CHAPTER V

DISCUSSION

The current study on biochemical evaluation and molecular identification of wild edible mushrooms was taken up to evaluate the nutritional properties and health benefits of mycophagy. The area is inhibited predominantly by tribal population and the forest serves as a natural source for many fruits and vegetables which also includes mushrooms. The knowledge about edibility of many wild mushrooms may not be ascertained only with traditional technique of identification. There shall always be a risk for the common people who collects and sells wild mushrooms in market. The awareness on the nutritional status and identification will be of immense help in growth of the mycophagal practice. These shall help to mitigate protein deficiency. The current study includes collection of five commonly consumed mushroom of the area with detail study on their taxonomy and nutritional attributes. The mushrooms which are often available in market include *Volvariella volvacea*, *Termitomyces heimii*, *Lentinus sajor-caju*, *Chlorophyllum hortense* and *Cantharellus subamethysteus*.

The *V. volvacea* is favoured for its taste and texture in this region gaining much popularity. There are 136 records (http://www.indexfungorum.org/) from the world which includes 89 species and the rest are synonym or subspecies. It is locally known as mushroom from straw. Paddy cultivation is an extensive agricultural practice in this region and the agro waste is stored as fodder after harvest. During rainy season the straw starts decaying and *V. volvacea* grows naturally on it. This mushroom is easily identified due to presence of volva and its umbrella like shape. *V. volvacea* is morphologically related to *Volvariella dunensis*, but can be distinguished due to difference in their growing seasons. *V. volvacea* grows during summer on organic rich substrate while *V. dunensis* grows on sand dunes during winter. The other differentiating characters include its cystidia, which is fusiform and clavate in *V. volvacea* whereas in *V. dunensis* it is clavate and obovoid. *Volvariella dunensis* have whitish volva compared to *V. volvacea* which have well developed grey volva (Justo & Castro, 2010).

Termitomyces genus has 92 records (http://www.indexfungorum.org/) from which 80 are ranked as species and the rest includes synonym. *Termitomyces heimii* is available during summer. It grows in association with termites. The locals believe that thundering induces its fruiting and are often collected after a rainy day. *Termitomyces heimii* have a long

pseudorrhiza which extends to the termite nest and often have umbo which helps them in growing through the soil. They have hollow stipe. *T. heimii* resembles *T. letestui* in many morphological characters but can be differentiated by the presence of greyish brown umbo and it can also be distinguished from *T. eurrhizus* by the presence of long dark and solid pseudorrhiza which is hollow and light coloured in *T. heimii*.

The genus Lentinus have 639 records worldwide (http://www.indexfungorum.org/) of which 293 are accepted as species. *Lentinus sajor- caju* is another mushroom which is found in dead and decaying logs during rainy seasons. They are wood rotting fungi, they have fleshy stipe often centrally located and sometimes accentric and hard fruiting body. *L. sajor-caju* is very closely related to *Pleurotus sajor-caju* morphologically and microscopically but it can be distinguished by the presence of dimetic hyphal system and hyphal pegs in hymenophoral trama. The basidiospores are also larger and elongated in *L. sajor-caju*. The other morphological difference includes the presence of annulus in *L. sajor-caju*.

Chlorophyllum includes 33 records (http://www.indexfungorum.org/) from which 28 are accepted as species. *Chlorophyllum hortense* is a medium sized mushroom having saprophytic mode of nutrition growing generally on dung. They are often found growing in kitchen gardens filled with manures. They are composed of thin and long stipe with pileus having bulbous structure which opens up with maturity and have whitish remnants of veil. The current specimen resembles with *Leucoagaricus carmenescens* with all the characters but the molecular data supports it to be *C. hortense.* It was first described by Murrill in 1914 later in 2002, Vellinga placed it in Chlorophyllum. It shares many characters with Macrolepiota and Leucoagaricas. Clamp connection was not observed in this specimen as described by Vellinga, 2003. It also differs from previous description in having tertasterigmate basidia (Peglar, 1983; Vellinga, 2003; Vizzini *et al.,* 2014).

