Antioxidant and Anti-inflammatory Properties of Cultivated Mushrooms Grown in Mekelle City Tigray Ethiopia

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Abstract: Traditionally mushrooms have been used for the prevention and treatment of a multitude disorders. Macro fungi regarding to the development of novel safe antimicrobials and antioxidants has become attractive source for researchers in the last decades. In the present study quantitative analysis of phytochemical constituents was carried out by using standard methods while 1,1 Diphenyl picryl hydroxyl(DPPH) Nitric oxide, Hydrogen peroxide free radical scavenging assay were used to evaluate the antioxidant properties of selected wild mushrooms. Anti-inflammatory capacity of samples was evaluated by HRBC membrane stabilization method. The results obtained from the study revealed that Agaricus Bosporus showed higher total phenol, mg flavonoid carotenoid, and lycopene and ascorbic acid contents of (617.9±1) mg/g, (62.52±1.13) mg/g, (74.2±0.057) µg / mg, (49.6±0.17) µg / and 28.8±0.34 mg / g. All the species showed antioxidant potential but Russula delica proved to be more active while Agaricus Bosporus. Proved to be least one.

Keywords: Wild Mushrooms, Scavenging Effect, Total Phenol, Anti-oxidant, Anti-inflammatory

1. Introduction

Mushrooms have received increasing attention, since the primers of human civilization; wild mushrooms have been part of human diet for centuries, mainly because of variety of flavors and tastes they provide. They possess great potential for nutrition, as a rich sources of minerals and having high amounts of water, protein, fiber, and carbohydrates. Lipids are present in low values, which make valuable foods due to low-calorie and low-fat content (Kalac 2009, 2012). This enhances their marketability and economic contribution by approximately two billion dollars (Wang and Hall 2004, Pettenella, Kloehn and 52- 68, 2007). Beyond the nutritional characteristics, a vast body of evidence indicates that mushrooms have been also extensively studied for their medicinal properties, mainly due to their richness in biologically active compounds that presented antioxidant, antimicrobial properties, strengthening the immune system and shielding against carcinogens (Ramesh and Patter 2010). For the effective treatment of infections the growing emergence of drug-resistant bacterial strains is a serious threat to mankind. It is important to choose the most appropriate antibiotics and to use them appropriately to minimize the development of drug-resistant strains as it is impossible to prevent bacterial evolution. (Klein Smith and Laxminarayan, 2007). Another significant health issue is stress on the body due to aging, obesity, and detrimental lifestyle choices is, which often takes the form of oxidative damage to tissues. In Human body free radicals are constantly formed, during energy production in the mitochondrial respiratory chain, phagocytosis, fertilization, arachidonic acid metabolism and xenobiotic metabolism. Free radicals such as Super- oxide radicals( ) and hydrogen peroxide ( ) damage DNA, impair enzymes and structural proteins, and provoke uncontrolled chain reactions including lipid peroxidation which leads to the development of cancer, cardiovascular and neurological diseases, cataracts, diabetes, and rheumatoid arthritis. (Circu and Aw, 2010, Jeong et al., 2012). ROS (Reactive oxygen species) are also responsible in development of systemic inflammatory response syndrome, and then they activate nuclear factors thus inducing the synthesis of cytokines. Later, inflammatory mediators and adhesion molecules are also formed. Free radicals react with different cell components at site of inflammation thus leading to loss of function and cell death (Closa and Puy, 2004). However to protect against free radical damage almost all organisms have defense systems which is not sufficient. Many synthetic antioxidants, such as butylated...
2. Material and Methods

2.1. Material

Four mushrooms were selected for the evaluation of antioxidant as well anti-inflammatory
I.e. Agaricus bisporous, PleurotusFlorida, Russula delica, Lyophyllum decaestosy were obtained from mekelle research centerMekelle Ethiopia

2.2. Determination of Bioactive Compounds

2.2.1. Standard and Reagents

All the Standards solvents other chemicals were of analytic grade.

