pentacosanoic acid, methyl ester	40.953	74	39991
33	9(11)-Dehydroergosteryl benzoate	45.113	251	150797
34	Ethyl iso-allocholate	45.699	207	2551

Table: 4.17: Bioactive compounds from *Termitomyces heimii*.

S.NO	NAME OF COMPOUND	RT	m/z	area
1	3-Pentanethiol	1.203	55	76610
2	Benzeneacetic acid, methyl ester	6.348	91	72842
3	2H-Pyran, 3,6-dihydro-4-methyl-2-(2-methyl-1-propenyl)-	7.699	67	10923
4	Benzenepropanoic acid, decyl ester	8.788	104	25555
5	Undecanoic acid, 10-methyl-, methyl ester	15.01	74	271351
6	[Bi-1,4-cyclohexadien-1-yl]-3,3',6,6'-tetrone, 4,4'-dihydroxy-2,2',5,5'-tetramethyl-	15.315	191	852
7	Nonanedioic acid, dimethyl ester	15.584	55	28010
8	1-Heptadecene	16.626	55	43514
9	2-Octanol, 8,8-dimethoxy-2,6-dimethyl-	16.78	75	30133
10	Triacontanoic acid, methyl ester	18.796	74	36849
11	Methyl tetradecanoate	19.605	74	1721755
12	Disulfide, di-tert-dodecyl	20.247	57	8794
13	1-Nonadecene	21.059	55	51037
14	Pentadecanoic acid, methyl ester	21.733	74	660516
15	Tetradecanoic acid, trimethylsilyl ester	22.297	73	166949
16	Pentadecanoic acid, 14-methyl-, methyl ester	23.342	74	350410
17	9-Hexadecenoic acid, methyl ester, (Z)-	23.793	55	4315450
18	l-(+)-Ascorbic acid 2,6-dihexadecanoate	24.543	73	265075
19	9-Tricosene, (Z)-	25.428	55	282015
20	Heptadecanoic acid, methyl ester	25.699	74	329706
21	Hexadecanoic acid, trimethylsilyl ester	26.136	117	468667
22	9,12-Octadecadienoic acid (Z,Z)-, methyl ester (CAS) Methyl linoleate	27.035	67	14829616
23	9,12-Octadecadienoic acid, methyl ester	27.775	67	1004093
24	9,12-Octadecadienoic acid (Z,Z)-, trimethylsilyl ester (CAS) LINOLEIC ACID-MONOTMS	29.111	73	380305
25	Oleic acid, trimethylsilyl ester	29.62	73	81826
26	6,9,12,15-Docosatetraenoic acid, methyl ester	29.558	79	28018
27	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- (CAS) Methyl linolenate	30.252	79	128115

28	8,11-Eicosadienoic acid, methyl ester	30.178	67	80311
29	Cyclopropane, 1-(1-hydroxy-1-heptyl)-2-methylene-3-pentyl-	30.578	167	79579
30	Eicosanoic acid, methyl ester (CAS) Arachidic acid methyl ester	31.027	74	85712
31	7-Hexadecenal, (Z)-	32.106	55	39966
32	Cyclopropaneoctanoic acid, 2-[[2-[(2-ethylcyclopropyl)methyl]cyclopropyl]methyl]-, methyl ester	32.968	81	96474
33	Docosanoic acid, methyl ester (CAS) Methyl behenate	34.247	74	127985
34	9-(2',2'-Dimethylpropanoilhydrazono)-3,6-dichloro-2,7-bis-[2-(diethylamino)-ethoxy]fluorene	34.567	149	85866
35	2-Dodecen-1-yl(-)succinic anhydride	35.317	55	70147
36	Tricosanoic acid, methyl ester	36.023	74	120312
37	9-Octadecenoic acid (Z)-, methyl ester (CAS) Methyl oleate	37.667	55	16797
38	Tetracosanoic acid, methyl ester	38.206	74	491022
39	9(11)-Dehydroergosterol-3,5-dinitrobenzoate	45.11	251	337018

Table: 4.18: Bioactive compounds from *Lentinus sajor-caju.*

S.NO	**NAME OF COMPOUND**	**RT**	**m/z**	**area**
1	Penicillamine	1.206	75	112337
2	Butanedioic acid, dimethyl ester	3.352	115	54674
3	Sulfurous acid, hexyl octyl ester	3.735	57	26311
4	Benzeneacetic acid, methyl ester	6.324	91	233262
5	2H-Pyran, 3,6-dihydro-4-methyl-2-(2-methyl-1-propenyl)-	7.688	68	11769
6	Benzenepropanoic acid, methyl ester (CAS) Methyl hydrocinnamate	8.767	104	152120
7	Tetradecane, 1-chloro-	8.822	57	44306
8	Benzenepropanoic acid, tridecyl ester	8.767	104	152120
9	1-Tridecene	11.713	83	18026
10	Citric acid, trimethyl ester	13.666	143	29914

11	Silane, 1-hexenyltrimethyl-, (Z)-	14.477	141	68696
12	Dodecanoic acid, methyl ester	15.006	74	158589
13	Nonanedioic acid, dimethyl ester	15.579	55	7323
14	Hexadecanoic acid, 3,7,11,15-tetramethyl-, methyl ester	15.932	74	8315
15	Cyclopentaneundecanoic acid, methyl ester	16.486	127	77969
16	1-Heptadecene	16.622	55	66730
17	2-Octanol, 8,8-dimethoxy-2,6-dimethyl-	16.775	75	64750
18	Methyl 9-methyltetradecanoate	20.961	74	1025713
19	1-Nonadecene	21.131	55	297989
20	Oleic acid, trimethylsilyl ester	21.547	73	34359
21	Pentadecanoic acid, methyl ester	21.733	74	1476930
22	Tetradecanoic acid, trimethylsilyl ester	22.295	73	60170
23	Phthalic acid, butyl undecyl ester	22.625	149	62597
24	Hexadecanoic acid, methyl ester	23.343	74	569804
25	Oleic acid, trimethylsilyl ester	24.254	117	163694
26	l-(+)-Ascorbic acid 2,6-dihexadecanoate	24.625	73	2323123
27	Heptadecanoic acid, methyl ester	24.997	74	157545
28	7-Hexadecenoic acid, methyl ester, (Z)-	25.246	55	227257
29	Cyclopropaneoctanoic acid, 2-hexyl-, methyl ester	25.704	55	95966
30	Hexadecanoic acid, trimethylsilyl ester	26.143	117	845916
31	9,12-Octadecadienoic acid (Z,Z)-, methyl ester (CAS) Methyl linoleate	27.07	67	2337792 1
32	9,12-Octadecadienoic acid (Z,Z)-	27.919	67	1025326
33	cis-9-Hexadecenal	27.922	55	6129682
34	Oleic acid, trimethylsilyl ester	29.127	73	184702
35	Z,Z-8,10-Hexadecadien-1-ol	30.298	55	62840
36	2-Methyl-Z,Z-3,13-octadecadienol	31.193	83	261011
37	17-Pentatriacontene	32.109	57	56232
38	2-Octylcyclopropene-1-heptanol	32.919	95	98943
39	9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-	34.25	55	58486
40	cis-1-Chloro-9-octadecene	35.308	55	75463
41	Tricosanoic acid, methyl ester	36.018	74	154790

