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Research Article

INVITRO ANTIOXIDANT ACTIVITIES, TOTAL PHENOLICS AND FLAVONOID OF WILD EDIBLE MUSHROOM *MACROLEPIOTA MASTOIDEA* (FR.) SINGER.

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ABSTRACT

Objective: Report the antioxidant activity, phenolic profile of the mushroom methanolic extract and find the correlation between the phenolics contents and their relative antioxidant activity to identified the natural antioxidant source of the wild edible mushroom *M. mastoidea*.

Methods: Total phenolics and flavonoids was estimated from dried methanolic extract of the mushroom. The mushroom phenolic compounds was identified by HPLC and free radical, radical scavenging, reducing power, of the mushroom extract was evaluated by adopting various standard methods.

Results: The methanolic extract of the mushroom *M.mastoidea* revealed the presence of total phenolics (5.5±0.151mg/g) and flavonoids (3.6±0.118mg/g). The phenolic acids such as p-hydroxy benzoic acid, vanillic acid, gentisic acid, and coumarin, p-coumaric acid of the mushroom *M.mastoidea* were identified by HPLC analysis. The results of free radical and radical scavenging activity showed very good superoxide scavenging activity at 5mg/ml (84.91±0.715%) than that with BHT. This may be due to the occurrence of phenolic acids in the mushroom. The correlation analysis results showed linear relation between phenolics content of mushroom methanolic extract and antioxidant assays (R^2=0.98, 0.89, 0.92, 0.84 and 0.88, significance at P<0.002 and P<.0.001).

Conclusion: The current observations suggested that the mushroom extract exhibited multiple antioxidant activities. So, it can be concluded that the wild edible mushroom *M. mastoidea* is a source of natural antioxidant.

Keywords: *Macrolepiota mastoidea,* HPLC analyzes, Reducing power, Antioxidant, Radical scavenging activity.

INTODUCTION

Reactive oxygen species (ROS) are generated under the situation of oxidative stress. The most common harmful reactive oxygen or nitrogen species existing in the body are peroxyl ($ROO\cdot$), hydroxyl ($HO\cdot$), hydrogen peroxide (H_2O_2), superoxide (O^{-}_2), singlet oxygen ($^1O_2$) and peroxynitrite ($ONOO^-$). These free radicals are leads to severe effects on the cardiovascular system either through lipid peroxidation or vasoconstriction and other ailments such as inflammation, cancer, diabetes mellitus etc., [1,2,3] There is considerable evidence that antioxidants could help to prevent these diseases because they have the capacity to quench free radicals. [4] Although some synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), exhibit potent free radical scavenging effects, they have been demonstrated to exert toxicological effects as compared with natural antioxidants. [4,5] Thus, the demand for alternative and safe antioxidants from natural sources has gradually grown. Epidemiological studies have demonstrated an inverse relationship between the intake of fruits and vegetables, rich in antioxidants and the occurrence of these diseases. [6] Commonly researched antioxidants are vitamin E, vitamin C, carotenoids, and more recently, phenolic compounds. [7,8] The phenolic antioxidant compounds, have gained importance due to their large array of biological actions that include free radical scavenging, metal chelating and enzyme modulation activities.

Moreover, in the last few years, an increasing interest in the consumption of mushroom has arisen, due to their elevated phenolic concentration, which correlates with an increased antioxidant activity. Several studies analyzing the total phenol and antioxidant activity of fresh and cooked wild and commercial mushroom. [9,10,11] Mushroom extracts contain various phenolic compounds, which were recognized as an excellent antioxidant due to their ability to scavenge free radicals by single – electron transfer. [12]

