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NUTRACEUTICAL PROPERTIES EVALUATION OF *Schizophyllum commune*

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ABSTRACT

***Schizophyllum commune* belongs to the large and remarkable group of mushroom and it is known as split gill mushroom. It is an unexplored and non-consumed by the human. Recent research revealed that *S. commune* emerges as a richness for various antioxidants and other pharma active component. In the present studied, hot water, methanol, and ethanol crude extracts of *S. commune* were investigated for their antioxidant capacity by using various assay methods and also determined antidiabetic property. Ethanolic extract showed ample potential for antioxidant activity, in the DPPH scavenging method recorded higher inhibition (IC_{50} =18.56 µg/ml), other than in hot water and methanol extracts. For the reducing power assay, hot water extract showed great scavenging activity recorded (IC_{50} =20.00 µg/ml) comparison to another extract. Ethanolic extract showed most potent H_2O_2 scavenging activity in the assay; which was showed (IC_{50} =19.79 µg/ml) and similarly total phenolic content was given significantly values for ethanolic extract (IC_{50} =11.86 µg/ml), comparatively methanol and hot water extracts. Positive correlation was found between to all extracts medium and their antioxidant activities. The studied revealed that split gill mushroom may have potential sources of natural antioxidant and antidiabetic stuff, therefore may be utilized as promising sources of therapeutics.**

KEYWORDS: Split Gill Mushroom, Antioxidant Capacity, Antidiabetic Assay

Mushrooms are an essential product of forest ecosystem that grows on the most abundant nutrients like cellulose. Mushrooms are a macro-fungus that has a distinctive fruiting body. It is suggested that only 2000 are safe for edible to human and about 650 of these possess medicinal value out of approximately 15,000 known species in the world (Petrovi*et al.*, 2015). Mushrooms have been mostly used as a human food for centuries and have been famous for texture and flavour as well as having various medicinal properties. However, in recently emerged the mushrooms as being an important source of biologically active material that has medicinal value. Wild-growing mushrooms contain a number of different secondary metabolites, carbohydrates, minerals, proteins, fibres, vitamins and fats that having antitumor, antifungal, antimicrobial and antioxidant property (Mehadi*et al.,* 2015). It is suggested that Mushrooms are also important sources for compounds alike beta-glucans, ascorbic acid, tocopherols, carboxylic acids, lectins, terpenoids, and various dietary fibres (Babu et al., 2013). Mushrooms are not accumulated sufficient amount of proteins or fats but taking mushroom in our regular diet or taking of their isolated bioactive constituents present in mushrooms that is also beneficial to health (Petrovi*et al.,* 2015).

In the present studied, taken *Schizophyllum commune*, which belongs to basidiomycete, it completes life cycle in -10 days. The ecotype nature is saprobic on dead wood, which is growing alone or more frequently, in clustered way. It's widely distributed in through the world. The fruiting body of *S. commune*, approx. 1-5 cm wide, fan-shaped when attached to the side of the log. The spore print depicts white colour (Kuo, M., 2003). According to Patel and Goyal (2012), Schizophyllan is a non-ionic, water-soluble homopolysaccharide consisting of a linear chain of β-d-(1-3)-gluco-pyranosyl groups and β-d-(1-6)-glucopyranosyl groups produced by *S. commune* ATCC 38548. The present scenario of pharmaceutical and drug development industry have focused for the preeminent option for immune-modulatory and anti-cancer agents. The antioxidant compounds, present in mushrooms that are capable of scavenging free radicals in the protection from oxidative damage in living organisms also play an important role in defensive and curing of unwanted physiological effects. The present study primarily focused on the selection of solvents for extraction and analysis of antioxidant potency. The results will be beneficial for pharma sector and drug developments. Consequently, it may be part of alternative antioxidant resources instead of synthetic antioxidant.

MATERIALS AND METHODS

Sample Collection

Fresh dried *Schizophyllum commune* spilt mushrooms collected from Charama forest, district Kanker, Chhattisgarh, at the session of October 2015. This collected sample was kept in polythene bags and packed loosely, also designated code (SC). Identification was made on the basis of critical observations of the specimens and examination of relevant literature (Kuo, M., 2005; Kuo, M., 2007). These identified mushrooms

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sample was deposited to School of Studies in Biotechnology Department, Pt. Ravishankar Shukla University, Raipur for further analysis.

