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Determination of Physicochemical, Phytochemical and Antioxidant activity of *Volvariella volvacea*

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ABSTRACT

Volvariella volvaceae is an edible mushroom occurring in both tropical and sub tropical regions of the world and is important for the prevention and treatment of various diseases. The physicochemical, phytochemical and antioxidant activity of hydroalcoholic lyophilized and oven dried extracts of *Volvariella volvaceae* were investigated. The total ash, water soluble, acid insoluble, alcohol soluble extractive, water soluble extractive, moisture content and fluorescence property of *Volvariella volvaceae* powder were evaluated. Phytochemical screening of lyophilized and oven dried extracts of *Volvariella volvaceae* were performed to check the presence of various phytoconstituents. The lyophilized and the oven dried extracts showed the presence of carbohydrates, glycosides, alkaloids, flavonoids, saponins and tannins. The protein was present in lyophilized extract; while steroid were absent in both extracts. The capacity of antioxidant property of the extracts was evaluated by using DPPH and Superoxide anion radical scavenging assay. Total phenolic and total flavonoid contents were estimated to quantify the presence of phenolic content in extracts. The lyophilized extract showed higher antioxidant activity compared to oven dried extract and thus, the outcomes of the present study suggest that the therapeutic activities of *Volvariella volvaceae* can be attributed to its antioxidant property.

Keywords: *Volvariella volvaceae*, Physiochemical, Phytochemical, Antioxidant, Lyophilized, Oven dried

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INTRODUCTION

Mushroom is known for their nutritive and therapeutic action from hundreds of years. As it contains a huge amount of carbohydrates especially polysaccharides, proteins, tannins, flavonoids, fibres, polyphenols, mineral content, low fat level and large amount of essential amino acid like leucine, glutamine, valine, gallic acid, aspartic acid, thus they are considered as Functional foods. It also contains an enormous amount of secondary metabolites, responsible for their therapeutic activity. These components serve as an immunomodulators, lipid lowering, antitumor and other valuable effect without any noteworthy adverse effect. Now a day, some mushrooms are being used in bioremediation, to absorb and digest waste and dangerous substances like agricultural and industrial waste. Mushrooms are cultivated in different agricultural wastes to increase the yield and their phytoconstitutents. In India, four mushrooms are chiefly cultivated and one of them is *Volvariella volvacea*.[1, 2]

Volvariella volvaceae is an edible mushroom which occurs in both tropical and sub tropical regions of the world. It is important for the prevention and treatment of various diseases in humans, which is evident from the biochemical analysis of the fruiting bodies of these mushrooms. This is also a good source of polypepetides, terpenes, steroids and phenolic compounds such as flavonoids, phenolic acids and tannins, which are responsible for its high antioxidant.[3-5] Its effectiveness has also been demonstrated for antitumor, cardiovascular, neurodegenerative diseases and for other applications.[6, 7]The aim of the present study was to evaluate the physiochemical, phytochemical and antioxidant activity of *Volvariella volvacea.*

MATERIALS AND METHOD

Collection of *Volvariella volvacea*

Culture of *Volvariella volvacea* was obtained from Mushroom Research Centre, Department of Plant Pathology, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India and it was also cultivated in Columbia Mushroom Centre, Raipur, Chhattisgarh, India.

Preparation of extract

Mushroom was collected and washed with water to remove soil and straw from base. The mushroom was shade dried and coarsely powered for further study. The powdered *Volvariella volvacea was* filled in stopper conical flask and mixed with hydroalcoholic solvent (70:30). The flask was placed in the orbital shaker incubator at 30°C for three days with continuous shaking. The extract was filtered, and filtrate was divided into two parts. First part of filtrate was subjected for Freeze drying by using Lyophilizer and second part for Oven drying.

PHYSIOCHEMICAL PARAMETERS:

The extracts were subjected to evaluate its total ash, water soluble ash, acid insoluble ash, water soluble extractive value, alcohol soluble extractive value, moisture content and fluorescence analysis.[8-10]

***Total Ash value*:**

About 2gm of the powdered drug was weighed accurately into a tarred crucible and spread in an even layer. Incinerated at 450°C in a muffle furnace until free from carbon. The crucible was cooled in a desiccator and weighed. Total ash content was calculated in mg per g of air-dried material.

