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Evaluation of the Phenolic Profiling, Flavonoid Content, Antioxidant and Antimicrobial activities of the Selected Three Edible Mushrooms.

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Abstract

Mushrooms are known to mankind and are gaining importance because of them of nutritional and medicinal properties,since early human civilization.Mushrooms are producing of antioxidant and antimicrobial properties that are known to have a potential sources variety of bioactive compounds. Antioxidants defenses through dietary supplementation of edible mushrooms are to reduce the level of oxidative stress, might be in enhancement of directly used.Mushrooms wild or cultivated bioactive compounds are polyphenols, polysaccharides, vitamins, carotenoids and minerals, due to their bioactive compounds, such as polyphenols. they have been related tosignificant antioxidant properties. Antioxidant and health benefits observed in edible mushrooms are seem an additional reason for their traditional use as a popular delicacy food.The methanolic extracts of *A. bisporous, L. edodus, T. heimii,* were analyzed for their antioxidant activities in different test systems namely, total phenolics, and flavonoid contents. To them in addition in activities DPPHfree radical scavenging, Hydroxy free radical, ABTS, ß-carotene/linoleic acid, reducing power and ß-glucans. *A. bisporous, L. edodus*, showed the strongest activity patterns. Their activities were as the positive controls as strong.The species was excellent of the DPPH, ABTS, reducing power.The extracts were increased of hydroxy radical scavenging capacity with the increasing concentration. On the other hand,*L.edodus*, found to have the highest phenolic content. Total flavonoid content of *A. bisporous*found the superior to the other mushroom. The antioxidant potential is dependent dose in all the assays carried out. It is concluded that the*L. edodus, A. bisporous* can be used as a medicine against free radical associated oxidative damage. Mushroom species natural demonstrated asa strong antioxidant and antimicrobial activity tested the present study shows that. It suggests that mushrooms may be used as good sources of natural antioxidant and for pharmaceutical purpose in treating of various diseases. Edible mushrooms may have potential as natural antioxidants. The antimicrobial activity was estimated by determination of minimal inhibitory concentration by using disc diffusion plate method against 3 species of bacteria. The ediblemushroom extracts had testedrelatively strong antimicrobial activity against generallythe tested microorganisms.

Keywords: Antioxidant activity, Antimicrobial activity, *A.bisporous, L.edodus, T.heimii.*

Introduction

Mushrooms have been utilized for medicinal purposes as well as being consumed as food in many parts of the world for centuries. Mushrooms are food sources that are poor in

fat while being quite rich in protein, dietary fiber, vitamins and minerals (1). In the structure of the mushrooms, some minerals such as calcium, phosphorus, potassium, iron, copper are found apart from thiamine, riboflavin, niacin, biotin, cobalamin, ascorbic acid, vitamin D and K(2). Mushrooms have been described as healthy foods due to their low calories, cholesterol, fat, and sodium content but rich in protein, carbohydrate, fibre, minerals and vitamins(3). Among the 2000 edible mushrooms, 35 species have gained much popularity worldwide due to their unique textures and aroma. Button mushroom *(Agaricus bisporus)* is the most widely cultivated species, followed by shiitake (*Lentinula edodes*), *A. bisporus* is well known to the Western countries since they are commonly found in Europe and North America. In contrast, *Auricularia spp., L. edodes and F. velutipe* are mostly cultivated in South Pacific and East Asian countries such as India, China, Taiwan, Singapore, Japan, Korea and Thailand (4).In Asia, mushrooms are recognized as functional foods as they contain a variety of biological active compounds with therapeutic potential such as alkaloids, b-glucan,lectins, peptides, phenolics, sterols (ergosterols) and terpenes (5).Thephytochemicals present in the mushrooms, particularly the antioxidants varied according to mushroom species, cell wall matrix, and the properties of phytochemicals such as their thermal stability (6). The present study is aimed to address this by investigating the water soluble phenolic and flavonoid content, total antioxidant activity and carbohydrate-digestive enzymes inhibition potential of three edible mushrooms; namely,*A. bisporus,L. edodes*, T*. heimii.* Consequently, mushrooms have always been appreciated as nutraceutical food across the globe due to their immense role in prevention of various disorders (7).Both edible and non-edible mushrooms are regarded as good sources of bioactive compounds in human diets for the antioxidant and anti-inflammatory purpose. Whichthesenutritional values include compoundslike they store secondary metabolites like flavonoids,polyphenols, polyketides, terpenes and steroids with pharmacological (8,9). They accumulate a variety of secondary metabolites, such as phenols, polyketides, steroids and terpenes. Among them, phenolics are one of the major groups of dietary components that have proved to be associated with antioxidant (10,11,12,13,14). Antimicrobial (15), and anticancer abilities (16). To investigate biological activities of macro fungi in order to discover novel therapeutic agent thus, researchers are showing increasing interest. Findings suggest that 158 species belonging to 88 genera have been recognized so far as new antibiotic sources effective against pathogenic microorganisms (17).Various antimicrobial compounds isolated from both mycelium and fruiting bodies of mushrooms have been reported. These secondary metabolites function just like antibiotics. Mushrooms need these antibiotics and vitamins to vegetate and reproduce. Synthetic antibiotics and antimicrobial medicines both threaten human health and cause antibiotic resistance. For this reason, nature are necessary substances obtained from antimicrobial. In this respect, mushrooms are important organisms (18).Free radicals and other reactive oxygen species that are continuously produced in cell oxidations may result in oxidative cell and tissue damage which has been associated to ageing and degenerative diseases, such as atherosclerosis, diabetes, cancer and cirrhosis(19). Most of organisms have defense systems against free-radical damage by oxidative enzymes including superoxide dismutase, glutathione peroxidase and catalase, or vitamins and chemical compounds antioxidants such as tocopherol, ascorbic acid, carotenoids phenolic acids or flavonoids (20). In recent years, several undesirable disorders have developed, due to the side-effects of the use of synthetic antioxidants commonly used in the food and food-flavoring industries. The reason for this study is that antioxidant activities of the mushrooms given here have not previously been reported in the literature Anatolian people have been using them as food for a long time. Therefore, the aim