Standard and Reagent

Present studied analyzed of antioxidant, using various solvent extracts. For following chemical reagents were used. Ethylenediaminotetraacetic acid (EDTA), Ascorbic acid, Gallic acid, Ethanol, Methanol, 1,1-Diphenyl-2-picryl-hydrazyl-hydrate (DPPH), Ferrozine, Sodium phosphate buffer, Potassium Ferricyanate, Trichloroacetic acid , Ferric chloride, Folin-ciocalteu (FC)- reagent, Sodium carbonate, Phosphate buffer, Sodium Chloride, Phenol red, Sodium Hydroxide, Fehling's solution, Chloroform, sulfuric acid, Glacial acetic acid, HCl, Alcohol, Ammonia. The entire Chemical reagents were purchased from HiMedia Pvt. Ltd. Mumbai and Sigma-Aldrich Bangalore, India.

EXTRACTION METHOD

Water

This procedure carried out according to the method of Abdullah *et al.,* (2011). Mushrooms were cut into small pieces then 25g of mushrooms were employed into the water bath at 100^0C for 2 hrs. After then water extract was filtered through what man filter paper no 1.

Methanol

Methanol extraction of mushroom was done by using the method of Elmastas*et al.,* (2007). All dried mushrooms were crushed with the help of motor pestle to making the fine powder. 25g of fine powder was stirring in shaking incubator with 250 ml of methanol at 25^0C at 150 rpm for 24 hrs and filtered through what man filter paper no 1. After then metabolic extract was evaporated at 40^0C for dryness.

Ethanol

This procedure was carried out according to the Hu *et al.,* (2009) 25g of mushroom powder was stirred in shaking incubator with 250 ml of 99.9% ethanol at 25^0C at 150 rpm for 24 hrs. After then centrifuged at 3000 rpm for 15 min, then a sample was filtered through what man filter paper no 1. Further ethanolic extract was evaporated at 40^0C for dryness.

ANTIOXIDANT ASSAY

DPPH Radical Scavenging Activity

Determination of scavenging ability using the stable radical DPPH will be carried out by using the method Menaga *et al.,* (2013). Various concentration (20, 40, 60, 80, 100, 120 µg/µl) of different extract (water, methanol, ethanol) from mushroom (4ml) was mixed with 1ml methanolic solution containing DPPH, mixture was shaking and left for 30 min in dark then absorbance was measured at 517nm using spectrophotometer. The absorbance of radical without DPPH was used as a control and Ascorbic acid was used as a standard. Graphs were plotted in which X axis showed concentration (µg/µl) and Y axis showed scavenging effect (%). The percentage of inhibition was calculated according to the formula:

$$\text{Percentage of DPPH assay} = \frac{(\text{Control OD} - \text{Sample OD})}{\text{Control OD}} \times 100$$

The activity was also expressed as the inhibition concentration at 50% (IC50), the concentration of solution for the test required to obtain 50% of radical scavenging capacity.

Total Phenolic Determination

Phenolic determination will be estimated by using the method of Slinkard and Singleton (1977). Initially, different concentration of mushroom extracts 50 µl and 50 µl folin-ciocalteu phenol reagent was mixed and then after 3 min saturated sodium carbonate solution 50 µl mixed and makeup solution up to 300 µl ml with distilled water. Solution kept in dark room for 90 min then absorbance was measured at 725nm using 96 well microtitre plate containing ELISA Reader. Gallic acid was used as the standard. Graphs were plotted against the standard in which X axis showed concentration (µg/µl) and Y axis showed inhibition (%).

$$\%\text{ of Total phenolic antioxidant capacity} = \frac{(\text{Control OD} - \text{Sample OD})}{\text{Control OD}} \times 100$$

Chelating Effect of Ferrous Ion

Chelating effect of ferrous ion determination will be carried out by using the method of Pal *et al.,* (2010). Different concentration of each extract 50µl was mixed with 180 µl ml of methanol and 10 µl of 2mM ferrous chloride then added 20 µl of 5 mMferrozine. After 10 min at room temperature, the absorbance was measured at 526 nm using ELISA reader. EDTA was used as a standard. Graphs were plotted against the standard in which X axis showed concentration (µg/µl) and Y axis showed chelating effect (%).

$$\%\ \text{of Chelating effect assay} = \frac{(\text{Control OD} - \text{Sample OD})}{\text{Control OD}} \times 100$$

Reducing Power

Reducing power assay determination will be done by using the method of Menaga *et al.* (2013). Firstly, we will take 25 µl methanolic extract, 25 µl of 200mM sodium phosphate buffer and 25 µl of 1% Potassium Ferricyante was mixed and incubated at 50^0C for 20 min and then added 25 µl 10% Trichloroacetic acid and centrifuged at 3000rpm for 10 min. After the centrifugation 100 µl of upper layer mixed with 100 µl of ultra-pure water and 10 µl of 0.1% ferric chlorides then absorbance was measured at 700nm against blank. Ascorbic acid was used as the standard. Graphs were plotted against the standard in which X axis showed concentration (µg/µl) and Y axis showed anti-radical scavenging activity (%).