***Water soluble Ash*:**

Ash obtained from the total ash was boiled with 25 ml of distilled water and insoluble matter was filtered through an ash less filter paper. The filter paper was transferred into a tarred silica crucible and was incinerated at 450°C in a muffle furnace until free from carbon, cooled and weighed. Percentage of water-soluble ash was calculated with reference to air-dried substance.

***Acid insoluble ash*:**

Ash obtained from the total ash was boiled with 25ml of 2N HCl and the insoluble matter was filtered through an ash less filter paper. The filter paper was transferred into a tarred silica crucible. It was washed with hot water, ignited in tarred crucible, cooled and the residue obtained was weighed. Percentage of acid insoluble ash was calculated with reference to air-dried substance.

***Alcohol soluble extractive value*:**

About 5gms of air dried coarse powdered drug was weighed and macerated alcohol in a closed flask for 24 hours, shaking frequently during the first 6 hrs & these allowed standing for 18 hrs. Thereafter, it was filtered taking precautions against loss of the solvent. 25 ml of the filtrate was evaporated to dryness, dried at 105°C & weighed. The % of the alcohol soluble extractive values was calculated with reference to the air-dried drug.

***Water soluble extractive value*:**

Coarsely powdered drug was macerated with 100 ml of chloroform water in a closed flask for 24 hrs shaking frequently during the first 6 hrs and then allowed to stand for 18 hrs. Thereafter, it was filtered taking precautions against loss of the solvent. 25 ml of the filtrate was evaporated to dryness, dried at 105°C & weighed. The percentage of the water soluble extractive value was calculated with reference to the air-dried drug.

***Moisture content*:**

Weigh about 1.5g of the powdered drug and dry it in the oven at 100^0C or 105^0C for about 30 min, cooled and loss in weight is usually recorded as moisture.

***Fluorescence analysis*:**

Fluorescence study is an essential parameter for first line standardization of crude drugs. The powder drug was treated separately with different reagents and exposed to visible and ultraviolet light (short & long) to study their fluorescence behavior.[11, 12]

PHYTOCHEMICAL SCREENING

The extracts were screened for the presence or absence of secondary metabolites such as alkaloids, steroidal compounds, glycosides, flavonoids, tannins, saponins, carbohydrates, amino acid and protein using standard procedures.[13-16]

Test for carbohydrate

Molisch test:

A small quantity of the extracts was dissolved separately in 4 ml of distilled water and filtered. The filtrate was then subjected to Molisch's reagent and formation of brick red colour confirmed the presence of reducing sugar.

Fehling's test:

Equal volume of Fehling A (copper sulphate solution) and Fehling B (potassium tartrate and sodium hydroxide in distilled water) reagents were mixed with few drops of extract and boiled, a brick red precipitate of cuprous oxide forms, reducing sugar are present.

Test for glycosides

Borntrager's test:

200 mg crude extract was mixed with 2 ml of dilute sulphuric acid and 2 ml of 5 % aqueous ferric chloride solution, boiled for 5 minutes which lead to oxidation to anthroquinones, indicating the presence of glycosides.

Kedde's test:

Crude extract was mixed with chloroform, one drop of 90% alcohol and 2 drops of 2% 3, 5 dinitrobenzoic acid in 90% alcohol and made alkaline with 20% sodium hydroxide. A purple color so produced, suggested the presence of glycosides.

Test for Alkaloids

Dragondroff's test:

Crude extract was mixed with Dragondroff's reagent (potassium bismuth iodide solution). Reddish brown precipitate was formed which suggested the presence of alkaloids.

Mayer's test:

Crude extracts were mixed with Mayer's reagent (potassium mercuric iodide solution). Cream colour precipitate was formed, indicating the presence of alkaloids.

