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Assessment of antioxidant activity of methanolic extract in *Volvareilla volvacea* (Paddy straw mushroom).

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Abstract

Some edible mushrooms are imperative sources of biologically active compounds. To screen of preliminary enzymatic composition of the methanolic extract of Volvareilla volvacea were evaluated. The enzyme analysis revealed that the extracts were rich sources of enzymatic constituents viz. MDA, GSH, SOD, CAT and GPx. The enzymatic screening was analyzed by biochemical parameters they are Lipid peroxides in terms of Malondialdehyde (MAD)[18], Reduced Glutathione (GSH)[15], Superoxide dismutase (SOD)[16], Catalase (CAT)[2], Glutathione peroxide (GPx)[19]. So it is a need of the time to explore the enzymatic properties of mushrooms especially for those used by society for nutritional and scientifically validates the use of the macro fungi in traditional antioxidant.

Key words : Volvareilla volvacea, MDA, GSH, SOD, CAT and GPx

Introduction

The genus *Volvariella* (paddy straw mushroom) comprises a group of several species, which can be found growing on a variety of substrates in tropical and subtropical regions. *V. volvacea* (Bull. Ex Fr.) Sing., is probably the best known species, as it has been traditionally cultivated in Southeast Asia since the eighteenth century[3]. Currently, *V. volvacea* is the third most important cultivated mushroom accomplishing a total world production of 287 metric tons[4]. In India, Su and Seth[20] first cultivated straw mushrooms; however, scientific cultivation using spawn was effectively demonstrated by Thomas et al.[21]. Mushrooms are considered to be a good quality source of a variety of antioxidants[12]. Methanol and water extracts from *Volvariella volvacea* were found to have rich antioxidative activities[5]. In recent years, the use of some synthetic antioxidants has been limited because of their possible toxic and carcinogenic effects[7]. Foods rich in antioxidants have been shown to play an essential role in the prevention of cardiovascular diseases[6], neurodegenerative diseases[9], inflammation, cytological problems, and aging[1]. Exogenous chemical and endogenous metabolic processes in the human body or in the food system might produce highly reactive free radicals, especially oxygen derived radicals, which are capable of resulting in cell death and tissue

damage. So organism cells are geared up with many more defense systems against free radical damage, including oxidative enzymes, namely Catalase and superoxide dismutase[17].
However, antioxidant supplements containing foods might be used to help human body cells to reduce oxidative scratch[8]. Thus the natural antioxidants present in foods and other biological materials have attracted considerable interest because of their presumed safety and potential nutritional and therapeutic effects[1]. Here, we report antioxidant assays on the analyzed biochemical parameters they are Lipid peroxides in terms of Malondialdehyde (MAD), Reduced Glutathione (GSH), Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxide (GPx) in methanolic extract of *Volvareilla volvacea* collected from Durg district of Chhattisgarh in India.

Materials and methods

For the study mushroom were considered, abundantly available in our area, Durg of Chhattisgarh, India (Latitude 21.1833◦N, Longitude 81.2833◦E, and Altitude 298.00 m), viz., *Volvareilla volvacea.* This mushroom has some enzymatic constituents and they are available abundantly in Chhattisgarh.

[A] Collection of mushrooms sample

Fresh fruiting bodies of *Volvariella volvacea* (paddy straw mushroom) were collected in the local farmhouse of Nikum in Durg district of Chhattisgarh.

[B] Drying and Extraction of mushrooms sample

After collection, the mushrooms sample and the fruiting bodies were cleaned, and remove residual compost on the sample surface. Consequently, mushrooms were left to shadow dry in the laboratory and in the oven at 40-50 °C for 6 hours to ensure complete dryness. Dried fruiting bodies were then kept in sterilized conditions for extraction and stored for further usage. Before the experiment, all the concerned dried parts of the mushroom were crushed and weighed. The fine dried mushroom powdered sample (15 g) was extracted with methanol (B.P 64–66 ºC, 150 ml) for 8 hours using a Soxhlet extractor apparatus. As per need concentration process was performed using a Rotatory evaporator. Subsequently, these extract was stored at 10º C for further usage.

