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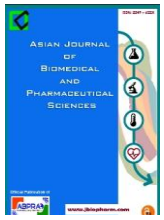

RESEARCH ARTICLE

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Free radical scavenging activity of fruiting body extracts of an edible mushroom, *Volvariella volvacea* (Bull. ex Fr.) Singer: an *in vitro* study

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Abstract
Mushrooms, being rich source of therapeutic and nutritional substances have received considerable attention for their antioxidant potential. In the present study, methanol (VV MET) and hot water (VV HW) extracts of fruiting bodies of *Volvariella volvacea* were investigated for free radical scavenging activity. Analysis of antioxidant content of the extracts showed that total phenolics were abundant with 53.13 mg GAEq/g and 36.67 mg GAEq/g in methanol and hot water extracts, respectively. Total flavonoid content in the methanol extract was higher (14.35 mg/g) than the hot water extract (12.54 mg/g). Ascorbic acid levels were 1.72 and 1.52 mg/g in methanolic and hot water extracts, respectively. Reducing power potential of methanol and hot water extracts were 2.11 and 2.38 at 250 mg/ml. 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay exhibited 80.32% inhibition at 250 mg/ml concentration. However, hot water extract showed 74.24% inhibition. ABTS assay showed 68.75 % inhibition for methanol extract and 62.56% for hot water. Hydroxyl radical scavenging activity of hot water extract was maximum (69.34%) when compared to methanol extract inhibition (62.45%). Superoxide and nitric oxide radical inhibition level for methanol extract were found to be 74.23 and 75.25%, respectively at 200ug/ml, whereas hot water extract showed 66.54 and 72.14% inhibition of superoxide and nitric oxide. The study reveals that both the extracts of *V. volvacea* are having better free radical scavenging potential with high levels of antioxidant compounds.

Keywords: mushroom, fruiting bodies, *Volvariella volvacea,* antioxidants, free radical scavenging

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INTRODUCTION

Free radicals are chemical species capable of independent existence, contain one or more unpaired electron in the outer orbit [1]. Though unstable they are highly reactive. Oxygen free radicals include superoxide, hydroxyl, peroxyl, alkoxyl and hydroperoxyl. Nitric oxide and nitrogen oxide are belonging to nitrogen radicals [2]. Being produced continuously in the body, they are necessary for some biological functions[3]. ROS and RNS are beneficial in certain cellular and immune functions at low level [4]. However, at higher concentrations they cause oxidative stress, a state of imbalance between production of free radicals and antioxidant defense, and are associated with damage to a wide range of molecules such as lipids, proteins, and nucleic acids [5] with potential impact on the whole organism[6]. Oxidative stress is often linked to either primary or secondary pathophysiologic mechanism of many acute and chronic diseases [7]. Free radical mediated oxidative stress has been associated with diseases such as atherosclerosis and cardiovascular complications [8,9], neurodegenerative disorder[10], diabetes[11], metabolic syndrome, skin disorders, and tumors[12]. There are several mechanisms by which the human body counteracts the oxidative stress. The primary defense is by the naturally produced endogenous antioxidant enzymes such as superoxide dismutase, glutathione peroxidase, and catalase or by the externally supplied foods rich in antioxidants [4, 13, 14]. However, these systems are overwhelmed during oxidative stress diminishing the endogenous antioxidant systems. In these circumstances, supplementation of antioxidant rich substances and foods would be of immense use to reduce the free radicals. Synthetic antioxidants like BHA and BHT are ill reputed with side effects [15] which prompted to search for safe natural antioxidants.

In recent years, mushrooms are gaining importance both as nutrient supplement and disease curing medicine. Mushrooms are unlimited source of therapeutically useful and biologically active agents. Compounds of mushrooms have been reported to have antifungal, anti-inflammatory, antibacterial, antiviral, antitumor, hepatoprotective, antidiabetic, anti-thrombotic and hypotensive [16]. They were shown to posses antioxidant capacity in *in vitro* systems [17,18]. Antioxidant properties of several culinary mushrooms [19], mycelia of 21 wild mushrooms[20], fruiting bodies of three edible mushrooms [21], *Ganoderma lucidum* [22] and four Indian medicinal mushrooms [23] were reported. Review by Asatiani *et al*., [24] reveals that mushrooms are promising antioxidant sources. The present study aimed at evaluating the antioxidant effect of an edible mushroom *Volvariella volvacea*. Commonly known as paddy straw mushroom or Chinese mushroom, *V. volvacea* (Pluteaceae) is one of the six species of edible mushrooms cultivated as food around the world [25] and the most favorite edible mushroom in south Asian countries because of its delicacy, rich protein, amino acid, vitamins and mineral contents [26].