2.2.2. Phenolic Compound Estimation

100µl of extract solution was mixed with 1.5ml of Folin-Ciocalteu reagent and incubated at room temperature for one minute followed by the addition of 1.5ml sodium carbonate (60g/l). The tubes were then vortex-mixed for 15s and allowed to stand for 90 minutes in dark at room temperature. Absorbance was then measured at 725 nm. Gallic acid was used as standard curve with concentration ranges from 1 to 100 µg/ml (R^{2}=0.996) and the results were expressed as mg Gallic acid equivalent (GAE)/100g DW. All experiments were performed in triplicates. (Singleton et al., 1999)

2.2.3. Flavonoid Contents

250 µl extract of mushrooms was mixed with 1.25 ml of distilled water and 75 µl of a 5% NaNO_{2} solution. 150 µl of 10% AlCl_{3}· H_{2}O of was added after 5 min. Then 275 µl of distilled water and 500 µl of 1 M NaOH were added to the mixture after 6 min. The solution was then mixed well and the intensity of pink colour was measured at 510 nm. The flavonoid compounds estimation was carried out in triplicate. Quercetin was used for calibration curve with concentration range of 20-100 µg/ml (R^{2}=0.9938). The results were mean values ± standard deviations and expressed as mil-ligrams of (+) quercetin equivalents (CEs) per gram of extract. (Jia et al., 1999)

2.2.4. Ascorbic Acid Determination

100 mg of dried extracts were extracted with 10 ml of 1% Meta phosphoric acid for 45 min at room temperature and filtered through Whatman No.4 filter paper. The filtrate (1 ml) was mixed with 9 ml of 2, 6-dichloroindophenol and then absorbance was measured within 30 min at 515 nm against a blank. Content of ascorbic acid would be calculated on the basis of the calibration curve of authentic L-ascorbic with concentration range of 2- 125 µg /ml (R^{2}=0.9929). All the experiments were performed in triplicates. (Klein and Perry, 1982)

2.2.5. β-Carotene and Lycopene Determination

The dried extract (100 mg) was vigorously shaken with 10 ml of acetone–hexane mixture (4:6) for 1 min and filtered through Whatman No.4 filter paper. The absorbance of the filtrate was measured at 453, 505, 645 and 663 nm (Nagata and Yamashita, 1992).

Content of β-carotene and lycopene were calculated by using following equations. Lycopene (mg/100mg) = -0.0458 A_{665}+ 0.372 A_{505} -0.0806 A_{453}

β-carotene (mg/100mg) = 0.216 A_{663}- 0.304 A_{505}+ 0.452 A_{453}

2.3. Determination of Antioxidant Capacity

2.3.1. DPPH Free Radical Scavenging Activity

Free radical scavenging activity of mushrooms extracts was determined by DPPH method.0.1mM of DPPH solution was prepared in methanol and 0.5 ml of it was added to 0.5 ml of extract. The mixture was vortexed thoroughly and left for 45 minutes in dark at room temperature and absorbance was measured at 515 nm against blank. A lower absorbance represents a higher DPPH scavenging activity. The capability of scavenging DPPH radical was calculated using following equation

DPPH scavenging effect (%) = (1-A_{S}/A_{C}) x 100

Where A_{C} is the absorbance of control containing DPPH; A_{S} is absorbance of extract solution containing DPPH. (Hung and Morita, 2009).

2.3.2. Nitric Oxide Scavenging Assay

The interaction of extract of macro fungi with nitric oxide (NO) was assessed by nitrite detection method. The chemical source of nitric oxide was sodium nitroprusside (10mM) in 0.5 M phosphate buffer pH 7.4 which spontaneously produced nitric oxide in aqueous solution. Nitric oxide interacted with oxygen to produce stable products, leading to the production of nitrates. After incubation for 5 hours at 3 C 0.5 ml of riess reagent (α-napthylethylenediamine 0 .1 % in water and sulphanilic acid 1 % in H_{2}PO_{4} 5 %) was added. Absorbance of solution was measured at 546 nm. (Tewariet al., 2014)

2.3.3. H_{2}O_{2} Radical Scavenging Assay

A solution of H_{2}O_{2} (40mM) was prepared in phosphate buffer (7.4pH). The concentration of H_{2}O_{2} was determined by absorption at 230nm using spectrophotometer. Extracts (10- 100µg/ ml) were then added to H_{2}O_{2} solution and after
10 minutes of incubation the absorbance was determined at 230nm. Phosphate buffer without \( \text{H}_2\text{O}_2 \) was used as blank. Ascorbic acid was used as standard curve. (Khaled-Khodjaa et al., 2014)

2.3.4. HRBC Membrane Sterilization Assay

Blood was collected from healthy volunteer who was not taken NSAID for two weeks. The collected blood was mixed with equal volume of sterilized AL sever solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid, and 0.42 % sodium chloride in water). The blood was centrifuged at 3000 rpm for 15 minutes. Packed cell were washed with isosalone. Different concentration of extract (100, 250, 500 and 1000 µg/ ml) were prepared in isosaline.

