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Research Article

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Study on *invitro* free radical scavenging activity of *Hypsizygus ulmarius* mushroom

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ABSTRACT

Antioxidant activity of methanolic extract of Hypsizygus ulmarius was studied for its free radical scavenging property on different models. The inhibition percentage increased in dose dependent manner. IC_{50} values for scavenging of 2,2-Diphenyl-1-Picrylhydrazyl (DPPH), nitric oxide(NO), hydroxyl(OH), superoxide(SO) and ferric reducing antioxidant power (FRAP) assay were found to be 74, 410, 450, 640, 152µg/ml respectively. This was compared with standards like ascorbic acid, quercetin and Butylated Hydroxytolune (BHT). The result presented has a good antioxidant property against free radical and it may serve as a good pharmacological property.

Keywords: Antioxidant, *Hypsizygus ulmarius*, Methanolic extract, Scavenging activity.

INTRODUCTION

Oxidation is essential for living organisms for biological processes to produce energy. The reactive oxygen species (ROS), free radical and reactive nitrogen species (RNS) are produced due to the oxidation of cell that leads to cell death and tissue damage. These ROS and Free radicals are responsible for aging and cause various human diseases such as atherosclerosis, diabetes, cancer, hypertension, alzheimer's disease, parkinson and cirrhosis [21]. Antioxidant deals with an important role in the prevention and treatment of a variety of diseases by removing free radical intermediate and inhibit other oxidation reactions by being oxidized themselves [25]. The antioxidants in the human diet are of great interest as it reduces oxidative damage. Many of food industries have long been using synthetic antioxidants such as butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) as preservatives in food products which are restricted due to their carcinogenic effects and has led to increased interest in antioxidant substances from natural resources [19].

There are thousands of medicines and natural products that have been closely linked through the use of traditional medicines. Natural products represent a wealthy source of biologically active compounds with recognized potential drug discovery and development [20]. In addition to the ancient utilization of plants, the medicinal use of mushrooms has also an incredibly long custom. Among the large resources of fungi, higher Basidiomycetes, particularly mushrooms are unlimited sources of remedially useful biologically active agents [30]. Since ancient times mushrooms have been used as folk medicine throughout the world and they are used as nutritionally functional food and beneficial nontoxic medicines [29]. Several species of mushroom contains a wide variety of free radicals or reactive oxygen species scavengers which have made mushrooms attractive as nutritionally beneficial foods and as a source for drugs development [8]. Mushroom flavonoids can act as free radical scavengers to terminate the radical chain reactions that occur during the oxidation of triglycerides in the food system [1]. The significant pharmacological effects and physiological properties of mushrooms are bioregulation (immune enhancement),

maintenance of homeostasis and regulation of biorhythm, cure of various diseases and prevention and improvement from life threatening diseases such as cancer, cerebral stroke and heart diseases. Mushrooms are also known to have effective substances for antifungal, antiinflammatory, antitumor, antiviral, antibacterial, hepatoprotective, antidiabetic, hypolipedemic, antithrombotic and hypotensive activities [31]. Great threat to human life by neoplastic diseases continues to increase and thus the pursuit for anti-tumor drugs takes a compelling urgency. Attempts have been made in many parts of the world to explore the use of mushrooms and their metabolites for the treatment of a variety of human ailments [12]. The most significant medicinal effect of mushrooms and their metabolites that have attracted the attention of the public is their antitumor property. Lucas and his collaborators first demonstrated the antitumor activity of the higher Basidiomycetes [15].

Elm oyster is a common name used for *Hypsizygus ulmarius* mushroom which is a high yielding edible mushroom for which commercial cultivation technology has been released and is gaining popularity. Earlier studies on the antioxidant activity of caps and stipes in *Hypsizygus ulmarius* by combined solvent extraction is reported [3]. Radical scavenging activity (RSA) by DPPH method is studied in *Pleurotus eryngii* [17]. So in the present study an attempt is made to study antioxidant activity of *Hypsizygus ulmarius* by using methanolic extract by different models.