of present work is to evaluate the antioxidant potentials of methanol extracts of *A. bisporous, L.edodes, T. heimii* by eight different antioxidant test systems namely Phenolic compounds, Flavonoid's, DPPH, ABTS, Reducing power, Hydroxy scavenging activity, ß-carotene/linoleic acid, ß-glucans.

Materials and Methods

Sample preparation:The fruiting bodies of *Agaricus bisporous*, (Button mushroom) and *Lentinusedodes* (Shiitake mushroom), *Termitomyces heimii* (Naturalrainy mushroom). Mushroom powders (25 g) were extracted with 250ml of 95% solvent methanol by using a Soxhlet apparatus for 6h. The extracts obtained were filtered through Whatman NO.41 filter paper. Then the filtrates were concentrated under vacuum using lyophilization. The concentrated extracts were stored at 4ºC for further investigation of methanol extracts were analyzed for their antioxidant activities.

Determination of total phenolic compounds: The concentration of phenolic compounds in the methanol extracts of mushrooms, expressed as gallic acid equivalent (GAE_S), were measured according to the method of(21). With some modifications. 1ml of sample was mixed with 1ml of Folin and Ciocalteu's reagent (Sigma). After 3 min, 1 ml of saturated Na_2CO_3 and it was made up to 10ml by adding distilled water. The reaction was kept in the dark for 90min, after which its absorbance was read to 600nm. A calibration curve was constructed with different concentrations of gallic acid (sigma) (0.01-0.1mM) as standard.

Total phenolic content (mg/GAE/Kg/g mushroom sample =

GAE (mg/L) ×total volume of methanol extract (ml)×10-3 (L/ml) ×dilution factor

Sample weight(g) ×10-3 (kg/g)

Determination of total flavonoid content: The total flavonoid content was determined by using the Dowd method(22). 2 ml of 2% aluminum trichloride ($AlCl_3$) in methanol was mixed with the same volume of the extract solution (1mg/ml). The mixture was incubated at room temperature for 10 min, and the absorbance was measured at 415nm in spectrophotometer against blank samples. The total flavonoid content was determined as microgram of quercetin equivalent (QE) per milligram of dry extracts by using the following formula was used to calculate the final total flavonoids content:

Total flavonoid content (mg QE/ kg/g mushroom sample) =

QE (mg/L) × total volume of methanol extract (ml)×10-3 (L/ml) ×dilution factor

Sample weight (g) ×10-3(kg/g).

Determination of DPPH radical scavenging activity: DPPH free radical scavenging activity of the extracts were measured in vitro using 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) assay described by Blois(23). 50mg of the extract and ascorbic acid (standard) were taken and dissolve in methanol and final volume make up to 50ml which was used as a stock solution with the concentration 1000µg/ml. Then different concentrations like 50,100, 200, 400, 800µg/ml were prepared by diluting with methanol from stock solution. 3ml of different concentration (50, 100, 200, 400, and 800µg/ml) of test solution and standard was taken in different test tubes. To this add 1ml DPPH working solution (0.1mM DPPH in methanol) and the mixtures were shaken vigorously and allowed to stand at room temperature for 30 min. The absorbance was measured against methanol as blank at 517nm using a UV-VIS spectrophotometer. The percentage inhibition was calculated by comparing the absorbance values of the test the capability of scavenging the DPPH radicals was calculated by using the following formula.

DPPH scavenging effect (% inhibition) = A_0-A_1/ A_0 × 100

Where, A0 refers to the absorbance of the DPPH control, and A1 refers to the absorbance of DPPH in the presence of extract/ ascorbic acid. The inhibitory concentration (IC50) value

was calculated. IC50 values denotes the concentration of extract/ standard required to scavenging 50% of free radicals.

Determination of hydroxyl radical scavenging activity: The effect of extracts on hydroxyl radical scavenging activity was assayed by using the deoxyribose method(24). With some modification.Stock solutions of EDTA (1mM), $FeCl_3$ (10mM), ascorbic acid (1mM), H_2O_2 (10mM) and deoxyribose (10mM), were prepared in deionized distilled water. 1ml of different concentration (50, 100, 200, 400 and 800µg/ml prepared with deionized distilled water of test solution and control (ascorbic acid) was taken in different test tubes. To this 0.1ml EDTA, 0.01ml of $FeCl_{3,}$ 0.1ml H_2O_2, 0.36ml of deoxyribose and 0.33ml of phosphate buffer (50Mm, pH 7.4) were added and the mixture was then incubated mixture was taken out and was mixed with 1ml of (10%) trichloroacetic acid and 1ml of (0.5% thiobarbituric acid (TBA) [in 0.025M NaOH containing 0.025% butylated hydroxy anisole (BHA)] to develop the pink chromogen. The absorbance of the test solution and thepercentage inhibition was calculated by comparing the absorbance values of the test sample with those of the controls (not treated with extracts). The hydroxyl radical scavenging activity of the extracts were reported as percentage inhibition of deoxyribose. The degradation is calculated by using the following equation.