MATERIALS AND METHODS

Mushroom preparation

Fruiting bodies of Paddy straw mushroom (*Volvariella volvacea*), procured from Directorate of Mushroom Research, Solan, India as dried sample was further dried at 50^0 C for 12 hrs and pulverized. The powdered mushroom was extracted with methanol and water in soxhlet apparatus successively for 12 hrs. The extract was concentrated using rotary vacuum evaporator and stored for the assays

Reagents

All the reagents used were of analytical grade procured from Himedia Laboratories, Mumbai, India. DPPH and ABTS were purchased from Sigma-Aldridge, USA.

Antioxidants analysis

Estimation of Total Phenol (TP): Total phenol content was estimated by Folin-Ciocalteau reagent method[27]. To 0.1 ml of Folin-Ciocalteau reagent 0.5 ml of the extract was added, mixed and incubated at room temperature for 15 min. Then, 2.5 ml of sodium carbonate was added and incubated again for 30 min. Absorbance was measured at 760 nm. Gallic acid was used as standard and the results are expressed in terms of gallic acid equivalent (GAEq) in mg.

Estimation of Total Flavonoid (TF): Flavonoid content in *V. volvacea* extracts was determined by aluminum chloride method [28]. A reaction mixture containing 1.0 ml of the extract, 0.5 ml of aluminum chloride (1.2%) and 0.5 ml of potassium acetate (120 mM) was incubated at room temperature for 30 min and was absorbance measured at 415 nm. Quercetin was used as positive control .The total flavonoid content is expressed in terms of quercetin standard equivalent (mg g-1 of extracted compound).

Estimation of Ascorbic Acid: Ascorbic acid estimation was done following the method given by Kumari *et al*., [29]. Extract of *V. volvacea* was re-extracted with metaphosphoric acid (1%, 10 ml) for 45 min at room temperature and filtered through Whatman No.4 filter paper. The filtrate (1 ml) was mixed with 2,6-dichlorophenol indophenols (9 ml) and the absorbance was measured at 515 nm against a reagent blank within 30 min. Total content of the ascorbic acid was calculated on the basis of the calibration curve of standard L- ascorbic acid.

Determination of free radical scavenging activity

Reducing power assay: The reducing power of the extract was measured as given by Athukorala *et al.,* [30]. Briefly, to 1 ml of the extract 2.5 ml of phosphate buffer (200 mM, pH 6.6) and 2.5 ml of potassium ferricyanide (30 mM) were added and incubated at 50 ^{0}C for 20 min. Thereafter, 2.5 ml of trichloroacetic acid (600 mM) was added to the reaction mixture and centrifuged for 10 min at 3000 rpm. The upper layer of the solution (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of $FeCl_3$ (6 mM) and absorbance was measured at 700. Curcumin as was used positive control.

a,a- Diphenyl–β-picryl-hydrazyl (DPPH) radical scavenging assay: DPPH radical scavenging assay was done according to McCunae and Johns[31]. The reaction mixture (3.0 ml) containing 1ml of DPPH in methanol (0.3 mM), 1ml of the extract and 1ml of methanol was incubated for 10 min in dark and the absorbance was measured at 517 nm. Gallic acid was used as positive control.

2, 2'-azino-bis 3-ethylbenzthiazoline-6-sulphonic acid (ABTS): ABTS radical scavenging activity of the mushroom extracts was done following the method given by Stratil [32]. The radical ion $ABTS^+$ was generated by persulphate oxidation of ABTS. A mixture (1:1 v/v) of ABTS (7.0 mM) and potassium persulphate was allowed to react overnight at room temperature to form radical cation, $ABTS^+$. A working solution diluted with phosphate buffer to absorbance values between 1.0 and 1.5 at nm 734 was prepared. An aliquot (0.1 ml) of the sample was mixed with 3.9 ml of working solution and decrease in absorbance measured after 10 min at 37^{0}C. Aqueous phosphate solution (3.9 ml) without ABTS was used as control. Quercetin was used as standard.