Cantharellus genus have 588 records worldwide (http://www.indexfungorum.org/) among which 337 have been given species rank and the rest includes synonym or subspecies. *Cantharellus subamethysteus* is also a commonly available mushroom. They are collected from the community managed forests dominated by *Shorea robusta*. They form ectomycorrhizal relation with *Shorea robusta* and fruits during summer. They are identified by their yellowish egg yolk colour. There is no proper identification, the collection comprises of many mixed species. *Cantharellus subamethysteus* was first described by Eyssartier and Stubbe from the rainforest of Malaysia in 2009. The present specimen can be differentiated

from *C. amethysteus* in having larger fruit body and smaller spore. *C. appalachienses* have larger spore and brownish disc. *Cantharellus subamethysteus* differs from *C. elongatipes* in having larger cap diameter, *C. himalayensis* can be differentiated from *Cantharellus subamethysteus* due of its smaller stipe and differs from *C. lateritius* for having smaller basidia.

A study on any species is incomplete without proper taxonomy. Fungi being among the most diverse and underexplored group with many overlapping characteristics, a detail and reliable taxonomic approach becomes much more important. The difficulty in fungal taxonomy due to their shared characters can be surmounted by using molecular techniques. With the development of molecular techniques, molecular systematic have become easily accessible. The ability of polymerase chain reaction to amplify any sequence and development of fungal specific universal primers has become very useful and important in such studies. Currently molecular markers and techniques like RAPD, AFLP, and RFLP along with analysis of DNA sequences from internal transcribed spacer (ITS) regions, nuclear small subunit (nSSU), large subunit (nLSU) and mitochondrial small subunit are widely used. The study employing morphological and molecular techniques will result in reliable fungal identification (Raja *et al.,* 2017). In the current study, the genomic DNA was amplified targeting Internal Transcribed Spacer (ITS) region and nuclear Large Subunit (nLSU) using PCR based assay. The amplified PCR products were then sequenced and the data obtained were submitted to GenBank. The PCR amplification of *V. volvacea* with primers ITS1 and ITS 4 resulted in a sequence length of 826 bp (SSU and LSU partial sequence and complete sequence of ITS1, 5.8S and ITS 2 region). ITS1 and ITS4 primer amplification of *Termitomyces heimii* resulted in 703 bp (SSU & LSU partial sequence and ITS1, 5.8S, ITS 2 complete sequence). In *Lentinus sajor-caju* PCR amplification resulted in 576 bp (ITS1 & LSU partial sequence and complete sequence of 5.8S and ITS 2 region). The sequence obtained was 488 bp with partial sequence of 5.8S & LSU and complete sequence of ITS 2 region in *Chlorophyllum hortense*. LR05 and ITS4R primers were employed for amplification of *Cantharellus subamethysteus*, sequence length of 906 bp with partial sequence of LSU was obtained.

The proximate analysis of the wild mushrooms *V. volvacea* showed to have moisture content of 90.27%, ash content of 5.08%, fat content of 2.14%, protein content of 29.76% and total sugar was within 33.2%. The moisture content of *V. volvacea* have been reported to range from 90-96% by different authors (Roy *et al.,* 2014; Adedokun *et al.,* 2013; Paisey *et*

al., 2015; Nhi *et al.,* 2012). The current sample showed lower fat and carbohydrate content than the report of Roy *et al.,* (2014). The report of Adedokun *et al.,* (2013) is higher in ash and moisture content and lower in protein, carbohydrate and fat content. The current study is comparable to the reports of Paisey *et al.,* (2015), except they found higher ash content.

The fat content of *V. volvacea* has been found to range from 1-10.6% from the works of different authors (Roy *et al.,* 2014; Adedokun *et al.,* 2013; Paisey *et al.,* 2015; Mshandete & Cuff, 2017; Crisan & Sands, 1987; Nhi *et al.,* 2012; Chang & Miles, 1997; Adejumo *et al.,* 2015; Salamat *et al.,* 2017; Brinda *et al.,* 2017).

From the available literature it has been seen that the ash content in *V. volvacea* falls within the range of 0.3-16.1% (Adedokun *et al.,* 2013; Paisey *et al.,* 2015; Mshandete & Cuff, 2007; Nhi *et al.,* 2012; Adejumo *et al.,* 2015; Salamat *et al.,* 2017; Brinda *et al.,* 2017).

The protein content of *V. volvacea* has been found to vary among the reports of many previous authors. Which falls within the range of 20-43% (Roy *et al.,* 2014; Adedokun *et al.,* 2013; Paisey *et al.,* 2015; Mshandete & Cuff, 2007; Crisan & Sands, 1987; Nhi *et al.,* 2012; Chang & Miles, 1997; Adejumo *et al.,* 2015; Salamat *et al.,* 2017; Brinda *et al.,* 2017)?