Hydroxyl radical scavenging activity= A_0-A_1/ A_0 × 100Where, A_0 is the absorbance of the control, and A_1 is the absorbance test samples and references.

Determination of ABTS radical cation scavenging activity: 2,2-Azinobis 3-Ethylbenzothiazoline 6-Sulfonate (ABTS) radical scavenging activity of *A. bisporous, L. edodus, T. heimii* extracts were measured by(25),method with some modifications. Unlike DPPH assay, the assay that involves scavenging of ABTS radicals required generation of the radicals. The ABTS radical cation ($ABTS^+$) was generated by mixing ABTS stock solution(7mM) with potassium persulfate (2.45mM). The reaction mixture left in the dark for 12h at room temperature and the resulting dark colored solution was diluted using ethanol to an absorbance of 0.70± 0.02 at 734 nm. 0.1ml of different concentrations (50, 100, 200, 400, and 800µg/ml prepared with methanol) of extracts and Trolox (reference standard) was mixed with3.9ml of radical solution in clean and labeled test tubes. The tubes were incubated in dark for 6min at room temperature followed by measuring the absorbance of the reaction mixture in spectrophotometer at 734nm. Methanol replacing the extracts /Trolox served as control(i.e.,0.1ml methanol+3.9ml ABTS radical solution).The ABTS radical scavenging activity of the extracts was calculated using the following formula and the results were expressed as Trolox equivalent antioxidant capacity (TEAC) values.

ABTS radical scavenging activity =A_0-A_1/A_0×100

Where,A_0 is the absorbance of the ABTS solution without extract/Trolox and A_1 absorbance the ABTS solution in the presence of extract/Trolox. The IC_{50} values was calculated. IC_{50} denotes the concentration of extract required to scavenging 50% of the radicals.

Reducing power: The reducing power of extracts were determined by the method of(26). 1ml of each extract of different concentration (50, 100, 200, 400, and 800µg/ml prepared with methanol) and standard (ascorbic acid) were mixed with 5ml 0f sodium phosphate buffer (0.2M, pH6.6) and 5ml of 1% potassium ferricyanide and the mixture was incubated at 50ºC for 20 min. After that, trichloroacetic acid (10%, 2.5ml) was added to the mixture and centrifuged. Finally, the upper layer was mixed with distilled water (2.5ml) and 0.5ml of 0.1% ferric chloride ($FeCl_3$). The absorbance of the solution was measured at 700nm is spectrophotometer. Higher absorbance of the reaction mixture indicated that the reducing power is increased. Ascorbic acid, BHA and

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ά-tocopherol were used as positive control.

ß-Carotene bleaching method: The antioxidant activity of Three edible mushroom extracts was determined according to the ß-carotene bleaching method described by(27). A reagent mixture containing 1ml of ß-carotene (sigma) solution (0.2mg/ml in chloroform), 0.02ml of linoleic acid (sigma), and 0.2ml of Tween 80 (Sigma) was evaporated to dryness under a nitrogen stream. Fifty milliliters oxygenated distilled water and 0.2ml of mushroom crude extracts (Methanol) with different concentrations (4-20mg/ml) were added. Pure methanol was used as the control, and the blank contained all the earlier chemicals except ß-carotene. All these mixtures were then shaken to form a liposome solution and then incubated at 50ºC for 2h. The absorbance of an aliquot (1ml) of these solutions' liposome at 470nm was monitored by a spectrophotometer at time intervals of 20min. All samples were assayed in triplicate. Tert-butyl-hydroquinone (TBHQ) 2mg/ml was used as the standard. The bleaching rate (R) of ß-carotene was calculated according to

Eq. (1) $R=\ln(a/b)/t$

Where: In=natural log, a=absorbance at time 0, b=absorbance at time t, and t=20,40,60,80, 100- or 120-min. The antioxidant activity (AA) was calculated in terms of percent inhibition relative to the control, using