[C] Experimental design and Enzymatic assay-

For the experimental design cultured lymphocytes of *Mus musculus* were divided into five groups and from each group five samples were further processed.

Group I	-	Only lymphocyte
Group II	-	Lymphocyte + 100µM H_2O_2 for 2 hours
Group III	-	H_2O_2 treated lymphocytes with 5µl / 10,000 cells with specific extracts
Group IV	-	H_2O_2 treated lymphocytes with 10µl / 10,000 cells with specific extracts
Group V	-	H_2O_2 treated lymphocytes with 20µl / 10,000 cells with specific extracts

The enzymatic assay was analyzed by biochemical parameters they are Lipid peroxides in terms of Malondialdehyde (MAD)[18], Reduced Glutathione (GSH)[15], Superoxide dismutase (SOD)[16], Catalase (CAT)[2], Glutathione peroxide (GPx)[19].

Observation and statistical validation

The freshly prepared methanolic extracts were subjected to standard enzymatic analysis. The results of enzymatic analysis were reported in the following Tables and The collected data for all parameters was statistically validated by ANOVA (One way).

Table-1[a]
showing effect of methanolic extract of *Volvariella volvacea* on MDA activity on H2O2 induced lymphocyte of *Mus musculus.*

S.N.	**Group- I (Control)**	**Group- II (H_2O_2 Treated)**	**Group- III (5µl/ME + H_2O_2)**	**Group- IV (10µl/ ME +H_2O_2)**	**Group- V (20µl/ME +H_2O_2)**
1	0.90	2.80	2.00	1.60	1.00
2	0.80	3.20	2.60	2.00	1.20
3	0.60	3.00	2.60	2.20	1.00
4	0.80	3.20	2.80	2.00	1.10
5	0.90	3.40	2.80	2.10	1.00
Mean	0.8000	3.1200	2.5600	1.9800	1.0600
S.D.	0.1225	0.2280	0.3286	0.2280	0.0894
S.E.	0.0548	0.1020	0.1470	0.1020	0.0400

Table-1[b]
Statistical analysis (ANOVA) of table-1[a]

Source	SSR	DF	MS	F	Significant
Between Groups	19.2296	4	4.8074	102.2851	P<0.05
Within Groups	0.9400	20	0.0470		

Table-1[a]-showed alteration in the activity of lipid peroxides in terms of Malondialdehyde (MDA) in controlled and H_2O_2 induced lymphocytes with treatment of methanolic extract of *Volvariella volvacea* (5µl, 10µl, 20µl / 10,000 cells). The MDA activity in control set was recorded 0.8000 ± 0.0548 which was increased up to 3.1200 ± 0.1020 after H2O2 exposure because of increased oxidative stress in cells. A mild recovery in MDA activity was noticed after exposure of *Volvariella volvacea* methanolic extract at 20µl / 10,000 cells concentration and it was recorded 1.6000 ± 0.0400. The *Volvariella volvacea* methanolic extract exposure has resulted good to reduce oxidative stress and statistically (F = 102.2851 > 2.87 at 5%P) found significant in table -1[b].

Table-2[a]
showing effect of methanolic extract of *Volvariella volvacea* on GSH activity on H2O2 induced lymphocyte of *Mus musculus.*

S.N.	Group- I (Control)	Group- II (H_2O_2 Treated)	Group- III (5µl/ME + H_2O_2)	Group- IV (10µl/ ME +H_2O_2)	Group- V (20µl/ME +H_2O_2)
1	5.80	2.00	2.20	2.80	3.40
2	6.00	2.40	2.80	3.20	4.00
3	6.40	2.40	2.80	3.60	4.20
4	6.20	2.20	2.60	3.40	4.00
5	5.80	2.00	2.80	3.60	4.40
Mean	6.0400	2.2000	2.6400	3.3200	4.0000
S.D.	0.2608	0.2000	0.2608	0.3347	0.3742
S.E.	0.1166	0.0894	0.1166	0.1497	0.1673

Table -2[b]

