ANTIOXIDANT AND ANTIMICROBIAL POTENTIAL OF WILD EDIBLE MUSHROOMS *LYCOPERDON PYRIFORME, ARIMILLARAIA TABESCENS* AND *AGARICUS BISPORUS* COLLECTED FROM FOOTHILLS OF EASTERN GHATS NEAR PONNAI VILLAGE, VELLORE DISTRICT

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Abstract : The extractive values for fruiting bodies of mushrooms *Lycoperdon pyriforme, Arimillaraia tabescens* and *Agaricus bisporus* in different solvents such as petroleum ether, benzene, chloroform, acetone, hexane ethyl acetate, water, ethanol and methanol were analysed to identify the suitability of solvents for extraction of maximum phytocompounds. The obtained results revealed the methanol gives maximum extractive value for all three mushrooms when compared to any other solvents. The qualitative analysis of phytocompounds for petroleum ether, benzene, chloroform, acetone, hexane ethyl acetate, ethanol, and methanol and water extracts of fruiting bodies of all three mushrooms showed the presence of tannins, saponin, flavonoids, glycosides, phenols, alkaloids and terpenoids in rich amount. However, their presence and absence is depends the solubility nature of phytocompounds in different solvents. The quantitative analysis of phytocompounds revealed the presence of flavonoid, alkaloids and tannins in maximum amount in all three mushrooms. Particularly, the flavonoid is in first place which were recorded as 2.4±0.19 mg/100g for *Lycoperdon pyriforme*, 2.0±0.15 mg/100g for *Arimillaria tabescens* and 2.7±0.21 mg/100g for *Agaricus bisporus.* The GCMS analysis exposed the existence of phytocompounds methyl 3-Bromo-1-adamantaneacetate (% of peak area 5.76 and 13.14 with RT 25.913 and 26.213), Trimethyl [4-(1,1,3,3,-Tetramethylbutyl) Phenoxy] Silane(% of peak area 7.913 with RT 26.773), Cyclotrisiloxane, Hexamethyl- (% of pea area 6.455 with RT 27.268) in *Lycoperdon pyriforme;* Bicyclo [2.2.1] Heptan-2-One,1,3,3-Trimethyl- at RT 15.472, 11-Hydroxy-11-Methyl-Tricyclo[4.3.1.1(2,5)] Undecan-10-One at RT 16.804, 13-Octadecenal at RT 19.560, 9,10-Secocholesta-5,7,10 (19) -Triene-1,3-Diol, 25-[(Trimethylsilyl) Oxy]- at RT 23.627,3-Isopropoxy-1,1,1,5,5,5-Hexamethyl-3-(Trimethylsiloxy) Trisiloxane at RT 26.028 and Hentriacontane at RT 26.278 and 26.938 in *Armillaria tabescens;* Paredrine (RT27.398 and area % 33.426), Cyclotrisiloxane, Hexamethyl (RT 27.483 and area % 30.769), Paredrine (RT 27.483 and area% 19.918) and Hexestrol Di-Tms (RT 28.769 and area %15.896) in *Agaricus bisporus.* The methanol extract of all three mushroom have considerable level of scavenging activity against DPPH,ABTS,FRAP, Hydroxyl radical, Superoxide and Nitric oxide at concentrations 25, 50,100,,250 and 400 µg/ml. These radical scavenging activities were dose dependent and directly proportionate to the concentration of plant extract.The antimicrobial activity of methanol extract of all three study mushroom tested against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Vibrio cholera* exhibited consider level of zone of inhibition.

Key words - Antioxidents, Free radicals scavenging, antimicrobial, mushrooms, phytocompounds.

I. Introduction

Mushrooms have been part of the human culture for thousands of years and a part of the human diet for thousands of years (Cooke, 1977) and have considerable interest in the most important civilizations in history because of their sensory characteristics. For centuries, they are valued as health food, an “elixir” of life in Chinese culture. By Romans mushrooms are believed to have properties that could produce super human strength, help in finding lost objects and lead the soul to the realm of the gods (Grube, et al. 2001). From the period of time immemorial, the ancient people has given a special place for mushroom in their day to day life because of having rich in protein, minerals and vitamins (Mattila, et al. 2001 and Rudawska and Leski, 2005), amino acids, ascorbic acid, glycogen, lipid, sugar and vitamins and minerals such as calcium, iron, phosphorus, potassium, sodium, magnesium and zinc (Moa and Jica, 2000, Oei, 2003, Jonathan, et al. 2011 and Kumari, et al. 2011) believed to refresh human body and promotes longevity (Ying, et al. 1986).

Ever growing world’s population meet need of food security. In the recent years, there a new trend is going among the mycologists to document the nutritional and medicinal properties of mushrooms around the globe. This is because of most of

mushrooms are highly prized for their utilization as nutritional and medicinal foods (Dutta and Acharya, 2014). Wild edible mushrooms are used as herbal medicine to treat wound healing, burning, itching, staunch bleeding eye disorders (pain, redness, conjunctivitis), fever and vomiting, typhoid, frost bites, ear puss, skin infections, curing stomach upset, brain tonic, against anger, constipation, weakness, eczema and mouth freshener (Devi, 2017, Dutta and Achariya, 2014, Kumar, et al. 2014, 2017, Lahiri, et al. 2010, Malik, et al, 2017, Manna, et al. 2014, Panda and Tayung, 2015, Rai, et al. 1993 and Thangaraj, et al. 2017) to cure common illness such as an antidepressant, treating lumbago, leg pains, numbness in limbs and tendon discomfort, nervous system colds, sore throats, sore eyes and stop bleeding (Malik, et al. 2017, Pala, et al. 2013 and Panda and Tayung, 2015) pneumonia, respiratory problems, asthma, jaundice, pox, goiters, diabetes, cancer, aphrodisiac, invigorative, revitaliser, anti-aging, kidney stones and partial paralysis (Kumar, et al. 2014 and 2017, Manna, et al. 2014, Pala, et al. 2013, Panda and Swain, 2011, Panda and Tayung, 2015 and Rai, et al. 1993). The disease curing potentiality of mushroom might be due to the presence of various phytocompounds such as polysaccharides, dietary fibers, selenium oligosaccharides, triterpenoids, peptides, proteins, alcohol, phenols, amino acids and mineral elements (Chang and Buswell, 1996; Chang and Miles, 2002, Lakhanpal, et al. 2010, Wani, et al. 2010 and Wasser, 2010) shikimic acid, aromatic phenols, fatty acid derivatives, polyacetylamine, polyketides, nucleosides, sesterterpenes, and many other substances from different mushrooms (Lorenzen and Anke, 1998, Wasser and Weis, 1999 and Mizuno, 1999). Hence, the present study has been programmed to evaluate the antioxidants and antimicrobial activities of three wild edible mushrooms collected from the foot- hills of Estern Ghats, near Ponnai village, Vellore District.

II. Materials and method

2.1. Collection and identification

The wild edible mushrooms for the present study were collected from foothills of Estern Ghates near Ponnai Village areas. The collections were done regularly from all above-mentioned collection areas during the rainy months from August to November for consecutive two years (2016 to 2018). The information about the edibility, poisonous impact on health, ritual usage and nutraceuticals potential gathered by discussion and direct interview with local people and by direct observation on the way different mushrooms were being collected and used. All the stories gathered during interview of local people were further verified to know their reliability by cross questioning the elders in the village about the key information and already documented literature. Both the poisonous and non-poisonous mushrooms were randomly collected and stored in perforated plastic bags. During collection, the damaged, infected and very young fruit bodies were avoided. Additions to these, during that time collection time, exact location on Google map with colour photograph were recorded. Mushrooms collected from different places or different species were kept separately. The information about the substrate such as decayed wood parts and dead plants remnant or growing ground, soil type, moisture level and air temperature of specimen collected areas were carefully noted. After the specimens were brought to the laboratory, the macroscopic and microscopic properties were analyzed by made a keen observation using Lica DMSL research microscope. All the collected mushrooms were approximately identified by using identification key and literature according to Ramsbottom (1965), Singer (1986), Bessey (1978), Thind (1961), Purkayastha and Chandra (1985) and Roy and De (1996). Then the specimen identification further confirmed and authenticated at species level by sending it to Pro.Dr.Jeyaraman, Department of Botany.

2.2. Preparation of mushroom extracts

The fruiting body of mushroom was washed in running tap water to remove the dirty particles and dust. Finally, they were chopped into small pieces and spread on the paper and dried under shadow places at room temperature. Then the dried pieces were grounder well with the help of electrical grinder. The dried powder (100 g) of each mushroom species was subjected to rigorous extraction soxhlet apparatus. For this extraction, the mushroom powder was packed inside the thimble and the bottom of the soxhlet apparatus filled with ethanol. The extract filled in the thimble was collected and were concentrated using rotary evaporator, which were later completely dried, weighed and kept for further usage in sterilized sealed vials at 4°C. This dried extract was re-diluted at the time of experimentation. The ethanol extract of mushroom was carefully packed inside the glass screwed vial and send to VIT (Vellore Institute of Technology), Vellore for GCMS and FTIR analysis.