Eq. (2) $AA=[(R_{control} - R_{Sample}) / R_{control}] \times 100$

Extraction and quantification of ß-glucans: Mushroom ß-glucans were isolated and quantified according to(28).750mg of dry mushroom powder was heated with 60 ml 1M KOH during 20 min at 60ºC under constant stirring. Then, the suspension was filtered, the filter cake was washed with distilled water and the filtrate was collected and neutralized with 6 M HCl. The neutralized filtrate volume was adjusted to 100 ml with distilled water in a volumetric flask. This fraction was called KOH-fraction. The filter cake was resuspended in 65ml 0f 0.58 M HCl and heated in an oil bath at 100ºC for 1h. The suspension was again filtered and the filter cake was washed with distilled water. The collected filtrate was neutralized with 6M NaOH, transferred to a 100 ml volumetric flask and the volume adjusted with distilled water. This fraction was named HCl-fraction. The filter cake was again resuspended with 60 ml of 1M NaOH and heated at 60ºC for 20min. The suspension was filtered and the filter cake was washed with distilled water. This fraction was called NaOH- fraction. The three fractions were used for the ß-glucans determination. For quantification of the ß-glucans 350µL of each fraction were mixed with 300µL of 0.2 M citric acid /sodium hydroxide buffer pH7 and50µL of dye solution (8mg of Congo red diluted in 10mL of buffer) wasadded. The mixture absorbances were read at 523 nm against 350 µLof distilled water, 300 µL of buffer and 50µL of dye solution as ablank. Because of the light brownish color of some of the fraction, a measurement of the back ground absorption at 523 nm was necessary. Therefore, 350µL the simple was mixed with 350 µL of the buffer,and the absorption was measured at 523 nm.The calibration curve was obtained with stock schizophyllan solutions in the range of 225-600µg/mL. All analyses were performed in triplicate.The total content of the ß-glucan is expressed as mg of ß-glucan per g of dry mushroom.

Antimicrobial activity

Microorganisms and media: The following bacteria were used as test organism in this study: *Chromobacterium violaceum* (MTCC2656), *P.aeruginosa*NCIM 2037, *B. subtilis* NCIM 2193. Bacterial cultures were maintained on Miller-Hinton agar substrates (Hi-Media). All cultures were stored at 4ºC and sub cultured every 15days.

Determination of inhibition zone diameter: The susceptibility of bacterial strains to the 3 edible mushrooms were tested by disc diffusion method on Muller Hinton agar plates. Sterile blank discs of 6 mm diameter (sigma) loaded with 10 µl and 20µl and 50 µl and 0.5 to 2mg/

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ml of mushrooms were placed on an agar plate previously inoculated with 10^5 bacteria. The plates were incubated at 37 C for 24 h. The diameter of inhibition zone was measured.

Statistical Analysis: All data presented in the study as mean± SD& SE, were statistically analysed by MS Excel for windows version 2019.Mean and standard deviation & standard error were calculated.

Results and Discussion

Determination of total phenolic compounds:The results of the determination of total phenolics are demonstrated in figure 1.*A. bisporous*, *L.edodus, T. heimii* samples were 244.56 mg GAE/g. The minimumdetected amount was 43.77mg GAE/g, while the maximum one was 388.51mg GAE/g.*L. edodus* mushroom methanolicextract of polyphenols content was388.51mg GAE/g. *T.heimii* methanolic extract of polyphenolscontent was 376.42mg GAE/g. &*A. bisporous* methanolic extracts of polyphenols content was 272.75mg GAE/g. *L. edodus* has the highest polyphenols content 388.51mg GAE/g.*A. bisporous* has the lowest polyphenols content 272.75mg GAE/g. The results of this study were comparable to other similar ones(29,30). These results indicated that the phenolic compounds had a major contribution to the antioxidant capacity of edible mushrooms.

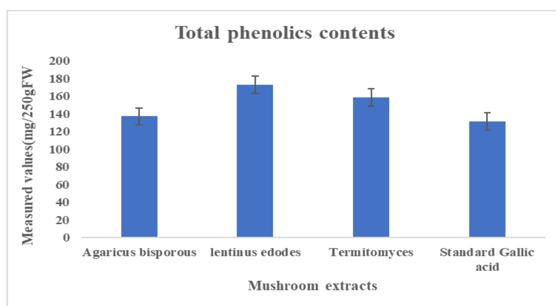

Figure 1: Determination of the number of phenolic compounds in the extracts of three edible mushrooms; Bars shown mean values of triplicates independent experiments and SD ± SE are shown.

Determination of total flavonoids content: The highest total flavonoid content was observed in *A. bisporous* 248.82mg QE/g. Which has higher than the reported value 57.06 mg QE/g (31).*T. heimii*had a value of 239.18 mg QE/g and *L. edodus* had a value of 213.48mg QE/g, higher than the reported value of 53.52 mg QE/g(32). It was served that *L. edodus* had the lowest total flavonoid content (213.48 QE/g) as shown infigure (2).

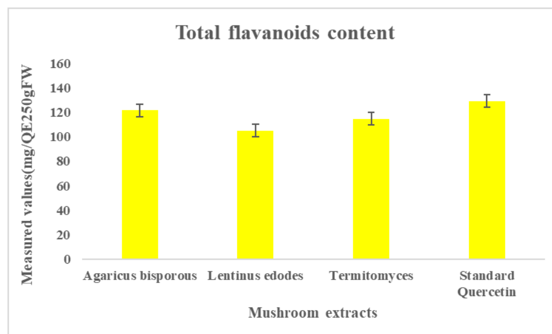

Figure 2: Determination of the flavonoids content in the extracts of three edible mushrooms; Bars shown mean values of triplicates independent experiments and SD ± SE are shown.