Mushroom have been used as traditional food and medicine in different parts of the world, including Asia, Africa, America and India. In Kerala (India), wild edible mushroom have been part of the diet, especially among rural and tribal people, because of the nutritional relevance due to their high fiber, minerals and protein content as well as low fat content [9]. Some of the common edible mushrooms, which are prevalently consumed in India such as *Agaricus bisporus, Pleurotus sajor-caju, P. euos, P. florida, P. platypus, P. djamor, Volveriela volvaciea and Calocybe indica,* are currently found to possess antioxidant activity, which is well correlated with their total phenolic content. [4,5] Puttaraju et al reported phenolic compounds in Indian mushroom *M. procera* [13]. Recently, we reported the antioxidant constituents, total phenol and flavanoid of bioleuminescent mushroom *Omphalotus nidiformis*. [14] Even though *M. mastoidea* was identified from different regions of Kerala, its characterization was not done till this date. In this present study we analyzed the free radical scavenging activity by reducing power assay and DPPH radical scavenging, ABTS radical scavenging and nitric oxide, superoxide, hydrogen peroxide scavenging activities. The correlation between total phenolic and antioxidant activities also carried out. This report on the evaluations of antioxidant potential of wild mushroom *M. mastoidea* will definitely improve the nutritional importance of wild mushrooms.

MATERIALS AND METHODS

Sampling site

The mushroom was collected from southern part of Kerala (India) during the period of May-December 2010. Regarding the climate of Kerala there is not much difference in the temperature of summer and winter months. The mean maximum temperature is 34 °C and the mean minimum temperature is 21 °C. The humidity is high and rises to about 90% during the monsoon season, which favor the mushroom growth.

Sample collection

Sample was collected using paper bags and packed loosely with provision of aeration. Field data sheets were maintained. The collection was transported to the laboratory at Department of Botany, University of Kerala. All the macro and micro characters including habit and morphological factors were recorded. The specimen was kept in the herbarium of Department of Botany, University of Kerala, Kariavattom (Coll. No: KUBH- 5801).

Sample preparation

The mushrooms were shade dried and powdered in an electric mixer grinder. The powdered mushroom (300g) was extracted with 500 ml methanol as a solvent by using soxhlet apparatus. After

extraction the extract was evaporated to dryness under reduced pressure in a rotary evaporator. This dried extract (7.2g) was used for following experiments.

Determination of total phenolics content

The amount of phenolic compounds in the methanol extract of *M. mastoidea* was determined by adopting Li et al method. [15] The dried extract was dissolved in distilled water (1 mg/ml). 0.5 ml of the dissolved extract was added to 2.5 ml of 10% folin-ciocalteu reagent and 2 ml of $NaCO_3$ (2% w/v). The resulting mixture was incubated at 45°C with shaking for 15 min. The absorbance of the samples was measured at 765 nm using UV/VIS spectrophotometer. Results were expressed as milligrams of gallic acid (mg/ml) dissolved in distilled water.

Determination of total flavonoids content

Aluminum chloride colorimetric Li et al method [15] was used for flavonoid determination. The dried extract was dissolved in (mg/ml) distilled water. The 1ml dissolved extract was added with 3ml methanol as taken in test tube and added 0.2ml of 10% aluminum chloride, 0.2 ml of 1 M potassium acetate and 5.6 ml of distilled water and kept at room temperature for 30 min. The absorbance of the reaction mixture was measured at 415 nm using UV-VIS spectrophotometer (UV-1700 Pharmaspec, Shimadzu). The content was determined from extrapolation of calibration curve which was made by preparing quercetin in distilled water (mg/ml). The concentration of flavonoid was expressed in terms of mg/ml extract.

Phenolic compound identification

HPLC analyses

The extract was analysed using a liquid chromatography (Simadzu corporation- Kyoto, Japhan). Separation was achieved on a reverse phase C_{18} column (250 x 4.6mm, 5micron) temperature at 24°C. The mobile phase of the optimized chromatographic method consisted of solvent A (methanol) and solvent B (0.5% (v/v) acetic acid in water). The elution profile was as follows: 0 min 10% A in B, 28.6 min 60% A in B, 30 min 10% A in B. All gradients were linear. The flow rate was 1 mL/min, and injection volume was 20µL. Absorption was measured at 290 nm. Detection was carried out in UV-visible detector. The eluted components were identified (System controller-CBM-20A/20Alite) based on the retention time by comparison with retention time of reference standard. The phenolic compounds present in the samples were characterized according to their UV-vis spectra and identified by their retention times in comparison with those of commercial standards(Himedia and Sigma-Aldrich).

