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In vitro evaluation of Phytochemical, antioxidant and preliminary antidiabetics activity of *Macrocybe* sps.

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Abstract

Mushrooms are considered as nutritious and delicious food and placed in between meat and vegetables. *Macrocybe* sps belongs to the family trichlomataceae is a wild, large, edible mushroom which is rich in protein, polysaccharides, vitamins and mineral salts and diateryfibre. The present study was conducted to evaluate the phytochemical, antioxidant and anti diabetic activities of ethanolic extract of *Macrocybe* sps. The phytochemical assay of the extract revealed the presences of alkaloids, carbohydrates, proteins, terpenoids, flavonoids and absence of saponins glycosides amino acids and phenols. DPPH, ABTS, Ferrous ion chelating assay and phosphomolybdenum assay were used to evaluate antioxidant capacity of the extract. *Macrocybe* sps. ethanolic extract had the dpph scavenging activity (IC_{50} value of 57.07µg/mL), ABTS value (IC_{50} value of 39.18µg/mL), Ion chelating value (IC_{50} value of 64.87µg/mL) and phosphomolybdenum assay (1.885). The antidiabetic effect were evaluated by invitro alpha amylase inhibitory assay. The *Macrocybe* sps extract exhibited the inhibitory assays potential with IC_{50} value of 78.1µg/ml.

Key words : *Macrocybe,* Phytochemical, antioxidant and antidiabetics.

Mushrooms are considered as "Elixir of life" because of their high nutritive value and medicinal values. Mushrooms are rich in various bioactive molecules which strengthen the immune function and protect against various disorders hence mushrooms are consumed during olden days as supplementary food[11]. They are rich in alkaloids, fibers, lectin, protein, polysaccharides and very less fat content[18]. Basidiomycetes are used for various treatments such as cancer, diabetes, inflammation, gastrointestinal disorder. Mushroom are rich in antioxidant that can inhibits and prevent oxidative stress which was caused by free radicals present in the body. The antioxidants from mushrooms contains decreased lipid

peroxidation, reduced post prandial triglyceride response and increases the enzymes of antioxidant such as catalase and superoxide dismutase**.** *Macrocybe* sps *(Massee) pegler and lodge* belongs to the family trichlomataceae is a wild edible mushroom. *Macrocybe* sps is rich in protein, polysaccharides, vitamins, minerals and dietary fibre.

Mushroom contains a variety of phytochemicals such as alkaloid, flavonoid, terpenoids, saponins, steroids, polysaccharides which enhances the immune system. The exopolysaccharides from mushroom shows high anticancer and antitumor property[8]. Edible mushrooms has many beneficiary properties. The phenolic compound present in the mushroom exhibits high antioxidant properties which reduces reduces oxidative damage in human beings[16]. Mushroom can be directly used in dietary supplementation for the enhancement of antioxidant to reduce the oxidative stress.

Diabetes mellitus is a metabolic abnormalities caused due to defect in secretion of insulin. The pancreatic β cells secretes defective insulin and there is a lack of insulin sensitive tissues to respond to insulin which leads to increase in blood glucose level[9]. Diabetes can be classified into four types, such as type 1 diabetes mellitus: pancreas does not secrete insulin and insulin dependent. Type 2 diabetes mellitus: combination of insufficient insulin secretion from the pancreatic islets and insulin resistance of target cells. Pancreatic beta-cell mass is reduced by ~50% in individuals with T2DM compared with non-diabetic subjects[3]. Gestational diabetes mellitus occurs during pregnancy. Type 4 (Non classical causes of diabetes mellitus) caused by resistance of insulin in old age. The α amylase and α glycosidae are the two enzyme responsible for the increase of glucose level in blood. The inhibition of these two enzymes may be useful in management of diabetes[2]**.**

The present study focuses on phytoconstituents of ethanolic extract of mushroom. The four different assays such as DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS ((2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid), ion chelating assay and phosphomolybdenum assay were performed to evaluate the antioxidant potential of mushroom *Macrocybe* sps. The antidiabetic potential of ethanolic extract of mushroom was studied using α amylase inhibition assay.

The cultivated *Macrocybe* sps. was dried using hot air oven at 30ºC for 3 to 4 days. The dried fruit body was homogenized using homogenizer. The homogenized sample was extracted with known solvent ethanol at room temperature on a shaker at 150 rpm for 24 hour and filtered through No. 4 whatman filter paper. The filtrate was evaporated to dryness at 40 ºC. The dried extract was stored at 4 ºC for further studies.