42	Tetracosanoic acid, methyl ester	38.202	74	317039
43	1,6,10,14,18,22-Tetracosahexaen-3-ol, 2,6,10,15,19,23-hexamethyl-, (all-E)-	40.852	69	242419
44	Pentacosanoic acid, methyl ester	40.852	69	242419
45	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)methyl]cyclopropyl]methyl]cyclopropyl]methyl]-, methyl ester	44.397	57	33327
46	Dehydroergosterol 3,5-dinitrobenzoate	45.107	251	582434
47	Ethyl iso-allocholate	45.619	69	29325

Table: 4.19: Bioactive compounds from *Chlorophyllum hortense.*

S.NO	**NAME OF COMPOUND**	**RT**	**m/z**	**area**
1	3-Pentanethiol, 2-methyl-	1.206	75	134323
2	Butanedioic acid, dimethyl ester	3.34	115	75726
3	Ritalin	6.32	91	318875
4	Benzaldehyde, 2,4-dimethyl-	7.34	133	55201
5	Razoxane	14.476	141	207099
6	Dodecanoic acid , methyl ester, (Z)-	15.005	55	29198
7	Tridecanoic acid, 3-methyl, methyl ester	15.927	74	227483
8	1-Heptadecene	16.625	55	87695
9	NSC 408941 4,6,6-trimethyl-bicyclo[3.1.1]heptan-2-ol	18.125	85	16784
10	7-Hexadecenal, (Z)-	18.629	57	7020
11	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)methyl]cyclopropyl]methyl]cyclopropyl]methyl]-, methyl ester	18.793	74	254106
12	9,9-Dimethoxybicyclo[3.3.1]nona-2,4-dione	20.959	57	126631
13	Methyl 9-methyltetradecanoate	21.131	74	913893
14	1-Nonadecene	21.131	55	341772
15	Pentadecanoic acid, methyl ester	21.732	74	1182484
16	Phthalic acid, butyl undecyl ester	22.625	149	101217
17	Hexadecanoic acid, methyl ester	23.027	74	511239
18	Oleic acid, trimethylsilyl ester	23.798	74	1039377

19	9-Octadecenoic acid (Z)- (CAS) Oleic acid	24.574	55	288944
20	Triacontanoic acid, methyl ester	24.998	74	201085
21	Cyclopropaneoctanoic acid, 2-hexyl-, methyl ester	25.427	55	382231
22	Heptadecanoic acid, methyl ester	25.704	74	1312543
23	Oleic acid, trimethylsilyl ester	26.158	73	366419
24	9,12-Octadecadienoic acid, methyl ester	27.142	67	37852468
25	Octadecanoic acid, methyl ester	27.582	74	4134869
26	10,13-Octadecadiynoic acid, methyl ester	27.98	91	3091940
27	9,12-Octadecadienoic acid (Z,Z)-, trimethylsilyl ester	29.111	55	144132
28	Cyclopropanecarboxylic acid, 2,2-dimethyl-3-(2-propenyl)-, ethyl ester, trans-	29.561	141	318287
29	6,9,12,15-Docosatetraenoic acid, methyl ester	30.252	79	71448
30	Z,Z-3,13-Octadecedien-1-ol	30.59	55	123861
31	8,11-Eicosadienoic acid, methyl ester	30.78	67	87072
32	Eicosanoic acid, methyl ester	31.028	74	278196
33	Emicymarin	31.056	163	199067
34	Oxirane, hexadecyl-	31.53	55	107112
35	17-Pentatriacontene	32.103	57	53860
36	Trilinolein	32.504	55	116433
37	9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-	34.246	55	103869
38	Ethyl iso-allocholate	36.021	55	43914
39	5-Hexadecenoic acid, 2-methoxy-, methyl ester	36.756	104	549184
40	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)methyl]cyclopropyl]methyl]cyclopropyl]methyl]-, methyl ester	38.209	55	590289
41	Cholesta-8,24-dien-3-ol, 4-methyl-, (3.beta.,4.alpha.)-	41.435	86	326482
42	Ergosterol	41.427	69	18035
43	9(11)-Dehydroergosteryl benzoate	45.109	251	856000
44	Ergosta-4,6,22-trien-3.alpha.-ol	46.532	69	111208

Table: 4.20: Bioactive compounds from *Cantharellus subamethysteus.*

S.NO	NAME OF COMPOUND	RT	m/z	area
1	3-Pentanethiol	1.206	75	134068
2	Butanedioic acid, dimethyl ester	3.337	115	74906
3	Octanoic acid, methyl ester	5.009	74	78814
4	Dodecane, 2,6,11-trimethyl-	8.822	57	68699
5	Eicosane	14.337	57	83007
6	Decanoic acid, 9-oxo-, methyl ester	14.893	111	79472
7	Undecanoic acid, 10-methyl-, methyl ester	15.002	74	235893
8	Nonanedioic acid, dimethyl ester	15.568	55	153248
9	1-Heptadecene	16.621	55	86122
10	Octanal dimethyl acetal	16.771	75	150212
11	Decanoic acid, 10-chloro-10-oxo-, methyl ester	17.919	55	18100
12	Eicosane	19.602	57	163955
13	1-Nonadecene	20.961	55	138460
14	Pentadecanoic acid, methyl ester	21.732	74	540503
15	Tetradecanoic acid , trimethylsilyl ester	22.301	73	24065
16	Phthalic acid, butyl undecyl ester	22.626	149	157733
17	Triacontanoic acid, methyl ester	23.345	74	254130
18	9-Hexadecenoic acid, methyl ester, (Z)-	23.79	55	4445956
19	Hexadecanoic acid, methyl ester	23.791	74	6660008
20	l-(+)-Ascorbic acid 2,6-dihexadecanoate	24.554	57	220820
21	Heptadecanoic acid, methyl ester	25.702	74	434427
22	Hexadecanoic acid, trimethylsilyl ester	26.146	117	257675
23	9,12-Octadecanoic acid, methyl ester	27.585	67	19404637
24	Pregnan-3,11-diol-20-one	29.251	71	282078
25	6,9,12,15-Docosatetraenoic acid, methyl ester	30.254	79	186586
26	8,11-Eicosadienoic acid, methyl ester	30.507	67	160014
27	Emicymarin	31.057	163	1571921
28	1,6-Octadiene, 3-ethoxy-3,7-dimethyl-	31.784	71	104977
29	Ethyl iso-allocholate	32.24	57	99273
30	10-12-Pentacosadiynoic acid	32.483	55	268048

31	2-Dodecen-1-yl(-)succinic anhydride	35.303	55	104121
32	Z,Z-8,10-Hexadecadien-1-ol	35.433	55	47679
33	Tetracosanoic acid, methyl ester	38.205	74	1403274
34	9(11)-Dehydroergosteryl benzoate	45.104	251	691111

4.8. Molecular docking Studies:

4.8.1. Absorption, Distribution, Metabolism, Excretion and Toxicity:

The bioactive compounds identified were subjected to Absorption, Distribution, Metabolism, Excretion and Toxicity test (ADME/T). Many of the detected compounds have been qualified to be drug. Few violations have been detected such as higher number of rotatable groups, molecular weight of more than 500 and octanol water partition coefficient. The compounds were further tested for drug like characters applying Lipinski rule of five and rule of three. Compounds with QPLogP $_{O/W}$ value of -2 -6.5, QPlog BB value of -3-1.2, Oral absorption >80%, Solvent accessible surface area of 300-1000, HB doner <5 and HB accepter <10, Molecular weight <500 are considered to be have good effectiveness in drug delivery.