Reducing power by Fe^{3+}-Fe^{2+} transformation

Reducing power was determined according to the method of Gulcin et al. [16] Different concentrations of the dried extract of M.mastoidea was dissolved in (mg/ml) distilled water. 1ml of dissolved extract was further mixed with 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide, and the mixture was incubated at 50°C for 20 min. Then, 2.5 ml of 10% trichloroacetic acid was added, and the mixture was centrifuged at 3000 rpm for 10 min (eppendorf-centrifuge 58042, Germany). The upper layer (2.5 ml) was mixed with 2.5 ml of deionized water and 0.5 ml of 0.1% ferric chloride. Finally, the absorbance was measured at 700 nm using UV-VIS spectrophotometer (UV-1700 Pharmaspec, Shimadzu). BHT was used as control.

DPPH free radical scavenging assay

DPPH·radical scavenging activity of the extract was measured by the method described by Gezer et al. [17] For this, different concentrations of methanol extracted and a positive control BHT (Sigma-Aldrich) was prepared with methanol as the test solutions (1mg-5mg/ml). One ml of each prepared concentrations of extract were taken into test tubes and 0.5 ml of 1 mM DPPH· (Sigma-Aldrich) solution in methanol was added. The test tubes were incubated for 30 min and the absorbance was read at 517 nm using UV-VIS spectrophotometer(UV-1700 Pharmaspec, Shimadzu). A control solution consists of all reagents except the extract. The DPPH· radical scavenging activity percentage was calculated by using the following formula.

$$\text{DPPH·radical scavenging activity (\%)} = [A_{Control} - A_{Extract}) / A_{Control}] \text{ X100}$$

Where $A_{Control}$ is the absorbance of the control reaction mixture, $A_{Extract}$ is the absorbance of the methanol extracted sample. All measurements were performed in triplicate.

ABTS free radical scavenging assay

Total antioxidant status was measured using 2, 2´-azinobis [3-ethylbenzthiazoline] -6-sulfonic acid (ABTS) assay by adopting Re et al., and Arumagam et al. [18,19] Dissolved $ABTS^{\cdot+}$ (Sigma-Aldrich) in deionized water to 7 mM concentration, and potassium persulphate (Sigma-Aldrich) added to a concentration of 2.45 mM. The reaction mixture was left to stand at room temperature overnight (12~16 h) in the dark before use. The resultant intensely-coloured $ABTS^{\bullet+}$ radical cation was diluted with ethanol and PBS (pH 7.4) to give an absorbance of 0.75 at 734 nm. Then, 1 ml of the extract was added to 1ml of diluted stock solution and the absorbance measured at 734 nm using UV-VIS spectrophotometer (UV-1700 Pharmaspec, Shimadzu), 5 min after the initial mixing, For control all solution consists of all reagents except the extract. The assay was performed in triplicate. The assay relies on the antioxidant capability of the samples to inhibit the oxidation of $ABTS^{\cdot+}$. The scavenging activities were expressed with positive control BHT (Sigma-Aldrich) equivalent scavenging capacity.

$$ABTS^{\cdot+} \text{ radical scavenging activity (\%)} = [A_{Control} - A_{Extract}) / A_{Control}] \text{ X100}$$

Where $A_{Control}$ is the absorbance of the control reaction mixture, $A_{Extract}$ is the absorbance of the methanol extracted sample. All measurements were performed in triplicate

Nitric oxide scavenging assay

Nitric oxide scavenging (NO) activity was measured spectrophotometrically by adopting Rice- Evans et al. [20] method. Sodium nitroprusside (5 mM) (SNP) (Sigma-Aldrich) in phosphate buffered saline was mixed with different concentrations of the extract (200–900µg/ml) prepared in methanol and incubated at 25°C for 30 min. A control without the test compound but with an equivalent amount of methanol was taken. After 30 min, 1.5 ml of the incubated solution was removed and diluted with 1.5 ml of Griess reagent (1% sulphanilamide, 2% phosphoric acid and 0.1% N-1-naphthylethylenediamine dihydrochloride). The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with N-1-naphthylethylene diamine dihydrochloride was measured at 546 nm and percentage scavenging activity was measured with reference to BHT(Sigma-Aldrich) standard.

$$\text{\% inhibition of Nitric oxide} = [(A_0\text{-}A_1)/A_0] \text{ x } 100$$

Where, A_0 was the absorbance of the control, and A_1 was the absorbance in the presence of the sample after reaction has taken place.