DPPH radical scavenging activity: Free radicals are molecules, usually of oxygen, which have lost an electron and are continuously generated during human body metabolism. DPPH isstable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule which is widely used to investigate to investigate radical scavenging activity. In DPPH radical scavenging assay, antioxidants react with DPPH (deep violet color) and convert it to yellow coloredά, ά-diphenyl-ß-picryl hydrazine.The degree of discoloration indicates the radical -scavenging potential of the antioxidant (23). The effect of three edible mushroom extracts of *A. bisporous, L. edodus, T. heimii* and standard ascorbic acid on DPPH radical scavenging activity were shown in figure 3. The DPPH radical scavenging activity of all the samples was highly depended on concentrations

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namely antioxidant activity increased with increase in extract concentrations. Among the tested extracts of *A. bisporous, L. edodus, T. heimii*. *L. edodus* exhibited high DPPH radical scavenging activity. Results show that 100µg/ml concentration of methanolic extract from the *L. edodus* exhibited the highest DPPH (92.64%) scavenging activity compared to the other extracts. The IC_{50} value of ascorbic acid was 41.50µg/ml; whereas, methanolic extract was found to contain 47.60µg/ml IC_{50}value.

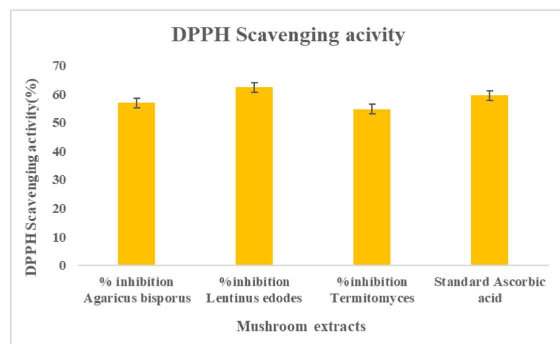

Figure 3: Detection of DPPH radical scavenging activity in the extracts of three edible mushrooms; Bars shown mean values of triplicates independent experiments and SD ± SE are shown.

Hydroxy radical scavenging activity: Hydroxyl radical scavenging activity was measured by studying the competition deoxyribose and the extracts for hydroxyl radicals generated from $Fe^{2+}/EDTA/H_2O_2$ system (Fenton reaction). The hydroxyl radicals attack deoxyribose, which eventually results in the formation of thiobarbituric acid substance (TBARS) (33). Among the reactive oxygen species, the hydroxyl radicals are the most reactive and predominant radicals generated endogenous during aerobic metabolism. A single hydroxyl radical results in the formation of many molecules of lipid hydroperoxides in the cell membrane which may severely, disrupts its function and leads to cell death (34). The methanol extracts of three edible mushrooms were found to possess concentration dependent scavenging activity on hydroxyl radicals and the results were given in figure (4).

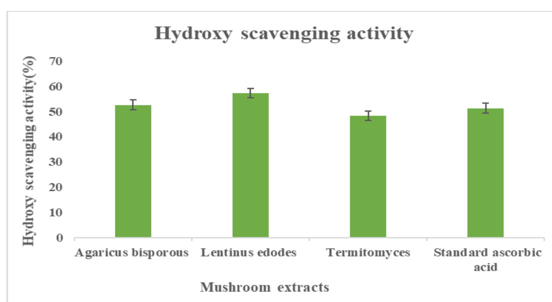

Figure 4: Detection of Hydroxy radical scavenging activity in the extracts of three edible mushrooms; Bars shown mean values of triplicates independent experiments and SD ± SE are shown.

Among all the tested extracts, methanol extracts of three edible mushrooms (100µg/ml) *L. edodus* showed high levels of hydroxy radical scavenging activity (80.10%) respectively.*A.bisporous* showed of hydroxyl radical scavenging activity (78.50%). *T. heimii* showed low levels of hydroxyl radical scavenging activity (71.69%). The IC_{50}Value of ascorbic acid was 45.66%; whereas, IC_{50}value of methanolic extract of *L.edodus* was found to be49.17%. &*A. bisporous* $IC_{50}$was found to be 46.15% and *T. heimii* $IC_{50}$was 47.66%.

ABTS radical cation scavenging activity: The effect of three edible mushroom extracts and standard ascorbic acid on ABTS radical cation were compared and shown in figure 3. At 100µg/ml concentration of methanolic extracts of *A.bisporous,L. edodus, T. heimii* possessed 87.20%, 73.16%, 75.32% scavenging activity on ABTS. All the concentration of *L. edodus* extract showed lower activity than the ascorbic acid 89.65%. The IC_{50}value of ascorbic acid was 46.85%. Whereas IC_{50}values of methanolic extracts of *A. bisporous* 45.61% &*T. heimii*42.89% showed this scavenging activity of ABTS radical by the edible mushroom extracts were found to be appreciable; this implies that the mushroom extracts useful for treating radical related pathological damage especially at higher concentration (35).

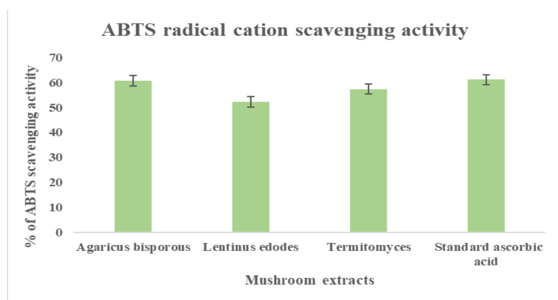

Figure 5: Detection of ABTS radical cation scavenging activity in the extracts of three edible mushrooms; Bars shown mean values of triplicates independent experiments and SD ± SE are shown.