4.8.2. Bacterial Efflux Pump:

PDB ID 5ENO is a transporter protein associated with bacterial efflux pump. X-ray diffraction structure with resolution of 2.2 Å and R-value of 0.247 was tested with the active components of mushrooms. The docking study showed that the compounds from mushrooms were interacting with the protein with high free energy for binding the best result was with 9-(2',2'-Dimethylpropanoilhydrazono)-3,6-dichloro-2,7-bis-[2-(diethylamino)-ethoxy]fluorene. It had a binding energy of -98.86 the main contributing energies were from lipophilic and Vander Wall interaction of -52.67 and -67.5. The docking score was also very favourable with -11.78. Compounds like 1,6,10,14,18,22-Tetracosahexaen-3-ol, 2,6,10,15,19,23-hexamethyl-, (all-E), Eicosanoic acid, methyl ester, Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)methyl] cyclopropyl]methyl] cyclopropyl] methyl]-, methyl ester, Phthalic acid, butyl undecyl ester, Ergosterol, Disulfide, di-tert-dodecyl, 2-[[2-[(2-ethylcyclopropyl)methyl]cyclopropyl]methyl]-, methyl ester, Heneicosanoic acid, methyl ester, 6,9,12,15-Docosatetraenoic acid, methyl ester and Dehydroergosterol 3,5-dinitrobenzoate were also found to have good docking scores.

4.8.3. Topoisomerase ATPase inhibitor.

Topoisomerase ATPase inhibitor from *Staphylococcus aureus* have been studied. The protein selected was (PDB ID 3TTZ) with X-ray diffraction deduced structure at resolution of 1.63Å, R free value of 0.194 and residue count of 396. The best docking score was found with Emicymarin at -5.66 which is nearly comparable to standard ligands previously used but the binding energy was not favourable with this molecule. The best molecule with high binding energy and also good docking score was Dehydroergosterol 3, 5-dinitrobenzoate with docking score of -5.05 and binding energy of -54.46. Other molecules with good binding energies were 9-(2',2'-Dimethylpropanoilhydrazono)-3,6-dichloro-2,7-bis-[2-(diethylamino)-ethoxy]fluorene and 1,6,10,14,18,22-Tetracosahexaen-3-ol, 2,6,10,15,19,23-hexamethyl-,.

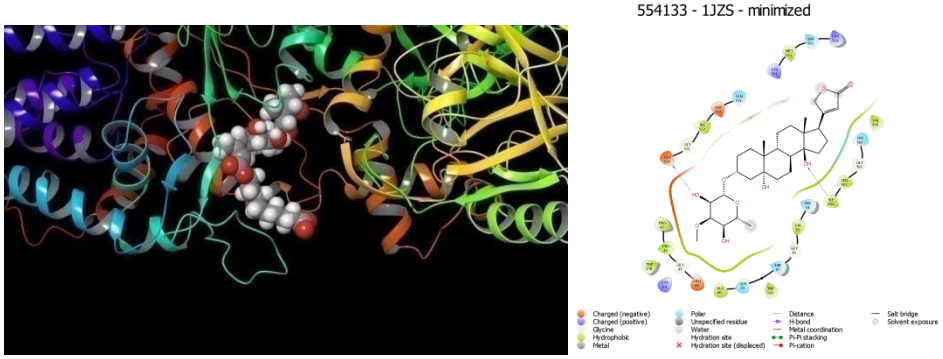


Figure: 4.27: 3D & 2D Ligand interaction diagram of Isoleucyl-tRNA synthetase (1JZS).

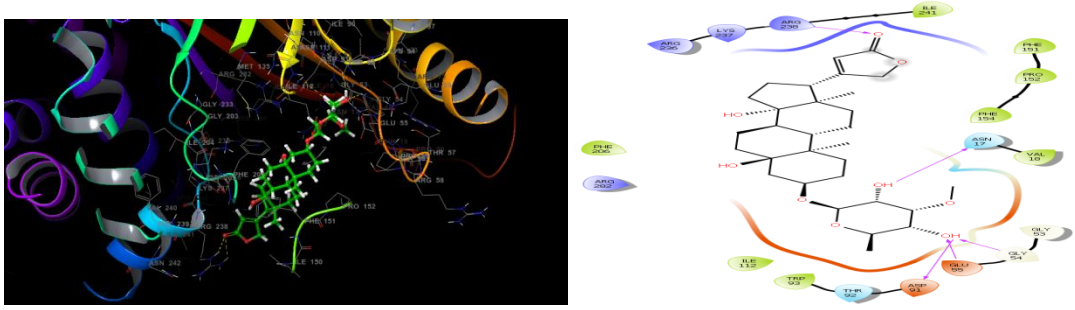


Figure: 4.28: 3D &2D Ligand interaction diagram of Dihydropteroate synthase (2VEG).

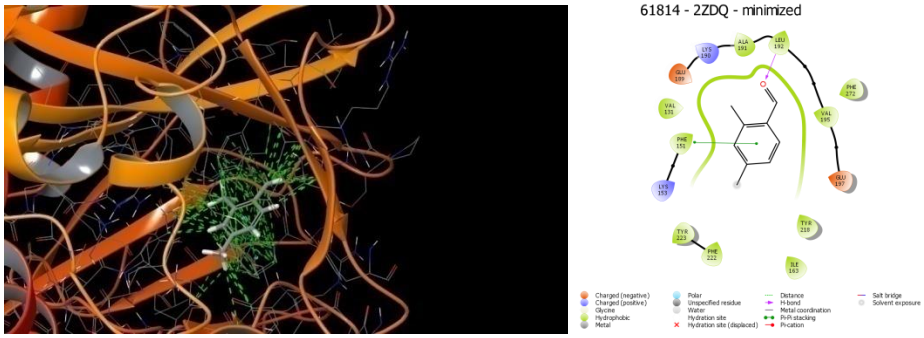


Figure: 4.29: 3D & 2D Ligand interaction diagram of D-alanine: D-alanine ligase (2ZDQ).