The assay mixture contained 0.5ml of extract 1ml phosphate buffer (0.15M, pH 7.4) 2ml of hypo saline (0.36 %) and 0.5ml HRBC suspension and incubated at 3 C for 30 minutes and then centrifuged at 3000 rpm for 20 minutes. Diclofenac sodium was used as reference standard. Instead of hypo saline 2ml of distilled water was used in control. The haemoglobin content in supernatant solution was estimated using spectrophotometer at 560 nm. (Gandhidasan et al., 1991, Chippada et al, 2011).

\[
\%\text{hemolysis} = \frac{\text{OD}_1 - \text{OD}_2}{\text{OD}_1} \times 100
\]

Where,

\( \text{OD}_1 = \) Optical density of hypotonic buffer saline alone (control).

\( \text{OD}_2 = \) Optical density of test sample in hypotonic buffer saline solution

3. Results

3.1. Quantitative Phytochemical Analysis

The determination of quantitative phytochemical content of methanolic extracts of selected mushrooms results as given in Table 1 that total contents of phenols ranged between (240.9±0.5)-(617.9±1) mg/ g, total flavonoid contents (39.55±0.65)-(62.52±1.13)mg/g, total ascorbic acid contents ( 19.20±0.005 )-(28.8±0.34) mg/g, total \( \beta \)-carotene contents (49.46±0.75 ) - (74.2±0.057)µg/mg and total lycopene content ( 31.4±0.17 )-(49.6±0.17) µg/mg. The mushrooms \textit{Agaricus bisporous} had the highest amount of phenol (617.9 ± 1mg GAE/g) while \textit{Pleurotus Florida} had least amount of phenol present (240.9±0.5mg GAE/g).

| S.No. | Mushrooms     | Phenols(mg/g) | Flavonoids(mg/g) | \( \beta \)- carotene(µg/mg) | Lycopene(µg/mg) | Ascorbic acid(mg/g) |
|-------|--------------|---------------|------------------|--------------------------|----------------|---------------------|
| 1     | \textit{Agaricus bisporous} | 617.9±1       | 62.52±1.13       | 74.2±0.057               | 49.6±0.17      | 28.8±0.34           |
| 2     | \textit{Pleurotus Florida}   | 240.9±0.5     | 39.55±0.65       | 49.46±0.75               | 31.4±0.17      | 19.20±0.005         |
| 3     | \textit{Russuladelica}       | 465.1±0.9     | 55.36±0.64       | 73.6±0.057               | 48.9±0.34      | 22.03±0.05          |
| 4     | \textit{Lyophyllumdecastes}  | 267±0.5       | 44.82±0.65       | 67.6±0.46                | 46.1±0.28      | 20.91±0.005         |

Results are presented as mean ±SEM

3.2. Anti-Oxidant Activity

3.2.1. DPPH Free Radical Scavenging Assay

The antioxidant activities of extracts were expressed as IC50 values of DPPH. The values varied from 342.72±1.21in Russuladelica. To 633.45±1.66µg extracted in \textit{Agaricus bisporous}. As shown in Table 2.

| S.no. | Macro fungi name | IC50 (DPPH) | IC50 (Nitric oxide) | IC50 (H2O2) |
|-------|-----------------|-------------|---------------------|-------------|
| 1     | \textit{Agaricus bisporous} | 633.45±1.66 | 439.8±1.8           | 312.74±1.97 |
| 2     | \textit{Pleurotus Florida}   | 649.6±2.41  | 436.9±2.4           | 310.34±1.87 |
| 3     | \textit{Russuladelica}       | 342.72±1.21 | 400.6±1.8           | 304.39±1.97 |
| 4     | \textit{Lyophyllumdecastes}  | 373.8±0.75  | 422.2±1.7           | 307.32±1.91 |

3.2.2. Nitric Oxide Scavenging Activity

Our finding suggest that all the extracts substantially inhibited nitric oxide production with IC50 value ranges from 400.6±1.8 (RussuladelicaRussuladelica.) to 439.8±1.8(Agaricusbisporous.) as shown in the above Table 2.

3.2.3. Hydroxyl Radical Scavenging Assay

Hydroxyl radical scavenging activity was quantified by measuring the inhibition of the degradation of 2-deoxyribose by free radicals generated by Fenton reaction.

