



Mycochemical profiling of selected wild Basidiomycetes mushroom fungi of Alagar Hills, Madurai, Tamilnadu

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Abstract

Mushrooms are large diverse group of fungi, forming basidiocarp of a macrofungus. Mushrooms possess a wide array of bioactive molecules such as Phenolics, Terpenoids, polysaccharides, tannins, flavanoids. They are extensively investigated and proved to exhibit many nutritional attributes and therapeutic values including anti-tumour, antimicrobial, hypoglycemic, antilipidemic and antihypertensive. The metabolites impart tremendous potential to mushrooms and established importance to mankind displaying a suitable alternative natural resource of phytochemicals with less toxic effects. The present study aimed at investigating the metabolites and quantification of hot water extracts of some wild basidiomycetes macro fungi present in the hilly regions of Alagarkovil, Madurai district. The exclusive geological and climatic conditions prevailing in the selected site greatly enhance the growth of variety of mushroom species. The macrofungi were collected, cultivated in vitro and mycelia were produced. The mycelial extracts were used for quantification of biochemicals available in mushrooms. Total Phenols, Total Flavanoids, Condensed Tannins were estimated. Total protein, Total carbohydrates, and crude fibre and lipid contents of the selected mushroom macrofungi were determined.

Keywords: Alagarhills, Basidiomycetes, Mycochemicals, wild mushroom fungi, Metabolites

1. Introduction

Mushrooms are fascinating group of fungi appearing in different shapes, size and color. Mushroom imparts diversification in farming to meet out the global needs such as food, health and environmental sustainability. Mushrooms are wide diversified macro-fungus (Isabel, 2016) with fleshy, distinctive fruiting body or sporophores or basidiocarp, that may be hypogeous or epigeous, can be seen through naked eye and to be

picked by hand (Chang & Miles, 1989). A mushroom is an example of a basidiocarp, a reproductive structure common to all species of fungi within the division Basidiomycota. The Basidiomycetes mushroom and some ascomycota species possess some characteristic features like stipe, pileus.

Mushrooms are well documented as source of nutrients as well as biopotential compounds beneficial to mankind. (Chand ST, 2011). Amidst the numerous varieties of mushrooms extensively studied, some local wild mushrooms are still mysterious. Wild mushrooms are utilized by the mycophilic society and documented in many countries due to their valid contribution to the forest ecosystem as a non timber source.(Chang & Lee, 2004). They are exploited abundantly for food and medicine. Mushrooms have been commercialized for cultivation and for therapeutic purposes. (Robert Garibay, 2005 and Adhikari et al., 2005). The mushrooms are seasonal organisms growing in their native habitat which limits the utilization of mushrooms and can be cultivated *in vitro*. The basidiocarp appear only during stipulated period while the *in vitro* cultures can be produced under controlled conditions. The compounds produced by in vitro cultures are same as of the fruiting bodies (Muszynska, 2017). The physical parameters like pH, temperature and moisture and chemical composition of carbon, nitrogen and minerals can be optimized in laboratory conditions. (Petre et al., 2010)

Most species of mushroom belonging to different genera are rich in various vital nutrients like carbohydrates, proteins, vitamins, lipids, mineral, crude fibre various amino acids (Okwulehie, 2004). Polysaccharides of mushrooms have wide spectrum of applications. They have beneficial therapeutic values and health promoting effects. Many mushroom species viz *Ganoderma*, *Flammulina*, *Cordyceps*, *Pleurotus* were documented for antiproliferative, immunomodulating, antimicrobial, antioxidant,

hypcholesterolemic, hypoglycemic bioactive properties (Wang et al., 2017). The mycelia and sporocarp of fungus are rich source of proteins. Lectins, antifungal proteins, ubiquitin like proteins, immunomodulatory proteins, peptides and enzymes such as laccases, peroxidises, proteases are found in mushrooms. Total polyphenols and dietary flavanoids are significant secondary metabolites showing antioxidant effects and health promoting properties respectively. (Jinting et al., 2017)

Poly unsaturated fatty acids are in high proportion in the mushrooms (Pe Dnueault et al., 2006), they reduce serum cholesterol. Ergosterol is a key component in mushrooms exhibiting antioxidant property (Guillam et al., 2010). The current investigation intended at determining the proximate composition of proteins, carbohydrates and lipids and analysis of phytochemicals such as Phenolics, flavanoids and tannins.

2. Materials and Methods

2.1 Samples

The samples used for the present investigation were cultivable wild mushrooms obtained from the hilly regions of Alagarkovil, Madurai district. The wild mushrooms were expressed as AMI –Alagarhill Mushroom Isolate. The number assigned was followed by their order of collection. The mushroom fungi used were enlisted in Table 1.