2.3. Determination of extractive value

Naturally all the solvents were classified into two types such as polar and non- polar based on their dielectric constant. In the present study both the polar and non-polar solvents were used to find the extractive value for all three wild edible mushrooms such as *Lycoperdon pyriforme, Arimillaria tabescens* and *Agaricus bisporus.* To determine the extractive value, 10 grams of dry powder of fruit body of all three mushrooms were taken separately and soaked in a beaker containing all polar and non- polar solvents such as water, ethanol, methanol, petroleum ether, benzene, chloroform, acetone, hexane and ethyl acetate. Left all the beakers for 48 hours, the supernatant were collected separately from each beaker. The same procedure repeated for three to five times until the soaked powder become discoloration, then, the extracts obtained were dried in room temperature and desiccated the extract under reduced pressure (Kokate, 1994).The extractive value (EV) (%) was calculated by using the following formula

$$EV\ (\%) = \frac{\text{Wt of dried extract}}{\text{Amount of dried powder}} \times 100.$$

2.4. Alkaloid determination

2.4.1. Procedure

1gm of dried mushroom powder was taken in a beaker, along with 6ml of dilute hydrochloric acid was added and boiled sometimes thereafter cooled down and filtered. The filtrate was divided into three portions. Each portion was separately poured into a separate test tube. Then, 2 drops of Dragendorff's reagent were added with the first portion of extract containing test tube. The formation of a red precipitate indicated the presence of alkaloids. Formation of a creamy white precipitate in the second portion of

solution when added 2 drops of Meyer's reagent is indicated the presence of alkaloids. Formation of reddish-brown precipitate in the third portion when added 2 drops of Wagner's reagent is indicated the presence of alkaloids.

2.5. Reducing sugar determination

2.5.1. Fehlings method

5 ml of mushroom extract containing test tubes; 5 ml of mixture of Fehlings solution (A and B) were added and boiled for 5 minutes. Red precipitate formed in the test tube indicated the presence of reducing action of sugar.

2.6. Benedicts Method

5 ml of mushroom extract was taken in a test tube, along with , 5 ml of Benedict's solution was mixed and heated for few minutes. Formation of brick red precipitate confirmed the presence of reducing sugars.

2.7. Flavonoids determination

5 ml of mushroom extract was taken in a test tube, along with 3 of 10% sulphuric acid was added and cooled down. Then this mixture was extracted with ether. Thereafter, the extracted solution was divided into three equal portion and poured into separate test tubes. Then in the test tube containing first portion 1 ml of dilute ammonia, in second portion 1ml of dilute sodium carbonate and in third portion 1ml of sodium hydroxide solution were added respectively. The yellow colour formation in all test tubes indicated the presence of flavonoids.

2.8. Polyphenols determination

5 ml of mushroom extract was taken in a test tube, along with 2ml of Ferric chloride (5%) was added in a test tube. The brown precipitate formation in a test tube indicated the presence of polyphenols.

2.9. Tannin test

The presence of polyphenol tannins in the mushroom was determined by followed the method of Jigna, et al. (2007). 5ml of extract was taken in a test tube along with few drops of ferric chloride was added. The brownish green colour formation in the test tube indicated the presence of tannins.

2.10. Steroids and triterpenoids

2.10.1. Lieberman- Burchard Test

5 ml of mushroom extract was taken in a test tube, along with 1 ml of chloroform, 1 ml acetic anhydride and 2 ml of concentrated sulphuric acid was added. The reddish-violet color developed in the test tube indicated the presence of steroids and triterpenoids.

2.10.2. Salkowski test

5 ml of mushroom extract was taken in attest tube, along with 1 ml of chloroform and 1 ml of concentrated sulphuric acid was added. Formation of reddish blue coloured chloroform layer and green coloured fluorescent acid layer indicated the presence of steroids.

2.11. Saponins test

A test tube containing 5ml of mushroom extract was boiled in a water bath for few minutes and shacked vigorously for 1minutes. The froth formed inside the test tube and emulsion formed when 3 drops olive oils added with this froth indicated the presence of saponin. (Harborne,1973)

2.12. Glycosides

5 ml of mushroom extracts was taken in a test tube which is treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. Along with 1 ml of concentrated sulphuric acid was added. A brown ring and violet ring appeared one above the other indicates a deoxy sugar characteristic of cardenolides. Harborne, (1973).

2.13. Quantitative analysis

2.13.1. Phenols

The phenol concentrations in the fruit body of mushroom were determined using the spectrophotometer method. The 5g of mushroom powder was taken in a beaker. Along with 50 ml of $(CH_3CH_2)_2O$ was added and boiled for 15 min. From this boiled sample 10 was taken in a 50ml conical flask and 10 ml of distilled water is added. Thereafter, 2 ml of NH_4OH solution and 5 ml of concentrated $CH_3(CH_2)_3CH_2OH$ was added to the mixture. The sample total volume was made up to the mark and left for 30 min. Then the colour developed was measured at 505 nm wavelength using a spectrophotometer.

2.14. Alkaloids

5 g of the mushroom powder was taken in a beaker. Along with, 200 ml of 10% CH_3CO_2H in C_2H_5OH is added. This mixture was covered with aluminium foil and allowed to stand for 4 h. This mixture was filtered and then filtrate was allowed to become concentrated in a water bath till it reaches 1/4 of the original volume. To form the precipitation in the concentrated filtrate, the concentrated NH_4OH was added until the completion of precipitation process. Thereafter, whole solution was allowed to settle. Finally, the precipitate was collected and washed with dilute NH_4OH and then filtered. The residue over the filter paper in the form of alkaloid was collected, dried and weighed.

2.15. Tannins

The tannins concentrations in the mushroom were determined by using the spectrophotometer method. 5 ml of mushroom extract was taken in a 25 ml conical flask. Along with 2 ml of 0.1 M $FeCl_3$ in 0.1 M HCl and 0.008 M $K_4Fe(CN)_6.3H_2O$ was added. Then the absorptions of this solution were measured with a spectrophotometer at 395 nm wavelength.

2.16. Saponins

20 g of mushroom powder was taken in a 200ml conical flask. Along with 100 ml of 20% C_2H_5OH was added. This solution was heated over a hot water bath for 4 h with continuous stirring at about 55ºC and then filtered. The residue formed was re-extracted with another 200 ml of 20% ethyl alcohol. This extract was kept in water bath at about 90ºC till to reduce 40%. Then this concentrated extract was transferred into a 250 ml separating funnel and 20 ml of $(CH_3CH_2)_2O$ was added and vigorously shaken. The aqueous layer was formed in the separating funnel was recovered ($(CH_3CH_2)_2O$ layer was discarded) and the purification process was repeated by adding 60 ml of n-C_4H_9OH. Finally, the n-C_4H_9OH extracts was washed twice with 10 ml of 5% NaCl. The remaining solution was then heated in a water bath and after complete evaporation. The sample obtained was dried in the oven up to a constant weight. Then the weight of the saponin was noted.

2.17. Flavonoids

10 g of mushroom powder was taken in a beaker which was repeatedly extracted with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered using No.1 Watman filter paper and then the filtrate was evaporated with the help of water bath. Finally, the moisture content was completely removed by kept the filtrate in oven up to constant and flavonoid was weighed.

2.18. DPPH scavenging activity

The free radical scavenging potential of mushroom extracts was measured on the basis of the scavenging activity of the stable 1, 1- diphenyl 2picrylhyorazyl (DPPH) free radical according to a modified method described by Brand-Williams, et al. (1995).1ml of plant extract with different concentrations such as was(10, 25, 50, 75, 100 µg/ml) taken in a separate test tube. In each test tubes, 1 ml of 0.1 mM DPPH solution in ethanol was added. Simultaneously, corresponding blank sample were prepared and L-Ascorbic acid (1-100 µg/ml) was used as reference standard. Mixer of 1 ml ethanol and 1 ml DPPH solution was used as control. The reaction was carried out in triplicate and the decrease in absorbance was measured at 517 nm after 30 minutes in dark using UV-Vis spectrophotometer. The % of inhibition of free radical formation was calculated using the following formula. Lower the absorption indicates the increase of FR scavenging.

$$\text{Inhibition \%} = \frac{\text{Absorption of the control - Absorbance of the sample}}{\text{Absorption of the control}} \text{ X100}$$

2.19. Lipid peroxidation inhibition

The lipid peroxidation inhibition activities of mushroom were analyzed by modified TBARS (Thiobarbituric acid reactive substances) method of Ruberto and Baratta (2000). The fresh egg was taken, homogenate and centrifuged in 10% ice-cold 1.15% KCl, w/v 1 at 2500g for 15minutes. From this, 500µL of supernatant was taken in a test tube and mixed it with 100µL of mushroom extract. The final solution was made upto 1ml by adding sterile distilled water. Thereafter, the lipid peroxidation formation was induced by adding 50 µL of 10 µM FeSO4 and 50 µLof 100 µM ascorbic acid and incubated at 37° C for 30 minutes.