In the present study IC50 value of Hydroxyl radical scavenging activity was calculated and it was observed that the effect on hydrogen peroxide was higher having IC50value 312.74±1.87in \textit{Agaricus bisporous} and lower in Russuladelica having IC50 value 304.39±1.97 as shown in the above Table 2.

3.2.4. HRBC Membrane Stabilization Assay

The extracts of different mushrooms were studied for their in-vitro anti-inflamatory activity and highest activity was observed in \textit{Agaricus bisporous} and least in Russuladelica as shown in Below Table 3.
phenols, flavonoids ascorbic acid and β-carotene and a better free radical scavenging effect due to their redox properties which can play an important role in absorbing in promising polyphenolic compound and therefore known by using FolinCiocalteu’s reagent. Excessive free radical generation and damage. Carotenoids like β-carotene and lycopene exert antioxidant functions therefore the mushrooms can be harnessed in the management of oxidative stress induced diseases. Nitric oxide is free radical present in mammalian cells which are responsible for regulation of various physiological processes but the excess production of NO is associated with several diseases (Ray et al., 2002). Nitric oxide under aerobic condition reacts with oxygen to produce stable products nitrate and nitrite through intermediate NO2, N2O4 and N2O3. Nitric oxide in higher concentration can be toxic therefore inhibition of nitric oxide is an important goal (Wang et al., 2005). H2O2 has strong oxidizing properties. It occurs naturally at low concentration levels in air, water, human body, plants, microorganism and food (Gulcin, 2005). It can be formed in vivo by oxidizing enzymes such as superoxide dismutase. It can cross membrane and slowly oxidize a number of compounds. H2O2 is rapidly composed into oxygen and water and this may produce hydroxyl radicals (•OH) that can initiate lipid peroxidation and cause DNA damage (Sahreen et al., 2011). Methanol fractions of mushroom’s mycelia cultures efficiently scavenged hydrogen peroxide which may be attributed to the presence of phenolic groups that could donate electrons to hydrogen peroxide, thereby neutralizing it into water.

In present study free radical scavenging activity assay of mushrooms were tested using methanol solution of stable free radical, and it was observed that Agaricusbisporous have more appreciable antioxidant activity while Russuladelica have least one. Thus other non-phenolic and flavonoids compound may be responsible for the antioxidant properties of Agaricusbisporous while other may have exhibited their antioxidant activity through different mechanism (Hamzahet al, 2014).
Hypotonicity induced HRBC membrane lysis i.e. HRBC membrane stabilization was taken as a measure of anti-inflammatory activity. Currently much interest have been paid in the searching of medicinal plants with anti-inflammatory activity which may lead to the discovery of new therapeutic agent that is not only used to suppress the inflammation but also used in diverse disease conditions where the inflammation response is amplifying the disease process.

The vitality of the cells depends on the integrity of their membranes. Red blood cell exposure to hypotonic medium results in lysis of its membrane accompanied by hemolysis and oxidation of haemoglobin (Feirrati et al., 1992). The hemolytic effect of hypotonic solution is related to excessive accumulation of fluid within the cell which results in the rupturing of its membrane. It is therefore expected that compounds with membrane-stabilizing properties, should offer significant protection of cell membrane against injurious substances. The lysosome enzymes released during inflammation produces a variety of disorders. Their extracellular activity is said to be related to acute or chronic inflammation. There is increasing evidence that lysosome enzymes play an important role in the development of acute and chronic inflammation (Prakash et al., 2010). Most of the anti-inflammatory drugs exert their beneficial effects by inhibiting either release of these enzymes or by stabilizing lysosome membrane, which is one of the major event responsible for the inflammatory process.

The extracts exhibited membrane stabilization effect by inhibiting hypo toxicity induced lysis of erythrocyte membrane. The erythrocyte membrane is analogous to the lysosome membrane (Mounnissamy et al., 2008) and its stabilization implies that the extract may stabilize the lysosome membranes. Lysosome membrane stabilization is important in limiting the inflammatory response by preventing the release of lysosome constituents of activated neutrophil such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extra cellular release (Chaitanya et al., 2011). Some of the NSAIDs are known to possess membrane stabilization properties which may contribute to the potency of their anti-inflammatory effect. Though the exact mechanism of the membrane stabilization by the extract is not known yet, hypo toxicity-induced hemolysis may arise from shrinkage of the cells due to osmotic loss of intracellular electrolyte and fluid components.