Hydroxyl radical scavenging assay: Hydroxyl radical scavenging effect of the mushroom extracts was done as described byKunchandy and Rao [33]. The reaction mixture (1ml) consisted of 100 ul of 2-deoxy-D-ribose (28 mM in 20 Mm KH_2PO_4-KOH buffer, pH 7.4) and 100 ul of the extract, 200 ul of EDTA (1.04 mM) 200 uM of $FeCl_3$(1:1v/v), 100 ul of H_2O_2 (1mM) and 100 ul of ascorbic acid (1mM) was incubated at 37^0 C for an hour. To this mixture 1 ml of thiobarbituric acid (1%) and 1.0 ml of trichloro acetic acid (2.8%) were added and incubated at 100^0 C for 20 min. The absorbance was measured after cooling at 532 nm against a blank sample. Quercetin was used as standard

Superoxide anion radical scavenging assay: The superoxide radical scavenging activity of the extract was assessed following the method of Robak and Gryglewski [34]. Brifely, 0.5 ml of NBT (0.3 Mm), 0.5 ml of NADH (0.936 Mm), 1 ml of the mushroom extract in concentrations ranging from 50 to 300 ug/ml and 0.5 ml Tris-Hcl buffer(16 mm, pH 8.0) were mixed with 0.5 ml PMS solution (0.12mM) and the reaction was started. After incubation at 25^0 C for 5 min, absorbance was measured at 560 nm against blank. Curcumin was used as standard.

Nitric oxide scavenging assay: The assay was done following the method of Green *et al*., [35]. To 3 ml of 10 mM sodium nitroprusside in phosphate buffer, 2 ml of the extract and reference compound in different concentrations (40-200 ug/ml) were added. This mixture was incubated at 25^{0}C for 60 min. To this 5 ml of the incubated samples, 5 ml of Griess reagent (1% sulphanilamide, 0.1% naphthy-ethylene diamine dihydrochloride in 2% H_3PO_4) was added and absorbance was measured at 540 nm. Ascorbic acid was used as standard. Percent inhibition of the nitric oxide generated was measured by comparing the absorbance values of mushroom extracts and standard solution preparations.

Statistical analysis

All the assays were done in triplicate and the Mean ± SE values were calculated using Graph Pad Prism 5. The percent inhibition or scavenging was calculated as follows:

% Inhibition = (A^0-A^1/A^0) X 100, where A^0 is the absorbance of control and A^1 is absorbance value of the test.

RESULTS AND DISCUSSION

Methanolic and hot water extracts of *V. volvacea* showed considerable amount of total phenolics, flavonoids and ascorbic acid, the major antioxidant compounds reported to be involved in free radical scavenging activity. In the present study, total phenolic content in the methanolic extract was 53.13 mg GAEq/g when compared to the 36.67 mg GAEq/g in hot water extract. Total flavonoid content was also higher (14.35 mg/g) in methanolic extract than the hot water extract (12.54 mg/g). On the other hand, ascorbic acid level in both the extracts was more or less equal, showing 1.72 and 1.52 mg/g in methanolic and ethanolic extracts, respectively. Mushrooms produce a variety of secondary metabolites including phenolic compounds, polyketides, terpenes and steroids[36]. They are also rich source of antioxidant phenolics, flavonones, ascorbic acid, beta carotenes and lycopenes[37]. Total phenols and flavonoid content in the aqueous extract of the mycelium of *V. volvacea* were 19.08 and 8.23 mg/g, respectively[38]. Higher phenolic compounds in hot water extract than in the methanolic extracts were reported by Puttaraju *et al.,* [39] in several edible Indian mushrooms. Total phenolic contents in *Lentinus edodes* and *V. volvacea* extracted with different solvents were higher in methanolic solvent extracts (4.79-15.0 mg/g) than in water extracts (1.33-1.34 mg/g). Total phenolic

content was three times higher in *V. volvacea* than in *L .edodes*[40].

The presence of these antioxidant compounds in the mushrooms is well associated with their free radical scavenging activity. In the present study, extracts of *V. volvacea* exhibited potential free radical quenching properties. Reducing power of the *V. Volvacea* for methanolic extract was 2.11 and of hot water extract was 2.38 at 250 mg/ml concentration (Figure 1). Studies show that reducing power was directly related to the antioxidant potential[41]. Reducing power was generally linked to the reductones, which by donating hydrogen break the chain reaction and exert antioxidant activity[42]. Reductones also prevent peroxides formation by reacting with precursors of peroxides and contribute to reducing power [43, 44]. The reducing power potential is the better indicator of antioxidant potential of any compound and thus supports the effectiveness of *V. volvacea* in reducing the radicals.