Butylated hydroxyanisole (BHA) was taken as reference control and distilled water was taken as oxidized control. The 50 µL of TCA (20%, pH 3.5), 1.5 ml of TBA (0.8%, w/v, in 1.1% SDS, w/v) and 1.5 ml of acetic acid (20%, w/v, pH 3.5) were added and heated at 100° C for 60 minutes to stop the reaction. Thereafter, 5.0 ml of 1-butanol was added in each tube to stabilize the formed colour. The absorbance was measured at 532 nm. The rate of inhibition was calculated by the following formula.

$$\text{Inhibition ratio (\%)} = \frac{\text{Absorption of oxidized control- Absorption of sample}}{\text{Absorption of oxidized control}} \text{X100}$$

2.20. Ferric reducing antioxidant power (FRAP)

The ferric reducing power was determined by modified method of Ramya and Lakshmi Devi, (2010). The fresh FRAP working solution (37°C) was prepared by mixing 25ml of acetate buffer, 2.5 ml of TPTZ and 2.5ml of $FeCl_3$ from the stock solution of 0.3M acetate buffer (pH 3.6), 0.01M 2, 4, 6-tripyridyl-s-tri-azine solution in 0.04M hydrochloric acid and 0.02M $FeCl_3$ solution. 1ml of the sample (25-500µg/ml) was mixed with 2.85ml of the FRAP solution and kept in dark for 30 min. the absorbance was recorded at 593nm using Butylated Hydroxytolune (BHT) as standard.

FRAP value = Absorbance (sample + FRAP reagent) – Absorbance (FRAP reagent)

2.21. **Hydroxyl radical scavenging**

Hydroxyl radical scavenging activities of the mushroom extracts was determined by method described by Kunchandy and Rao (1990). 100 ul of mushroom was taken in a test tube, Along with extract The 100 ul of 2-deoxy-D-ribose (28 mM in 20 Mm KH_2PO_4-KOH buffer, pH 7.4) and, 200 ul of EDTA (1.04 mM) 200 uM of $FeCl_3$(1:1v/v), 100 ul of H_2O_2 (1mM) and 100 ul of ascorbic acid (1mM) were added. This mixture was incubated at 370 C for an hour. Thereafter, 1 ml of 1% thiobarbituric acid and 1.0 ml of 2.8% trichloro acetic acid were added and incubated at 1000 C for 20 min. The absorbance of final volume was measured at 532 nm against a blank sample. Quercetin was used as standard.

2.22. Superoxide anion radical scavenging assay

The superoxide radical scavenging activity of mushroom extract was estimated by using the method of Robak and Gryglewski (1988). For this estimation, 1ml of mushroom was taken in a test tube, along with 0.5 ml of N0.3 Mm BT and 0.5 ml of 0.936 Mm NADH was added. Thereafter, 0.5 ml Tris-Hcl buffer (16 mm, pH 8.0) were mixed with 0.5 ml PMS solution (0.12mM) to start the reaction and incubate at 250 C for 5 min. Finally, the absorbance was measured at 560 nm against blank. Curcumin was used as standard.

2.23. Nitric oxide scavenging assay

The nitrous oxide scavenging activity was estimated by the method of Green, et al. (1982). 2 ml of mushroom extract was taken in a rest tube. Along with, 3 ml of phosphate buffer containing 10 mM sodium nitroprusside was added and this mixture was incubated at 250C for 60 min. To this,5 ml of Griess reagent (mixture of 1% sulphanilamide and 0.1% naphthy-ethylene diamine dihydrochloride in 2% H_3PO_4) was added and absorbance was measured at 540 nm. Ascorbic acid was used as standard. The percent of nitrous oxide scavenging was calculated by using the following formula.

$$\text{Nitrous oxide \% Inhibition} = \frac{\text{Absorbance of control - Absorbance of test}}{\text{Absorbance of control}} \times 100$$

2.24. Antimicrobial activity

2.24.1. Glassware cleaning

Chromic acid is a commonly used glassware cleaning reagent. All the glassware required for the present antimicrobial screening were cleaned well in chromic acid solution then rinsed in deionized double distilled water and dried in oven at 100c for1hour.

2.24.2. Sterilization procedures in autoclave

The chromic acid washed all glassware were transferred to autoclave cylinder and close the lid and put on the electric heater and then adjusted the safety value to the required pressure. After the water boils, allow the steam and air mixture to escape through the discharge tap till all the air has been displaced. This can be tested by passing the steam-air mixture liberated from the discharge tap into a pail of water through a connecting rubber tube. When the air bubbles stop coming in the pail, it indicates that all the air has been displaced by steam. Close the discharge tap. The steam pressure rises inside and when it reaches the desired set level e.g. at 121ºC and 15 pounds (lbs) pressure. Finally, the safety valve opens and excess steam escapes out, counted the holding period from this point of time about 15 minutes. After the holding period, stop the electrical heater and allow the autoclave to cool until the pressure gauge indicates that the pressure inside is equal to the atmospheric pressure. Open the discharge tap slowly and allow the air to enter the autoclave. Open the lid of the autoclave and remove the sterilized materials. The culture medium was also autoclaved in the same above-mentioned method.

2.24.3. Antimicrobial susceptibility test

To prove the antimicrobial penitential of selected wild edible mushroom was determined by using antimicrobial susceptibility test. For this, the inhibition of microbial growth was observed in different concentrations of extract mixture containing culture medium. From the observed results, the minimum inhibitory concentration (MIC) of the mushroom extract and the minimum bactericidal concentrations (MBC) were calculated. For this study, two principal methods were used they were given below.

2.24.4. Microorganism used in this test

The gram negative bacterial species such as *Bacillus subtilis* (ATCC-6051) , *Staphylococcus aureus* (ATCC-9144), *Pseudomonas aeruginosa* (ATCC-25619) and *Vibrio cholerae* (MTCC-3906) were obtained from the MTTC , Chandigarh, India.

2.24.5. Minimum inhibitory concentration (MIC)

The MIC was defined as the lowest concentration of the compounds at which the growth of the microorganisms inhibited. The MIC values were studied for the bacterial strains; From the stock solutions of the extracts initially prepared a concentration of 250µ/mL of extract was added into the first wells. Then, dilutions were made so as to decrease the concentration of extract by 1 mg at each dilution. The different concentrations of mushroom extracts such as 1 µg/ml, 2.5 µg/ml, 5µg/ml, 10 µg/ml, 25 µg/ml, 5 µg/ml were prepared to detect the MIC. All four microbial species were cultured in the culture medium containing all concentrations. Positive and negative controls and incubation conditions were the same as in the zone of inhibition assay. Visible growth (turbidity) in the dilution tubes was the criterion to determine MIC values for the tested microorganisms at the given concentration of each extract. The extract in this study was tested in triplicate against each test organism.

2.24.6. Agar disc diffusion assay

Antimicrobial activity of methanolic extract of mushrooms was determined by the agar well diffusion method. For this study, the methanol extracts of mushroom were dissolved in dimethylsulfoxide (DMSO) to a final concentration of 10 mg/mL, and filter sterilized through 0.45-µm membrane filter. Small wells (6 mm in diameter) were made in the agar plates by sterile 10mm diameter glass rod. In each agar medium containing Petri plate, 5 wells were formed i.e., One center well surrounded by four peripheral wells. In all four peripheral wells, one hundred microliters of the extract with different concentration of mushroom such as 50µg, 100 µg, 250 µg and 500 µg were loaded into the different wells. The well located in the central region was filled by antimicrobial synthetic compound (Ampicillin:10 µg/mL). Before loading of bacteria, the concentration of target microbial cells suspensions was adjusted to 10^4 cfu/mL. In order to determine the antimicrobial efficacy of the fractions, aliquot of test culture (100 µL) was evenly spread over the surface of the solidified agar. Bacteria were cultured on Mueller Hinton agar. Ampicillin (10 µg/mL) was used as positive control and DMSO was used as negative control for test microorganisms. All the preloaded plates with respective extract and test organism were incubated at 37° C, for 24 hours for bacteria and at 27° C. After incubation period, zone of inhibition was measured in millimeters. All the tests were carried out in triplicate and their means recorded.