Superoxide scavenging assay

The superoxide scavenging assay was carried by adopting Elmastas et al method. [11] The reaction mixture consisting of 1ml of nitro blue tetrazolium (NBT) (Sigma-Aldrich) solution (156 mM NBT in phosphate buffer, pH 7.4), 1 ml NADH solution (468 mM NADH reduced nicotinamide adenine dinucleotide in phosphate buffer, pH 7.4), and 1ml of sample solution(mg/ml) of extract was mixed. The reaction was started by adding 100 ml of phenazine methosulfate (PMS) (Sigma-Aldrich) solution (60 mM PMS in phosphate buffer, pH 7.4) to the mixture. The reaction mixture was incubated at 25°C for 5min and the absorbance was measured at 560 nm using UV-VIS spectrophotometer(UV-1700 Pharmaspec, Shimadzu) against blank sample and compared with standards. Decreased absorbance of reaction mixture indicated increased superoxide anion scavenging activity. The percentage inhibition of superoxide anion generation was calculated using the following formula.

$$\text{\% inhibition} = [(A_0\text{-}A_1)/A_0] \text{ x100}$$

Where A_0 was the absorbance of the control and A_1 was the absorbance of *M mastoidea* after reaction.

Hydrogen peroxide scavenging assay

Hydrogen peroxide scavenging was determined according to the method of Ali et al. [21] A solution of hydrogen peroxide (40mM) was prepared in phosphate buffer (pH 7.4). Different concentrations of *M. mastoidea* (1-5 mg/ml) were added to a hydrogen peroxide solution (0.6 ml, 40mM). Absorbance of hydrogen peroxide at 230 nm was determined after 10 min. against a blank (Control) solution containing phosphate buffer without hydrogen peroxide. The percentage scavenging of hydrogen peroxide of extract and standard compounds was calculated using the following formula.

$$\% \text{ scavenged } [H_2O_2] = [(A_0-A_1)/A_0] \times 100$$

Where A_0 was the absorbance of the control, and A_1 was the absorbance in the presence of the sample and standards.

Statistical analysis

The analysis of phenolic, flavonoid and scavenging activity of the mushroom was carried out in triplicate and the results expressed as mean ± standard deviation (SD). Data was analysed by one-way analysis of variance (ANOVA) followed by Duncon, Posthoc test with $\alpha = 0.05$, using SPSS v. 11.0 program.

RESULTS

The total phenolics content of the methonolic extract of the *M. mastoidea* (Fig 1.) was estimated by folin – ciocalteau method. The amount of 5.5±0.151 mg gallic acid equivalent /g of extract was found.

Fig. 1: The wild mushroom *Macrolepiota mastoidea*

Phenolic compound identification

HPLC analyses

The composition profile of phenolic compounds was analysed by HPLC analysis. According to Barros et al. [22] phenolic compounds include different subclasses and they displayed a large diversity of structure. Similarly in our observation we found both benzoic acids derivatives and cinnamic acid derivatives are presents in the samples. The benzoic acids derivatives of p-hydroxy benzoic acid (2.4min), vanillic acid (2.5min), gentisic acid (2.6min), and cinnamic acid derivatives like coumarin (3.1min) and p-coumaric acid (3.2min) were identified (Fig. 2) from *M.mastoidea* by comparison of their chromatographic characteristic with the standards compounds.

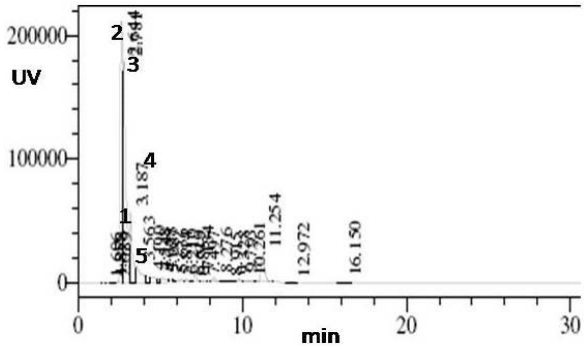

Fig. 2: HPLC chromatogram recorded at 290nm of *M. mastoidea* extract, peaks related to phenolic compounds are indicated. 1- p-hydroxy benzoic acid; 2- vanillic acid; 3- gentisic acid; 4- coumarin; 5- P-coumaric acid.