$$\%\ \text{ofReducingpowerassay} = \frac{(\text{Control OD} - \text{Sample OD})}{\text{Control OD}} \times 100$$

Hydrogen Peroxide Scavenging

This method is carried out according to Sroka*et al.,* (2003). Different concentrations of mushroom extract 20 µl were added to 20 µl of 0.002% hydrogen peroxide. After then added 160 µl of 0.1 M phosphate buffer and 100 mMNaCl were added, the reaction mixtures was incubated for 10 min at 37^0C. Then 1 ml 0.2 mg/ml phenol red was added. After 15 min 10 µl of 1M NaOH were added and absorbance was measured at 610 nm. Graphs were plotted against the standard in which X axis showed concentration (µg/µl) and Y axis showed inhibition (%).

$$\%\ \text{of Hydroxyl radical scavenging assay} = \frac{(\text{Control OD} - \text{Sample OD})}{\text{Control OD}} \times 100$$

Statistical Analysis

All the experimental results were the mean of three parallel measurements. Data were evaluated by ANOVA, SPPS 16.0 package and graph created by Origin Pro Lab 4.0.

RESULTS AND DISCUSSION

Yield of Mushroom Extracts

The *S.commune* showed significant yield contains concentration (Table,1). The extraction ranges recorded from 14.00 to 19.60% respectively, calculated from quantity determination method. The highest extractable yield was obtained by hot water extraction, which gave (19.06 g/100 dw). This was followed by ethanol as well as methanol (14.00 g/100 dw) respectively.

Table 1: Yield of Mushroom Extracts

Mushroom samples	Extraction solvent	Yield of Extract (% dry mushroom)
S. commune (SC)	Hot Water	19.6
	Methanol	14.00
	Ethanol	14.00

Antioxidant Capacity of Mushroom Extract

In this study, various methods have been employed for evaluate the in vitro antioxidant capacity of the mushroom extract, extracted by the various solvent. Basis the methodology, based on different reaction mechanisms and results revealed. So, therefore it is recommended to employ at least two methods for the reliable antioxidant capacity of the mushroom extract. In this study, five different methods were used to evaluate the antioxidant capacity of the mushroom.

DPPH Scavenging Activity

DPPH radical scavenging activity of different extracts was presented in figure 1. The different extracts of mushrooms showed increasing scavenging effect while increasing the concentration. DPPH is a free radical compound that has been mostly used for scavenging free radical from food components. The extract obtained by 100 % ethanolic yielded the highest scavenging activity at the concentration ranging from 20-120 (µg/ml). Among the solvents used, extraction with ethanol showed the highest value at (120µg/ml) 80.73±0.00, followed the similar amount of another extract, water extract showed 80.73 ± 0.0 and the methanolic extract was 62.46 ± 0.08 respectively. In the present study, methanolic extraction gave least scavenging activity, compare to ethanol and water solvents. Radical scavenging activity was found to exhibit the IC5value of various extracts; lower IC50 values indicate higher antioxidant activity. The IC50 value was presented in Table 2. These are represented in the following order, SCEE< STANDARD< SCHE< SCME respectively. Among all mushrooms and their different extracts, SEE showed lowest IC50 value that was 18.56 (µg/µl). It is better than ascorbic acid 19.24 (µg/µl) that was used as a standard. It has been reported

that IC50 value of methanolic extracts of *Lentinula edodes* was 110 ± 1.24µg/ml (Chowdury*et al.*, 2015). Sheikh *et al.,* (2015) was worked on two mushrooms *Ganoderma lucidum*&*Trameteshirsuta*, the IC50 value was found to be 46.354 and 77.69µg/ml respectively. The present work reported a very significant IC50 value of extracted mushroom samples.