Reducing power: Reducing power reflects the electron donating capacity of its bioactive compounds, which serves as a significant indicator of its antioxidant activity. Reduced Fe^{3+} /ferricyanide complex to the ferrous form, which indicated existence of reductants in the sample solution. The reductants have been exhibiting antioxidative potential by breaking the free radical chain and donating a hydrogen atom (36). The reducing ability of the extract increased with increasing concentration; our results were in accordance to studies done by(37). In the present study, 100μg/ml concentration of *A. bisporous* showed the highest reducing power than the ascorbic acid.

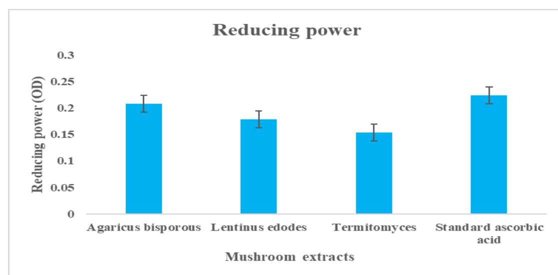

Figure 6: Detection of Reducing power in the extracts of three edible mushrooms; Bars shown mean values of triplicates independent experiments and SD ± SE are shown.

ß-Carotene bleaching method: The antioxidant activities of mushrooms methanol extracts with the coupled oxidation of ß-carotene and linoleic acid. The methanol extracts of *A. bisporous*, *L. edodus,* and *T. heimii*. Thehigher antioxidant activities showedof *L. edodus*, and *T. heimii.* The methanol extract of *L. edodus* showed a strong correlation between its antioxidant activity and concentration (R^2=0.99). The methanol extract of *L. edodus* 100mg/ml exhibited 86.70% antioxidant activity which was comparable to that of ascorbic acid at 60mg/ml 87.14%. It is probable that the antioxidative components in the three edible mushroom extracts can reduce the extent of ß-carotene destruction by neutralizing the linoleate free radical and other free radicals formed in the system.

Table 1: IC_{50} values of methanolic extracts of three edible mushrooms

Sample Name	DPPH	Hydroxyl radicals	ABTS
A. bisporous	46.55	46.15	45.61
L. edodus	47.60	49.17	46.85
T. heimii	45.76	47.66	42.89

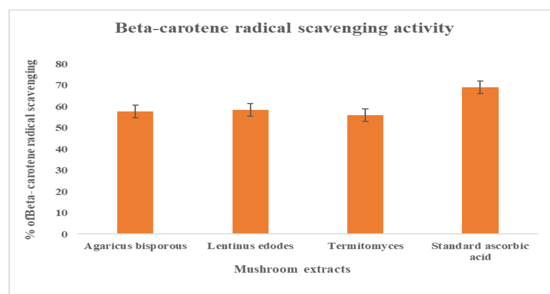

Figure 7: Detection of Beta- carotene radical scavenging activity in the extracts of three edible mushrooms; Bars shown mean values of triplicates independent experiments and SD ± SE are shown.

ß-glucans

ß-glucans are the most abundant polysaccharide on the fungal cell wall (38),and the spectrophotometric method with Congo red detects ß-1,3-1,6 glucans from mushrooms with high precision and without extensive clean-up (39). Thus, the ß-glucans content determined by

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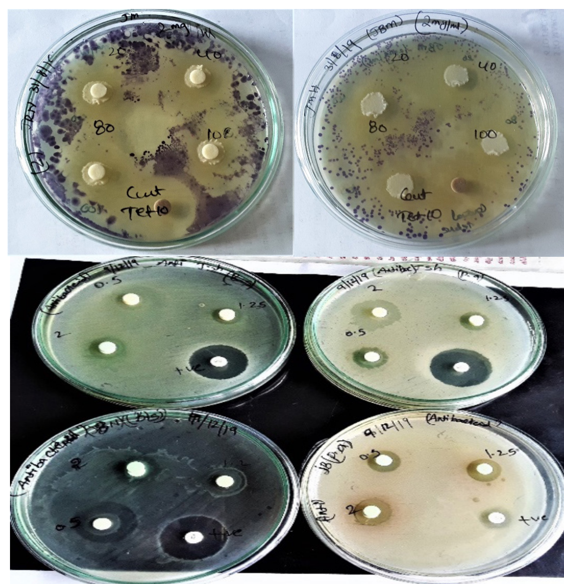
this study showed highly significant differences between the mushroom species. The highest ß-glucans content was detected in *L. edodus* with 247.57 mg/g of dry mushroom, while *A. bisporous* sample had a ß-glucans content of 117.75mg/g and *T. heimii* sample had a ß-glucans content of 97.69mg/g. It seems that *L. edodus* is a rich source of ß-glucans, which will produce health benefits can have consumed. The presence of these biologically active molecules in the mushroomsstudied reveals the nutraceutical potential of the mushrooms.

Antimicrobial activity: Methanolic extracts of three edible mushroom samples were subjected to antimicrobialscreening and the results were promising *C. Violaceum* (MTCC2656),*P. aeruginosa* (NCIM 2037), *B. subtilis* (NCIM 2193), were observed disc diffusion method. The mushroom extracts concentration of 0.5 to 2mg/ml used to demonstrate the antibacterial activity of disc diffusion method.Gram-negative bacteria *C. violaceum, P. aeruginosa* which were inhibited by the mushroom extracts. The mushroom extracts which were inhibited by Gram-positive bacteria of *B. subtilis*. Methanolic extract of *L. edodus*, producing *C. Violaceum*, *P. aeruginosa, B. subtilis*which were inhibited the largest clear zones on the agar.