The carbohydrate content of *V. volvacea* have been reported to fall within the range of 20-66% (Roy *et al.,* 2014; Adedokun *et al.,* 2013; Paisey *et al.,* 2015; Mshandete & Cuff, 2007; Nhi *et al.,* 2012; Chang & Miles, 1997; Adejumo *et al.,* 2015; Salamat *et al.,* 2017; Brinda *et al.,* 2017).

The carbohydrate and ash content is lower in the current study when compared to Mshandete and Cuff, (2007). The current study agrees with the range of protein content but have lower fat content than the reports of Crisan and Sands, (1987). Except for carbohydrate and ash content the results are similar to the reports of Nhi *et al.,* (2012). The protein and ash content falls within the range but carbohydrate content is lower in the current study compared to the report of Chang and Miles (1997).

The result of current study varies significantly from the reports of Adejumo *et al.,* (2015). The results are comparable except for low carbohydrate content in the current study from the report of Salamat *et al.,* (2017). The current study found higher carbohydrate content and low protein and fat content as compared to Brinda *et al.,* (2017).

Termitomyces heimii have moisture content of 88.32%, ash content of 4.07%, fat content of 1.54%, protein content of 35.37% and carbohydrate content of 20.4%. Singha *et al.,* (2017) reported Termitomyces species to have 76-88% moisture, protein content of about 33.2-38.3%, fat content of 0.8-6.2%, ash content of 15% and carbohydrate content of 33.2-48.4%. The current study is low in ash and carbohydrate content. The current results also differs from the results reported by Ijioma *et al.,* (2015) and Atri *et al.,* (2014) where it was reported carbohydrate content of 36.2%, fat content of 1.65%, protein content of 40.95% and ash content of 8.6%. Protein, fat, ash and moisture content are comparable but carbohydrate content differs from the results reported by Johnsy *et al.,* (2011). The current result also differs from the results presented by Due *et al.,* (2016) with regard to protein, carbohydrate, fat and ash. The protein content is higher in current study and all other parameters are lower in current study.

Lentinus sajor-caju was found to have 89.5% moisture, 4.5% ash, 1.02% fat, protein content of 52% and carbohydrate content of 46.6%. The results are significantly different from the reports of Sharma *et al.,* (2015). The results also differ in protein and carbohydrate content from the reports of Reneses *et al.,* (2016). The ash and fat content are similar to the current report. The reports of Arvind *et al.,* (2014) vary from the current result. Apart from carbohydrate content all other result differs from the reports of Oyeleke *et al.,* (2017). All other results except the ash content differ in the current study from the reports of Singdevsachan *et al.,* (2013).

The protein content of *Lentinus sajor-caju* has been reported within the range of 1-28 % by various authors (Sharma *et al.,* 2015; Reneses *et al.,* 2016; Arvind *et al.,* 2014; Oyeleke *et al.,* 2017; Singdevsachan *et al*., 2013). The carbohydrate content of *Lentinus sajor-caju* have been reported to range from 47-85% by previous authors (Singdevsachan *et al.,* 2013; Oyeleke *et al.,* 2017; Arvind *et al.,* 2014; Reneses *et al.,* 2016; Sharma *et al.,* 2015).

The ash content of *Lentinus sajor-caju* have been reported to range from 1.91-5.30% by different authors (Singdevsachan *et al.,* 2013; Oyeleke *et al.,* 2017; Arvind *et al.,* 2014; Sharma *et al.,* 2015). Fat content in *Lentinus sajor-caju* ranged from 0.8-2.78 % as reported by various authors (Singdevsachan *et al.,* 2013; Oyeleke *et al.,* 2017; Arvind *et al.,* 2014; Sharma *et al.,* 2015). The moisture content of *Lentinus sajor-caju* have been reported to fall within the range of 80-90% from the reports of Singdevsachan *et al.,* 2013 & Oyeleke *et al.,* 2017.

Chlorophyllum hortense is found to have 90.11% of moisture, 5.02% of ash content, 3.21% of fat content, protein content of 36.6% and carbohydrate content of 22.4% in the present study. There are no records on the biochemical and proximate analysis of this species.

Cantharellus subamethysteus is found to have 91.7% moisture, 5.3% ash content, 3.18% fat content 36.6% protein and 22.4% and carbohydrate content in the present study. This is the first report on biochemical evaluation of this species and the results are comparable to the results of same genus as reported by Kumari, (2011).