Statistical Analysis (ANOVA) of Table-2[a]

Source	SSR	DF	MS	F	Significant
Between Groups	45.3280	4	11.3320	132.3832	P<0.05
Within Groups	1.7120	20	0.0856		

Table-2[a]- showed alteration in activity of reduced glutathione(GSH) in controlled and H2O2 induced lymphocytes with treatment of methanolic extract of *Volvariella volvacea* (5µl, 10µl, 20µl / 10,000 cells). The GSH activity in control set was recorded 6.0400 ± 0.1166 which was increased up to 2.2000 ± 0.0894 after H2O2 exposure because of increased oxidative stress in cells. A mild recovery in GSH activity was noticed after exposure of *Volvariella volvacea* methanolic extract at 20µl / 10,000 cells concentration and it was recorded 4.0000 ± 0.1673. The *Volvariella volvacea* methanolic extract exposure has resulted good to reduce oxidative stress and statistically (F = 132.3832 > 2.87 at 5%P) found significant in table -2[b].

Table-3[a]
showing effect of methanolic extract of *Volvariella volvacea* on SOD activity on H2O2 induced lymphocyte of *Mus musculus.*

S.N.	Group- I (Control)	Group- II (H_2O_2 Treated)	Group- III (5µl/ME + H_2O_2)	Group- IV (10µl/ ME +H_2O_2)	Group- V (20µl/ME +H_2O_2)
1	3.60	1.40	1.80	2.40	3.20
2	3.80	1.60	1.90	2.60	3.00
3	3.60	1.60	1.80	2.40	3.40
4	4.00	1.80	2.60	3.00	3.80
5	3.80	1.40	2.00	2.80	3.40
Mean	3.7600	1.5600	2.0200	2.6400	3.3600
S.D.	0.1673	0.1673	0.3347	0.2608	0.2966
S.E.	0.0748	0.0748	0.1497	0.1166	0.1327

Table-3[b]
Statistical Analysis (ANOVA) of Table-3[a]

Source	SSR	DF	MS	F	Significant
Between Groups	16.5984	4	4.1496	64.0370	P<0.05
Within Groups	1.2960	20	0.0648		

Table-3[a] showed alteration in activity of superoxide dismutase (SOD) in controlled and H2O2 induced lymphocytes with treatment of methanolic extract of *Volvariella volvacea* (5µl, 10µl, 20µl / 10,000 cells). The SOD activity in control set was recorded 3.7600 ± 0.0748 which was increased up to 1.5600 ± 0.0748 after H2O2 exposure because of increased oxidative stress in cells. A mild recovery in SOD activity was noticed after exposure of *Volvariella volvacea* methanolic extract at 20µl / 10,000 cells concentration and it was recorded 3.3600 ± 0.1327. The *Volvariella volvacea* methanolic extract exposure has resulted good to reduce oxidative stress and statistically (F = 64.0370 > 2.87 at 5%P) found significant in table -3[b].

Table-4[a]
Showing effect of methanolic extract of *Volvariella volvacea* on CAT activity on H2O2 induced lymphocyte of *Mus musculus.*

S.N.	Group- I (Control)	Group- II (H_2O_2Treated)	Group- III (5µl/ME+ H_2O_2)	Group- IV (10µl/ ME +H_2O_2)	Group- V (20µl/ME +H_2O_2)
1	5.60	2.30	3.00	3.40	4.20
2	5.80	2.40	3.20	3.80	5.00
3	6.00	3.00	3.80	4.20	5.60
4	6.20	2.60	3.00	3.80	4.80
5	5.80	2.80	3.20	4.00	5.00
Mean	5.8800	2.6200	3.2400	3.8400	4.9200
S.D.	0.2280	0.2864	0.3286	0.2966	0.5020
S.E.	0.1020	0.1281	0.1470	0.1327	0.2245

Table -4[b]
Statistical Analysis (ANOVA) of Table-4[a]

Source	SSR	DF	MS	F	Significant
Between Groups	34.1920	4	8.5480	73.4364	P<0.05
Within Groups	2.3280	20	0.1164		