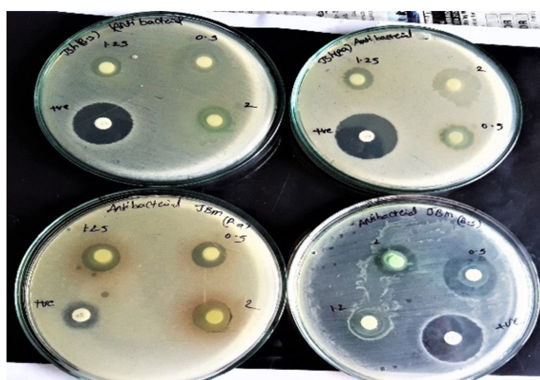


Figure 8 (a): Antibacterial activity of C. Violaceum

(b) Antibacterial activity of P. aeruginosa

(d)Antibacterial activity B. subtilis bacterium

Conclusion

Mushrooms are commercial substrate are a good source of natural antioxidants. In the present study, it can be concluded that methanolic extracts of *A. bisporous, L. edodus, T, heimii*. *L. edodus* has the highest polyphenols content 388.51mg GAE/g. *A. bisporous* has the lowest polyphenols content 272.75mg GAE/g. *L.edodus*have wide range of antioxidant and / or free radicals scavenging activity.Literature survey reveals that flavonoids(40), and phenolic compounds (41), are responsible for antioxidant activity. More detailed studies on chemical composition of the mushroom extracts, as well as *in vivo* assays are essential to characterize them as biological antioxidant. It should be kept in mind that antioxidant activity measured by *invitro* methods may not reflect *in vivo* effects of antioxidant (42).Many other factors such as absorptionmetabolism are also important.

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References

1. P. Manzi L, Gambelli S, Marconi V, Vivanti

L,Pizzoferrato. (1999). Nutrients in edible mushrooms: an inter-species comparative study. Food chem,65:477-482.

2. Surekha Ch, Kaladhar DSVGK, Raju Srikakarlapud HaseenaJR. (2011). Evaluation of antioxidant and antimicrobial potentiality of some edible mushrooms. Int J Advan Biotech Res, 2:130-134.
3. Aida FMNA, Shuhaimi M, Yazid M, Maaruf AG.(2009). Mushroom as a potential source of prebiotics: a review. Trends Food Sci Technol ,20(11–12):567–575.
4. Ghorai S, Banik SP, Verma D, Chowdhury S, Mukherjee S, Khowala S.(2009). Fungal biotechnology in food and feed processing. Food Res Int, 42(5–6):577–587.
5. Bach F, Helm CV, Bellettini MB, Maciel GM, Haminiuk CWI. (2017).Edible mushrooms: a potential source of essential amino acids, glucans and minerals. Int J Food Sci Technol, 52(11):2382–2392.
6. Ng ZX, Tan WC. Impact of optimised cooking on the antioxidant activity in edible mushrooms (2017). J Food Sci Technol, 54(12):4100–4111.
7. Valverde ME, Hernande-Perez T, Paredes-López O. (2015). Edible mushrooms: improving human health and promoting quality life, Int J Microbiol doi: 10.1155/376387.1.
8. Abugri DA, Mcelhenney WH. (2013). Extraction of Total Phenolic and Flavonoids from Edible Wild and Cultivated Medicinal Mushrooms as Affected by Different Solvents. J Nat Pro Plant Resour, 3(3):37-421.
9. Nguyen TKI, Kyung HC, Jaehyuk S, Pyung GL, Tae S. (2016). Evaluation of antioxidant, anticholinesterase and anti-inflammatory effect of culinary mushroom Pleutorus pulmonarius Microbiol,44(4):291-301.
10. Acharya K, Bera I, Khatua S, Rai M. (2015).Pharmacognostic standardization of Grifola frondosa: A well-studied medicinal mushroom. Der Pharmacia Lettre, 7(7):72–78.
11. Acharya K, Khatua S, Sahid S. (2015). Pharmacognostic standardization of Macrocybe crassa: an imminent medicinal mushroom. Res J Pharm Technol, 8(7):860–866.
12. Acharya K, Ghosh S, Khatua S, Mitra P. (2016).Pharmacognostic standardization and antioxidant potentiality of an edible mushroom, *Laetiporus sulphureus*.J Verbrauch Lebensm,11(1):33–42.
13. Acharya K, Das K, Paloi S, Dutta AK, Hembrom ME, Khatua S, Parihar A. (2017). Exploring a new edible mushroom Ramaria subalpina: Chemical characterization and antioxidant activity. Phcog J,9(1): 30–34.
14. Acharya K, Khatua S, Ray S. (2017). Quality assessment and antioxidant study of *Pleurotus djamor* (Rumph. ex Fr.) Boedijn. J Appl Pharmaceutic Sci, 7(6); 105–110.
15. Khatua S, Dutta AK, Acharya K. (2015). Russula senecis: A delicacy among the tribes of West Bengal. Peer J, 3: e810.
16. Yaltirak T, Aslim B, Ozturk S, Alli H. (2009). Antimicrobial and antioxidant activities of Russula delica Fr. Food Chem Toxicol, 47: 2052–2056.
17. Shen H, Shao S, Chen J, Zhou T. (2017). Antimicrobials from mushrooms for assuring food safety. Compr Rev Food Sci Food Saf, 16: 316–329.
18. Akyuz M, Onganers AN. (2010). Antimicrobial activity of some edible mushrooms in the eastern and southeast Anatolia region of Turkey. Gazi University J Sci. 2010; 23:(2)125-130.
19. Ferreira ICFR, Barros L, Abreu RM. (2009). Antioxidants in wild mushrooms. Curr Med Chem, 16: 1543-1560.
20. Halliwell B. (1996). Antioxidants in human

health and disease. Annual Review of Nutrition. 1996; 16: 33–50.