III. Results and Discussion

The constituents and composition of phyto-compounds are differed in different biomaterials. The water is a universal solvent, even though; all the phytoconstituents are not dissolved in water. Some compounds can dissolve in polar solvent; some others are in nonpolar solvent and few are in dipolar solvents. Hence in the present study, the extraction study was made in different polar and non-polar solvents for fruiting bodies of mushroom *Lycoperdon pyriforme, Arimillaria tabescens* and *Agaricus bisporus* to find out the maximum solubility nature to select the appropriate solvents to extract the maximum number of phytocompounds. Several previous researches on extractive value give an indication about the nature of the chemical constituents present in the biomaterial, biomass or drug. Alcohol soluble extractive (2.8%) was higher as compared to water soluble extractives (0.4%) for *Hypsizygus ulmarius* because of having more alcohol soluble polar constituents (Shivashankar and Premkumari, 2014).

Lyophilized fruit bodies of *P. ostreatus, P. sajor-caju, A. bisporus*, and *A. campestris* were extracted by water, ethanol, ethyl acetate, acetone, chloroform, hexane, and petroleum ether. The yields of water extract apparently were the highest, regardless of the mushroom species used, and the amounts of yield among the 4 mushrooms were very similar, having 17.6, 16.6, 19.5, and 18.2 g for *P. ostraetus, P. sajor-caju, A. bisporus*, and *A. campestris*, respectively. Ethanol gave rise to the next-highest yield, whereas hexane extract produced the lowest yields, having 3.5, 2.86, 3.4, and 3.46 g for *P. ostraetus, P. sajor-caju, A. bisporus*, and *A. campestris*, respectively. Chloroform and petroleum ether also produced low yields, whereas acetone and ethyl acetate gave moderate yields. These results indicate that the majority of the substances in fruit bodies of mushrooms are extractable by solvents with high polarity (Elbatrawy, et al. 2015), which supports, the present study showed the maximum extraction in methanol solvents for fruiting bodies of *Lycoperdon pyriforme, Arimillaraia tabescens* and *Agaricus bisporu* when compared to the solvents petroleum ether, benzene, chloroform, acetone, hexane ethyl acetate, water, ethanol and methanol. Because, among all these nine solvents, the methanol has come in first place because of their high yielding of extractive value for all three mushroom species which were recorded as 36.2±4.1%, 34.5±2.7% and 33.6±2.2% respectively. The solvent ethanol has come in second position and the universal solvent water has come in third position in their yield. Based on these study results, the methanol was selected as a suitable solvent to extract phytocompounds for present all three study mushrooms, further, the selection of methanol and ethanol as a extraction solvent is safety, easy accessibility and high affinity for antioxidants compared with other organic solvents (Dailey and Vuong, 2015, Xu and Bao, 2014, and Ramic, et al. 2015). Different methanol and ethanol concentrations had different polarities. Lower concentration of methanol and ethanol is suitable for extracting the polar antioxidant compounds, whereas higher concentration of methanol and ethanol favors the less polar ones (Chen, et al. 2015).

The quantitative analysis of phytocompounds in the study mushroom *Lycoperdon pyriforme, Armillaria tabescens* and *Agaricus bisporus* exposed the presence of several phytocompounds such as Tannins, Saponin, Flavonoids, Glycosides, Phenols, Alkaloids and Terpenoids in rich amount in their fruiting bodies. But their presence and absences were varied based on what type of solvents was used in the extraction process. For example, totally eight variety of solvents were used to extract the phytocompounds from fruiting bodies of all three mushrooms. In the solvent n-hexane, the phytocompounds such as tannins, flavonoid, terpenoids and steroids were obtained for mushroom *Lycoperdon pyrifoem***,** tannins, flavonoid and terpenoids for *Armillaria tabescens* and tannins, flavonoid, phenols and terpenoids for *Agaricus bisporus.* In solvent ethanol, tannins, saponin, phenol, alkaloids and steroids were identified from *Lycoperdon pyrifoem*, tannins, saponin, flavonoids, glycosides, phenols, alkaloids and terphenoids were identified from *Armillaria tabescens* and tannins, saponin, flavonoids, glycosides, phenols from *Agaricus bisporus.* In chloroform solvent, tannins, flavonoids, glycosids, alkaloids, terpenoids, steroids were identified for *Lycoperdon pyrifoem*, tannins, saponins**,** flavonoids, glycosids, alkaloids, terpenoids, steroids were for *Armillaria tabescens* and tannins, saponins, flavonoids, glycosids, alkaloids, phenols and steroids for *Agaricus bisporus.* In acetone solvent, all eight phytocompounds were noted for *Lycoperdon pyrifoem*, among all eight compounds, six compounds were existed except phenols and alkaloids for *Armillaria tabescens.* The same six compounds except two compounds such as alkaloids, terpenoids were observed for *Agaricus bisporus.* In solvent petroleum ether, saponin, glycosides, phenols and alkaloids were missing for both, *Lycoperdon pyrifoem* and *Armillaria tabescens* and saponin, glycosides and alkaloids were missing for *Agaricus bisporus.*In universal solvent water the phytocompounds flavonoid and terpenoids were absent for *Lycoperdon pyrifoem*, phytocompounds flavonoids, terpenoids and steroids were missing for both *Armillaria tabescens* and *Agaricus bisporus.* In ethanol solvent, all eight phytocompounds are existed for *Lycoperdon pyrifoem*, terpenoid alone is absent in *Armillaria tabescens* and alkaloids, terpenoids and steroids were absent in *Agaricus bisporus.* In methanol solvent extract, the phytocompounds flavonoids, alkaloids, terpenoids and steroids were missing for *Lycoperdon pyrifoem***,** favanoids terpenoids alone were missing for both *Armillaria tabescens and Agaricus bisporus.* The above mentioned results are supported by the results of Ikon,

et al. (2019) exhibited that P. ostreatus extract contains glycosides, alkaloids, saponins, flavonoids, reducing compounds, polyphenol, steroids/triterpenoids, proteins and amino acids. The similar results were also observed by Awala, et al. (2015) that the presence of saponins, tannins, steroids, terpenoids, flavonoids and cardiac glycosides in all the *Trametes* species extracts, while anthraquinones, alkaloids and phlobatannins were absent. Qualitative phytochemical analysis of *M. esculenta* extracts showed the presence of alkaloids, anthraquinone, glycosides, tannins, saponins, flavonoids, terpenoids, phenolics, carbohydrates, anthocyanins as well as proteins and amino acids and steroids were found (Monika Thakur, 2015). Tannin, saponin, terpenoid, alkaloid, steroid, phlobatannin, anthraquinone and cardiac glycoside, were found in the *Rigidoporus microporus* mushroom extracts while anthraquinone, phlobatannin and alkaloids were absent (2017).

Quantitative analysis of phenols, alkaloids, tannins, saponins, flavonoids glycosides and terpenoids were observed in all three study mushroom mushroom *Lycoperdon pyriforme, Armillaria tabescens* and *Agaricus bisporus*. The obtained results exhibited flavonoid was in first place which was noted as 2.4±0.19 mg/100g for *Lycoperdon pyriforme*, 2.0±0.15 g/100g for *Arimillaria tabescens* and 2.7±0.21 mg/100g for *Agaricus bisporus.* The phytocompond alkaloids were in second place and tannins in third place for all three mushrooms. The other phytocompounds in all three mushrooms were quantitatively sequenced in the following order phenols > glycosides > terpenoids. The above results showed a nearby similarities with the phenolic content of *Macrolepiota dolichuala*, *Macrolepiota procera* and *Macrolepiota rhacodes* were 5.5 mg/g and total flavonoids of *Macrolepiota mastoidea* was 3.6 mg/g (Shirmila and Radhamany, 2013). Total phenols of *Macrolepiota dolichuala*, *Macrolepiota procera* and *Macrolepiota rhacodes* were .90 mg/g, 11.0 mg/g and 16.0 mg/g and average of total flavonoids were 1.7 mg/g, 1.46 mg/g and 1.30 mg/g (Babita and Narender, 2014). The acetone extract of macrofungus *Rigidoporus microporus* has the highest amount of cardiac glycosides (23.682mg/g), flavonoids (7.290mg/g), saponins (18.000mg/g), tannins (11.943mg/g), steroids (14.546mg/g) and terpenoids (22.285mg/g)(Awala, *et al.*, 2017).