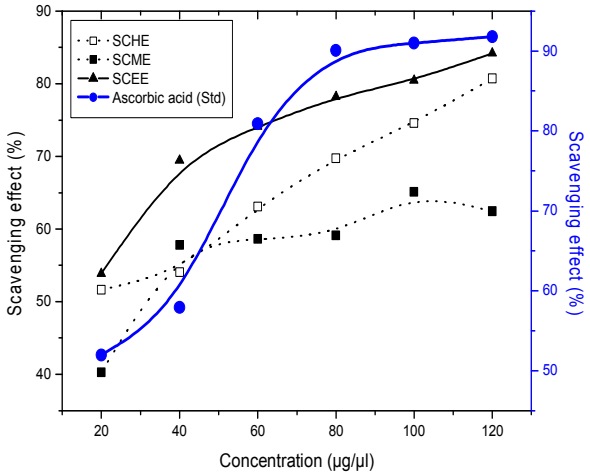

Figure 1: Scavenging effect of various extracts from *S. commune*, on 1, 1-diphenyl-2-picrylhydrazyl radical

Total Phenolic Determination

Mostly phenolic, a natural antioxidants derived from plants are of considerable as food preservatives or dietary supplements. In the present study, the total phenolic content was showed in figure 2. The water extracts showed highest total phenolic activity was recorded at (120µg/ml) 81.97±0.00, the followed by methanol extract (75.11±0.02), whereas ethanol extract showed (47.08±0.01) showed lowest total phenolic content. It has been reported that phenolic compounds are suggested to be powerful chain-breaking antioxidants and possess scavenging ability due to their hydroxyl groups. The IC50 values ((Table,2). of mushroom extracts are following: STANDARD< SCEE< SCME< SCHE respectively. The lowest IC50 of among all extract, recorded for SCEE was 11.86 µg/µl. Keles *et al.,* (2011) were found 19.51, 24.71 and 16.2mg/ml IC50 values of *Agaricus bisporus*, *Pleurotousdryinus* and *Lepistanuda* respectively.

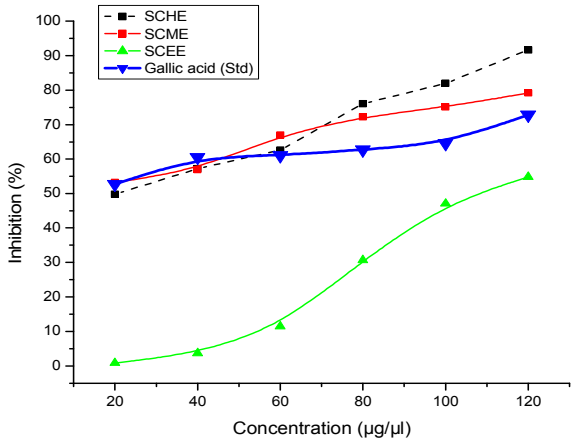

Figure 2: Total phenolic antioxidant capacity of various extracts from *S. commune*

Table 2: IC_{50} value of different mushrooms from different extracts

Mushroom samples	DPPH (µg/µl)	Total pheolic determination (µg/µl)	Chelating effect of ferrous ion (µg/µl)	Reducing power (µg/µl)	Hydrogen peroxide scavenging (µg/µl)
SHE	19.36	20.07	10.69	20	37.53
SME	24.82	18.80	20.05	21.68	19.85
SEE	18.56	11.86	18.87	20.15	19.79
Standard	19.24	16.52	10.15	63.04	18.18

Chelating Effect of Ferrous Ion

In this assay, water, methanolic and ethanolic extracts of mushroom species and standard antioxidant compounds inhibited the formation of ferrous and ferrozine complex, showed that they have chelating activity and capture ferrous ion before binding with ferrozine. Chelating effects of different mushrooms were showed in figure 3. Chelating effect of ferrous ion is following: SCME< SCHE< SCEE< STANDARD respectively. Among these SCEE was found highest 85.06±0.032 and SMEE were found lower 58.19 ± 0.02 chelating effect. The results of IC50 are following (Table no.2).: STANDARD< SCHE< SCEE< SCME. Lowest IC50 10.69 µg/µl showed by water extract, Another researcher was found 355 µg/ml IC50 showed for methanolic extracts of *Pleurotusfloida* mushroom (Menaga *et al.,* 2013) and mushroom *Coprinuscomatus* showed 2.27mg/ml (Vamanu E., 2014).