21. Singleton VL, Rossi JA. (1965). Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. Am J Enol Viticult, 16: 144-158.

22. Meda, A., Lamien, C. E., Romito, M., Millogo, J., & Nacoulma, O. G. (2005). Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. *Food Chemistry*. 2005; 91(3):571-577.

23. Bilos MS. (1958). Antioxidant determinations by the use of a stable free radical. Nature, 26:1199-1200.

24. Halliwell B, Gutteridge JMC, Aruoma OI. (1987). The deoxyribose method: a simple test to be assay for determination of rate constants for reaction of hydroxyl radicals. Analyt Biochem,165:215–219

25. Huang MH, Huang SS, Wang BS, Wu CH, Sheu MJ, Hou WC. (2011). Antioxidant and anti-inflammatory properties of Cardiospermum halicacabum and its reference compounds ex vivo and in vivo. J Ethnopharmacology, 133:743-750.

26. Kumar RS, Hemalatha S. (2011).In vitro antioxidant activity of alcoholic leaf extract and sub-fractions of Alangium lamarckii Thwaites. J Chem Pharm Res, 3:259–267.

27. Velioglu YS, Mazza G, Gao L, Oomah BD. (1998). Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. J Agric Food Chem, 46:4113.

28. Nitschke J, Modick H, Busch E, Wantoch VR, Altenbach MH. (2011). A new colorimetric method to quantify β-1,3-1,6-glucans in comparison with total β-1,3-glucans in edible mushrooms. Food Chem, 127:791.

29. Proestos C, Boziaris IS, Nychas GJE, Komaitis M. (2006). Analysis of flavonoids and phenolic acids in Greek aromatic plants: Investigation of their antioxidant capacity and antimicrobial activity. Food Chem, 95:664–671.

30. Viuda-Martos M, Navajas YR, Zapata ES Fernandez-Lopez J, Perez-Alvarez JA. (2009). Antioxidant activity of essential oils of five spice plants widely used in a Mediterranean diet. Flavour Fragr J, 25:13–19.

31. Raseta M, Karaman M, Jaksic M, Sibul F, Kebert M, Novakovic A, Popovic M. (2016). Mineral composition, antioxidant and cytotoxic biopotentials of wild-growing Ganoderma species (Serbia): G. lucidum (Curtis) P. Karst vs. G. applanatum (Pers.) Pat. Int J Food Sci Technol,51(12):2583-2590.

32. Awala, S.I., and Oyetayo, V.O. (2015). Assessment of free radical scavenging potentials of extracts of Trametes lactinea collected from Akure. Research Journal of Phytochemistry,10(1), 10-20.

33. Abirami B, Gayathri P, Uma D.(2012). In vivo antioxidant potential of Pterocarpus marsupium bark. Int J Chem Pharm Sci, 3:17-24.

34. Battu GR, Kumar BM. (2012). In-vitro antioxidant activity of leaf extract of Aerva lanata Linn. Int J Pharm Sci, 2:74-78.

35. Karthika K, Paulsamy S, Jamuna S. (2012). Evaluation of in vitro antioxidant potential of methanolic leaf and stem extracts of Solena amplexicaulis (Arm) Gandhi. J Chem Pharm Res, 4:3254-3258.

36. Gordon MH. (1990). The mechanism of antioxidant action in vitro. In: BJF Hudson Ed. Food antioxidants. London: Applied Science, 1-18.

37. Vijaya G, Doss A, Parthipan B, Mohan, VR. (2018). Assessment of in-vitro antioxidant activity of various bark extracts of Crateva manga (Lour) DC. (Capparaceae). J Pharmacogn Phytochem, 7:1596-1599.

38. Fesel P.H. and Zuccaro A. (2015). β-glucan: crucial component of the fungal cell wall and

elusiveMAMP in plants, Fungal Genetics and Biol,12.004.

39. Zhu F, Du B, Bian Z, Xu B. (2015). Beta-glucans from edible and medicinal mushrooms: Characteristics, physicochemical and biological activities. J Food Comp Anal, 41:165.

40. Lamson, DW, Bringnal MS. (20000. Antioxidants and cancer therapy II. Quick reference guide. Alternative Medicine Review, 5:152-163.

41. Stratil P, Klejdus B, Kuban V. (2006). Determination of total content of phenolic compounds and their antioxidant activity in vegetables evaluation ofspectrophotometric methods. J Agric Food Chem, 54:607-616.

42. Sini KR, Sinha BN, Karpagavalli M. (2011). Determining the antioxidant activity of certain medicinal plants of Attapady (Palakkad), India using DPPH assay. Curr Bot, 1:13-16.