The GCMS analysis of *Lycoperdon pyriforme* showed three compounds such as methyl 3-Bromo-1-adamantaneacetate (% of peak area 5.76 and 13.14 with RT 25.913 and 26.213), Trimethyl [4-(1,1,3,3,-Tetramethylbutyl) Phenoxy] Silane(% of peak area 7.913 with RT 26.773), Cyclotrisiloxane, Hexamethyl- (% of pea area 6.455 with RT 27.268). The similar GCMS study on *Lycoperdon pyriforme* by Asgharpoura, *et al*., (2020) observed the phytocompounds Toluene, n-decanoic acid, Tetradecanoic acidethylester, Di-sec-butylphthalate, Dibutylphthalate, Methylenebis(2,4,6triisopropylphenylphosphine), dimethyl{bis(tridecyloxy)}silane, Ergosta-5,7-dien-3-ol, and 7,22-Ergostadienone. The phytocompounds methyl 3-Bromo-1-adamantaneacetate observed in the present study has potent larvicidal and antibacterial activity which is isolated from plant *Caulerpa racemosa* (Nagaraj and Osborne, 2014). The compound Trimethyl [4-(1,1,3,3,-Tetramethylbutyl) Phenoxy] Silane of *Lycoperdon pyriforme* also observed in plant S*olanum villosum (mill) (solanaceae)*by Venkatesh, et al. (2014) suggested its acts vitamin D derivatives to cure rickets and antioxidents. The compound Cyclotrisiloxane, Hexamethyl- used as fragrances or odor agents, can be used in home products (cleaners, laundry products, air fresheners) or similar industrial products; usage indicated when known; more specific modifiers.

The GCMS study of mushroom *Armillaria tabescens* showed the presence of eight compounds such as Bicyclo[2.2.1] Heptan-2-One,1,3,3-Trimethyl-.,11-Hydroxy-11-Methyl-Tricyclo[4.3.1.1(2,5)]Undecan-10-One.,.13-Octadecenal.,10-Secocholesta-5,7,10(19)-Triene-1,3-Diol, 25-[(Trimethylsilyl)Oxy]-., 3-Isopropoxy-1,1,1,5,5,5-Hexamethyl-3-(Trimethylsiloxy)Trisiloxane and Hentriacontane. The compounds Bicyclo[2.2.1]Heptan-2-One,1,3,3-Trimethyl- has immune enhancement and antimicrobial activities (Al-Marzogi, *et al*., 2015), 11-Hydroxy-11-Methyl-Tricyclo[4.3.1.1(2,5)]Undecan-10-One.,.13-Octadecenal has antiinflamatory and aniallergic activities (Kamal, et al. 2017), 9,10-Secocholesta-5,7,10(19)-Triene-1,3-Diol, 25-[(Trimethylsilyl)Oxy]- has anticancer activity and regulate calcium in blood (Aballah, et al. 2019), the silica compound 3-Isopropoxy-1,1,1,5,5,5-Hexamethyl-3-(Trimethylsiloxy)Trisiloxane no any biological activities. The compound Hentriacontane inhibit the production of tumor necrosis (TNF)-a, interleuin (IL)-6 and Prostoglandin E(2)PGE(2) (Kim, et al. 2011), useful to develop new drugs to treat inflammatory diseases and also has antioxidant, antitumor and antibacterial activity.

In Mushroom *Agaricus bisporus* only four compounds such as Paredrine, Cyclotrisiloxane, Hexamethyl., Paredrine and Hexestrol Di-Tms were identified. The compound paredrine was used medically in eye drops to dilate the pupil (a process called mydriasis), so that the back of the eye can be examined. It is used to diagnostic test for Horner's Syndrome. Patients with Horner's syndrome exhibit anisocoria brought about by lesions on the nerves that connect to the nasociliary branch of the ophthalmic nerve (Walton and Buono,2013). Cyclotrisiloxane is a unsaturated cyclic compounds, hence play a major role in the free radical scavenging (Alok Prakash and Suneetha, 2014). Cyclotrisiloxane, Hexamethyl used as conditioning agent, emollient, solvent in personal care products, defoaming agent and lubricant. It has antioxidant activity (Alok Prakash and Suneetha, 2014). Hexestrol has been used in estrogen replacement therapy, for the treatment of breast cancer in women and prostate cancer in men, and for the treatment of certain gynecological disorders(Morton and Hall, 2012).

Free radical mediated oxidative stress has been associated with diseases such as atherosclerosis and cardiovascular complications (Ballinger, 2005 and Cherubini, et al. 2007) neurodegenerative disorder (Aruoma, et al. 2007), diabetes (Maritim, et al. 2003), metabolic syndrome, skin disorders, and tumors (Valko, et al. 2006). The antioxidant properties of mushroom was evaluated by several previous studies i.e., The antioxidant properties of several culinary mushrooms (Abdullah, et al. 2012), mycelia of 21 wild mushrooms (Kalyoncu, et al. 2010), fruiting bodies of three edible mushrooms (Liu, et al. 2001], *Ganoderma lucidum* (Rajasekaran and Kalaimagal, 2011) and four Indian medicinal mushrooms (Ajith and Janardhanan,2007). Mushrooms produce a wide range of secondary metabolites with high therapeutic value (Demain, 1999). Both fruiting body and the mycelium contain compounds with wide-ranging antioxidant and antimicrobial activities (Barros, et al. 2007 and Ferreira, 2007). Recently, Ferreira et al. (Ferreira, et al.

2007) reported the antioxidative properties of two mushrooms, *Lactarius deliciosus* (L.) Gray and *Tricholoma portentosum* (Fr.) obtained from northeast Portugal. Antioxidant and antimicrobial potentials of extracts obtained from four wild mushrooms, Termitomyces clypeatus (TCE), Termitomyces robustus (TRE), Lentinus subnudus (LSE) and Lenzites species (LZE) exhibited that LSE and LZE displayed good scavenging activity against 2, 2-Diphenyl-1Picrylhydrazyl (DPPH) and ferrous ion radicals at concentration of 2 mg/mL. However, TRE and TCE exhibited better superoxide anion scavenging effect at 2 mg/mL. All extracts (TCE, TRE, LSE and LZE) had comparable scavenging effect on hydroxyl radicals as butylated Hydroxytoluene (BHT) used as control (Oyetayo, 2009). The free radical scavenging activities of fruiting bodies of present study mushroom *Lycoperdon pyriforme w*ere increased with increasing concentrations of the *Lycoperdon pyriforme* extracts (25 µg/ml, 50 µg/ml ,100 µg/ml,250 µg/ml and 400 µg/ml) which were observed as 29.63±0.74%, 45.93±1.48%, 51.73±0.93 % and 66.54±1.67% for DPPH, 12.28±0.81%, 21.16±1.54%, 37.72±2.1%, 56.15±3.8% and 72.22±5.7% for ABTS, 10.61±0.51%, 18.31±1.2%, 35.57±2.7%, 69.35±4.8% and 87.24±7.3% for FRAP, 18.31±0.70%, 27.16±1.62%, 41.04±3.61%, 71.22±5.52% and 80.16±6.74% for hydroxyl radicals, 04.15±0.14%, 12.56±0.92%, 27.29±1.76%, 74.18±6.28% and 88.30±7.51% for superoxide, 28.63±0.60%, 47.05±0.84%, 53.99±0.84%, 62.84±1.08% and 74.8±1.12% for nitric oxide. The same trends of concentration based free radical scavenging activities were observed in both mushroom species such as *Arimillaria tabescens* and *Agaricus bisporus.* Hence all three mushrooms have considerable level of free radical scavenging activities. These results has some resemblances with the edible mushroom *Sparassis crispa* showed a significant antioxidant efficiency on inhibition of 2,2-diphenyl-1-picrylhydrazyl(DPPH) when compared to standard antioxidant like L-ascorbic acid(Madhavi Joshiand, 2014). The metanolic extract of edible mushroom, Volvariella volvacea exhibited 62.45% Hydroxyl radical scavenging activity at 250 ug/ml, 74.23% superoxide scavenging activity at 300 ug/ml concentration, 75.52% of Nitric oxide radical inhibition at 200 ug/ml concentration (Carmel Punitha and Rajasekaran, 2014). Methanolic extract four hydrocotyle species exhibited a maximum of 68.78% of ABTS inhibition and hot water extract showed 62.56% inhibition at 125 mg/ml concentration (. Huang, et al. 2008). The free radical FRAPS scavenging activites were 32.71%, 63.38%, and 73.25% at 0.1%, 0.5%, and 1% *Morchella esculenta* mycelia concentrations of respectively (Nitha, et al. 2010). The above results indicated that all three mushroom having free radical scavenging activities were concentration dependent (Oyetayo, 2009). These free radical scavenging activity of the present study could be attributed may be due to the existence of phenolic, flavonoids and ascorbic acid content in the study mushroom species (Carmel Punitha and Rajasekaran, 2014).