EXPERIMENTAL SECTION

Chemicals

The chemical procured were of analytical grade, from Sigma Chemicals Co. (St. Louis, USA), Merck (Darmstall, Germany) and Himedia, Mumbai.

Methanol extraction

Fresh mushroom sample (1kg) were randomly selected, air dried in shade and powdered. About 50gms of the powdered mushroom was extracted with 400ml of methanol (40-50°C), using soxhlet apparatus (9-cycles) and filtered through muslin cloth. The filtered extract was evaporated under reduced pressure and vacuum dried to get viscous residue. The sample obtained is used to study the antioxidant activity.

DPPH radical scavenging assay

Hydrogen donating radical scavenging ability of methanolic extract of *Hypsizygus ulmarius* mushroom was determined according to the method [24]. 1ml of 100µM DPPH solution in methanol was mixed with 1ml of extract (20-100µg/ml) and incubated in dark for 30 min. The absorbance was measured using a spectrophotometer at 517 nm (Shimadzu) using methanol as control and ascorbic acid as reference compound.

Nitric oxide radical scavenging assay

The interaction of *Hypsizygus ulmarius* methanolic extract with nitric oxide was spectrophotometrically assessed [6]. The chemical source of nitric oxide was Sodium nitroprusside(5mM) in 0.1M phosphate buffer of pH 7.4. The generated nitric oxide reacts with oxygen to produce nitrite ions. After incubation at 25^0C for 2hrs Greiss reagent (1% sulphanilamide, 2% O-phosphoric acid and 0.1% of N-(1- naphthyl) ethylenediamine dihydrochloride) was added and absorbance was measured at 546nm. Ascorbic acid was used a standard along with control.

Hydroxyl radical scavenging assay

Hydroxyl radical scavenging activity of *Hypsizygus ulmarius* methanolic extract was measured according to the method explained [11]. To 1ml of extract, 0.1ml 1mM EDTA, 0.01ml 10mM of $FeCl_3$, 0.1ml 10mM H_2O_2, 0.36ml of 10mM deoxyribose, 0.33ml of 50mM phosphate buffer (pH 7.4) and 0.1ml of 1mM ascorbic acid was added and incubated at 37°C for 1h. To 1ml reaction mixture, 1ml of 10% TCA and 1ml of 0.5% TBA (in 0.025M NaOH containing 0.025% butylated hydroxyl anisole) was added and absorbance was recorded at 532nm.

Superoxide radical scavenging assay

Superoxide radical scavenging activity of *Hypsizygus ulmarius* methanolic extract was measured according to the method explained [5]. To 1ml of extract (200-1000µg/ml), 1 ml of NADH (468µM) and 0.1ml of PMS (60µM) was added and incubated at 25°C for 5min and absorbance was recorded at 560nm. Quercetin was used as a standard.

Ferric reducing antioxidant power (FRAP) assay

The ferric reducing power was determined [22]. The fresh FRAP working solution (37°C) was prepared by mixing 25ml of acetate buffer, 2.5 ml of TPTZ and 2.5ml of $FeCl_3$ from the stock solution of 0.3M acetate buffer (pH 3.6), 0.01M 2, 4, 6-tripyridyl-s-tri-azine solution in 0.04M hydrochloric acid and 0.02M $FeCl_3$ solution. 1ml of the sample (50-250µg/ml) was mixed with 2.85ml of the FRAP solution and kept in dark for 30 min. the absorbance was recorded at 593nm using Butylated Hydroxytolune (BHT) as standard.

RESULTS AND DISCUSSION

Mushroom serves as a dietary supplement for proteins, vitamins, minerals as well a cheap and easily accessible source for natural antioxidant for humans and livestock. Antioxidant properties, especially radical scavenging activity is very important, due to the deleterious role of free radical in biological systems. Antioxidant activity has become one of the studies on mechanisms of the nutraceutical and therapeutical effects if traditional medicines. The present investigation reveals the radical scavenging activity of *Hypsizygus ulmarius* mushroom methanolic extract with various models like DPPH, NO, OH, SO and FRAP. The results of the study showed the maximum extent inhibition of free radical generation and maximum amount of antioxidant capacity.