Table-4[a]-showed alteration in activity of catalane (CAT) in controlled and H2O2 induced lymphocytes with treatment of methanolic extract of *Volvariella volvacea* (5µl, 10µl, 20µl / 10,000 cells).The CAT activity in control set was recorded 5.8800 ± 0.0020 which was increased up to 2.6200 ± 0.1281 after H2O2 exposure because of increased oxidative stress in cells. A mild recovery in CAT activity was noticed after exposure of *Volvariella volvacea* methanolic extract at 20µl / 10,000 cells concentration and it was recorded 4.9200 ± 0.2245. The *Volvariella volvacea* methanolic extract exposure has resulted good to reduce oxidative stress and statistically (F = 73.4364 > 2.87 at 5%P) found significant in table- 4[b].

Table-5[a]
Showing effect of methanolic extract of *Volvariella volvacea* on GPx activity on H2O2 induced lymphocyte of *Mus musculus.*

S.N.	Group- I (Control)	Group- II (H_2O_2 Treated)	Group- III (5µl/ME + H_2O_2)	Group- IV (10µl/ ME +H_2O_2)	Group- V (20µl/ME +H_2O_2)
1	9.80	6.00	6.80	7.60	8.40
2	9.60	5.80	6.60	7.40	8.40
3	9.60	5.60	6.80	7.80	9.00
4	9.80	5.40	6.00	7.10	9.10
5	9.90	6.00	7.10	8.60	9.40
Mean	9.7400	5.7600	6.6600	7.7000	8.8600
S.D.	0.1342	0.2608	0.4099	0.5657	0.4450
S.E.	0.0600	0.1166	0.1833	0.2530	0.1990

Table-5[b]
Statistical Analysis (ANOVA) of Table-5[a]

Source	SSR	DF	MS	F	Significant
Between Groups	51.7136	4	12.9284	83.7332	P<0.05

Within Groups	3.0880	20	0.1544		

Table-5[a] -showed alteration in activity of glutathione peroxide (GPx) in controlled and H2O2 induced lymphocytes with treatment of methanolic extract of *Volvariella volvacea* (5µl, 10µl, 20µl / 10,000 cells). The GP_X activity in control set was recorded 9.7400 ± 0.0600 which was increased up to 5.7600 ± 0.1166 after H2O2 exposure because of increased oxidative stress in cells. A mild recovery in GP_X activity was noticed after exposure of *Volvariella volvacea* methanolic extract at 20µl / 10,000 cells concentration and it was recorded 8.8600 ± 0.1990. The *Volvariella volvacea* methanolic extract exposure has resulted good to reduce oxidative stress and statistically (F = 83.7332 > 2.87 at 5%P) found significant in table -5[b].