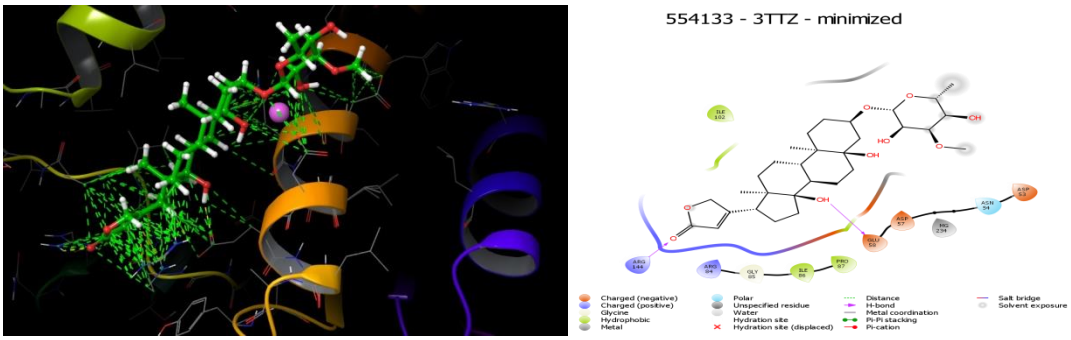


Figure: 4.30:2D&3D Ligand interaction diagram of Topoisomerase ATPase inhibitor (3TTZ).

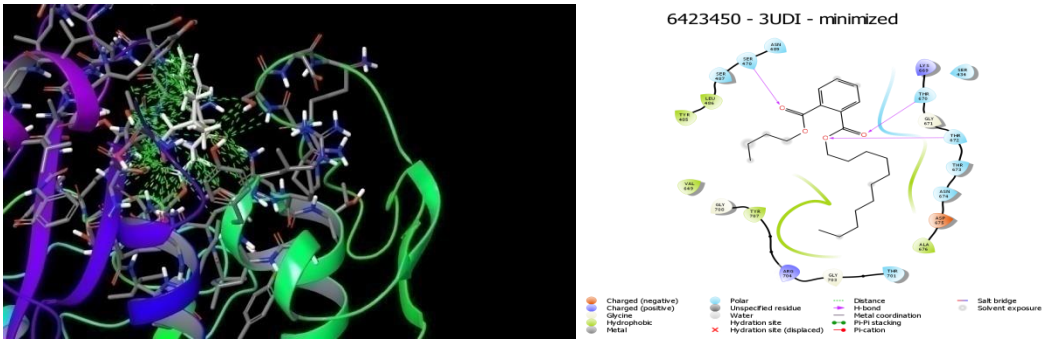


Figure: 4.31:3D &2D Ligand interaction diagram of Penicillin binding protein (3UDI).

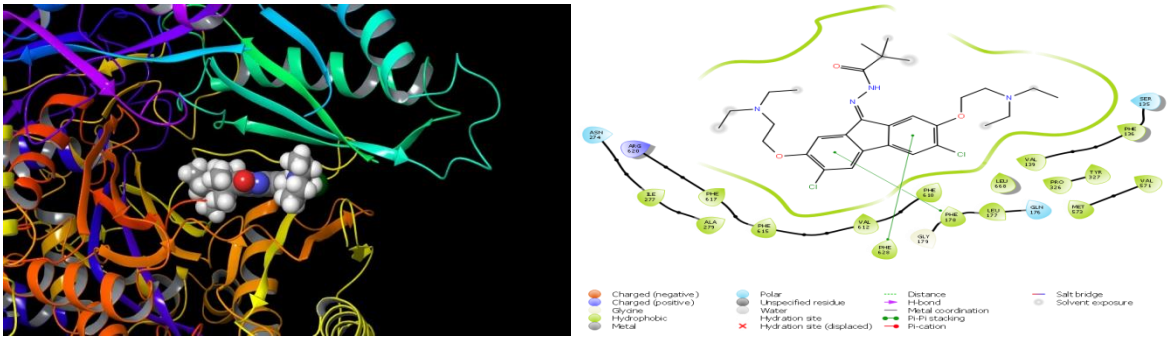


Figure: 4.32: 2D& 3D Ligand interaction diagram of Bacterial efflux pump (5ENO).

4.8.4. Penicillin Binding Protein:

Penicillin binding protein (PDB ID 3UDI) from *Acinetobacter baumanni* determined by X ray diffraction at resolution of 2.6 Å, R value of 0.250 sequence length of 731 and residue count of 1462 was docked with the compounds from mushrooms. The results were comparable taking docking score into account but the binding energies were much lower than penicillin. The compound Phthalic acid, butyl undecyl ester and Emicymarin had the docking score of -5.78, -5.69 and -4.76 respectively compared to -6.33 of penicillin.

4.8.5. Isoleucyl-tRNA synthetase:

Isoleucyl-tRNA synthetase (PDB ID 1JZS) from *Thermus thermophilus* determined by X ray diffraction at the resolution of 2.5 Å, R value of 0.274, sequence length of 821 and residue count of 821 was subjected to docking analysis with compounds from mushrooms. Results as tabulated the best docking score was -6.83 for compounds compared to -9.74 of standard ligand. However the binding energies are better in compounds like 1,6,10,14,18,22-Tetracosahexaen-3-ol, 2,6,10,15,19,23-hexamethyl-, (all-E)-. and Tricosanoic acid, methyl ester with -57.23 and -57.27 as compared to standard ligand with binding energy of -52.76. Vander wall and lipophilic interactions were the main contributing factors.

4.8.6. Dihydropteroate synthase:

Dihydropteroate synthase (PDB ID 2VEG) from *Streptococcus pneumonae* having R value of 0.297, sequence length of 314, residue count of 628 determined by X ray diffraction at resolution of 2.4 Å was docked with compounds from mushrooms. The result has been in favour of the compounds from mushrooms, though the docking score was lower compared to the standard ligand. The free energy of binding was higher with the compounds from mushrooms. The best docking score of -5.40 was found in Emicymarin compared to -7.36 in the standard ligand. The binding energy was -41.04 with few penalties compared to -28.76 in standard ligand. Other compounds with good docking scores were Razoxane, Ethyl iso-allocholate, Ritalin, Ergosta-4,6,22-trien-3.alpha.-ol with docking scores of -4.05, -3.08, -3.47,-3.68 and binding energy of -33.04, -34, -31.57 and -31.89 respectively.

4.8.7. D-alanine: D-alanine Ligase:

D-alanine:D-alanine Ligase (PDB ID 2ZDQ) from *Thermus thermophilus* determined by X ray diffraction at the resolution of 2.3 Å having residue count of 638 and sequence length of 319 was taken for docking analysis with bioactive compounds identified from mushrooms. The results revealed that the compounds from mushrooms were better than the standard ligand previously studied. The docking score was lower than standard but with higher binding energy, the docking score ranged from -7.20 to -5.52 compared to -12.77 of standard ligand while the binding energy of tested compounds were from -6.54 to -41.18 compared to 22.58 in the standard ligand. The compounds were Benzaldehyde, 2,4-dimethyl-, Razoxane , Benzenepropanoic acid, methyl ester, Ritalin, Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)methyl]cyclopropyl]methyl]cyclopropyl]methyl]-, methyl ester , cis,cis-Linoleic acid, Penicillamine and 6,9,12,15-Docosatetraenoic acid, methyl ester.