Phenols are secondary metabolites in many plants and mushrooms. Chemically phenols are compounds with aromatic ring having one or more hydroxyl substitutes with derivative such as esters, glycosides and methyl esters (Harborne, 1989). The phenolic content found in the current study is 33.71 µg/mg, 27.38 µg/mg and 25.54 µg/mg in aqueous, ethanolic and methanolic extracts of *V. volvacea* respectively. Punitha *et al.,* (2014) reported 53.13 µg GAE/mg in methanolic extract and 36.67µg GAE/mg in aqueous extract which is higher than the current study. Bedi *et al.,* (2017) reported total phenolic content of 10.74 µg GAE/mg in extract of *V. volvacea*. Nhi *et al.,* (2012) reported free and bound phenolic of 4122.7 µg GAE/mg and 190.6 µg GAE/mg from *V. volvacea*. Suddha *et al.,* (2012) also reported 10.05 mg/g dry weight of phenolic compounds which is much lower than the current results.

In *Termitomyces heimii* the phenolic content in different extracts were 24.41 µg GAE/mg, 18.09 µg GAE/mg and 12.47 µg GAE/mg in aqueous, ethanolic and methanolic extracts respectively. Puttaraju *et al.,* (2006) reported phenolic content of 37.0 mg GAE/g in aqueous and 11.2 mg GAE/g in methanolic extract. The results are similar in methanolic extract but differ in aqueous extract. Atri *et al.,* (2014) reported phenolic content of *Termitomyces heimii* to be 21.32 mg/g of extracts which is nearly similar to the current result. From all the results it is evident that phenolic content are higher in aqueous extract.

The highest phenolic content was found in *Lentinus sajor-caju* among the studied samples with 59.13 µg GAE/mg, 53.46 µg GAE/mg and 32.3 µg GAE/mg in aqueous, ethanolic and methanolic extracts respectively. Singdevsachan *et al.,* (2013) reported phenolic content of 36.26 mg/g, 22.25 mg/g and 18.6 mg/g in aqueous, methanolic and ethanolic extracts respectively the results are lower than the current study. Sharma *et al.,* (2014) reported 8.83 mg/g of phenolic contents. Acharya *et al.,* (2017) reported 7 µg GAE /mg of phenolic content which is lower than the current result.

The phenolic content of *Chlorophyllum hortense* was found to be 37.12 µg GAE/mg 21.25 µg GAE/mg and 23.5 µg GAE/mg in aqueous, ethanolic and methanolic extracts respectively and *Cantharellus subamethysteus* have the phenolic contents of 24.66 µg GAE/mg, 17.57 µg GAE/mg and 18.5 µg GAE/mg in aqueous ethanolic and methanolic extracts respectively.

Flavonoids are phenolic compounds with strong antioxidant activity. They are widespread in many plants and vegetables. Flavonoids are the main compounds in colour and aroma of plants. There are six main subclasses and are known to have potential health benefits like UV protection, antibacterial, anti-lipoperoxidant, anti-mutagenic, anti-carcinogenic and their ability to amend many cellular functions. The studied mushrooms are also found to hold certain flavonoids content. The flavonoid content was higher in ethanolic extracts in *V. volvacea, L. sajor-caju* and *C. hortense*. The aqueous extracts had higher content in *T. heimii* and *C. subamethysteus*. The highest content of flavonoid was recorded in *L. sajor-caju* ethanolic extract with 44.8 µg QE/mg of dried extract and the least was found in *C. subamethysteus* with 6.5 µg QE/mg of dried extract. Bedi *et al.,* (2017) reported 1.43 mg QE/g in *V. volvacea*. Punitha *et al.,* (2014) reported flavonoid content of 14.35 mg/g in methanolic extract and 12.54 mg/g in aqueous extract in *V. volvacea*. Sudha *et al.,* (2008) reported flavonoid content of 6.92 mg/g dry weight in *V. volvacea*. Singdevsachan *et al.,* (2013) reported flavonoid content of 8.51 mg QE/g in *L. sajor-caju*.

Reactive oxygen species are required for normal cellular functions but when in higher concentration they are harmful causing mutations, cancer, aging, degenerative diseases etc (Gulcin *et al.,* 2004; Sachindra *et al.,* 2010).