Table 1: List of cultivable wild mushrooms collected from Alagar hills

S.No	Mushroom	Source
1	AMI 2	Alagarhills, Madurai
2	AMI 3	Alagarhills, Madurai
3	AMI 5	Alagarhills, Madurai
4	AMI 8	Alagarhills, Madurai
5	AMI 10	Alagarhills, Madurai
6	AMI 11	Alagarhills, Madurai
7	AMI 12	Alagarhills, Madurai
8	AMI 14	Alagarhills, Madurai

AMI-Alagarhill Mushroom Isolate



Fig 1: List of wild basidiomycetes mushroom fungi

2.2. Maintenance of stock culture

The pure cultures of mushrooms isolated from the wild were maintained both in slant and plates. The cultures were stored in refrigerator at 15degree Celsius. Subcultures of each macrofungi was done periodically at one month interval to ensure the viability and sustenance. 7mm mycelial agar plugs from the stock cultures were transferred under sterile conditions to Potato Dextrose Agar medium and incubated at room temperature ($28\pm 2^{\circ}\text{C}$) until confluent growth of mycelia occurs.

2.2.1 Broth culture and mycelial biomass

50ml of Potato Dextrose Broth was distributed in Erlenmeyer flasks and plugged properly with cotton wool. The flasks were autoclaved at 121.1°C for 15 minutes and the medium was cooled to room temperature. Antibiotics such as streptomycin were added at a concentration of 1mg/ml in order to avoid bacterial contamination. 8mm mycelia agar plugs taken from the growing edge of 7 days old culture were transferred aseptically into each flask and incubated statically at room temperature ($28\pm 2^{\circ}\text{C}$) for a period of 28 days for growth and biomass production. The mycelia were taken from the growing edge after mat formation over the medium. The harvesting was done once in 7days till growth was achieved.

2.3 Hot water extracts of mycelia

Mycelia were separated from the broth by vacuum filtration using Whatman No-1 filter paper. The mycelial biomass was rinsed with sterile water, blot dried and weighed. With minor modifications of the procedure described by Yim et al., (2009) and Turkmen et al., (2006) extraction was done. One gram of mycelia powder was mixed with 10ml of distilled water and extracts were prepared at 80°C . The mixture was kept in shaker at 150rpm for 3 hours at $28\pm 2^{\circ}\text{C}$ and for 10minutes at 15°C . The extract was filtered using Whatman No.1 filter paper. The filtrates obtained were free from cultures and used for further analysis. The aqueous mycelia extracts' were utilized for studying different bioactive compounds of mushrooms and their biological potential.

2.4 Quantification of metabolites

2.4.1 Total Carbohydrates

Phenol-Sulphuric acid method (Dubois et al., 1956) was followed for quantification of carbohydrate content. 100mg of pulverized mycelia was dissolved in 5ml of 0.5N HCl and kept in boiling water bath for about 3 hours. The mixture was neutralized with 0.5N NaOH and the volume of the solution was made up to 100ml with distilled water.

0.1ml aliquots of the solution was used for estimating the total carbohydrates. 1mg/ml of D-Glucose stock was used as standard. Different concentrations of standard and test sample were taken in test tubes and the volume was made up to 1ml with distilled water. 1ml of 5% phenol followed by 5ml of 96% phenol was added to each tube. The samples were shaken well and incubated at room temperature $28\pm 2^{\circ}\text{C}$ for 20 minutes. Absorbance of the samples were read at 490nm. The total carbohydrate content was expressed in terms of percentage. (Rao and Pattabiraman, 1989)

2.4.2 Total Protein

2.4.2(a) Protein extraction (Lowry et al., 1951)

1 g of fresh mycelium was ground using 5ml of Tris HCl buffer with pH 6.8 and allowed to stand at 4°C for 10 minutes. The mixture was homogenized completely. The homogenate was centrifuged at 3000rpm for 5 minutes and the clear supernatant was collected in a fresh screw cap tube and the volume was made up to 10ml with the Tris buffer. 2ml of 10% ice cold TCA was added to 1ml of the extract incubated for an hour and centrifuged at 10000 g or 10minutes to precipitate protein. The pellet was redissolved in 2ml of 0.1N NaOH.

2.4.2(b) Estimation of Protein

The total protein content of the mushrooms was estimated as per the method proposed by Lowry et al., 1951. 5ml of alkaline CuSO_4 was added to 1ml of the protein extracts of all the mushroom samples. The suspensions were shaken vigorously and kept in dark for 15minutes at room temperature $28\pm 2^{\circ}\text{C}$. Folin phenol reagent (0.5ml) was added to each tube. Then the mixture was incubated in dark at room temperature for 10minutes. The optical density was measured at 660nm. The same procedure was adopted for estimation of standard too. Bovine Serum Albumin (BSA) at a stock concentration of 1mg/ml was used as standard.