Test for Steroids

Libermann-Buchard test:

Crude extracts were mixed with few drops of acetic anhydride, boiled and cooled, conc. H_2SO_4 was then added from the sides of the test tube. A brown ring at the junction of two layers was formed. The upper layer turned green which showed the presence of steroids.

Salkowski test:

Crude extracts were mixed with chloroform and a few drops of conc. H_2SO_4, shaked well and allowed to stand for some time. Red color appeared at the lower layer indicated the presence of steroids.

Test for Flavonoids

Alkaline reagent test:

Crude extracts were mixed with few drops of sodium hydroxide solution. An intense yellow colour was formed and turned to colorless on addition of few drops of diluted acid, which marked the presence of flavonoids.

Lead acetate test:

1ml of 10% lead acetate solution was added to 0.5 ml of extracts, yellow precipitate is formed, indicated the presence of flavonoid.

Test for Saponins

Froth test:

0.5g extracts were dissolved in 10ml of distilled water for about 30 seconds. The test tube was stoppered and shaken vigorously for about 30 seconds and allowed to stand and observed after 30 min. If a "honey comb" froth above the surface of liquid persists after 30 minutes the sample is suspected to contain saponin.

Test for Tannins

Ferric chloride test:

Crude extract was mixed with ferric chloride. Blue green colour appeared, suggested the presence of tannins.

Test for Amino acids and proteins

Millons test:

Crude extract was mixed with 2 ml of Millon's reagent (mercuric nitrate in nitric acid containing traces of nitrous acid), white precipitate appeared, which turned red upon gentle heating.

Ninhydrin test:

Crude extract when boiled with 0.2 % solution of ninhydrin (Indane 1, 2, 3, trione hydrate), violet color appeared. Suggesting the presence of amino acids and protein.

ANTIOXIDANT ACTIVITIES

Total Phenolic content:

Total phenolic content (TP) was determined by Folin-Ciocalteu colorimetric method. 200 µl of each extract solution (1mg/ml) was mixed with 1800 µl of distilled water and 2ml of Folin-Ciocalteu reagent was added. After 5 minutes at room temperature, 2ml of 10% sodium carbonate was mixed and shaken. Thereafter, the reaction mixture was allowed to stand for 1 hour at 37˚C and the absorbance at 760 nm was taken. Results were expressed as gallic acid equivalents per gram dry extract weight, with the use of the standard curve [17]

***Total Flavonoid content*:**

Total flavonoids were determined by Aluminium chloride colorimetric method. Briefly, 1 ml of each extract solution (1mg/ml) was mixed with 4 ml of distilled water and 300 µl of 5% sodium nitrite. After 5 min, add 300 µl of 20% aluminium chloride, allowed it to stand for 6 min and then add 2 ml of 1 M NaOH. Absorbance was taken at 510 nm and the results were expressed as mg quercetin equivalent per gram dry extract weight with the use of standard curve [17]

***DPPH Assay (1,1-diphenyl-2-picryl-hydrazyl*):**

1.2 ml of the extract was added to 0.1 ml of 1 M tris-HCl buffer (pH 7.9) and mixed with 1.2 ml of 5 mM DPPH in methanol. The reaction mixture was allowed to stand for 30 min in dark and the absorbance was measured at 517 nm.

Radical scavenging assay is expressed as (%) = $[(A_0-A_S)/A_0\times100]$

Where A_O=Absorbance of control, A_S= Absorbance of Sample. Gallic acid was taken as standard solution. DPPH radical scavenging activity was expressed as mg gallic acid equivalent per 1 gm of sample [18]

***Superoxide anion scavenging assay*:**

The activity was performed from the literature given in Sathisha *et al*, 2011.[19] The reaction mixture containing Phenazine methosulphate (0.1 mmol/L), Nicotinamide adenine dinucleotide reduced (1 mmol/L), Nitroblue tetrazolium (1 mmol/L) in phosphate buffer (0.1 mol/L, pH 7.4) with different concentrations of the extract were incubated at room temperature for 5 min

and the colour was read at 560 nm against a blank. The scavenging effect was calculated using the following equation:

Effect of scavenging (%) = $[(1-A_S/A_0) \times 100]$

Where, A_O=Absorbance of control, A_S= Absorbance of Sample

RESULTS AND DISCUSSION

Physiochemical analysis:

Percent weight loss on drying or moisture content was found to be 12.56±1.6 for *Volvariella volvacea*. The less moisture content of drugs prevents from fungal, bacterial or yeast growth during storage. The ash values, acid insoluble ash and water soluble ash were found to be 5.4±0.39, 1.03±0.38 and 2.1± 0.42 respectively. Ash values are used to find out the authenticity, quality and purity of crude drug. Through acid insoluble ash impurities of silicates and other earthy materials are indicated. Water soluble ash value indicates the presence of inorganic elements present in drugs. Furthermore, the extractive values are important to estimate the chemical constituents present in the drug.[20]The observational values are given in Table 1.

Table 1. Physiochemical characterization of *Volvariella volvaceae*

Parameters	***Volvariella volvacea***
Loss on drying	12.56±1.6
Ash Value (%w/w)	5.4±0.39
Acid insoluble ash (%w/w)	1.03±0.38
water soluble ash (%w/w)	2.1 ± 0.42
Water soluble extractive value (%w/w)	14.2 ±1.4
Alcohol soluble extractive value (%w/w)	8.5 ± 1.5

Values are expressed in mean± standard deviation

Further analytical parameters like Fluorescence studies were also performed and summarized in **Table 2**.

Table 2: **Fluorescence Value of mushroom *Volvariella volvacea* under visible and ultraviolet light**

Solution	***Volvariella volvacea***	
	Visible light	**Ultraviolet light**
Powder + water	Clear	Clear
Powder + methanol	Light yellow	Greenish yellow
Powder + 1N HCl	Light yellow	Greenish colour
Powder + aq. NaOH	Clear	Clear
Powder + 80% HNO_3	Not found	Not found
Powder + alcoholic 1N NaOH	Light yellow	Yellow
Powder + 50% H_2SO_4	Light green	Green

Phytochemical Screening:

Table 3 indicates the phytochemical constituents of *Volvariella volvacea* extracts. The Lyophilized and the oven dried extracts showed the presence of Carbohydrates, Glycosides, Alkaloids, Flavonoids, saponins, tannins and does not indicated the presence of steroids and the oven dried extract indicated the absence of proteins.

Table 3: Preliminary Phytochemical screening of lyophilized and oven dried hydroalcoholic extract of *Volvariella volvacea*

S. No	Plant Constituent	Test	Volvariella volvacea	
			LHA extract	OHA extract
1.	Carbohydrate	Molisch test	+	+
		Fehling's test	+	+
2.	Glycosides	Borntrager's test	+	+
		Kedde's test	+	+
3.	Alkaloids	Mayer's test	+	+
		Dragondroff's test	+	+
4.	Steroids	Libermann-Buchard test	-	-
		Salkowski test	-	-
5.	Flavonoid	Alkaline reagent test	+	+
		Lead acetate test	+	+
6.	Saponin	Froth test	+	+
7.	Tannin	Ferric chloride test	+	+
8.	Amino acid & Protein	Ninhydrin test	+	-
		Millon's test		

LHA: Lyophilized hydroalcoholic extract; OHA: Oven dried hydroalcoholic extract

+: Found to be present

-: Found to be absent

Total phenolic content:

It was reasonable to investigate the total phenolic content of natural extracts because plant phenol content constitutes one of the major groups of compounds acting as free radical terminators or primary antioxidants.[17] The total phenol content in lyophilized and oven dried extracts of *Volvariella volvacea* were found to be 97.35±0.59 and 75.14±1.32 mg GAE/gm dry extract. These phenolic compounds possess redox properties, which allow them to act as antioxidants and their free radical scavenging ability is due to the presence of hydroxyl groups, thus determination of phenolic concentration in plant extracts could be used as rapid screening for their antioxidant activity.[21]The extracts which have higher content of phenol also indicated that they have better antioxidant activities. So, antioxidative activity may be correlated with total phenolic contents of the extracts.[22, 23]