The mushroom extracts were studied for its antioxidant activity by ABTS radical scavenging activity, DPPH radical scavenging activity, ferric reducing antioxidant potential, nitric oxide scavenging activity and superoxide radical scavenging activity. These are the standard techniques to analyse the potential of extracts to scavenge free radicals. The results suggested that mushrooms are good antioxidant and this attributes to the therapeutic value of mushrooms apart from its nutritional values. The results also suggest that *Lentinus sajor-caju* has the best antioxidant potentials among the studied species. The aqueous extracts were better in FRAP assay and DPPH scavenging activity except in *Chlorophyllum hortense* where methanolic extract showed better inhibition of DPPH radicals.

Lentinus sajor-caju showed better results in ABTS assay, *V. volvacea* had better activity against superoxide radicals and nitric oxide free radicals. *V. volvacea* is favoured the most among the wild species in this region and thus will be helpful in combating oxidative stress.

To be a good nutritious diet a food should include all the essential components in a balanced amount apart from protein and carbohydrate content. The presence of adequate amount of essential amino acid is necessary. Mattila *et al.,* (2002) reported that mushrooms are good source composed of roughly all essential amino acids. The wild mushrooms in the current study comprises all the essential amino acid except lysine and valine in *V. volvacea*, valine in *Termitomyces heimii*, isoleucine in *Chlorophyllum hortense* and leucine in *Cantharellus subamethysteus*. Among the samples *Cantharellus subamethysteus* had the highest percent of essential amino acids. Arora *et al.,* (1991) and Crisan and Sands, (1987) reported all the essential amino acids from *V. volvacea*. Sharma *et al.,* (2014), Afiukwa *et al.,* (2015) and Atri *et al.,* (2017) reported the amino acid content of *Lentinus sajor-caju*. Histidine, arginine, glutamic acid and serine were not detected in any of the samples.

Mushrooms have low fat content and the fatty acid is dominated by unsaturated fatty acids (Huang *et al.,* 1989; Sharma *et al.,* 2014). In the present study except in *Termitomyces heimii* all other samples were having higher content of unsaturated fatty acid with higher content of linoleic acid except in *Chlorophyllum hortense* where oleic acid was present in higher content. Some fatty acid like lauric acid, palmitic acid, linoleic acid, linolenic acid, oleic acid, stearic acid and myristic acids have also been reported to have antibacterial and antifungal activities (Agoramoorthy *et al.,* 2007; McGaw *et al.,* 2002; Seidel & Taylor, 2004). Ergosterol from *Volvariella volvacea* has been reported to have resistance against *E. coli* (Perera *et al.,* 2001). Mushroom fatty acid profile is predominated by palmitic acid, stearic acid, linoleic acid and oleic acid, (Kalac, 2009; Lee *et al.,* 2011; Ruess *et al.,* 2002; Senatore *et al.,* 1988) which is in compliance with the results of the present study. Linoleic acid is an essential fatty acid which is a precursor of prostaglandins, thromboxanes and leukotriene. It is known to have anti-inflammatory, anti-hypertensive and anti-artherosclerotic activities (Das, 2006). Oleic acid enhances wound healing and helps in drug absorption. It has anti-inflammatory, antimicrobial, anticancer properties. It also plays an important role in attenuation of autoimmune diseases (Sales- Campos *et al.,* 2013). The presence of these three types of fatty acids i.e. SFA, MUFA and PUFA in wild edible mushrooms, namely *Agaricus bisporus*, *A. silvaticus*, *A. silvicola*, *Boletus edulis*, *Calocybe*

gambosa, *Canthrellus cibarius*, *Craterellus cornucopioides* and *Marasmius oreades* has also been demonstrated by Barros *et al.,* (2008). Wani *et al.,* (2010) documented that the fats present in mushrooms are dominated by unsaturated fatty acids. As documented by Barros *et al.,* (2008) in these species the percentage of SFA ranged from 14.52 - 22.63%, MUFA ranged from 1.52 – 59.85% while the percentage of PUFA ranged from 23.79 – 76.95%.