Preliminary phytochemical analysis[4] *:*

Test for saponins :

To 5.0ml of distilled water was mixed with ethanolic mushroom extract in a test tube and it was mixed vigorously. The foam appearance showed the presence of saponins.

Test for flavonoids:
Alkaline Reagent Test:

To 2ml of 2.0% NaoH mixture was mixed with ethanolic mushroom extract, concentrated yellow colour was produced. This result showed the presence of flavonoids.

Test for terpenoids

The 2.0ml of chloroform was added with the 5ml ethanolic mushroom extract and evaporated on the water bath and then boiled with 3ml of concentrated H_2SO_4. A grey colour formed which showed the entity of terpenoids.

Test for glycosides

A solution of glacial acetic acid (4.0ml) with 1 drop of 2.0%of fecl3 mixture was mixed with the 10ml of ethanolic mushroom extract and 1ml of concentrated H_2SO_4. A brown ring formed between the layers which showed the entity of cardiac steroidal glycosides.

Test for alkaloids:
Dragendorff test:

To 5mg of ethanolic extract was taken in test tube. Add one drop of dragendorff´s reagent and orange red colour precipitate shows the presence of alkaloids.

Test for aminoacids:

Ninhydrin test: To 5mg of ethanolic extract of mushroom was mixed with 2ml of 0.2% solution of Ninhydrin and boiled for 2 min on water bath, the violet colour appearance indicates the presence of amino acids

Test for proteins :

Biuret´s test: To 5 mg of ethanolic extract of mushroom was added with the few drops of biuret´s reagent. The obtained mixture was shaken well and allowed to warm for 1-5 min. Appearance of red or violet colour indicated presence of proteins.

Test for carbohydrates:

Fehling´s test: To 5 mg ethanolic extract of mushroom was mixed with few drops of benedict´s reagent, than allowed to boiled, the reddish brown precipitate are formed with the presence of the carbohydrates.

Test for phenols:

To 20ml of distilled water in a test tube, the powdered sample of mushroom was boiled and then filtered. Add 3-4 drops of 0.1% v/v ferric chloride to the filtered sample and the colour changes to brownish green or blue, it indicates presences of phenols.

DPPH radical scavenging activity:

The percentage of antioxidant activity of each substance was assessed by DPPH free radical assay. The measurement of the DPPH radical scavenging activity was performed according to methodology described by Szabo *et al*.[17]. The samples were reacted with the stable DPPH radical in methanol solution. The reaction mixture consisted of adding 0.5 mL of sample, 1 mL of methanol and 1 mL of DPPH radical solution 0.5 mM in methanol. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in color (from deep violet to light yellow) were read [Absorbance (Abs)] at 517 nm after 100 min of reaction using a UV-VIS spectrophotometer. The control solution was prepared by mixing methanol (1.0 mL) and DPPH radical solution (1.0 mL) and 1 mL of methanol serves as blank. The scavenging activity percentage

was determined according to
% of inhibition = Control O.D – Sample O.D/ Control O.D X 100.

ABTS•+ free radical scavenging activity [14]

ABTS•+ was dissolved in deionised water to 7 mM concentration and potassium persulphate was added to a concentration of 2.45 mM. The reaction mixture was left to stand at room temperature overnight (2 h) in the dark before usage. The resultant intensely-coloured ABTS•+ radical cation was diluted with 0.01 M PBS (phosphate buffered saline), pH 7.4, to give an absorbance value of ~0.70 at 734 nm. The sample was diluted with methanol, treated with ABTS•+ solution and made up to a total volume of 1 mL. Absorbance was measured spectrophotometrically at time intervals of 10 min after addition of a range of 10-100 µg/mL concentrations for each extract. The assay was performed in triplicates. Controls without ABTS•+ were used to allow for any absorbance of the extracts themselves, and 990 µL of PBS was added to these control samples. Fresh stocks of ABTS•+ solution were prepared every five days due to self-degradation of the radical. The assay was first carried out on ascorbic acid, the water-soluble analogue, which served as a standard. The results of the assay were expressed relative to ascorbic acid.