2.4.3 Content of Crude fibre (Maynard 1970)

2g of fresh ground material was defatted with petroleum ether. The dried matter was boiled with 200ml of concentrated H_2SO_4 for 3 minutes with bumping chips. The solution was filtered through linen cloth and repeatedly washed with water till it became acid free. The resultant residue was underwent to alkali digestion by boiling with 200ml of NaOH

solution for 30 minutes, filtered again with linen cloth and subjected to wash with 25ml of boiling 1.25% H_2SO_4 . Then the mixture was washed with 50ml of water and 25 ml of alcohol thrice. The residue was transferred to pre weighed ashing dish (W1). The residue was kept in hot air oven at $130\pm 2^{\circ}\text{C}$. Then it was allowed to cool and weighed (W2). The residue was then ignited at $600\pm 15^{\circ}\text{C}$, cooled in a dessicator and weighed again (W3). The content of the crude fibre was calculated by the formula

% of crude fibre =

$$\frac{\text{Loss in weight on ignition } (W2-W1)-(W3-W1) \times 100}{\text{Weight of the sample}}$$

2.4.4 Total lipid content

Lipid content was determined by homogenizing mycelia in 20ml chloroform: methanol 2:1(v/v) mixture for 10 minutes in a homogenizer. The homogenate was shaken vigorously and filtered. The residue was re-stirred with 25ml chloroform: methanol solvents for 30 minutes and filtered. The combined filtrate was then dissolved with 0.9% NaCl to exclude non-fat components (Folchet al., 1991). The solvent layer was dried in vacuum and the total fat content was estimated by the method of Itoch and Kaneko, (1974).

2.5 Quantification of phytochemicals

2.5.1 Total Phenolics (TP)

The total content of the phenolic compound present in the mycelial extract was determined by Folin-Ciocalteu method with minor modifications (Zhao and Hall, 2008). 1ml of sample was mixed with equal volume of Folin-Ciocalteu reagent. 1ml of 7.5% of sodium carbonate was added to the mixture and after 3 minutes the final volume was made upto 10ml with distilled water. The preparation was kept at dark for 90minutes at room temperature. Optical density was measured at 725nm using spectrophotometer (Systronics, India). Gallic acid was used at a concentration of 50-1000 $\mu\text{g/ml}$ for calibration. The phenolic content was expressed as gallic acid equivalents (mg gallic acid equivalents/100g or mg/g). The experiment was carried out in triplicates for each sample.

2.5.2 Total Flavanoids (TF)

The total flavanoid content was estimated by colorimetric method with slight variations (Xu and Chang, 2007 and Yoo et al., 2008). 0.25ml of sample was mixed with 150µl of 10% AlCl₃.6H₂O. Then the sample was incubated for 5 minutes at room temperature. 0.5ml of 1M NaOH and 2.5ml of distilled water were added to the mixture after incubation. The mixture was then subjected to brief vortexing and developed pink color was read at a wavelength of 510nm using colorimeter (Systronics, India) against the blank. Catechin at a concentration of 10-1000µg/ml was used as a standard for calibration. All the determinations were carried out in triplicates. The results were expressed as catechin equivalents (mg (+)-catechin equivalent)/100g) or mg/g.

2.5.3 Condensed Tannins (CT)

Condensed Tannins of the mycelial extracts were analyzed by colorimetric analysis by the method described by Chavan et al., (2001) and Xu and Chang (2007) with minor modifications. 50µl of the sample was added with 3ml of 4% methanolic vanillin solution. 1.5ml of Concentrated HCl was then added to the mixture, vortexed and allowed to stand for

15minutes at room temperature. 5ml of 4% concentrated HCl in methanol was taken as blank. Optical density of the samples were measured at 440nm. Catechin was used as a standard for plotting the calibration curve. The experiment for each sample was carried out in triplicates. The tannin content was shown in terms of mg/g.

3. Results

3.1 Metabolites estimation

Carbohydrates, proteins, lipid, crude fibre contents contribute substantially to the nutritional component of mushrooms. The percentages of each component in selected wild mushroom species were determined.

3.1.1 Carbohydrate content in mycelia

The total carbohydrate content of the mycelia of the selected wild mushroom fungi ranged between 8.46 % to 55%. AMI 14 contains less carbohydrates than the other mushroom species. AMI 8 (55.03%) had the highest content of carbohydrates followed by AMI 2(45.03%). AMI 12 has significant low content of mycelial carbohydrate next to AMI 14.