Table 4: Determination of Total Phenol and Total Flavonoid content of various extracts

Extract	Total phenol content(GAE mg/gm)	Total flavonoid content (QE mg/gm)
Lyophilized *V. volvacea* extract	97.35±0.59	63.59±1.53
Oven dried *V. volvacea* extract	75.14±1.32	49.37±1.24

Total Flavonoid content:

Total flavonoid content in lyophilized and Oven dried extracts of *Volvariella volvacea* were found to be 63.59±1.53 and 49.37±1.24 mg QE/gm dry extract. Flavonoids are one of the major and widespread groups of natural compounds and are one of the most important natural phenols. They also possess several biological and chemical activities and are having free radical scavenging properties too. Its antioxidant property depends primarily on their hydroxyl group position in the molecule and their ability as electron donor to a free radical.[24, 25] Flavonoids are plant secondary metabolites ad their antioxidant activity depends on the presence of free OH groups, especially 3-OH.[26]

DPPH Assay:

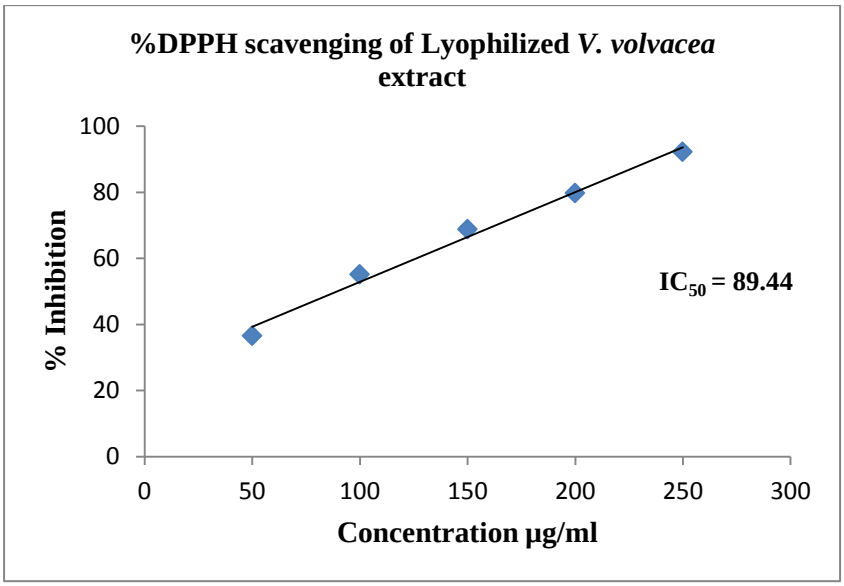
The antioxidant activities of the prepared extracts were determined by using DPPH radical scavenging assay and Superoxide anion scavenging assay. DPPH radical scavenging assay is a sensitive method to determine the antioxidant activity of the extracts. Hydrogen donating ability of the antioxidant molecule contributes to its free radical scavenging nature and was measured from the bleaching of violet colored DPPH solution at 517nm.[19, 17] The DPPH assay method is a sensitive method based on the reduction of DPPH, a stable free radical. When plant extracts having antioxidants react with DPPH, DPPH becomes paired off in the presence of a hydrogen donor (e.g., a free radical scavenging antioxidant) and is reduced to the DPPH-H and as a result, the absorbance gets decreased from the DPPH. Radical to the DPPH-H form, results in decolorization (yellow colour) with respect to the number of electrons captured. More is the decolorization, more is the reducing ability. DPPH assay has now been the most accepted model for evaluating the free radical scavenging activity of any new drug. [27-31]The concentration of extracts required to inhibit 50% DPPH free radicals are shown in Table 5. Among the lyophilized and oven dried of both extracts, the higher scavenging activity was shown by lyophilized extracts.