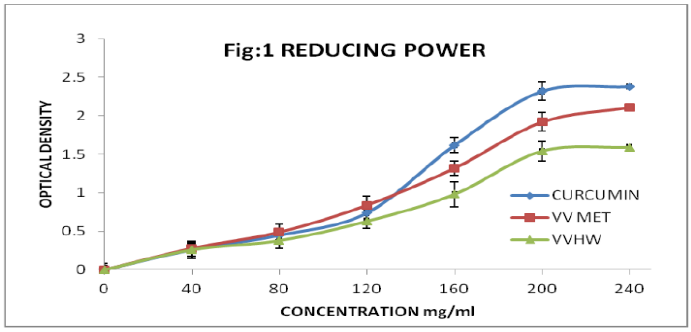

Figure 1: Reducing power assay of the methanol (VV MET) and hot water (VV HW) extracts

DPPH radical scavenging assay revealed that methanolic extract is more effective with 80.32% inhibition and hot water extract showing 74.24% inhibition at 250mg/ml concentration. A dose dependent increase in inhibition was noted in both the extracts with 36.26% and 26.27% at 50 mg/ml concentration of methanol and hot water extracts, respectively (Figure 2). The IC^{50} value of methanolic extract was 110.40 mg/ml and hot water IC^{50} concentration for DPPH was 142.45 mg/ml. DPPH is a stable radical and is a popular assay for free radical scavenging of any antioxidant as it can accept an electron or hydrogen to become a stable diamagnetic molecule [45]. Similarly ABTS assay is used to evaluate the total antioxidant capacity of the compounds [46]. Methanolic extract exhibited a maximum of 68.78% inhibition and hot water extract showed 62.56% inhibition at 125 mg/ml concentration (Figure 3). However, quercetin inhibition of ABTS at this concentration was 75.54%. DPPH and ABTS radical scavenging activity can be attributed to the phenolics and flavonoids. These properties in general depend on the ability to donate hydrogen or electron to the radicals. In flavonoids, the hydrogen position in the molecules determines the properties[47].

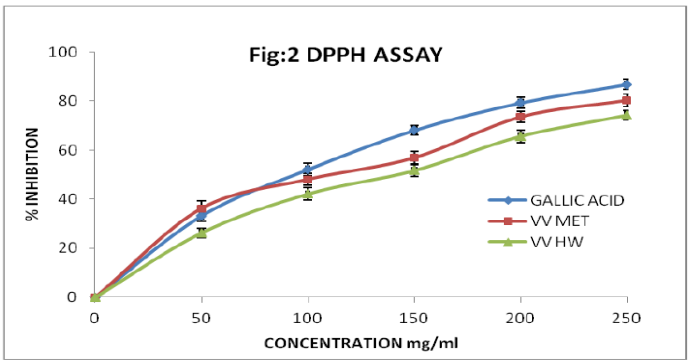

Figure 2: DPPH radical scavenging activity of methanol(VV MET) and hot water(VV HW) extracts

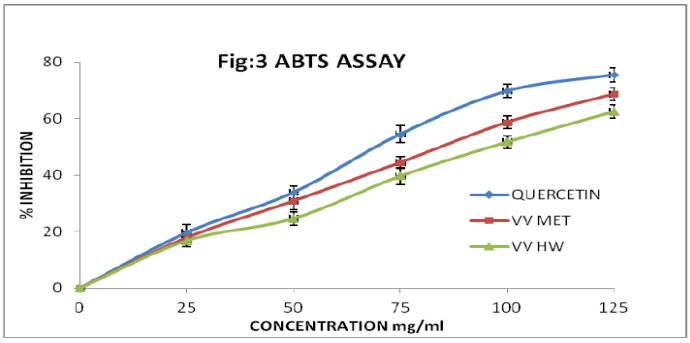

Figure 3: ABTS scavenging activity of methanol (VV MET) and hot water (VV HW) extracts

Hydroxyl, superoxide and nitric oxide radicals were also considerably scavenged by both methanolic and hot water extracts. Hydroxyl radical scavenging activity of hot water extract was 69.34% whereas of methanolic extract was 62.45% at 250 ug/ml (Figure 4). On the other hand, methanolic extract showed a maximum of 74.23% superoxide scavenging activity and hot water extract exhibited 66.54% at 300 ug/ml concentration. The curcumin standard superoxide scavenging activity, however, was 82.34% (Figure 5). Nitric oxide radical inhibition of methanolic extract was 75.52% and of hot water extract was 72.12% at 200 ug/ml concentration (Figure 6).