Table 2 : Total Carbohydrates content (%dry weight) of mycelia of wild mushroom fungi

S.No	Mushroom	Total carbohydrates (%)
1	AMI 2	45.04±0.25
2	AMI 3	28.06±0.20
3	AMI 5	19.07±0.40
4	AMI 8	55.04±0.25
5	AMI 10	18.50±0.20
6	AMI 11	25.37±0.15
7	AMI 12	9.04±0.35
8	AMI 14	8.47±0.35

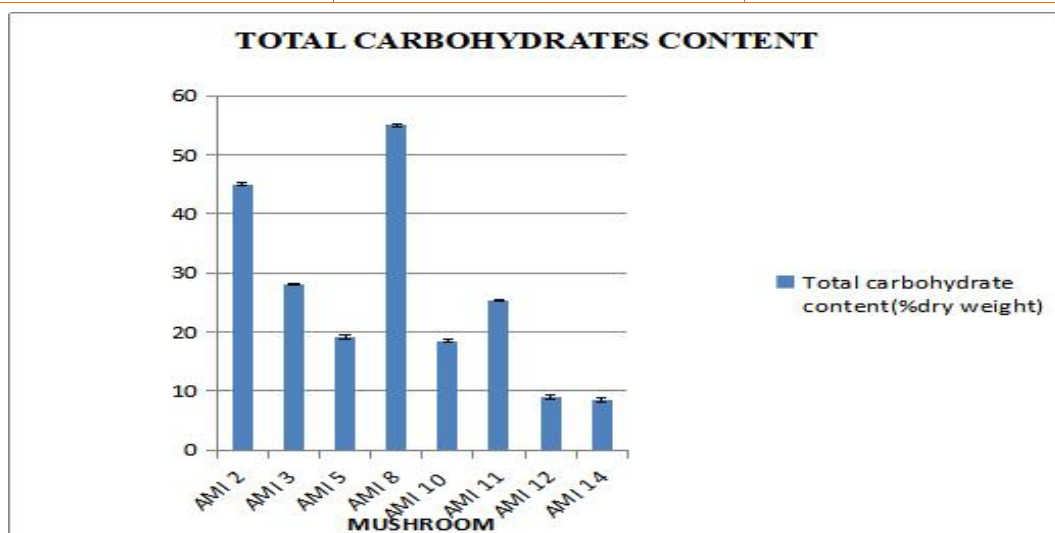


Fig 2: Content of total carbohydrates (%dry weight) in the selected mushroom fungi

3.1.2 Mycelial protein content

Total mycelial protein was analyzed by Lowry method and the results were expressed in terms of percentage dry weight. AMI 2 possess highest content mycelial

protein (29.2%) followed by AMI 14(23.5%) and AMI 14(23.2%). The percentage of total protein in other mushrooms falls in the range of 12.47% to 21.72%. (Table 3, Fig.2)

Table 3: Total Protein content (%dry weight) of mycelia of wild mushroom fungi

S.No	Mushroom	Total protein (%)
1	AMI 2	29.20±0.21
2	AMI 3	21.72±0.30
3	AMI 5	17.77±0.13
4	AMI 8	13.70±0.34
5	AMI 10	12.47±0.06
6	AMI 11	23.24±0.35
7	AMI 12	17.23±0.10
8	AMI 14	23.51±0.33