Antibiotic resistance has become a global concern in recent years (Westh, et al. 2004). In the recent scenario, the world is facing significant challenges in modern healthcare services because many antimicrobial agents have lost their effectiveness in treating infectious diseases primarily due to the development of microbial resistance (Balouiri, et al, 2016). Exploration of new antimicrobial properties containing bioactive compounds from plants, fungi, bacteria to treat the day by day resistant gained pathogenic microorganisms is a new trend drug discover (Freire-Moran, et al. 2011, Chaudhary and Singh, 2016 and Alves, et al. 2012). The bioactivities of mushrooms include antibacterial, antifungal, antioxidant, antiviral, anti-tumor, cytostatic, immunosuppressive, antiallergic, antiatherogenic, hypoglycemic, anti-inflammatory and hepatoprotective activities (Wasser and Weis, 1999 and Li ndequist, et al. 2005). Mushroom is a macrofungi release various antiviral, antifungal antibacterial bioactive compounds such as terpenoids, flavonoids, tannins, alkaloids, and polysaccharides to survive in the wild against competing or pathogenic agents ((Vamanu, et al. 2018, Elisashvili, 2012 and Sorimachi, et al. 2001). The hot water extracts of five mushrooms (*Trichaptum sp, Flammulina sp, Boletus sp, Tricholoma sp, and Psalliota campestris*) showed zone of inhibition in the culture of Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus pyogenes, Eschericia coli, and Candida at 37oC within 24hrs (Udu-Ibiam, et al. 2014). The antimicrobial potential of methanolic extracts of six wild macromycetes (*Boletus lupinus, Flammulina velutypes, Phellinus igniarius, Sarcodon imbricatus, Tricholoma aurantium, Xerocomus ichnusanus*) was evaluated against six strains of bacteria *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 9027, *Bacillus pumilus* NCTC 8241, *Sarcina lutea* ATCC 9341 and *Bacillus subtilis* ATCC 6633, and 5 species of fungi: *Saccharomyces cerevisiae* ATCC 16404, *Candida albicans* ATCC 10231, *Aspergillus niger* I.N. 1110, *Aspergillus sojae* CCF and *Penicillium spp*. FNS FCC 266 exhibiting more potent inhibitory effects on the growth of bacteria than on fungi (Nedelkosk, 2013). In the present study, the different concentrations of mushroom *Lycoperdon pyriforme, Arimillaria tabescens* and *Agaricus bisporus* extract was tested against the bacterial strain *Bacillus subtilis, Staphylococcus aureus, Escherichia coli and Vibrio cholerae* to check their antibacterial potentiality. The obtained results validated that the mushroom extract of *Agaricus bisporus* was not have any zone of inhibition against bacterial strain *Bacillus subtilis, Staphylococcus aureus, Escherichia coli and Vibrio cholerae* at 50 µg concentration and exhibited considerable inhibition such as 8mm, 7mm, 10mm and 8mm at 100 µg, 11mm, 12mm, 12mm and 10mm at 250 µg, 16mm, 16mm, 18mm and 17mm at 500 µg respectively. These results give a definite confirmation about that zone of inhibition increased with increasing concentration of mushroom extract. The zone of inhibition results observed for higher concentration 500 µg of mushroom extract was showed a nearby maximum similarity with zone of inhibition obtained for Ciproflaxin at 20 µg. The above results of the present study has some resemblance with six wild edible mushrooms (*Lycoperdon perlatum, Cantharellus cibarius, Clavaria vermiculris, Ramaria formosa, Marasmius oreades, Pleurotus pulmonarius*) isolated from the Western Ghats of Karnataka has considerable antibacterial activity against gram positive *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 6633); gram negative species *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and antifungal activity unicellular *Candida albicans* (ATCC 60192) (Ramesh, et al. 2010). The 192 extracts obtained from 19 mushroom *(Morchella semilibera, Agaricus bisporus, Cantharellus tubaeformis, Polyporus lepideus, Sarcodon imbricatus, Boletus badius, Suillus granulatus, Suillus variegates, Lycoperdon perlatum, Calocybe gambosa, Hygrophorus cossus, Hygrophorus russula, Lepista luscina, Lepista nuda, Tricholoma albobrunneum, Tricholoma terreum, Lactarius deliciosus, Lactarius quieticolor, and Lactarius salmonicolor)* were tested against nine foodborne pathogenic bacterial strains (*Escherichia coli O157:H7, Salmonella Enteritidis, Shigella sonnei, Vibrio parahaemolyticus, Yersinia enterocolitica, Bacillus cereus, Clostridium perfringens, Listeria monocytogenes, and Staphylococcus aureus*) showed more than half (50.2%) them have some antibacterial activity (Venturini, et al. 2008).

Table 3.1: Qualitative analysis of extractive value in the fruiting of *Lycoperdon pyriforme* at different solvents

Mushroom species	*Lycoperdon pyriforme*	*Arimillaraia tabescens*	*Agaricus bisporus*
Solvents	Yield of extractive materials (%)		
Petroleum ether	3.5±0.25	3.1±0.19	3.9±0.28
Benzene	1.6±0.11	2.7±0.19	1.3±0.12
Chloroform	3.9±0.27	4.2±0.31	3.8±0.29
Acetone	5.1±0.35	4.8±0.31	5.4±0.41
Hexane	2.8±0.13	2.4±0.12	3.1±0.25
Ethyl acetate	5.8±0.39	6.1±0.46	4.9±0.34
Water	18.9±1.2	20.4±1.7	19.3±1.3
Ethanol	27.5±2.4	30.2±2.8	29.1±2.6
Methanol	36.2±4.1	34.5±2.7	33.6±2.2

Table 3.2: Qualitative analysis of phytocompounds in the fruiting of *Armillaria tabescens* at different solvents

Solvent / Phytocompounds	NH	EA	CH	AC	PE	WA	ET	MT
Tannins	+	+	+	+	+	+	+	+
Saponin	-	+	-	+	-	+	+	+
Flavonoids	+	-	+	+	+	-	+	-
Glycosides	-	-	+	+	-	+	+	+
Phenols	-	+	-	+	-	+	+	+
Alkaloids	-	+	+	+	-	+	+	-
Terpenoids	+	-	+	+	+	-	+	-
Steroids	+	+	+	+	+	-	+	-

NH-N-Hexane, EA-Ethyl acetate, CH-Chloroform, AC-Acetone, PE-Petroleum ether, A-Water, ET-Ethanol and MT-Methanol

Table 3.3: Qualitative analysis of phytocompounds in the fruiting of *Armillaria tabescens* at different solvents

Solvent / Phytocompounds	NH	EA	CH	AC	PE	WA	ET	MT
Tannins	+	+	+	+	+	+	+	+
Saponin	-	+	+	+	-	+	+	+
Flavonoids	+	+	+	+	+	-	+	-
Glycosides	-	+	+	+	-	+	+	+
Phenols	-	+	-	-	-	+	+	+
Alkaloids	-	+	+	-	-	+	+	+
Terpenoids	+	+	+	+	+	-	-	-
Steroids	+	+	+	+	+	-	+	+

NH-N-Hexane, EA-Ethyl acetate, CH-Chloroform, AC-Acetone, PE-Petroleum ether, A-Water, ET-Ethanol and MT-Methanol

Table 3.4: Qualitative analysis of phytocompounds in the fruiting of *Agaricus bisporus* at different solvents

Solvent / Phytocompounds	NH	EA	CH	AC	PE	WA	ET	MT
Tannins	+	+	+	+	+	+	+	+
Saponin	-	+	+	+	-	+	+	+
Flavonoids	+	+	+	+	+	-	+	-
Glycosides	-	+	+	+	-	+	+	+
Phenols	+	+	+	+	+	+	+	+
Alkaloids	-	-	-	-	-	+	-	+
Terpenoids	+	-	-	-	+	-	-	-
steroids	+	+	+	+	+	-	-	+

NH-N-Hexane, EA-Ethyl acetate, CH-Chloroform, AC-Acetone, PE-Petroleum ether, A-Water, ET-Ethanol and MT-Methanol

Table 3.5: Phytocompounds concentration in the mushrooms available in the waste land around Poannai Village at Vellore District

Mushroom species	*Lycoperdon pyriforme*	*Arimillaria tabescens*	*Agaricus bisporus*
Phytocompounds mg/100g			
Phenols	0.34±0.051	0.38±0.041	0.27±0.012
Alkaloids	1.9±0.15	1.2±0.18	1.5±0.13
Tannins	0.57±0.067	0.44±0.054	0.65±0.079
Saponins	0.04±0.004	0.05±0.007	0.03±0.005
Flavonoids	2.4±0.19	2.0±0.15	2.7±0.21
Glycosides	0.14±0.062	0.17±0.053	0.11±0.042
Terpenoids	0.06±0.005	0.09±0.007	0.07±0.009

Qualitative Report

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Sample ID: B1-(18ES-0035)-1 Vial Number: 24