Total flavonoids content

The total flavonoids content of *M. Mastoidea* estimated by using aluminium chloride colorimetric technique, and the amount was observed as 2.84±0.12 mg quercetin equivalent /g of extract.

Reducing power by Fe^{3+}-Fe^{2+} transformation

The reducing capacity of the mushroom extract may serve as a significant indicator of its potential antioxidant activity. In the reducing power assay, the presence of antioxidants which present in the sample would result in the reduction of Fe^{3+} to Fe^{2+} by donating an electron. The amount of Fe^{2+} complex can be monitored by measuring the formation of blue color at 700 nm and the increasing absorbance indicates an increase in reducing ability (Fig. 3). The result shows that there is increase in reducing power of the mushroom extract as the extract concentration increases. However the control synthetic antioxidant BHT exhibited the same result, that the increase in reducing power with increasing concentration.

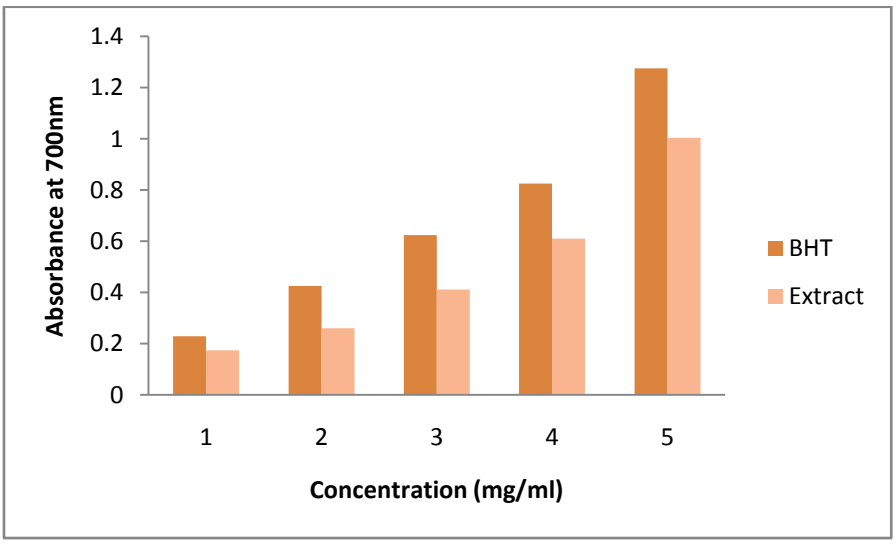

Fig. 3: Reducing power of mushroom methanolic extract.

Antioxidant activity

The DPPH·and ABTS·+ radicals had been used widely to investigate the scavenging activities of several natural compounds such as phenolic compounds, anthocyanins, or crude extracts of plants. The model of scavenging the stable DPPH· radical is widely used to evaluate antioxidant activities over a relatively short time compared to other methods. Kalyoncua et al [23] reported the antioxidant activity of mycelia from 21 wild mushrooms by two methods; free radical scavenging (DPPH) and the scavenging activity of 2,2'-azinobis-(3-ethylbenzthiazoline-6-sulphonic acid) radical cation (ABTS·+). DPPH· radical was scavenged by antioxidants through the donation of hydrogen, forming the reduced DPPH-H•. The color changed from purple to yellow after reduction, which can be quantified by its decrease of absorbance at wavelength 517 nm (Fig. 4). IC_{50} value for DPPH scavenging of the mushroom extract was found to be 3.7mg/ml extract. The mushroom extracts showed appreciable DPPH scavenging activities (70.87±0.503%) at the highest concentration of 5mg/ml. About the ABTS scavenging activity of mushroom, the methanolic extract of the fruiting bodies of *M.mastoidea* significantly scavenged 68.27±0.682, 73.78±0.850, 82.39±1.113 % of ABTS* radicals at3,4 and 5mg/ ml respectively. IC_{50} value of the mushroom extract was found to be 1.8 mg/ ml. The results of DPPH, ABTS radical scavenging activity were compared with standard BHT (Fig. 5), they showed significance ($p< 0.01$). Nitric oxide plays an important role in various types of inflammatory processes in the animal body. The mushroom extract directly competes with oxygen to react with nitric oxide and there by inhibits nitride formation. The present study proved that the nitric oxide scavenging activity of *M.mastoidea* at different concentration (1-5mg/ml), the IC_{50} to be 1.8 mg/ ml. The maximum scavenging activity 70± 2.053% was observed at 5mg/ml (Fig. 4). Scavenging of 84± 0.513% at 5mg/ml was observed in BHT(Fig. 5). These results were found to be significant ($p< 0.05$). Like reducing power the antioxidant activity of both extract and BHT was increased with increasing concentration.