Mushrooms are reported to have good content of minerals by different authors (Kreula *et al., 1976;* Bano *et al.,* 1982; Chang *et al.,* 1982; Li and Chang, 1982; Bano and Rajarathanam, 1982; Chang, 1982). In the current study magnesium was found to be in higher content which agrees with the previously reported ranges. Apart from Mg, the other minerals like Fe, Zn and Mn have been found to be in considerable amount with trace amount of Co and Ni. Ni has been known to increase iron absorption and prevent anaemia. It is also known to strengthen bones (Kumar *et al.,* 2016). Several enzyme complex uses nickel for its function (Krajewska, 2012). Nickel is also helpful in radical scavenging and its deficiency causes liver and kidney diseases in some individuals (Kumar *et al.,* 2016).

GC-MS analysis of the wild samples was carried out to access the bioactive components of mushroom species. The results reveals that the mushrooms have many compound which includes fatty acids, terpenoids, phenolic acids, alcohols, steroids and compounds with variable biological activities. Bioactive components from mushrooms have antioxidant properties (Ferreira *et al.,* 2009). The volatile compounds from mushrooms have been reported to have antimicrobial activity (Johnsy *et al.,* 2015).

Fungi and animals share a close relation (Redecker, 2000). Fungi is gifted with innate protection against common antagonists microbes of human by producing antibiotics against organisms like *E. coli, S. aureus and P. aeruginosa* (Ranadive *et al.,* 2013). Four extracts viz, aqueous, ethanolic, methanolic and petroleum ether extracts were studied against five bacterial species with three gram negative and two gram positive bacterial species. The *Escherichia coli* was inhibited maximum by ethanolic extract of *Termitomyces heimii* with zone of inhibition of 16.15 mm. *Lentinus sajor-caju* showed highest activity with petroleum ether extract against *Bacillus cereus* with zone of inhibition of 13.3 mm. *Proteus vulgaris* was inhibited maximum by petroleum ether extract of *Chlorophyllum hortense* with zone of inhibition of 13.06 mm. *Lentinus sajor-caju* ethanolic extract had maximum potential against the growth of *Klebsiella pneumonae* with zone of inhibition of 14.04 mm. Petroleum ether extract of *Chlorophyllum hortense* was most potent against *Staphylococcus aureus* with zone of inhibition of 12.99 mm. Many authors have reported the antimicrobial potential of

mushrooms against different microbes. Iwalokun *et al.,* (2007) studied *Pleurotus ostreatus* against eight gram positive and eight gram negative bacteria. Benedict *et al.,* (1972) studied mushroom metabolites against five bacterial species. Kalyonchu *et al.,* (2010) studied mycelia of ten mushrooms against eleven microbes. Jonathan *et al.,* (2005) studied six mushrooms against six bacterial species. Alvis *et al.,* (2012) in his review reported antimicrobial activity of different mushroom extracts against microbes and concluded that extracts were having greater potential against gram positive bacteria than gram negative bacteria. Smolskaite *et al.,* (2015) studied eight mushroom species against two microbes. Ramesh *et al.,* (2010) reported the antimicrobial potential of six mushrooms against four microbes.

Drug discovery is an expensive, comprehensive & cumbersome process. It includes identification of molecules, target selection, target validation, optimization and clinical trials. Computer aided drug design have been helpful by reducing cost and time required for a new drug design. It involves simulation of structure of biomolecules with different compounds available. It can screen numerous molecules against a specific target using mathematical models. The compound or ligand that are complimentary in their structure or charge will tend to interact and bind to target which will in turn activate or inhibit its function. The screening of molecules involves its properties of drug likeliness, toxicity, metabolism, absorption and distribution. When molecules qualify with these properties, they are docked with the target to find its affinity and structural relatedness with the binding site or interaction on the basis of different bonding and energy involved. Thus knowledge about target molecules and functions associated with it can be used for drug discovery.

AcrAB-TolC is a tripartite protein of resistance nodulation cell division (RND) family efflux pump of gram negative bacteria. It's over expression results in efflux of multiple drugs out of the cell developing multi drug resistant strains. AcrB subunit is a periplasmic unit and acts by binding the foreign compounds. The recognition is through deep binding pocket with hydrophobic trap. Thus it becomes an important target for drug discovery. In the current study the compound 9-(2',2'-Dimethylpropanoilhydrazono)-3,6-dichloro-2,7-bis-[2-(diethylamino)-ethoxy]fluorine which was detected from *T. heimii* interacted with the protein with docking score of -11.78 and the binding free energy of -98.86. There was no hydrogen bond but the interacting force included lipophilic interaction and VdW force. The interacting residues were 787, 620, 591,575,329,175, 668, 666, 630-625, 620, 617- 608, 574-571, 328-324, 292-277, 179-176, 142-134 which falls in the active site of the protein. Previous study

has developed a molecule MBX3135 which was effective with binding energy of -52.7. The interaction involved residues 136, 139, 178, 277, 279, 287, 326, 327, 331, 333, 616, 612, 615, 617 and 620 (Sjuts *et al.,* 2016). This compound can be a good target for further evaluation for drug designing. .