From this result, it is suggested that anti-inflammatory activity observed in this study, may be due to the ability of the extracts to interfere with the early phase of inflammatory reactions, which may stimulate or enhance the efflux of these intracellular components there by exhibiting the anti-inflammatory activity.

5. Conclusion

The present study focuses on antioxidant and anti-inflammatory effects of wild mushrooms collected from Mekelle Tigray Ethiopia. These mushrooms are not only consumed but also utilized for their medicinal properties hence there is a great need to exploit these wild occurring mushrooms from the dense and well expanded forests of Tigray to new drug discovery.

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References

[1] Agaricus bisporus harvested at different stages of maturity. Food Chem. 103, 1457-1464.
[2] Amarowiez R, Pegg R B, Rahimi Moghddam P, Barl B and Weil J A. Free radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. Food chemistry, 2004; 84: 551-562.
[3] Amarowiez R, Pegg R B, Rahimi Moghddam P, Barl B and Weil J A. Free radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. Foodchemistry, 2004; 84: 551-562.
[4] AOAC, 1995. Official methods of analysis (16th Ed.). Arlington VA, USA: Association of Official Analytical Chemists.
[5] Barros, L., Baptista, P., Ferreira, I. C. F. R. 2007. Effect of Lactarius piperatus fruiting body maturity stage on antioxidant activity measured by several biochemical assays. Food. Toxicol. 45, 1731-1737. 325-331. 325-331.
[6] Barros, L., Correia, D. M., Ferreira, I. C. F. R., Baptista, P., Santos-Buelga, C. 2008a. Optimization of the determination of tocoherols in Agaricus sp. edible mushrooms by a normal phase liquid chromatographic method. Food. Chem. 110, 1046-1050.
[7] Barros, L., Cruz, T., Baptista, P., Estevinho, L. M., Ferreira, I. C. F. R. 2008b. Wild and commercial mushrooms as source of nutrients and nutraceuticals. Food. Chem. Toxicol. 46, 2742-2747.
[8] Beluhan, S., Ranogajec, A. 2011.Chemical composition and non-volatile components of Croatian wild edible mushrooms. Food Chem. 124, 1076-1082.
[9] Gandhidasan R, Thamaraichelyan A, Baburaj S. Anti-inflammatory action of Lannea coromandelicabPLICATE HRBC membrane stabilization. Fitoterapia, 1991; 62: 81-83.
[10] Gandhidasan R, Thamaraichelyan A, Baburaj S. Anti-inflammatory action of Lannea coromandelicabPLICATE HRBC membrane stabilization. Fitoterapia, 1991; 62: 81-83.
[11] Bonatti, M., Karnopp, P., Soares, H. M., Furlan, S. A., 2004. Evaluation of PreutusostreatusandPleurotussajor- cajunutritional characteristics when cultivated indifferent lignocellulosic wastes. Food Chem. 88, 425-428.
[12] Braaksma, A., Schampa, D. J. 1996. Protein analysis of the common mushroom Agaricusbisporus. Postharvest Biol. Tech. 7, 119-127.
[13] Çağırirmak, N.2007. The nutrients of exotic mushrooms (Lentinula edodes andAida, F. M. N. A., Shuhaimi, M., Yazid, M., Maaruf, A. G. 2009. Mushroom as a potential source of prebiotics: a review. Trends Food Sci. Tech. 20, 567-575.
[14] Chaitanya R, Sandhya S, David B, Vinod KR, Murali S. HRBC Membrane Stabilizing Property of Roor, Stem and Leaf of Glochidionvelutinum. Int J Res Pharmacist Biomed Sci., 2011; 2(1): 256–259.

[15] Chaitanya R, Sandhya S, David B, Vinod KR, Murali S. HRBC Membrane Stabilizing Property of Roor, Stem and Leaf of Glochidionvelutinum. Int J Res Pharmacist Biomed Sci., 2011; 2(1): 256–259.

[16] Chippada SC, Volluri SS, Bammidi SR, Vanglapati M. In vitro anti-inflammatory activity of methanolic extract of Centellaasiatica by HRBC membrane stabilization. Rasayan J Chemistry, 2011; 4(2): 457-460.

[17] Chippada SC, Volluri SS, Bammidi SR, Vanglapati M. In vitro anti-inflammatory activity of methanolic extract of Centellaasiatica by HRBC membrane stabilization. Rasayan J Chemistry, 2011; 4(2): 457-460.