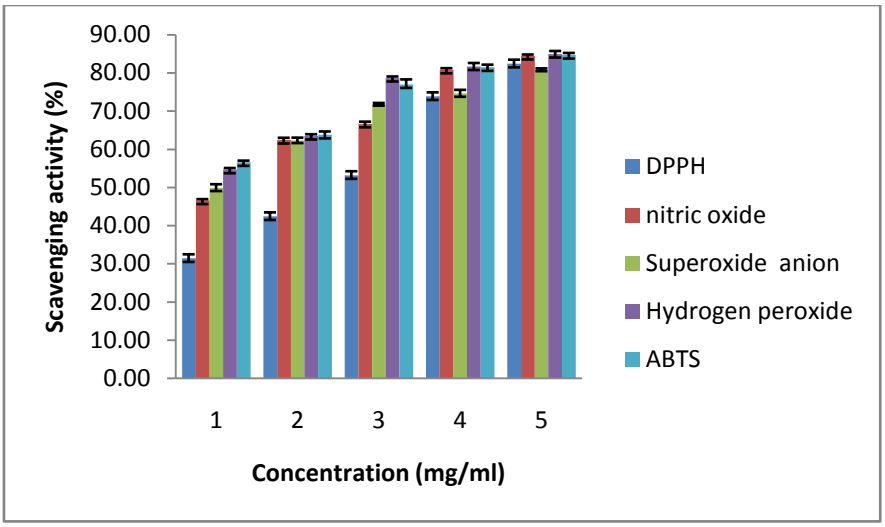

Fig. 4: Antioxidant activity (%) on DPPH radical, Nitric oxide, Superoxide anion, H_2O_2 and ABTS radical of mushroom methanolic extracts.

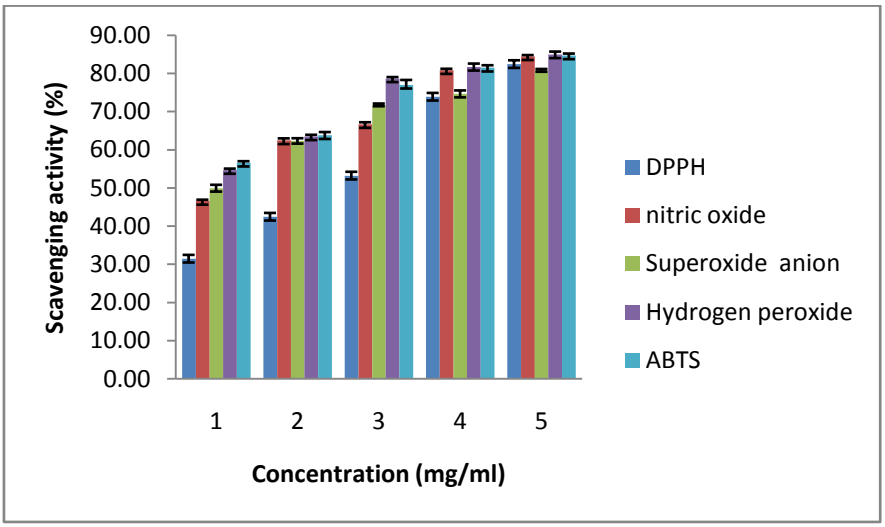

Fig. 5: Antioxidant activity (%) on DPPH radical, Nitric oxide, Superoxide anion, H_2O_2 and ABTS radical of BHT.