DPPH model is widely used for the evaluation of antioxidant capacity in short time and frequently applied for testing food products. The decrease in absorbance at 517nm is an indication of DPPH radical reduction [13]. The sample (*Hypsizygus ulmarius* ethanolic extract) at a concentration of 100µg/ml showed demonstrated excellent antioxidant capacity with percentage of inhibition of 68.35% which is lesser than *pleurotus florida* [16] (Figure-1). At the same concentration standard (ascorbic acid) showed 70.31%. The IC_{50} value of *Hypsizygus ulmarius* extract and ascorbic acid was found to be 74 µg/ml and 68µg/ml (Table 1). As reported by [13], the decrease in absorbance at 517nm is proportional to reduction capability of the DPPH radical and it also signifies the antioxidant capacity [14]. DPPH radical are unaffected by enzyme inhibition and metal chelation [21].

A 64.63% of nitric oxide free radical scavenging activity is due to scavenging ability of *Hypsizygus ulmarius* mushroom methanolic extract at a concentration of 500µg/ml when compared to standard ascorbic acid 67.07%(Figure-2). The IC_{50} value of *Hypsizygus ulmarius* extract and vitamin C was found to be 410µg/ml and 360µg/ml (Table 1). Scavenging of nitric oxide radical is due to the generation of nitric oxide from sodium nitroprusside in buffered saline, which reacts with oxygen to produce nitrite ions that can be measured by using Griess reagent. Nitric oxide is an unstable free radical that is recognized to be an inter and intra-cellular mediator of several cell functions. It acts as a signal molecule in immune, nervous and vascular system [23]. It reacts with oxygen to produce stable product nitrate and nitrite through intermediates and high concentration of nitric oxide can be toxic and inhibition of over reduction is an important goal [18].

Hydroxyl radicals are the major active oxygen species that cause lipid oxidation and severe damage of adjacent biomolecules at diffusion controlled rates. It is highly reactive with half life of approx. 10^{-9} s *in vivo* and is generated in the human body at sites of inflammation in oxidative stress-originated diseases [28, 26]. Hydroxyl radical-scavenging activity of methanolic extract and standard at a concentration of 1000µg/ml was found to be 76.17% and 80.67%(Figure-3) along with IC_{50} value of 450µg/ml and 370µg/ml respectively (Table 1).

Dose dependent superoxide radical scavenging was seen in *Hypsizygus ulmarius* extract with percentage inhibition of 69.01 and 76.76 at a concentration of 1000µg/ml (Figure-4). Superoxide anion is weak oxidant and highly toxic species that can generate singlet oxygen and hydroxyl radical which could cause damage to tissues, numerous biological and photochemical reactions [27, 7]. The antioxidant activity is expressed as the IC_{50} values of 640 and 400µg for extract and standard respectively (Table 1). .

FRAP assay is based on the ability of anti-oxidants to reduce Fe^{3+} to Fe^{2+} in the presence of 2, 4, 6-tri (2-pyridyl)-s-triazine (TPTZ) at optimum P^H of 3.6 forming an intense blue Fe^{2+} -TPTZ complex with an absorption maximum at 593 nm. The decrease in absorbance is proportional to the anti-oxidant content [2]. The high reducing power is indicative of the hydrogen donating ability of the active species present in the extract. In the present study, the reducing power of the standard and methanolic extract of *Hypsizygus ulmarius* was found to be 80.32% and 76.22% at a concentration of 250µg/ml (Figure. 5). IC_{50} value of *Hypsizygus ulmarius* was found to be 152µg/ml while that of BHT was 120µg/ml (Table 1). The antioxidant activity of putative antioxidants have been attributed to various

mechanisms, among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstractions and reductive capacity [4, 9, 10].