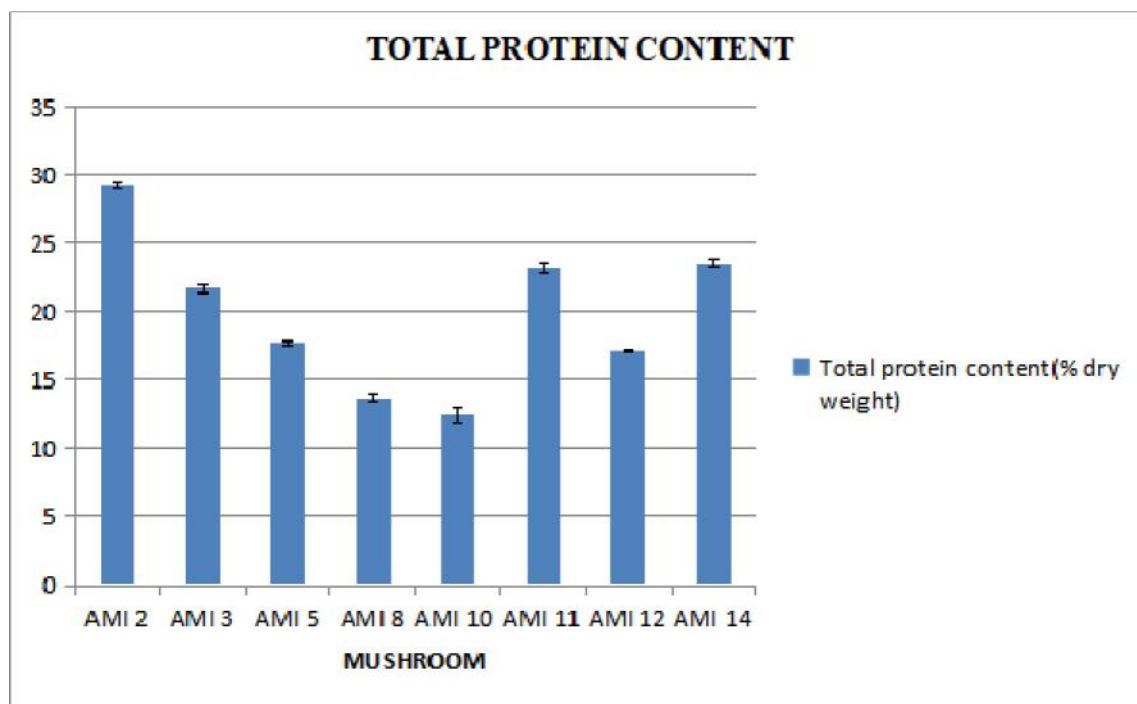


Fig 3: Content of total protein (%dry weight) in the selected mushroom fungi

3.1.3 Lipid content in mycelia

Fig. 3 shows the estimated values of lipid present in each mushroom species. AMI 11 and AMI 8 contained the maximum lipid content of 3.14% and 2.93%

respectively. AMI 2 had slightly lower lipid percentage (2.87%) than AMI 8. The result of other mushroom species had a relatively low content of lipids in mycelia. The lipid content of mushrooms like AMI 3, AMI 5, AMI 10, AMI 12 was below 2%.

Table 4: Total lipid content (%dry weight) of mycelia of wild mushroom fungi

S.No	Mushroom	Total Lipid(%)
1	AMI 2	2.87±0.55
2	AMI 3	1.57±0.32
3	AMI 5	1.27±0.35
4	AMI 8	2.93±0.61
5	AMI 10	1.67±0.15
6	AMI 11	3.13±0.60
7	AMI 12	1.40±0.30
8	AMI 14	2.63±0.31

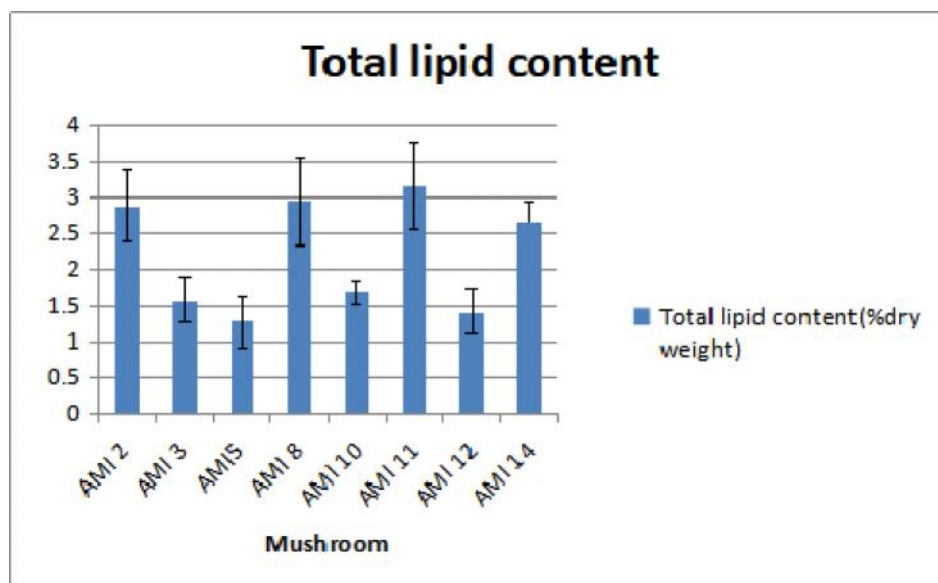


Fig 4: Content of total lipid (%dry weight) in the selected mushroom fungi

3.1.4 Crude fibre content

The crude fibre content was expressed as percentage dry weight. AMI 5 had minimal percent of crude fibre

as 8.7 %.The crude fibre content of the selected mushrooms ranged between 8.7 and 12.3%. AMI 14 possess maximum percentage dry weight of crude fibre.

Table 5: Crude fibre content (%dry weight) of mycelia of wild mushroom fungi

S.No	Mushroom	Crude fibre (%)
1	AMI 2	11.70±0.75
2	AMI 3	9.03±0.61
3	AMI 5	8.70±0.50
4	AMI 8	7.63±0.59
5	AMI 10	10.40±0.36
6	AMI 11	11.41±0.30
7	AMI 12	9.30±0.60
8	AMI 14	12.37±0.15