Superoxide anion is a harmful reactive oxygen species as it damage cellular components in biological system. It was therefore proposed to measure the ability of mushroom extract to scavenging superoxide anion.In the PMS-NADH-NBT(phenazine methosulfate - reduced nicotinamide adenine dinucleotide - nitro blue tetrazolium) system, super oxide anion, derived from dissolved oxygen and from the coupling reaction of PMS-NADH, reduces NBT. The decreases in absorbance at 560nm with antioxidants indicates the consumption of superoxide anion in the reaction mixture. The mushroom extract shows the 84.91±0.715 % scavenging potential (Fig. 4) of superoxide radical at 5mg/ml compared to the same doses of BHT(80.78 ±0.631%) (Fig. 5). IC_{50} value of mushroom extract on superoxide radical scavenging activity was found to be 1.54mg/ml. The results were found to be significant ($p< 0.05$). When compared with BHT the mushroom *M.mastoidea* extract showed high scavenging activity at 4 and 5 mg/ml (79.33± 1.065%,84.91±0.751%).

The ability of the extract to effectively scavenge hydrogen peroxide was determined, where it is compared with that of BHT as standards. Hydrogen peroxide itself is not very reactive, it can some time cause cytotoxicity by giving rise to hydroxyl radical in cell. Thus, removing H_2O_2 is very important throughout the system of human. [24] The mushroom extracts were capable of scavenging hydrogen peroxide in a concentration dependent manner. The methanolic extract (4&5mg/ml) exhibits 70.45±2.877 and 73.95 ±2.987% hydrogen peroxide scavenging activity (Fig. 4) respectively while at the same concentration BHT(Fig. 5) showed 81.72±1.444% and 84.98 ± 1.2987%activity. IC_{50} value of mushroom extract on H_2O_2 scavenging activity was found to be 1.42mg/ml. Over all the scavenging assay results revealed that the methanolic extract of *M. mastoidea* have higher free radical scavenging activity compared with BHT. Especially it showed very excellent superoxide radical scavenging activity at 5mg/ml (84.91±0.715%). The antioxidant activity of natural antioxidants has been shown to be involved in termination of free radical reaction. So, the results of reducing capacity and scavenging potential of the extract may serve as a significant indicator of its possible antioxidant activity.

Correlation between antioxidant activity and phenolics content

Correlation study between phenolics content and antioxidant activity of the mushroom was carried out. Fig. 6 shows the linier correlation between the phenolic content and scavenging activity of the mushroom methanolic extract.

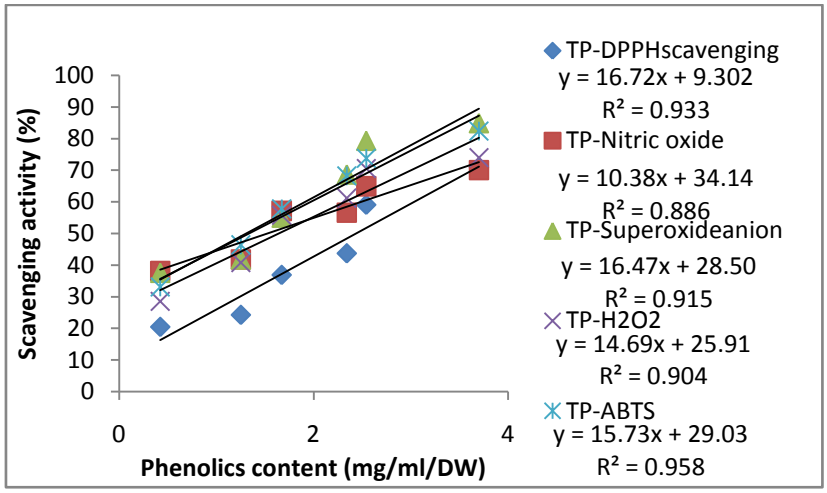

Fig. 6: Correlation between phenolics content of mushroom extract and antioxidant activity.