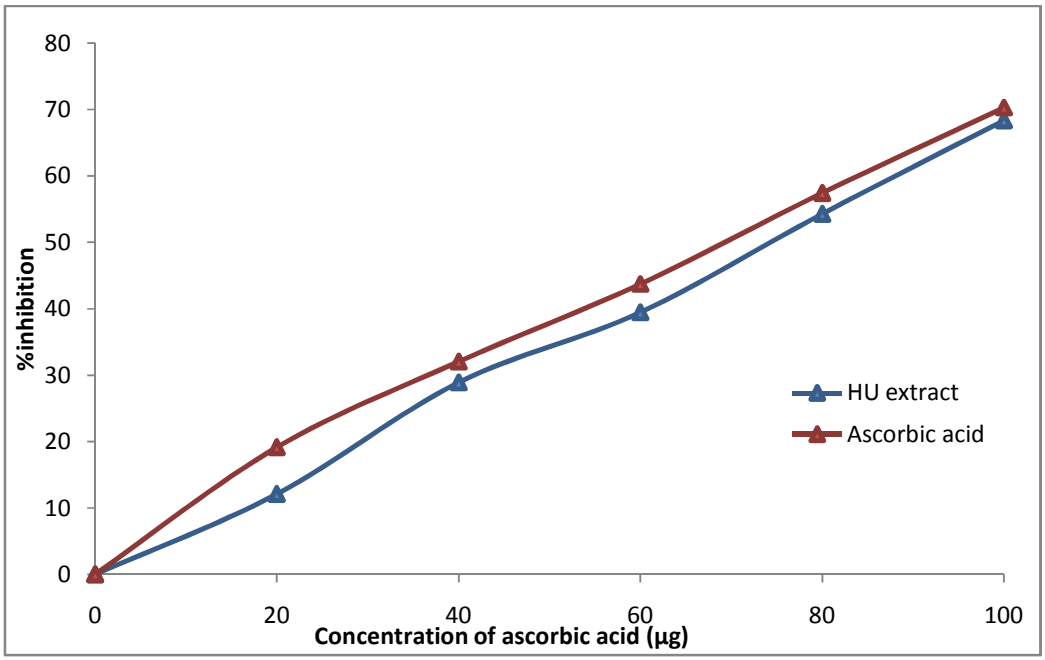

Figure 1- DPPH radical scavenging activity

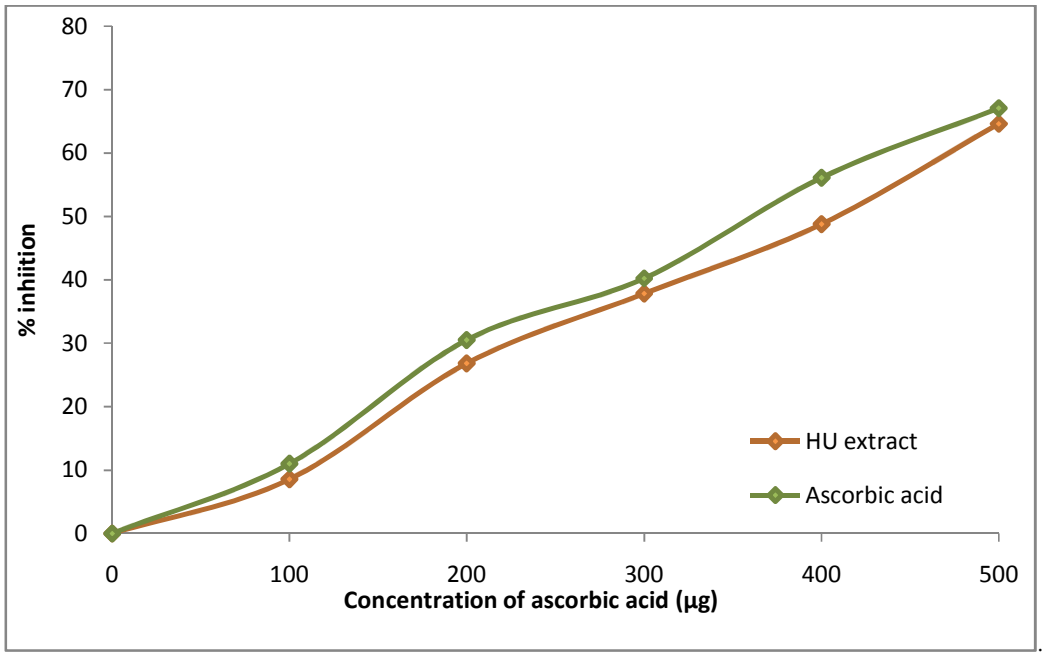

Figure 2- Nitric oxide radical scavenging activity

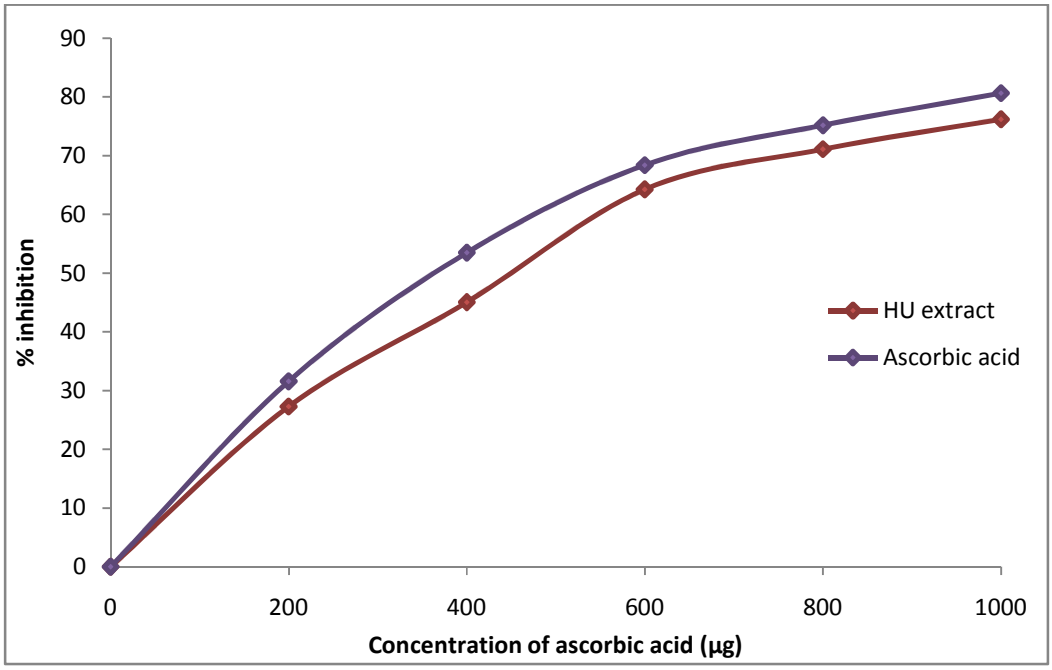

Figure 3- Hydroxyl radical scavenging activity

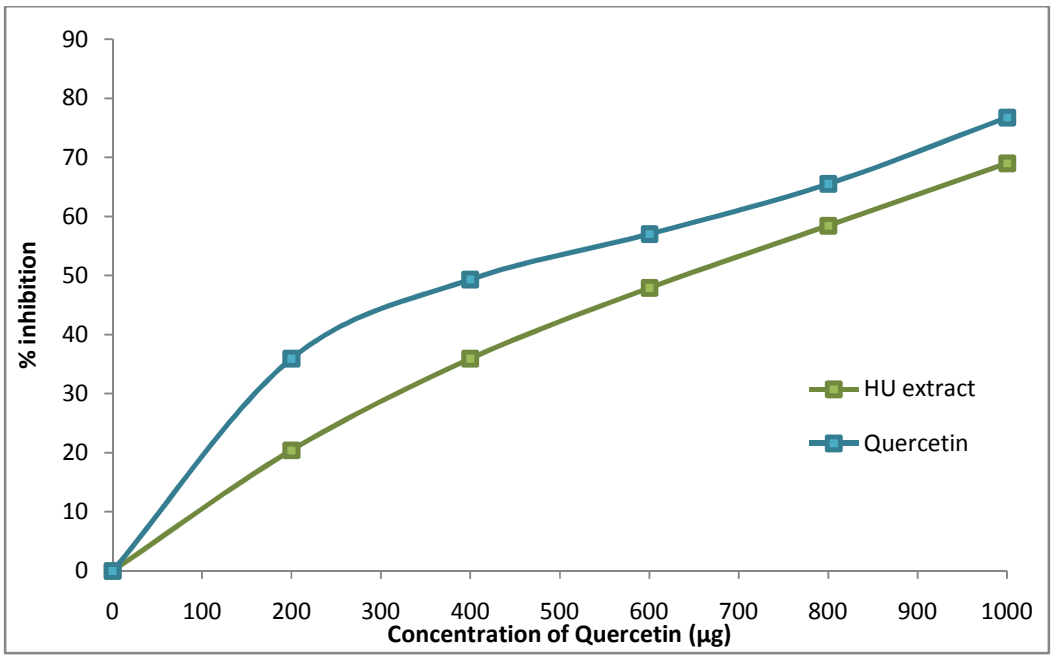

Figure 4- Superoxide radical scavenging activity