Table 4.21: Absorption, Distribution, Metabolism Excretion.

Title	Entry Name	#rotor <15	CNS -2-2	mol MW <500	SASA 300-1000	donorHB <5	acceptHB <10	QPlog Po/w -2.0-6.5	QPlog BB- -3-1.2	#metab 1-10	Human Oral Absorption 80-100 %	Rule Of Five	Rule Of Three
75519	Tricosanoic acid, methyl ester.1	21	-2	368.642	949.366	0	2	8.527	-1.517	1	100	1	1
4158	ritalin.1	3	1	233.31	513.298	0	2.5	0.384	0.36	2	82.054	0	0
30623	Razoxane.1	3	-1	268.272	465.144	2	10	-2.368	-0.921	6	19.218	0	1
6423450	Phthalic acid, butyl undecyl ester.1	15	-2	376.535	806.177	0	4	6.284	-1.364	0	100	1	1
41431	Pentacosanoic acid, methyl ester.1	23	-2	396.696	1019.625	0	2	9.351	-1.673	1	100	1	1
5852	Penicillamine.1	4	-1	149.207	327.768	3	2.5	-1.471	-0.105	4	46.403	0	0

13584	Methyl behenate.1	20	-2	354.615	920.275	0	2	8.16	-1.446	1	100	1	1
75546	Lignoceric acid methyl ester.1	22	-2	382.669	981.276	0	2	8.96	-1.528	1	100	1	1
22434	Heneicosanoic acid, methyl ester.1	19	-2	340.588	886.881	0	2	7.759	-1.37	1	100	1	1
6452096	Ethyl iso-allocholate.1	8	-2	436.631	752.815	3	7.1	3.866	-1.517	4	94.141	0	1
444679	Ergosterol_444679.1	5	0	396.655	736.255	1	1.7	7.164	-0.224	6	100	1	1
5379712	Ergosta-4,6,22-trien-3.alpha.-ol_5379712.1	5	0	396.655	729.672	1	1.7	7.179	-0.196	4	100	1	1
554133	Emicymarin.1	7	-2	550.688	807.307	4	13	2.526	-1.504	6	72.125	1	0
14259	Eicosanoic acid, methyl ester.1	18	-2	326.562	857.966	0	2	7.393	-1.297	1	100	1	1
117981	Disulfide, di-tert-dodecyl.1	19	2	402.779	876.282	0	1	8.252	1.417	0	100	1	1
2115	Dehydroergosterol 3,5-	8	-2	588.742	978.88	0	4	8.063	-2.701	8	80.123	2	2

9991	dinitrobenzoate.1												
554084	2-[[2-[[2-[(2-pentylcyclopropyl)methyl]cyclopropyl]methyl]cyclopropyl]methyl]-, methyl ester.1	14	-1	374.606	808.069	0	2	7.563	-0.887	1	100	1	1
74112	Citric acid, trimethyl ester.1	6	-2	234.205	504.344	0	5.75	0.783	-1.261	3	76.664	0	0
5280450	cis,cis-Linoleic acid.1	14	-2	280.45	625.105	1	2	5.299	-1.301	4	87.757	1	0
7643	Benzenepropanoic acid, methyl ester (CAS) Methyl hydrocinnamate.1	3	0	164.204	424.138	0	2	2.344	-0.154	2	100	0	0
61814	Benzaldehyde, 2,4-dimethyl-.1	1	0	134.177	356.31	0	2	1.767	-0.013	2	100	0	0
5366124	9(11)-Dehydroergosteryl benzoate.1	6	0	498.747	901.719	0	2	9.561	-0.236	6	100	1	1
590814	9-(2',2'-Dimethylpropanoilhydrazono)-3,6-dichloro-2,7-bis-[2-(diethylamino)-	15	1	577.593	959.982	1	7.5	6.712	0.052	4	81.131	2	1

	ethoxy]fluorene.1												
590814	9-(2',2'-Dimethylpropanoilhydrazono)-3,6-dichloro-2,7-bis-[2-(diethylamino)-ethoxy]fluorene.1	15	1	577.593	941	1	7.5	6.589	0.139	4	80.539	2	1
5365673	8,11-Eicosadienoic acid, methyl ester.1	16	-2	322.53	833.45	0	2	7.17	-1.162	4	100	1	1
5362672	6,9,12,15-Docosatetraenoic acid, methyl ester.1	16	-2	346.552	892.204	0	2	7.828	-1.199	6	100	1	1
29772	2-Dodecen-1-yl(-)succinic anhydride.1	10	-2	266.38	626.053	0	4.5	3.209	-1.272	4	96.953	0	0
534619	2-[[2-[(2-ethylcyclopropyl)methyl]cyclopropyl]methyl]-, methyl ester.1	13	-1	334.541	760.282	0	2	6.822	-0.889	1	100	1	1
5366014	1,6,10,14,18,22-Tetracosahexaen-3-ol, 2,6,10,15,19,23-hexamethyl-, (all-E)-.1	17	-1	426.724	836.372	1	1.7	8.799	-0.83	17	100	1	2

Table 4.22: Docking score of ligands with contributing energies of Bacterial efflux pup (PDB ID 5ENO) AcrB protein.

Sl No	Pubchem ID	dG bind	dG Hbond	dGlipo	dGvdw	Docking score
1	590814	-98.86	0	-52.67	-67.5	-11.78
2	5366014	-72.04	-0.52	-50.73	-49.8	-10.82
3	554084	-49.59	-2.02	-47.13	-44.3	-10.24
4	6423450	-60.98	0	-40.33	-46.3	-9.79
5	444679	-57.86	-0.52	-47.47	-35.6	-9.73
6	117981	-63.50	0	-48.59	-45.1	-9.42
7	534619	-36.04	-0.55	-37.3	-29.8	-9.35
8	22434	-52.86	0	-38.04	-52.3	-9.25
9	5362672	-48.3	-0.51	-33.8	-50.8	-9.17
10	14259	-50.46	-0.36	-36.7	-51.3	-8.99
11	21159991	-59.27	-0.57	-46.0	-45.1	-8.87

Table 4.23: Docking score of ligands with contributing energies of Topoisomerase ATPase inhibitor (PDB ID 3TTZ).

Sl No	Pubchem ID	dG bind	dG Hbond	dGlipo	dGvdw	Docking score
1	Std lig	-64.6	-2.86	-18.3	-49.0	-7.02
2	554133	4.59	-1.88	-10.6	-30.4	-5.66
3	6452096	13.8	-1.93	-10.2	-18.7	-5.33
4	5852	-6.61	-1.61	-1.7	-14.2	-5.20
5	21159991	-54.46	-0.97	-20.4	-57	-5.05
6	5366014	-46.79	-1.3	-20.1	-40.3	-5.04
7	534619	-22.31	-0.47	-20.4	-39.3	-5.02
8	590814	-52.12	-0.58	-24.9	-50.4	-4.75
9	534619	-10.77	0	-21.6	-29.7	-4.55
10	7643	-28.06	-0.52	-12.9	-22.6	-4.30
11	554084	-13.97	-1.2	-20.9	-35.7	-4.28

Table 4.24: Docking score of ligands with contributing energies of Penicillin Binding protein (PDB ID 3UDI).