[18] Circu ML, Aw TY. Reactive oxygen species cellular redox system and apoptosis. Free Radical Biology and Medicine, 2010; 48: 749-762.

[19] Circu ML, Aw TY. Reactive oxygen species cellular redox system and apoptosis. Free Radical Biology and Medicine, 2010; 48: 749-762.

[20] Feirrali M, Signormi C, Ciccilili L, Comporti M. Iron release and membrane damage in erythrocytes exposed to oxidizing agents, phenyl hydrazine, devicene and iso-uranil. Biochem Journal, 1992; 285: 295–301.

[21] Feirrali M, Signormi C, Ciccilili L, Comporti M. Iron release and membrane damage in erythrocytes exposed to oxidizing agents, phenyl hydrazine, devicene and iso-uranil. Biochem Journal, 1992; 285: 295–301.

[22] Grice HC. Safety evaluation of butylated hydroxyanisole from the prospective of effects on forestomach and oesophageal squamous epithelium. Food and Chemical Toxicology, 1988; 26: 717-723.

[23] Grice HC. Safety evaluation of butylated hydroxyanisole from the prospective of effects on forestomach and oesophageal squamous epithelium. Food and Chemical Toxicology, 1988; 26: 717-723.

[24] Gulcin I. The antioxidant and radical scavenging activity of black pepper (Piper nigrum) seeds. International Journal of Food Sciences and nutrition, 2005; 56: 491-499.

[25] Gulcin I. The antioxidant and radical scavenging activity of black pepper (Piper nigrum) seeds. International Journal of Food Sciences and nutrition, 2005; 56: 491-499.

[26] Hamzah RU, Jig am A A, Makun HM, Egwim E C. Phytochemical screening and antioxidant activity of methanolic extract of selected wild edible Nigerian mushrooms. Asian Pacific Journal of Tropical Disease, 2014; 4(1): 153-157.

[27] Hamzah RU, Jig am A A, Makun HM, Egwim E C. Phytochemical screening and antioxidant activity of methanolic extract of selected wild edible Nigerian mushrooms. Asian Pacific Journal of Tropical Disease, 2014; 4(1): 153-157.

[28] Hung PV, Morita N. Distribution of phenolic compounds in the graded flours milled from

[29] Hung PV, Morita N. Distribution of phenolic compounds in the graded flours milled from

[30] Jeong EM, Liu M, Sturdy M, Gao G. Varghese ST, Sovari AA. Metabolic stress, reactive oxygen species and arthythmia. Journal of Molecular and Cellular Cardiology, 2012; 52: 454-463.

[31] Jeong EM, Liu M, Sturdy M, Gao G. Varghese ST, Sovari AA. Metabolic stress, reactive oxygen species and arthythmia. Journal of Molecular and Cellular Cardiology, 2012; 52: 454-463.

[32] Jia ZS, Tang MC, Wu JM. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry, 1999; 64: 555-99.

[33] Jia ZS, Tang MC, Wu JM. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry, 1999; 64: 555-99.

[34] Kalac P. Chemical composition and nutritional value of European species of wild growing mushrooms. In S. Andres and N. Baumann (Eds), Mushrooms: Types properties and nutrition Nova Science Publishers, 2012; 130-151.

[35] Kalac P. Chemical composition and nutritional value of European species of wild growing mushrooms. In S. Andres and N. Baumann (Eds), Mushrooms: Types properties and nutrition Nova Science Publishers, 2012; 130-151.

[36] Ashagrie Z. Woldegiorgis, Dawit Abate, Gulelat D. Haki, Gregory R. Ziegler. Proximate and Amino Acid Composition of Wild and Cultivated Edible Mushrooms Collected from Ethiopia Journal of Food and Nutrition Sciences Vol. 3, No. 2, 2015, pp. 47-54. doi: 10.11648/j.jfns.20150302.14

[37] Masumi Kamiyama, Takayuki Shibamoto, Masahiro Horisuchi, Katsumi Umano, Kazuo Kondo, Yuzuru Otsuka, Antioxidant/Anti-Inflammatory Activities and Chemical Composition of Extracts from the Mushroom Trametes Versicolor, International Journal of Nutrition and Food Sciences. Vol. 2, No. 2, 2013, pp. 85-91. doi: 10.11648/j.jfns.20130202.19.