Table 5. Free radical scavenging capacity of Hydroalcoholic extract of *Volvariella volvacea*

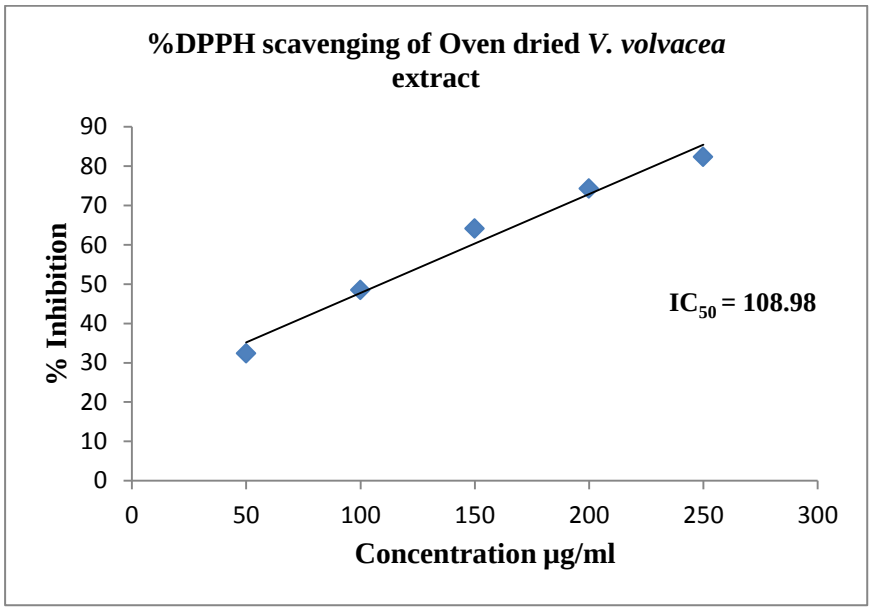
Concentration (µg/ml)	DPPH Scavenging %		
	Lyophilized	Oven dried	Ascorbic Acid
50	36.52±0.43	32.4±0.5	93.51±0.61
100	55.1±0.62	48.44±0.81	-
150	68.8±0.76	64.12±0.46	-

200	79.72±0.91	74.25±0.43	-
250	92.23±0.81	82.32±0.24	-
IC_{50}	89.44	108.98	-

Values are expressed as mean ± SEM



Graph 1:% DPPH scavenging of Lyophilized *V. volvacea* extract



Graph 2: % DPPH Scavenging activity of oven dried *V. volvaceae* extract

Superoxide anion radical scavenging assay:

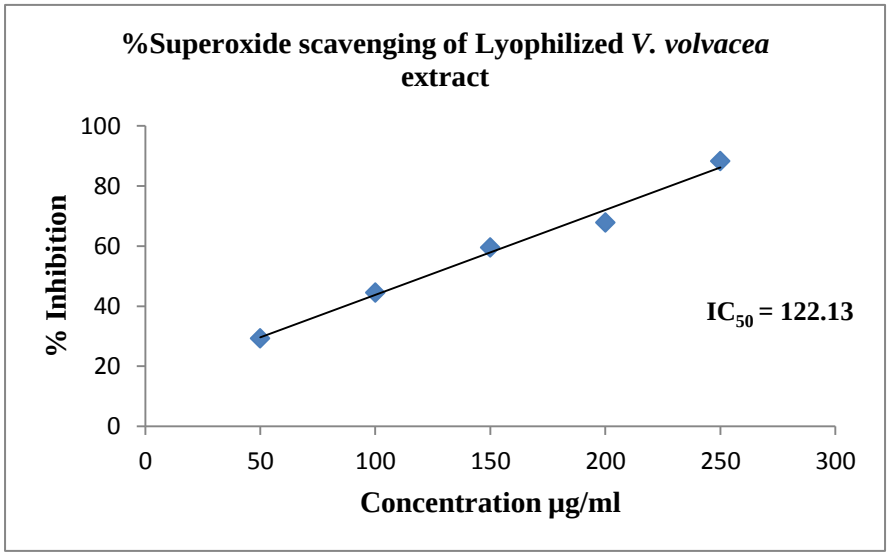
This is quite a weak oxidant but it results to the generation of powerful and dangerous hydroxyl radical as well as singlet oxygen both of which contribute to oxidative stress. [19, 32]