Sl No	Pubchem ID	dG bind	dG Hbond	dGlipo	dGvdw	Docking score
1	Std lig	-146.6	-11.73	-0.7	4.59	-6.33
2	6423450	-16.49	-14.65	-0.01	12.84	-5.78
3	554133	-20.79	-15.97	0	7.14	-5.69
4	6452096	-29.75	-17.02	-0.16	4.0	-4.76
5	5852	-18.01	-3.38	0	2.5	-4.71
6	590814	-11.33	-20.92	0	13.4	-4.62
7	74112	-25.26	-5.32	0-1.27	2.06	-4.58
8	4158	-22.06	-12.01	0	4.7	-4.16
9	519592	-16.04	-11.5	-137	2.3	-4.08

Table 4.25: Docking score of ligands with contributing energies of Isoleucyl-tRNA synthetase (PDB ID 1JZS).

Sl No	Pubchem ID	dG bind	dG Hbond	dGlipo	dGvdw	Docking score
1	Std lig	-52.76	-5..03	-17.91	-56.64	-9.74
2	554133	-44.78	-1.73	-19.48	-53.88	-6.83
3	6452096	-43.53	-2.51	-15.29	-53.87	-6.67
4	5366014	-57.23	-0.53	-25.59	-59.77	-6.45
5	444679	-34.08	-0.63	-18.5	-50.71	-6.63
6	5352860	-39.5	-0.57	-19.78	-52.14	-6.08
7	5366124	-47.25	-0.16	-27.91	-59.93	-5.87
8	5379712	-44.35	-1.04	-18.25	-36.3	-5.82
9	534619	-21.49	-0.55	-19.36	-49.3	-5.08
10	75519	-57.27	-0.54	-24.22	-59.8	-5.07
11	554084	-17.54	-0.73	-20.31	-52.0	-5.06

Table 4.26: Docking score of with contributing energies of Dihydroteroate synthase (PDB ID 2VEG).						
Sl No	Pubchem ID	dG bind	dG Hbond	dGlipo	dGvdw	Docking score
1	Std lig	-28.76	-6.79	-2.96	-27.31	-7.36
2	554133	-41.04	-3.6	-13.16	-37.44	-5.40
3	30623	-33.04	-3.34	-5.07	-30.95	-4.05
4	5852	-11.04	-1.56	-1.61	-14.23	-3.70
5	5379712	-31.89	-1.28	-20.31	-36.07	-3.68
6	74112	-23.93	-3.83	-3.5	-15.19	-3.63
7	4158	-31.57	-2.56	-8.83	-26.42	-3.47
8	6452096	-34	-1.57	-11.49	-36.49	-3.08

Table 4.27: Docking score of with contributing energies of D-Alanine:D-alanine ligase (PDB ID 2ZDQ).						
Sl No	Pubchem ID	dG bind	dG Hbond	dGlipo	dGvdw	Docking score
1	Std lig	22.58	-12.79	-11.97	-26.17	-12.77
2	61814	-35.57	-0.54	-16.09	-25.26	-7.20
3	30623	-36.1	-2.67	-10.96	-40.44	-7.10
4	7643	-41.18	-0.53	-17.36	-27.31	-6.83
5	4158	-39.18	-0.55	-24.9	-27.67	-6.65
6	554084	-28.56	-2.42	-27.74	-43.67	-6.38
7	5280450	-36.73	-3.25	-27.7	-44.05	-5.84
8	5852	-6.54	-2.33	-6.75	-16.9	-5.54
9	5362672	-32.46	-1.26	-23.53	-40.99	-5.52

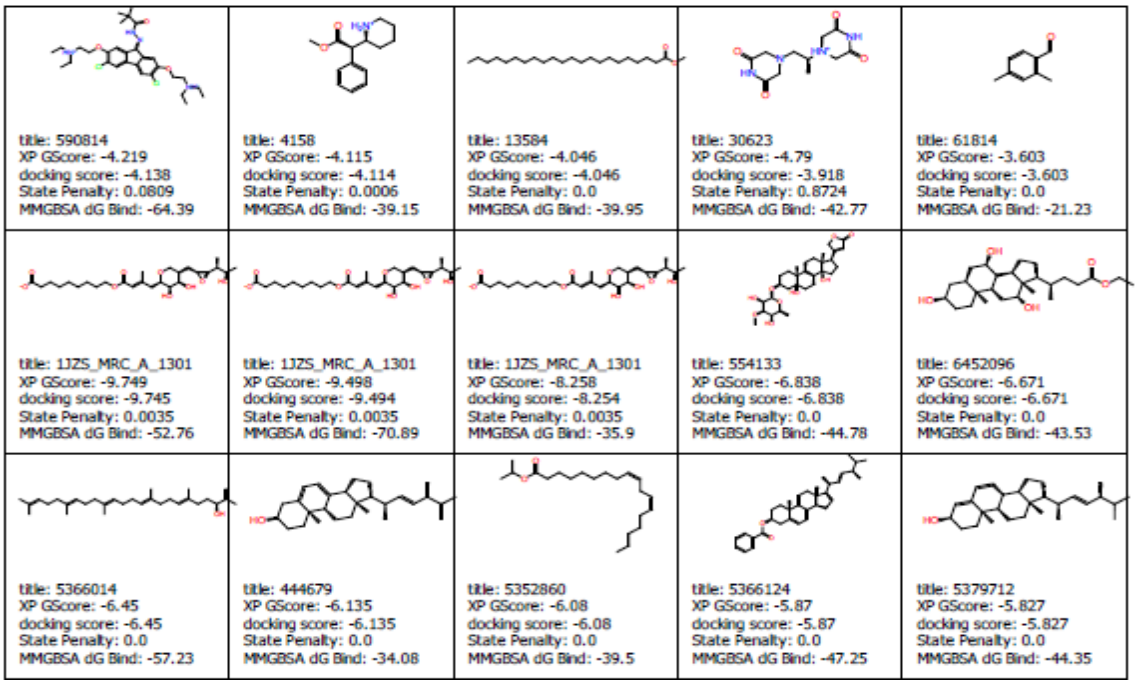


Figure 4.33: Glide score, Docking score, State penalty and ΔG of Ligands binding with Isoleucyl-tRNA Synthetase (PDB ID 1JZS).