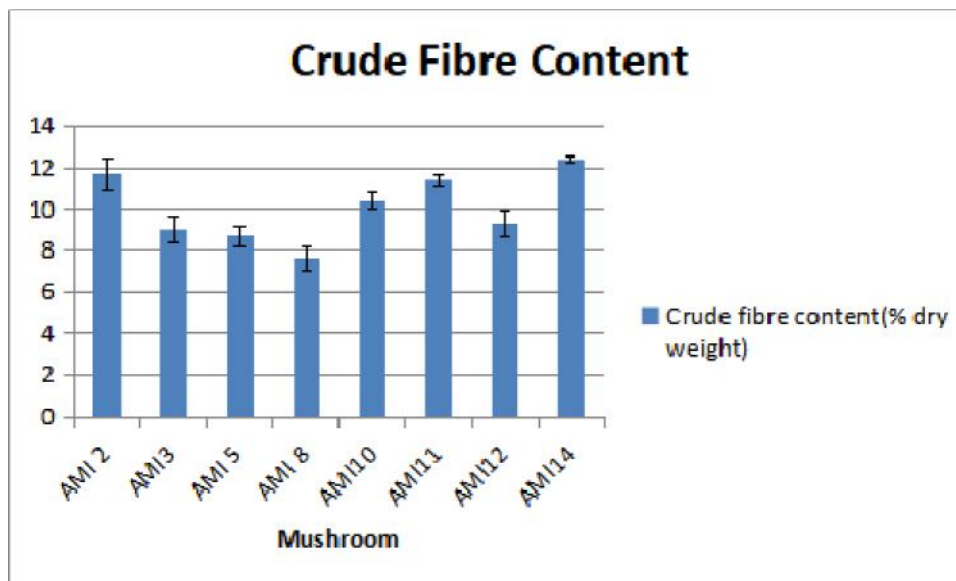


Fig 5: Content of crude fibre (% dry weight) in the selected mushroom fungi

3.2 Phytochemicals quantification

Secondary metabolites such as phenolics, flavanoids and condensed tannins were quantified for the selected wild basidiomycetes mushroom fungi. They were expressed in terms of mg/g dry weight with respective equivalents.

3.2.1 Phenolic compounds

The total phenolic compounds were estimated colorimetrically as gallic acid equivalents. The content of phenolics ranged between 2.2 to 42.6 mg/g dry weight. AMI 11 shows least quantity whereas AMI 10 has maximum amount of phenolics.

Table 5: Total Phenolics content (mg/g dry weight) of mycelia of wild mushroom fungi

S.No	Mushroom	Total Phenolics(mg/g)
1	AMI 2	30.17±0.76
2	AMI 3	10.67±5.03
3	AMI 5	7.80±1.15
4	AMI 8	11±3.61
5	AMI 10	42.67±4.16
6	AMI 11	2.17±0.59
7	AMI 12	6.47±0.50
8	AMI 14	17±3.61

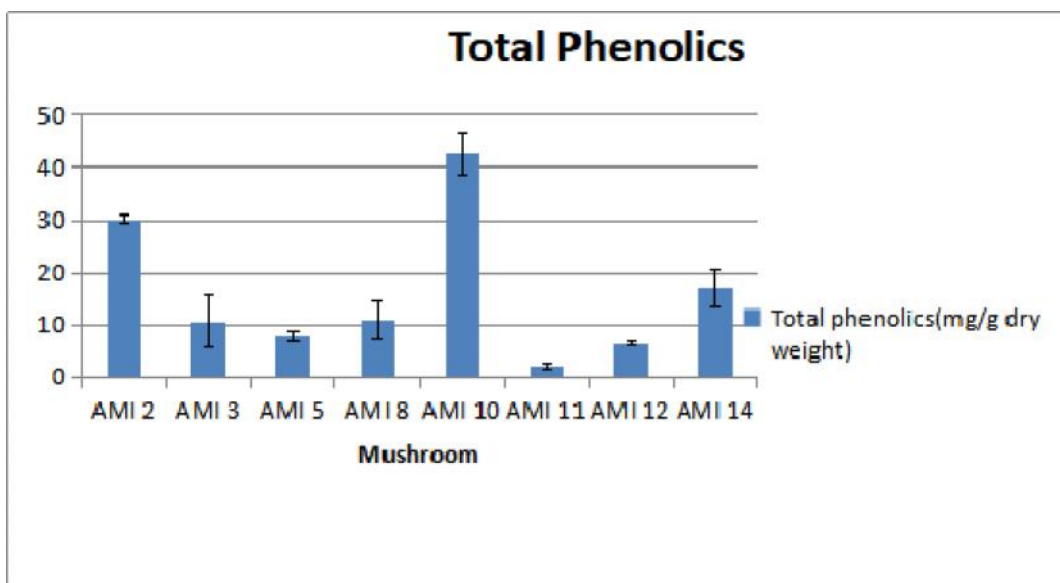


Fig 6: Content of total phenolics (mg/g) in the selected mushroom fungi

3.2.2 Flavanoids

The total flavanoid content of all the selected mushroom fungi were determined

spectrophotometrically as catechin equivalents. AMI 11 possess relatively low quantity of flavanoids and AMI 14 has high quantity of flavanoids when compared with other selected mushrooms.