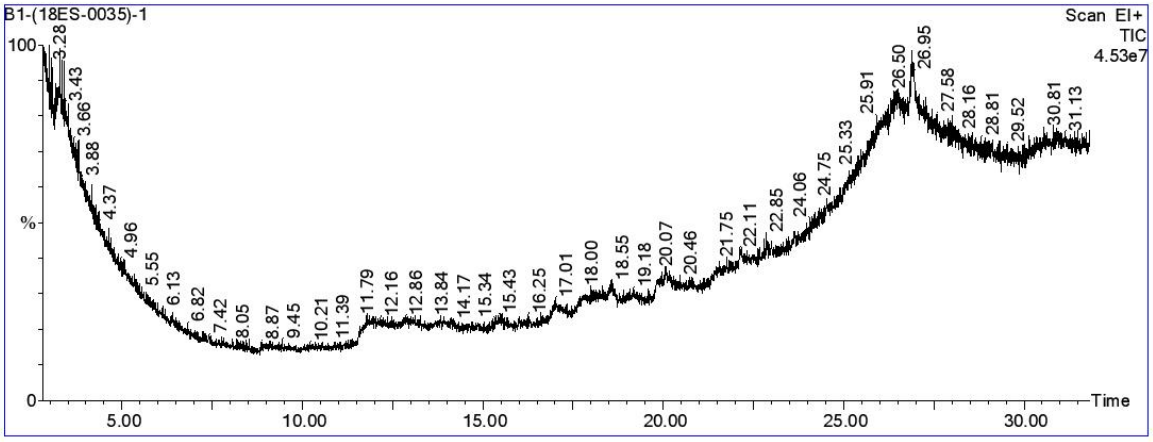
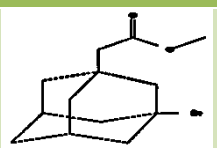
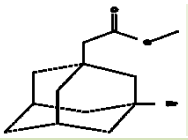
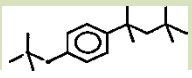
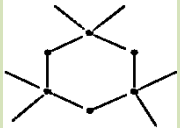


Table 3.6: GCMS analysis showed retention time related bioactive phytocompound identified for the methanol extract of fruiting bodies of mushroom *Lycoperdon pyriforme*

S. NO	R/T	Name of the Compound	Molecular Formula	MW	Structure
1.	25.913	Methyl 3-Bromo-1-Adamantaneacetate	$C_{13}H_{19}O_2Br$	286	
2.	26.213	Methyl 3-Bromo-1-Adamantaneacetate	$C_{13}H_{19}O_2Br$	286	
3.	26.378	No Compound	-	-	-
4.	26.503	No Compound	-	-	-
5.	26.773	Trimethyl[4-(1,1,3,3,-Tetramethylbutyl)Phenoxy]Silane	$C_{17}H_{30}OSi$	278	

6.	26.878	No Compound	-	-	-
7.	27.268	Cyclotrisiloxane, Hexamethyl-	$C_6H_{18}O_3Si_3$	222	

Qualitative Report

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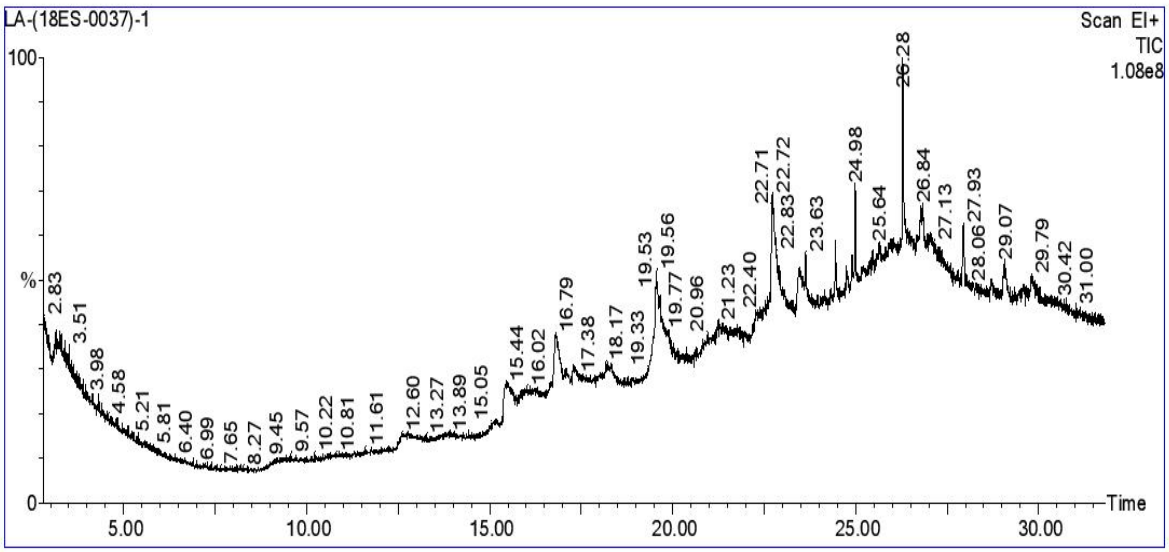
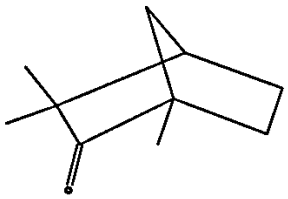
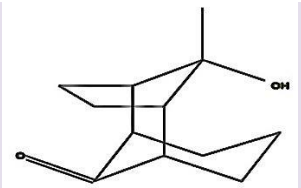
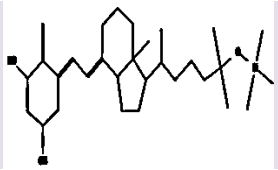
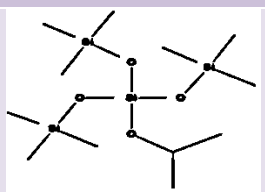
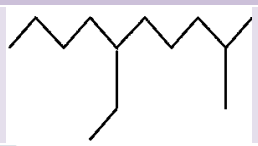


Table 3.7: GCMS analysis showed retention time related bioactive phytocompound identified for the methanol extract of fruiting bodies of mushroom *Armillaria tabescens*

S. NO	R/T	Name of the Compound	Molecular Formula	MW	Structure
1.	**15.478**	Bicyclo[2.2.1]Heptan-2-One, 1,3,3-Trimethyl-	$C_{10}H_{16}O$	152	
2.	**16.804**	11-Hydroxy-11-Methyl-Tricyclo[4.3.1.1(2,5)]Undecan-10-One	$C_{12}H_{18}O_2$	194	
3.	**19.560**	13-Octadecenal	C18H34O	266	
4.	**22.721**	**No Compound**	-	-	-
5.	**23.477**	**No Compound**	-	-	-

6.	**23.627**	9,10-Secocholesta-5,7,10(19)-Triene-1,3-Diol, 25-[(Trimethylsilyl)Oxy]-,	$C_{30}H_{52}O_3Si$	488	
7.	**25.642**	**No Compound**	-	-	-
8.	**26.028**	3-Isopropoxy-1,1,1,5,5,5-Hexamethyl-3-(Trimethylsiloxy)Trisiloxane	$C_{12}H_{34}O_4Si_4$	354	
9.	**26.278**	Hentriacontane	$C_{31}H_{64}$	436	
10.	**26.838**	**No Compound**	-	-	-
11.	**27.043**	**No Compound**	-	-	-
12.	**27.938**	Hentriacontane	$C_{13}H_{28}$	184	

Qualitative Report

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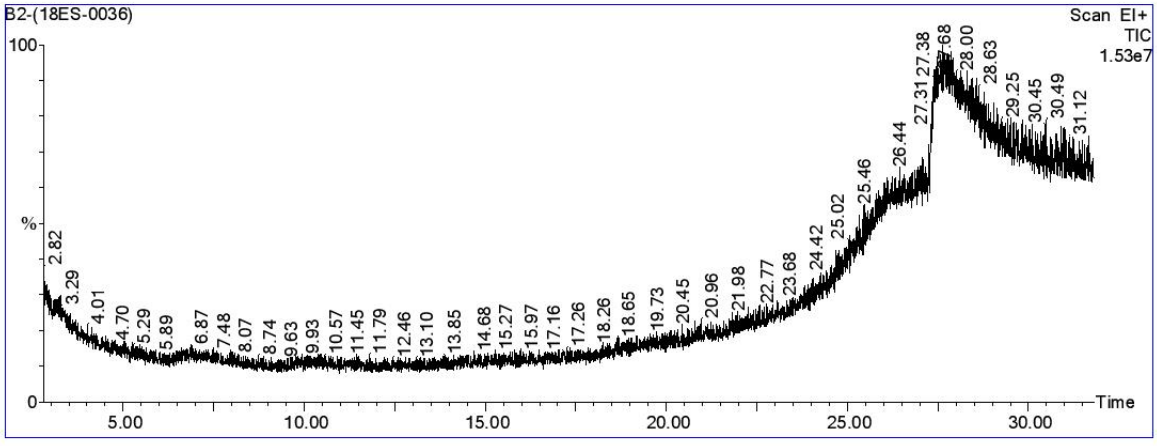