Table -1 IC_{50} values of the methanolic extract from *Hypsizygus ulmarius*.

Sl. No	Antioxidant profile	IC_{50} values (standard) µg/ml	IC_{50} values (methanolic extract) µg/ml
1	DPPH	68	74
2	Nitric oxide	360	410
3	Hydroxyl	370	450
4	Superoxide	400	640
5	FRAP	120	152

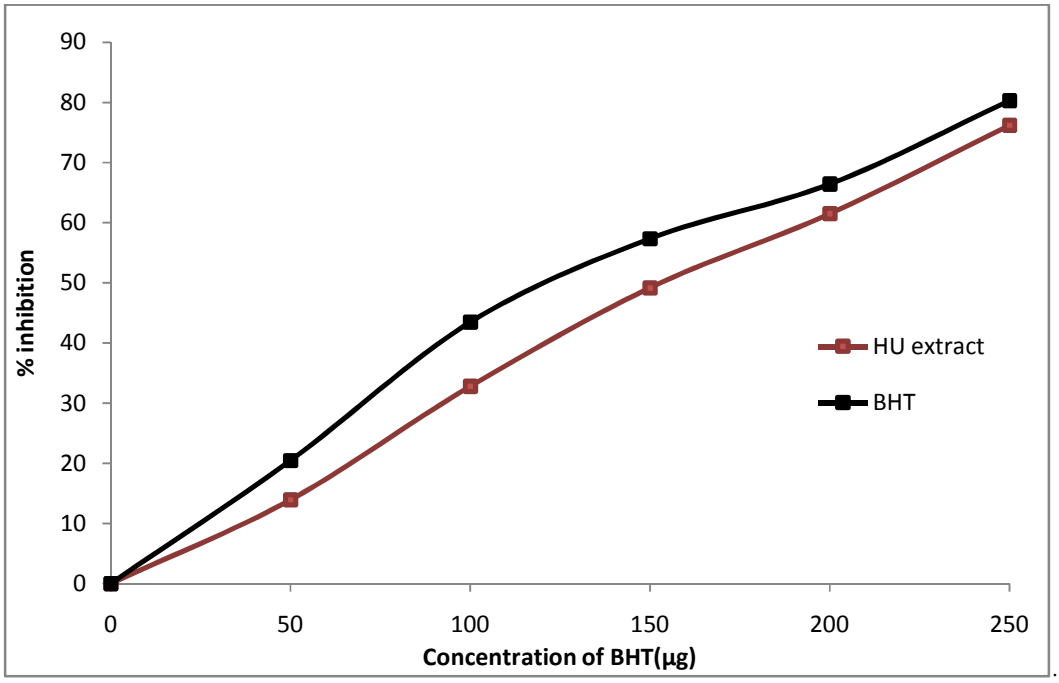

Figure 5- FRAP radical scavenging activity

CONCLUSION

The data recorded in the above experiments showed that the methanolic extract of *Hypsizygus ulmarius* mushroom has significant antioxidant activity and free radical binding ability, that could be served as an easily accessible product of natural rich antioxidant with high biological value in pharmaceutical industry to cure diseases.

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