Table 6: Total Flavanoids content(mg/g dry weight) of mycelia of wild mushroom fungi

S.No	Mushroom	Total Flavanoids(mg/g)
1	AMI 2	27.17±2.84
2	AMI 3	34.67±3.06
3	AMI 5	26±3.61
4	AMI 8	28.67±6.67
5	AMI 10	31±2.65
6	AMI 11	24.33±3.21
7	AMI 12	32.66±6.11
8	AMI 14	38.33±4.73

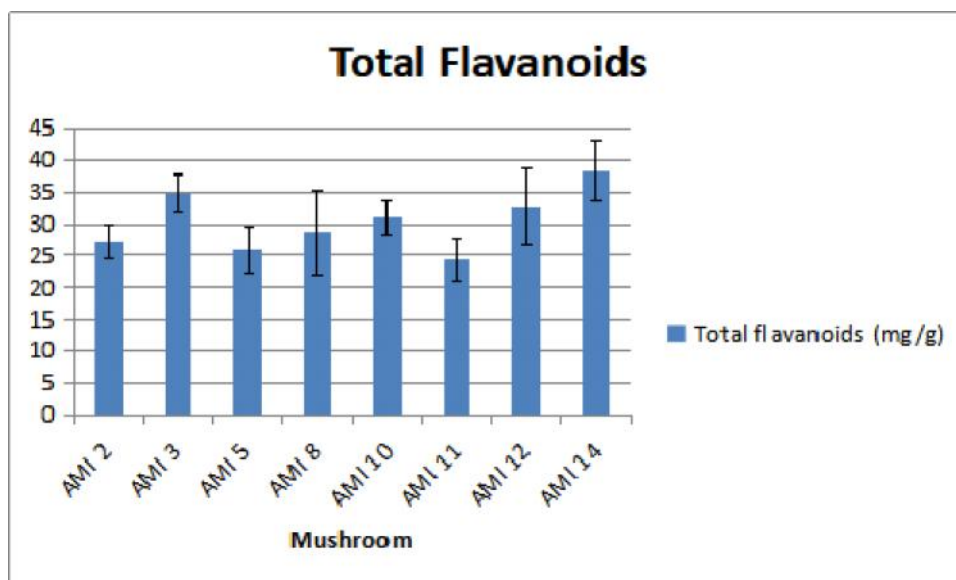


Fig 7: Content of total flavanoids (mg/g) in the selected mushroom fungi

3.2.3 Condensed Tannins

The quantity of condensed tannins in the selected mushroom fungi were determined colorimetrically as catechin equivalents and expressed in mg/g.

The quantity of tannins ranged between 2.16 ± 0.58 mg/g and 10.0 ± 0.49 mg/g. AMI 10 has maximum tannin content while AMI 11 has the least amount of tannins among the selected macro fungi.

Table 7: Condensed Tannins content (mg/g dry weight) of mycelia of wild mushroom fungi

S.No	Mushroom	Condensed Tannins(mg/g)
1	AMI 2	6 ± 0.4
2	AMI 3	8.07 ± 0.80
3	AMI 5	7.93 ± 0.31
4	AMI 8	7.17 ± 0.76
5	AMI 10	10.01 ± 0.49
6	AMI 11	2.17 ± 0.59
7	AMI 12	5.97 ± 0.45
8	AMI 14	7.47 ± 0.35