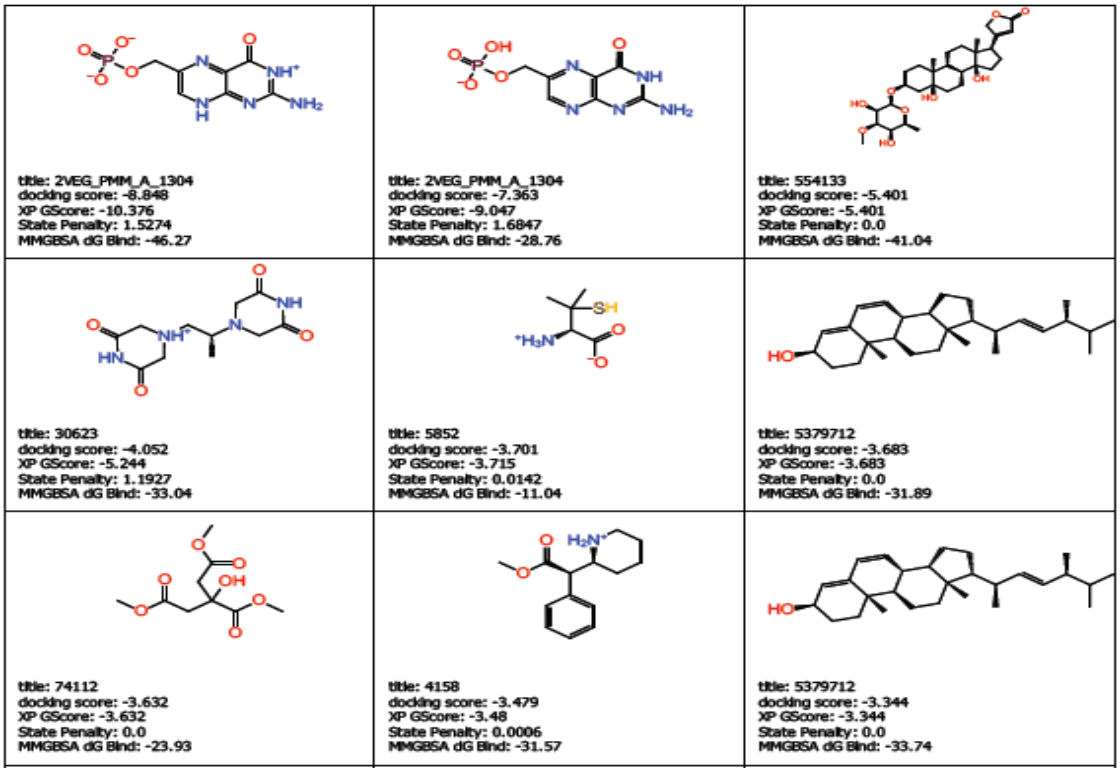


Figure 4.34: Glide score, Docking score, State penalty and ΔG of Ligands binding with Dihydropteroate Synthase (PDB ID 2VEG).

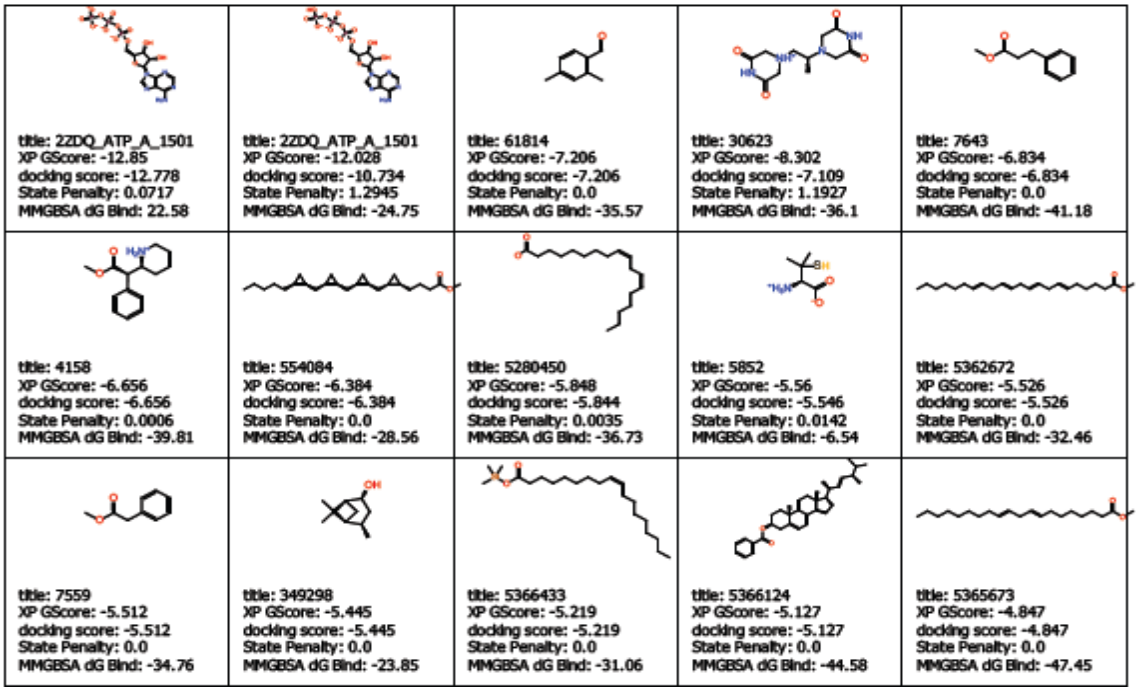


Figure 4.35: Glide score, Docking score, State penalty and ΔG of Ligands binding with D-Alanine: D-Alanine Ligase (PDB ID 2ZDQ).