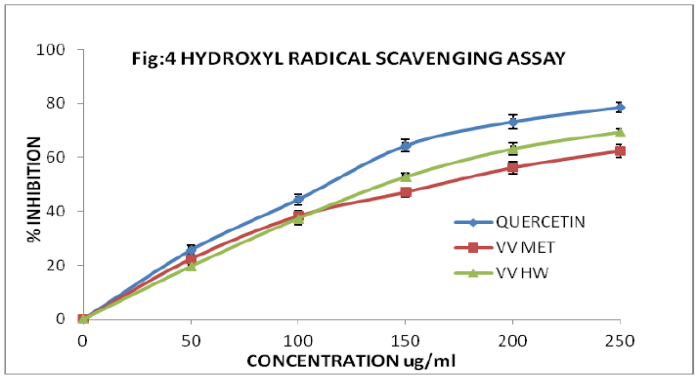

Figure 4: Hydroxyl radical scavenging activity of methanol (VV MET) and hot water (VV HW) extracts

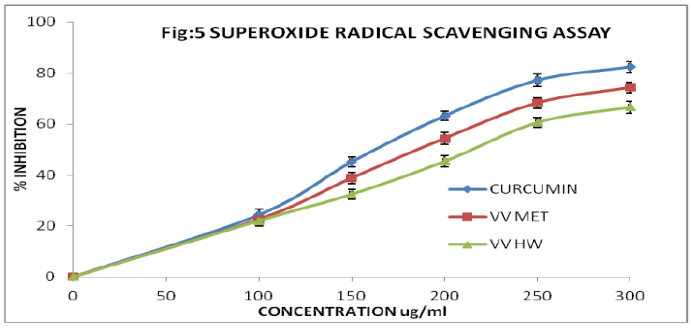

Figure 5: Superoxide radical scavenging by methanol(VV MET) and hot water(VV HW) extracts

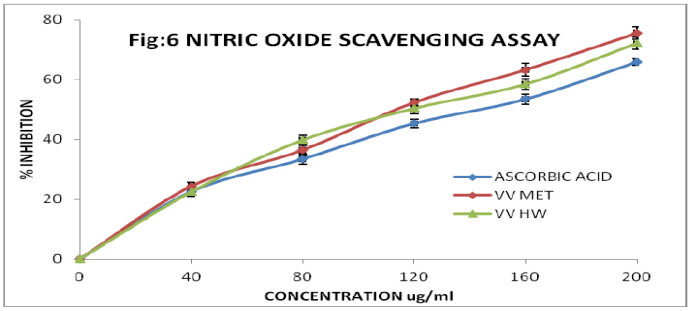

Figure 6: Nitric oxide scavenging activity of methanol(VV MET) and hot water(VV HW) extracts

The antioxidant properties of the *V. volvacea* can be undoubtedly attributed to the rich total phenolics, flavonoids and vitamin C. Antioxidants found in mushrooms are mainly phenolic acids flavonoids, followed by tocopherols, ascorbic acid and carotenoids[48]. Several studies have confirmed that the antioxidant property of the mushrooms is due to their abundant phenolics which are composed of one or more hydroxyl groups contributing to the antioxidant activity [49]. Polyphenols are multifunctional antioxidants by acting as reducing agents, hydrogen donating antioxidants and singlet oxidant quenchers [50]. Flavonoids are effective scavengers of most of oxidizing molecules [51] stabilize the reactive oxygen species by reacting with the reactive compound of the radical. Because of the high reactivity of the hydroxyl group of the flavonoids, radicals are made inactive [52]. Vitamin C, one of the simplest vitamins is reported in several mushrooms such as *Armillaria mellea, Calocybe gambosa and Clitocybe odora* [53]. Ascorbic acid contents of flesh samples of *Pleurotus ostreatus* and *V. volvacea* were 35-58 mg/100g and 8-120 mg/100 g, respectively [54]. Vitamin C is effective against superoxide, hydroxyl radical, hydrogen peroxide, peroxyl radical and singlet oxygen [55]. Free radical scavenging activity observed in the present study could be attributed to the phenolic, flavonoids and ascorbic acid content.

CONCLUSION

Antioxidant activity of the methanolic and hot water extracts of the edible mushroom *V. volvacea* observed in the present study demonstrates that this mushroom contains considerable amount of phenolics, flavanoids and ascorbic acid. Known for its delicacy, nutritional and therapeutic values *V.volvacea* can also be a promising medicine against several oxidative stresses mediated disorders.

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