Table 3.8: GCMS analysis showed retention time related bioactive phytocompound identified for the methanol extract of fruiting bodies of mushroom *Agaricus bisporus*

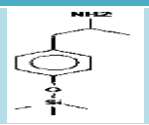
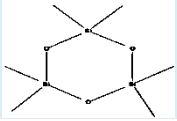
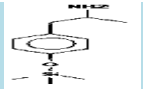
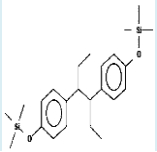
S. NO	R/T	Name of the Compound	Molecular Formula	MW	Structure
1.	27.398	Paredrine	$C_{12}H_{21}ONSi$	223	
2.	27.483	Cyclotrisiloxane, Hexamethyl	$C_6H_{18}O_3Si_3$	222	
3.	27.553	Paredrine	$C_{12}H_{21}ONSi$	223	
4.	28.769	Hexestrol Di-Tms	$C_{24}H_{38}O_2Si_2$	414	

Table 3.9: Zone of inhibition of *Lycoperdon pyriforme* extract against bacterial strain *Bacillus subtilis, Staphylococcus aureus, Escherichia coli and Vibrio cholerae* at different concentrations

Bacterial Strains	Zone of inhibition at different concentration of mushroom extract				
	50µg	100µg	250µg	500µg	Ciproflaxin 20
B.subtilis	-	-	-	-	38mm
S.aureus	-	-	-	-	27mm
E.coli	-	-	-	-	25mm
V.cholerae	-	-	-	-	44mm

Table 3.10: Zone of inhibition of *Arimillaria tabsenes* extract against bacterial strain *Bacillus subtilis, Staphylococcus aureus, Escherichia coli and Vibrio cholerae* at different concentrations.

Bacterial Strains	Zone of inhibition at different concentration of mushroom extract				
	50µg	100µg	250µg	500µg	Ciproflaxin 20
B.subtilis	-	8	12	16	42mm
S.aureus	-	8	10	14	22mm
E.coli	-	6	7	9	25mm
V.cholerae	-	-	6	8	37mm

Figure 3.1: Culture plate not showing any zone of inhibition for different concentration of *Lycoperdon pyriforme* extract against bacterial strain *Bacillus subtilis and Escherichia coli*

Bacillus subtilis ***Escherichia coli***

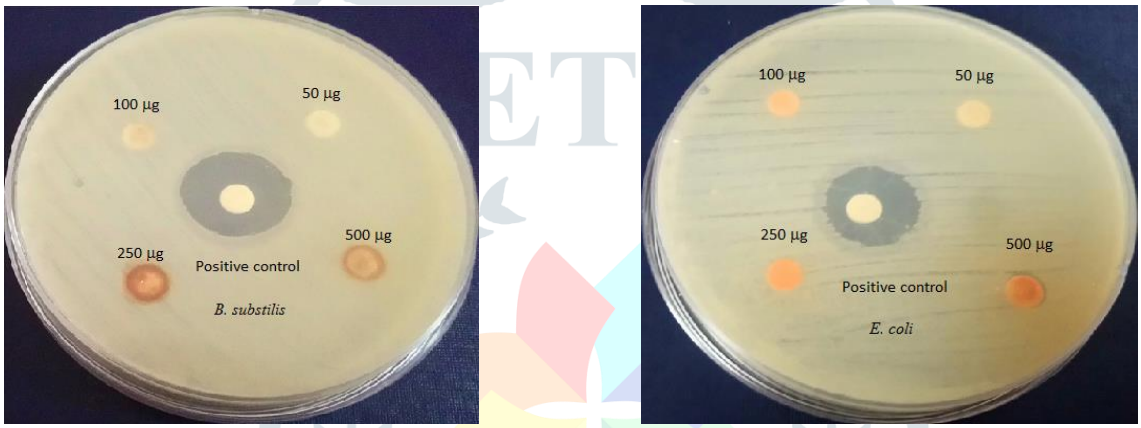
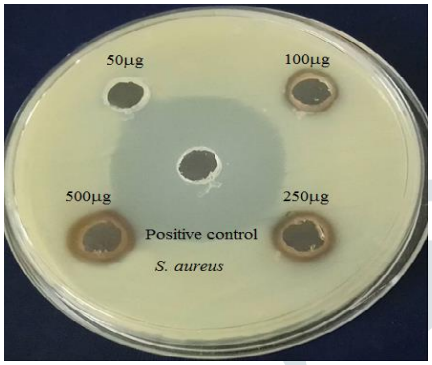
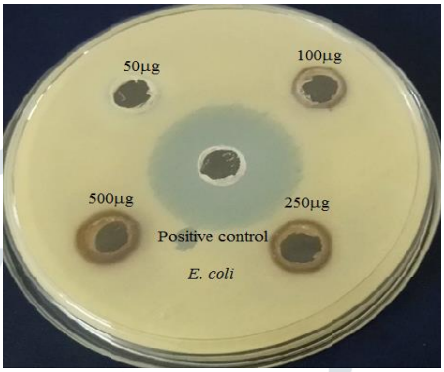


Figure 3.2: Culture plate showing any zone of inhibition for different concentration of *Arimillaria tabsenes* extract against bacterial strain *Bacillus subtilis and Escherichia coli*

B.subtilis



S.aureus



E.Coli

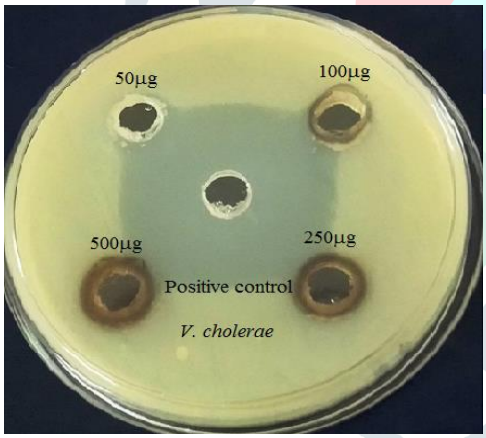
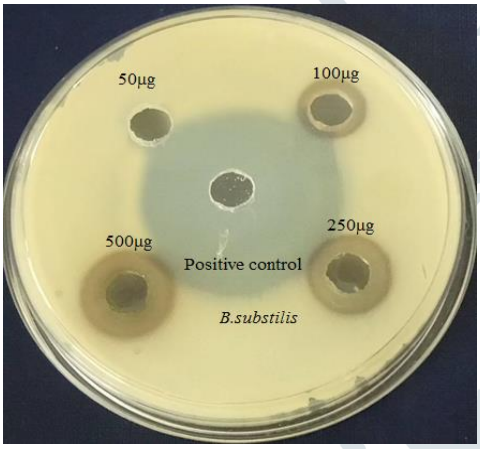


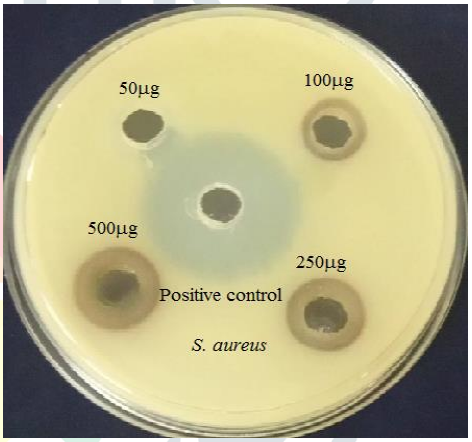
Table 3.11. Zone of inhibition of *Agaricus bisporus* extract against bacterial strain *Bacillus subtilis, Staphylococcus aureus, Escherichia coli and Vibrio cholerae* at different concentrations

Bacterial Strains	Zone of inhibition at different concentration of mushroom extract				
	50µg	100µg	250µg	500µg	Ciproflaxin 20 µg
B.subtilis	-	8	11	16	40mm
S.aureus	-	7	12	16	28mm
E.coli	-	10	12	18	24mm
V.cholerae	-	8	10	17	43mm

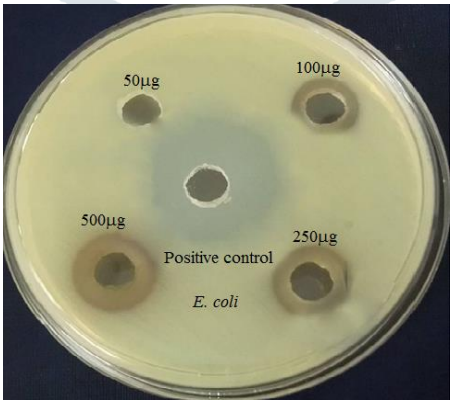
Figure 3.3: Culture plate showing any zone of inhibition for different concentration of *Arimillaria tabsenes* extract against bacterial strain *Bacillus subtilis and Escherichia coli*

B.subtilis ***S.aureus***





E.Coli



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