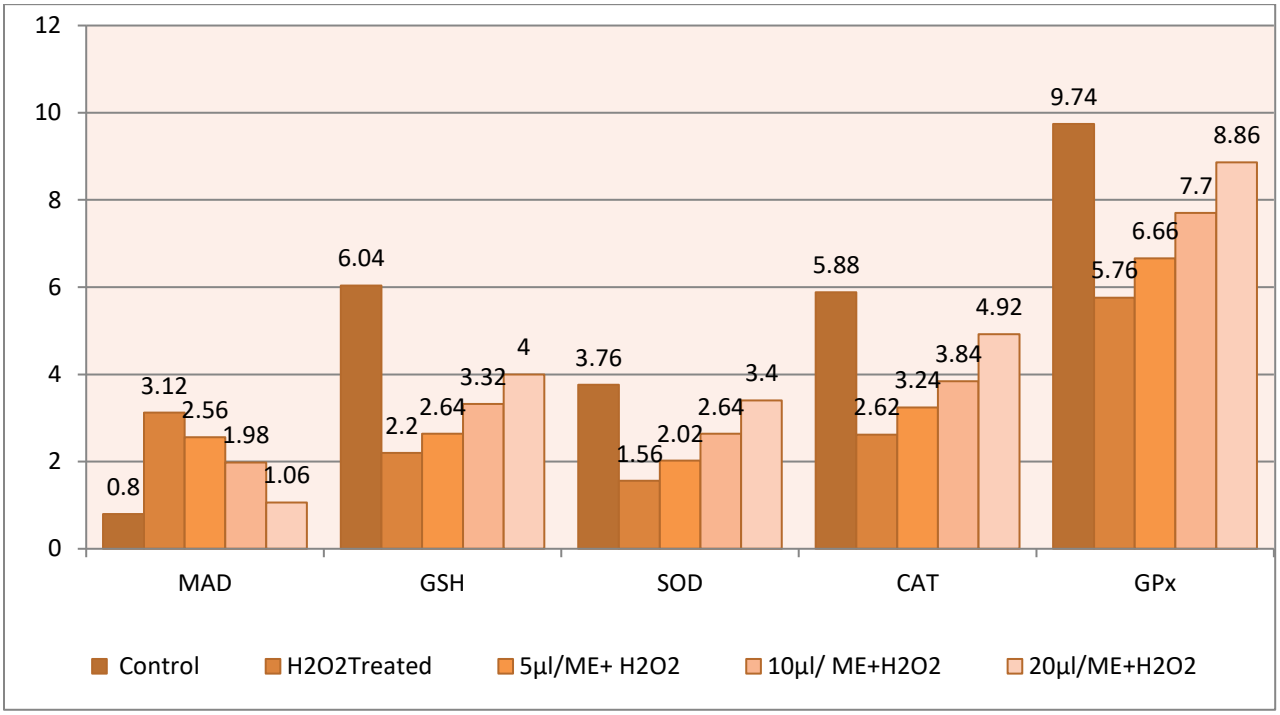

Fig-1, Pictographic comparison of antioxidant features indicated by MDA, GSH, SOD, CAT and GPx in methanolic extract of *Volvariella volvacea*.

Result and discussion

The occurrence and allocation of different macro and micro elements in fruiting bodies of certain mushrooms is not only an important problem for physiology and ecology of fungi, but also has practical environmental and toxicological aspects. As evident in this and several other studies[14]. The successful commercial cultivation of paddy straw mushroom depends to a large extent on quality and purity of spawn[11]. Some food are rich in antioxidative substances have been shown to play an essential role in the prevention of several diseases[6&9]. The use of synthetic antioxidants has been restricted because of their possible toxic and carcinogenic effects[7].

Methanol extract of *P. florida* have potent hydroxyl radical scavenging and lipid peroxidation inhibition (antioxidant) activities. The antioxidant activities in *Ganoderma lucidum*, *P. florida*, and *P. sajor-caju*, as scavenging activity were demonstrated by Lakshmi et al,[13]. Cheung et al.[5] reported that the four mushroom extracts from *L. edodes* showed the most potent radical scavenging activity in each assay, showing 75.9% (at 20 mg/ ml) in the β-carotene bleaching method, 55.4% in the DPPH radical scavenging method (at 6 mg/ ml), and 94.0% of inhibition of erythrocyte heamolysis (at 5 mg/ ml).

Methanol and water crude extracts from shiitake and paddy straw mushroom were investigated for their antioxidant capacity in three different assays, the β-carotene and linoleic acid system, DPPH, radical scavenging activity, and inhibition of haemolysis of rat erythrocyte induced by peroxyl radicals[5].
The present study clearly showed that the methanolic extract of *Volvariella volvacea paddy straw* mushroom) recorded highest levels of antioxidants; Lipid peroxides in term of Malondialdehyde (MAD) (1.6000 ± 0.0400), Reduced Glutathione (GSH) (4.000 ± 0.1673), Superoxide dismutase (SOD) (3.3600 ± 0.1327), Catalase (CAT) (4.9200 ± 0.2245), Glutathione peroxide (GPx) (8.8600 ± 0.1990).

Conclusion

In this study, methanolic extract of *Volvariella volvacea* the antioxidant features indicated by MDA, GSH, SOD, CAT and GPx was found significant but their antioxidant features indicated by GSH, CAT, GPx, SOD was found more effective and MDA activity are satisfactory.

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