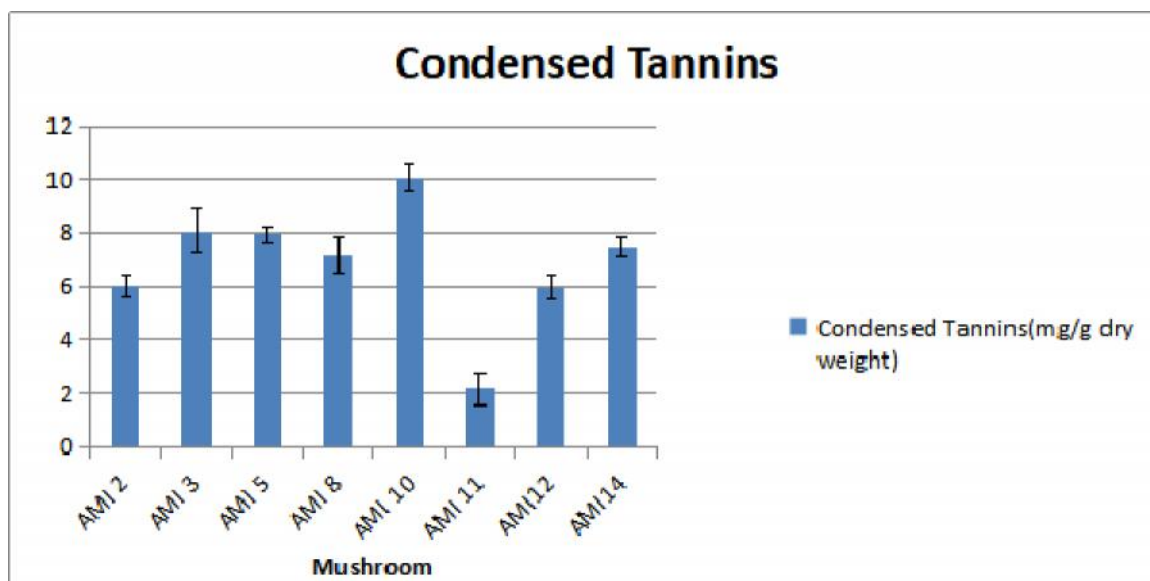


Fig 8: Content of condensed tannins (mg/g) in the selected mushroom fungi

4. Discussion

Mycelial protein content of the selected macro fungi were estimated from $13.7 \pm 0.3\%$ dry weight to $29.2 \pm 0.2\%$ dry weight. AMI 2 possessed significantly high amount of mycelial protein followed by AMI 14. Lower mycelia protein content was noticed in AMI 10 and AMI 8 as $12.46 \pm 0.05\%$ and $13.7 \pm 0.3\%$ dry weight respectively.

Mushrooms are documented as natural sources of nutraceuticals and are rich in proteins, minerals and vitamins but have low lipid content (Pathak et al.,

1997). The content of mycelia protein are correlated well with Rashad et al., (2009) citing that the protein content of mycelia ranged between 10.5 and 30.4% dry weight. The high protein content validates the complement “mushrooms as vegetarians meat”. Proteins are vital for maintainance of structure and function and crucial for growth and development. Significantly higher mycelia protein content makes them as promising component in increasing food value or a bioactive protein could also be isolated. (Thomsen, 1991)

The reports of the present study was in agreement with the results of previous studies which showed that mushroom macrofungi are low in crude lipid content (Bahl, 1998 and Raghunathan, 2003). The proportion of lipid generally varies from 1.75% to 15.5 % (Hong et al., 1988). Mycelial lipid content of the present study was on par with the results of Ogowok et al., (2017) citing that lipids ranged between 0.25% -5.23% dry weight .Many macrofungi are documented as rich sources of polyunsaturated fatty acids (Ergonul et al., 2013). Edible mushrooms found to contain upto 27.4% polyunsaturated fatty acids (Barros et al., 2007).The low fat content of mushrooms suggests them to be a component contributing dietary as well as health benefits.

Dietary fibres are important indigestible carbohydrates resistant to human enzymes which are components of cell wall of mushrooms (Cheung, 2013). Secondary metabolites such as Phenolic compounds, flavanoids and tannins were quantified in the selected basidiomycetes fungi. These metabolites are not essential for the actual growth of the macrofungi but they exhibit biological activity (Wynk.). The most reknown bioactive metabolites include phenolics, alkaloids, saponins, tannins (Lingaro, 2011).

Significant content of total phenolics was observed in the selected mushroom fungi. The earlier studies on phenolic compounds reveal their importance as antioxidants, antimicrobials .Considerable variations exist in the quantity of total phenolic content among the mushrooms of interest. Polyphenols are the widely distributed compound among the secondary metabolites. Phenolics exhibit high level of antioxidant activity, act as potent radical scavengers, provide defense against UV radiation or pathogen aggression (Shirwaikar, 2003).

Aqueous extracts of macrofungal mycelia possess higher TPC, TFC and CT (Yim et al., 2006).The chemical constituent of the mushrooms are revealed by the quantitative analysis of primary and secondary metabolites. The total flavanoids are in the range of 24.33±3.21 to 38.34±4.72 mg/g. Flavanoids exert wide spectrum of biological potentials such as anti-inflammatory, antiviral, antibacterial, anti allergic (Cushnie TP, 2005 and Cook, Nc, 1996) cytotoxic, antitumour, treatment of neurodegenerative diseases, vasodilatory action (Tsuchiya,2010 and Chebil L et al., 2006). Flavonoids retard lipid peroxidation, platelet aggregation, capillary permeability and fragility, cyclo-oxygenase and lipoxygenase enzyme

activities. These activities are exerted as free radical scavenging, chelation and antioxidant effects (Middleton, 2000). Flavanoids bear inhibitory activity against hydrolases, hyaluronidase, alkalinephosphatase, arylsulphatase, cAMP phosphodiesterase, lipase, -glucosidase, kinase (Narayana, KR, 2001).

Condensed tannins inhibit the growth of many fungi, virus, bacteria, yeast (Bajaj YPS, 2001).Tannins contribute astringent property (Akiyama H, 2001).The content of tannins of the selected mushroom fungi falls between 2.16±0.58 mg/g and 10.0±0.49mg/g. The results are in conformity with the reports of Renugadevi et al., (2015). The results obtained from the present study indicates that the selected wild mushrooms will serve as a source of potent bioactive compounds which have pharmacological benefits. The presence of considerable amount of phytochemicals reveal their significance as biopotent molecules.

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