The development of drug resistance outraced new antibacterial drugs. Study on the mechanism of inhibition can be of great importance. A new drug or chemical compounds that can inhibit the drug targets in the binding site, one of such target for antimicrobial drug is DNA gyrase which belongs to Type II family of Topoisomerase that controls replication. It is a tetramer of subunits GyrA and GyrB. GyrB couples with ATP hydrolysis to negatively super coil DNA. Inhibition of GyrB results in disruption of replication and cell death (Sherer *et al.,* 2011). Till now two classes of antibiotics are administered this includes fluoroquinolones and aminocoumarins. In the current study the compounds Emicymarin, Ethyl iso-allocholate, Penicillamine, Dehydroergosterol 3,5-dinitrobenzoate, 1,6,10,14,18,22-Tetracosahexaen-3-ol,2,6,10,15,19,23-hexamethyl-, 2-[[2-[(2-ethylcyclopropyl)methyl] cyclopropyl] methyl]-, 9-(2',2'-Dimethylpropanoilhydrazono)-3,6-dichloro-2,7- bis- [2-(diethylamino)-ethoxy]fluorine have been found to be interacting with the protein with docking scores of -5.66 ΔG 4.59, -5.33 ΔG 13.8, -5.20 ΔG -6.61, -5.05 ΔG -54.46, -5.04 ΔG -46.79, -5.02 ΔG -22.31 and -4.75 ΔG 52.12 respectively compared to docking score of -7.02 ΔG -64.6 with the standard ligand **2-[(3S,4R)-4-{[(3,4-dichloro-5-methyl-1H-pyrrol-2-yl)carbonyl]amino}-3-fluoropiperidin-1-yl]-1,3-thiazole-5-carboxylic acid. The docking scores were lower but considerable free energy of binding have been found and** due to the presence of many interacting compound with their additive and synergistic effect wild edible mushrooms will prove helpful in combating infection.

Dehydrofolate reductase is an enzyme in metabolic process. Currently compounds like methotrexate, pyrimethanine, trimethoprim are used. Dehydrofolate reductase helps in reducing dihydrofolic acid to tetrahydrofolic acid. The inhibition of this enzyme disrupts the production of thiamine and stops rapidly dividing cells. Apart from good target for antimicrobial drugs, it is also a good target for anticancer drugs (Withlow *et al.,* 1997). Dihydropteroate synthetase is an intermediate enzyme in this metabolic pathway which catalyses the condensation of 6-hydroxymethyl-7, 8-dihydropteridine pyrophosphate to para-aminobenzoic acid to form 7, 8-dihydropteroate which further forms 7, 8-dihydrofolate and is also a good target for antimicrobial drugs (Levy *et al.,* 2008). The protein Dihydropteroate synthetase which is an intermediate enzyme in this pathway is inhibited by compounds

Emicymarin with docking scores of -5.40 ΔG -41.04 compared to -7.36 in the standard ligand. The binding energy was -41.04 without penalties compared to -28.76 in standard ligand. Other compounds were Razoxane with docking scores of -4.05 ΔG -33.04, Penicillamine with docking scores of -3.70 ΔG -11.04, Ergosta-4,6,22-trien-3.alpha.-ol with docking scores of -3.68 ΔG -31.89, Citric acid , trimethyl ester with docking scores of –3.63 ΔG –23.93, Ritalin with docking scores of –3.47 ΔG -31.57, Ethyl iso-allocholate with docking scores of -3.08 ΔG –34 compared to **pterin-6-yl-methyl-monophosphate with docking score of -7.36 and binding energy of -28.76.** The standard ligands had a state penalty whereas Emicymarin did not have state penalty and can be useful to block the Dihydropteroate synthetase.