Superoxide anion radicals are produced endogenously by flavoenzymes like Xanthine oxidase which converts hypoxanthine and subsequently to uric acid. Significant decrease in absorbance at 560nm with extracts indicates the consumption of superoxide anion

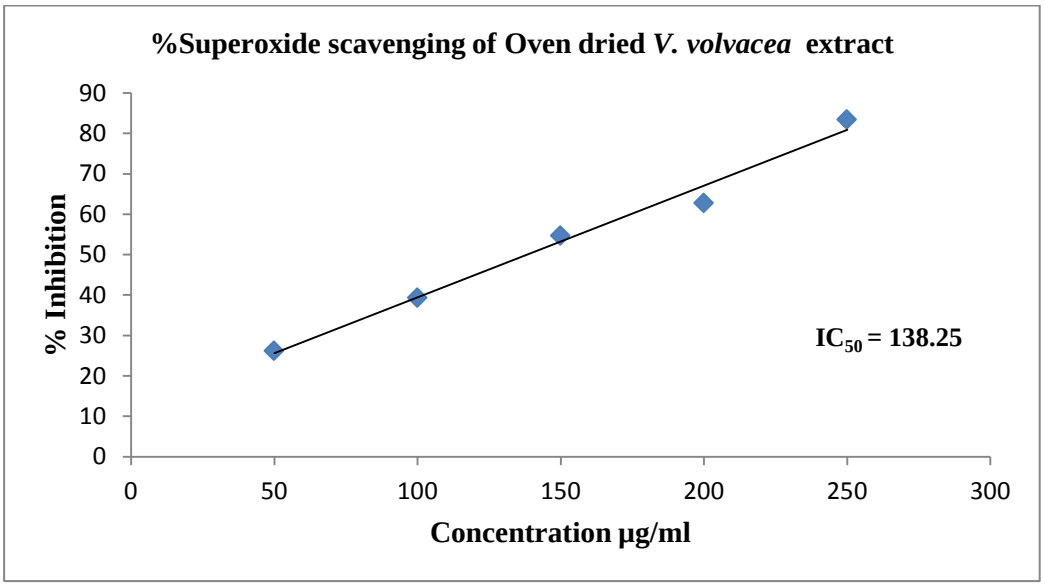
in the reaction mixture and thereby exhibiting a dose dependent increase in superoxide scavenging activity.[33] The results are summarized in Table 6

Table 6: Superoxide scavenging capacity of Hydroalcoholic extract of *Volvariella volvacea*

Concentration (µg/ml)	Superoxide Scavenging %		
	Lyophilized	Oven dried	Ascorbic Acid
50	29.21±0.32	26.1±0.43	85.62±0.54
100	44.52±0.45	39.31±0.42	-
150	59.52±0.34	54.67±0.71	-
200	67.85±0.76	62.73±0.77	-
250	88.31±0.37	83.42±0.65	-
IC_{50}	122.13	138.25	-



Graph 3:% Superoxide scavenging activity of Lyophilized V. volvaceae extract



Graph 4: % Superoxide scavenging activity of oven dried V. volvaceae extract

CONCLUSION

In the present study, phytochemical, physiochemical and antioxidant activities of *Volvariella volvaceae* extracts were evaluated. The lyophilized extracts of *V. volvaceae* showed maximum antioxidant activity; and their total phenolic and flavonoid content are also high. The phytochemical screening of *Volvariella volvacea* extracts demonstrated presence of Carbohydrates, Glycosides, Alkaloids, Flavonoids, saponins, tannins and does not indicated the presence of steroids. Likewise the physiochemical properties were also demonstrated. Thus, from the data obtained it can be concluded that the lyophilized extract will be the best for quantitative phytochemical analysis and further pharmacological evaluation.

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