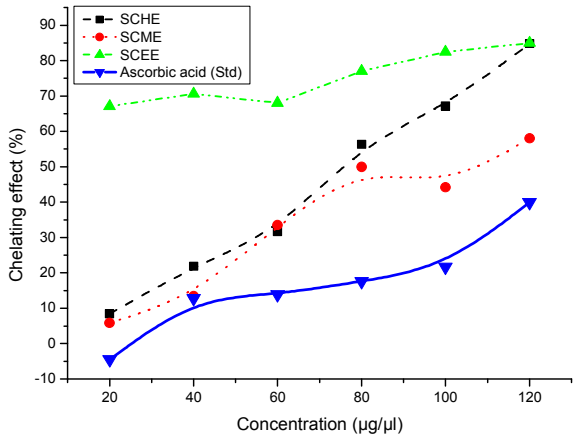

Figure 3: Chelating effect of various extracts from *S. commune*

Reducing Power

The result of reducing the power of different mushrooms from different extracts was plotted in figure 4. Ethanolic extract showed highest reducing ability at 100 µg/µl (74.80±0.01), similar result reveled by hot water extract (74.03±0.00) and methanolic extract gave (70.15±0.02) lower reducing ability.According to the IC505 (Table no.2) value increased the order of the different mushrooms from a different sample are following: SCHE< SCEE< SCME< STANDARD etc. The hot water extract showed lowest 20.00 µg/µl IC50 value. Vaz *et al.,* (2011), *Clitocybeodora* showed 0.94 mg/ml and *Coprinuscomatus* showed 0.72 mg/ml IC50 value.

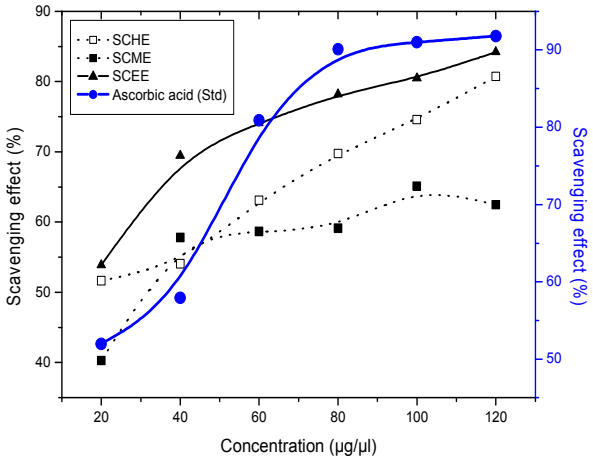

Figure 4: Reducing power of various extracts from *S. commune*

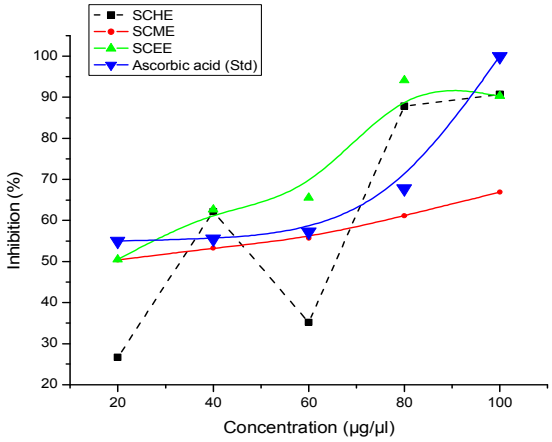

Figure 5: Hydroxyl radical scavenging activity of various extracts from *S. commune*

Hydrogen Peroxide Scavenging

Results of H_2O_2 reducing capacity are presented in Figure 3. H_2O_2 is considered as one of the main inducers of cellular ageing and could attack many cellular energy-producing systems (Liu *et al.,* 2013).The results indicate that the hot water extract showed highest H_2O_2 scavenging activity at (100 µg/µl), 90.72±0.00, as well as ethanolic extract, had given 90.29±0.00, but lowest H_2O_2 scavenging activity was recorded 66.91±0.00 for methanolic extract. The increasing order of the mushrooms extracts according to the IC50 values are STANDARD <SCEE < SCME< SCHE respectively. According to the Babu*et al.,* (2013), mushrooms *Hypsizygusulmarius* (cap), *Hypsizygusulmarius* (stipe), *Agaricus bisporus* (cap), *Agaricus bisporus* (stipe), *Calocybe indica* (cap) and *Calocybe indica* (stipe) showed 2.920, 2.995, 0.908, 2.770, 0.993 and 3.252mg/ml IC50 value respectively. Therefore, obtained result was profound antioxidant capacity.

CONCLUSION

Oxidation is essential processes of living organism that produces energy for their survival but the excess amount of oxidation causes the formation of reactive oxygen species and reactive nitrogen species like free radical that causes may disease like Alzheimer's diseases, diabetes, cancer, Parkinsonism, and ageing. An antioxidant is a compound that controls or removes free radical formation but the synthetic antioxidant has costly so the need of the natural source for extracts antioxidant is evaluated. In the present work, we have analyzed an effect of solvents, extractions efficacy though their

antioxidant capacity typing. The *S. commune* showed profound a nutritional and important medicinal mushroom. It can be used as a therapeutic agent and used instead of synthetic antioxidant resources.

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