Penicillin binding protein (PBP) is a periplasmic enzyme of gram negative bacteria which works in cross linking peptidoglycan matrix to form cell wall and maintain integrity, PBP1a is a transglycosylase and PBPb is a transpeptidase. Access to these periplasmic targets is complicated due to the presence of outer wall which excludes β-lactam type antibiotics, if the expression of specific porin channel is down regulated. Thus a fatty acid like molecules will have greater access to the protein which can penetrate the cell wall (Hans *et al.,* 2011). In the current study the compounds like Phthalic acid, butyl undecyl ester with docking score of -5.78 ΔG -16.49, Emicymarin with docking score of -5.69 ΔG -20.79, Ethyl iso-allocholate with docking score of -4.76 ΔG -29.75, Penicillamine with docking score of -4.71 ΔG -18.01, 9-(2',2'-Dimethylpropanoilhydrazono)-3,6-dichloro-2,7-bis-[2-(diethylamino)-ethoxy]fluorine with docking score of –4.62 ΔG -11.33, Citric acid, trimethyl ester with docking score of -4.58 ΔG -25.26, Ritalin with docking score of -4.16 ΔG -22.06 were found to be interacting with the protein with interacting residues 712, 711, 701, 693, 682, 465, 710-702, 681-669, 649, 540, 528-523, 492, 490-483, 472-467, 441, 438-431. The standard ligand previously reported had docking score of -6.33 which is comparable to the compounds from mushrooms but the free energy of binding is very high in **Penicillin G with -146.6. Due to the fatty acid nature of some compounds from mushrooms that are interacting, will be able to penetrate the lipid bilayer more easily and with further synergistic effect of many different compounds with considerable docking score and binding energy it will be helpful in blocking this protein.**

Isoleucyl-tRNA synthetase is a cytoplasmic enzyme which catalyzes the aminoacylation of tRNA. Its inhibition disrupts the ability to link amino acid to the growing

chain and stalling translation finally killing the cells. Currently Mupirocin is in use (Nakama *et al.,* 2001). The standard ligand **Mupirocin previously studied was found to have docking score of -9.74 and ΔG free energy of binding of -52.76 compared to compounds from mushrooms** Emicymarin with docking score of -6.83 ΔG of -44.78, Ethyl iso-allocholate with docking score of -6.67 ΔG of -43.52, 1,6,10,14,18,22-Tetracosahexaen-3-ol with docking score of -6.45 ΔG of -57.23, Ergosterol with docking score of -6.63 ΔG of -34.08, 9(11)-Dehydroergosteryl benzoate with docking score of -5.87 ΔG of -47.25, Tricosanoic acid, methyl ester with docking score of -5.07 ΔG of -57.27. The study found that though the docking scores are lower than the standard drug currently in use, few compounds have higher free energy of binding and those compounds can be useful in potential drug designing approach.

D-Alanine-D-Alanine ligase is an enzyme that condenses two D-Ala molecules using ATP to produce D-Ala-D-Ala which is a terminal peptide in peptidoglycan monomers. Previously β-lactam antibiotics were used but with development of Multi Drug Resistant strains like MRSA which resist β-lactam family drugs, Vancomycin was used later. Vancomycin forms hydrogen bond with D-Ala-D-Ala inhibiting peptidoglycan synthesis. Bacteria subsequently evolved to replace D-Ala-D-Ala with D-Ala-D-lactate which resisted the binding of vancomycin to the terminal peptide (Katamura *et al.,* 2009). The evaluation of compounds from mushroom for their affinity and ability to bind to this enzyme showed that compounds like Benzaldehyde, 2,4-dimethyl- with docking score of -7.20 and Δ G of -35.57, Razoxane with docking score of -7.10 and Δ G of -36.1, Benzenepropanoic acid, methyl ester with docking score of -6.83 and Δ G of -41.18, Ritalin with docking score of -6.65 and Δ G of -39.18, 2-[[2-[[2-[(2-pentylcyclopropyl) methyl] cyclopropyl] methyl] cyclopropyl] methyl]- , methyl ester with docking score of -6.38 and Δ G of -28.56, Linoleic acid with docking score of -5.84 and Δ G of -36.73, Penicillamine with docking score of -5.54 and Δ G of -6.54, 6,9,12,15-Docosatetraenoic acid, methyl ester with docking score of –5.52 and Δ G of -32.46 as compared to adenosine-5'-triphosphate with docking score of -12.77 and free energy for binding of 22.58. The affinity for binding was greater with the standard ligand but the free energy is very low and the compounds from mushrooms had higher free energy and also considerable binding affinity to the protein and the compounds can be better option in blocking this enzyme.