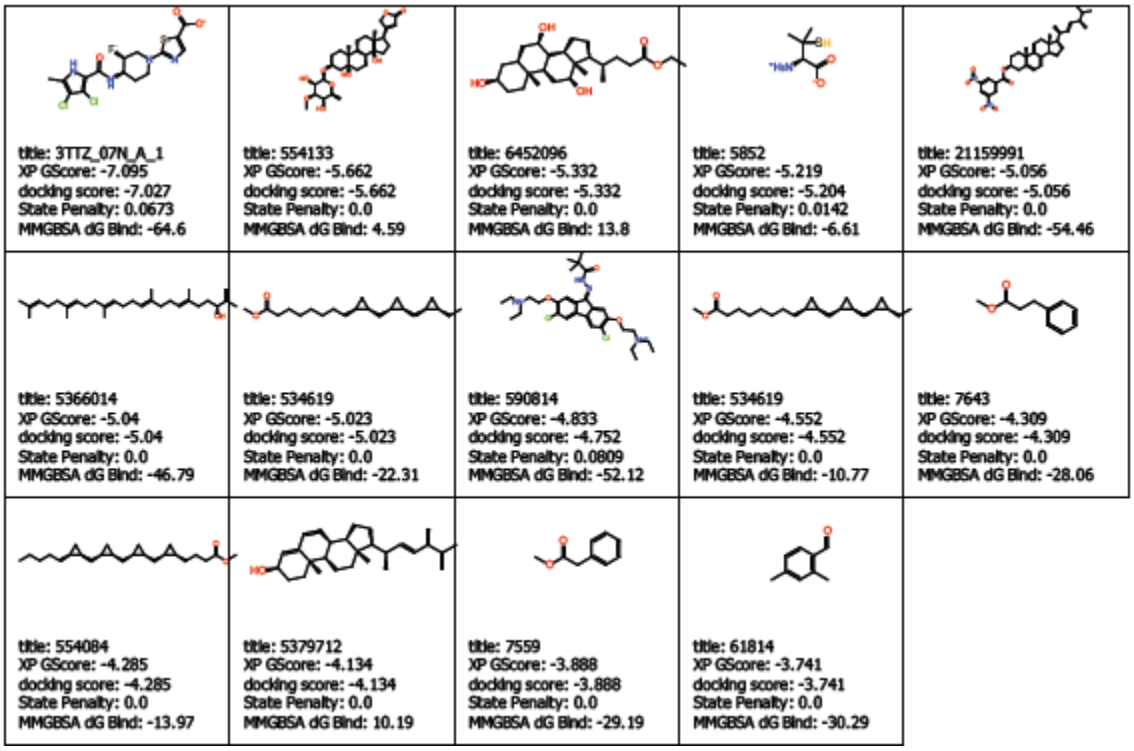


Figure 4.36: Glide score, Docking score, State penalty and ΔG of Ligands binding with Topoisomerase ATPase inhibitor (PDB ID 3TTZ).

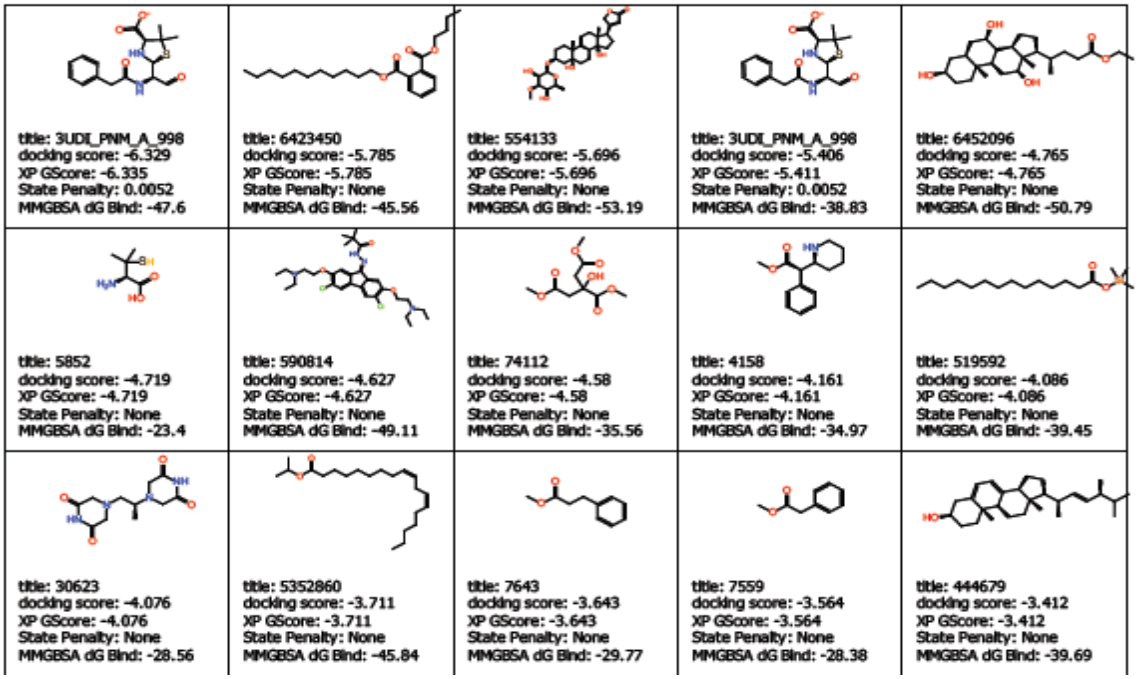


Figure 4.37: Glide score, Docking score, State penalty and ΔG of Ligands binding with Penicillin Binding Protein (PDB ID 3UDI).

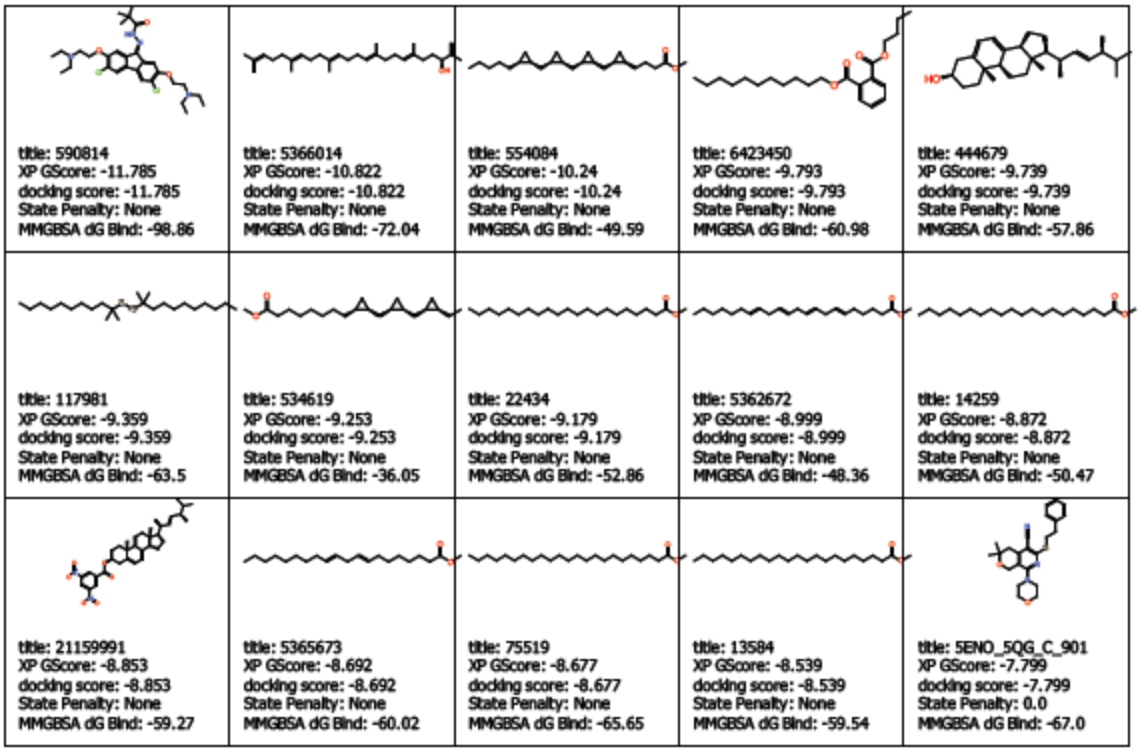


Figure 4.38: Glide score, Docking score, State penalty and ΔG of Ligands binding with Bacterial efflux pump (PDB ID 5ENO).