This result indicates that phenolic compounds are one of the antioxidant compounds found in mushroom, in agreement with Ferreira et al. [25] The results show significance ($p< 0.002$, $p< 0.001$). Positive correlations were established between phenolic content of the mushroom extract and their antioxidant activities. Similar results were reported in *Lentinus edodes* and *Volvariella volvacea.* [26]

DISCUSSION

In mushroom *M. mastoidea* total phenolics and flavanoids were mostly similar to the reported flavonoids content per gram of Portuguese wild mushroom *M.mastoidea and M. procera* (2.16±0.15, 1.99± 0.5mg /gm). [27] Total flavonoids showed significant correlation with antioxidant action through scavenging or chelating process. [28] Also, which was comparatively higher than that reported for Portuguese wild mushroom *M.mastoidea, M. procera* (3.08± 0.17, 3.17± 0.92 mg/g). [27] The HPLC phenolic results are not means that only these phenolic compounds are in *M.mastoidea*, because some of which may escape the usual methodologies of analysis, commonly carried out by HPLC. Various reasons exist for that, like the existence of isomers, difficulty for chromatographic separation of some compounds, lack of commercial standards, or structure not yet elucidated [29]. Phenolic acids are generally considered as nontoxic and are often found in many traditional herbal medicine. Most of them showed excellent scavenging activity of active oxygen such as superoxide anion radical, hydroxyl radicals and singlet oxygen. Some phenolic components such as *p*-hydroxybenzoic acid reported in *Amanita rubescens*, *Tricholoma equestre*, and *Russula cyanoxantha*. [13] The presence of caffeic acid, *p*-coumaric acid, chlorogenic acid and rutin was also reported in *Cantharellus cibarius.* [28,30] Puttaraju et al [14] reported that phenolic compounds such as galic acid, gentisic acid, vanillic acid, syringic acid, p-coumaric acid, caffeic acid, ferullic acid and tannic acid were found in *M. procera.* In this present study we identified p-hydroxy benzoic acid, vanillic acid, gentisic acid, and cinnamic acid derivatives like coumarin and p-coumaric acid *in M. mastoidea*. Phenolic compounds have attracted much interest recently because *in vitro* and *in vivo* studies suggest that they have a variety of beneficial biological properties like antioxidant, anti-inflammatory, antitumor and antimicrobial activities. [23,31] Reports suggested that the reducing power are generally associated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom. [22] Kalyoncua et al [23] reported the antioxidant activity of mycelia from 21 wild mushrooms. According to Mau et al [12] the DPPH scavenging effects of *Termitomyces albuminosus*, *Grifola frondosa* and *Morchella esculenta*. Antioxidant properties of mushrooms were usually related to low-molecular-weight compounds, in particular to the phenolic fractions. [2,4] Phenolic compounds such as flavonoids, phenolic acids, and tannins are considered to be major contributors to the antioxidant capacity of plants, these antioxidants also possess diverse biological activities as anti-inflammatory,anti-atherosclerotic and anticarcinogenic activities. [26] Phenolic compounds seem to have important, when the results obtained from the assay are compared with the literature. [32,33]

CONCLUSION

This study demonstrated that the methanolic extract derivative has various extents of antioxidant properties, including radical scavenging activities and reducing power. These antioxidant effects may vary with the concentration, In general, the antioxidant activity increased with the concentration. The mushroom *M.mastoidea* extracts have significant radical scavenging potential. Moreover this can be attributed a strong abilities as a reducer, and higher superoxide scavenger than BHT. The phenolic acids p-hydroxy benzoic acid, vanillic acid, gentisic acid, and coumarin, p-coumaric acid of the mushroom *M.mastoidea* also contribute the radical scavenging ability particularly the excellent super oxide scavenging ability of this mushroom. Now a day's attention has focused on to natural antioxidants because the use of synthetic antioxidant has been failing off due to their suspected action as cancer inducer. On the basis of the above results, it is suggested that *M. mastoidea* can be used as a source of natural antioxidants. Further studies are required to identify active principles of this mushroom for the significant antioxidant compound.

Abbreviations

1,1- Diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), nitro blue tetrazolium (NBT), reduced nicotinamide adenine dinucleotide (NADH), phenazine methosulfate (PMS), sodium nitroprusside (SNP), sodium carbonate (Na_2CO_3), butylated hydroxytoluene (BHT).

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