Ferrous iron chelating assay :

The chelating activity of the ferrous ions (Fe 2+) was measured according to the method of Dinis *et al*.[5]. Briefly, 0.5 mL of different concentrations (10-100 µg/mL) of the extracts were added to a solution of 2 mM FeCl 2 (0.05 mL). The reaction was initiated by the addition of 5 mM ferrozine (0.2 mL). The mixture was shaken vigorously and left at room temperature for 10 min. Ferrozine reacted with the divalent iron to form stable magenta complex species that were very soluble in water. Absorbance of the solution was then measured spectrophotometrically at 562 nm. The percentage inhibition of ferrozine-Fe 2+ complex formation was calculated as:

Percentage of inhibition (%) = [(A0 – A1)/ A0] × 100

Phosphomolybdenum assay :

Extracts in different concentration ranging from 10 to 100 µg/mL were added to each test tube individually containing 1 ml of distilled water and 1 ml of Molybdate reagent solution, 1mL of Sodium phosphate and 1 mL of Sulphuric acid were added separately. These tubes were kept incubated at 95 °C for 90 min. After incubation, these tubes were normalized to room temperature for 20-30 min and the absorbance of the reaction mixture was measured at 695 nm. The values were recorded.

α-amylase inhibitory assay :

The pancreatic porcine α-amylase assay was adapted from (Sudha *et al*.,[17]) with modifications (Parmar *et al*.,[12])

Preliminary phytochemical analysis :

The preliminary phytochemical analysis of ethanolic extract of *Macrocybesps* have been studied. Totally nine compounds such as alkaloids, saponins, glycosides, carbohydrates, proteins, amino acids, phenols,

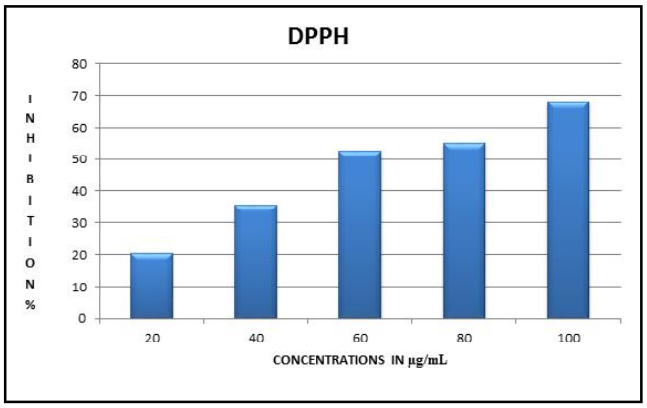

Figure 1 Dpph Radical Scavenging Activity

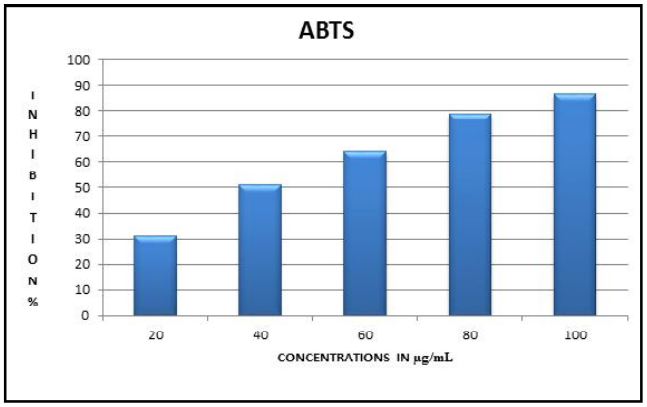

Figure 2 Abts Activity

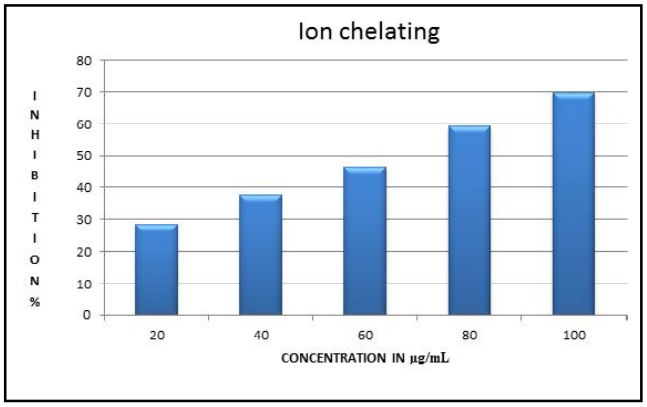

Figure 3. Ion Chelating Assay

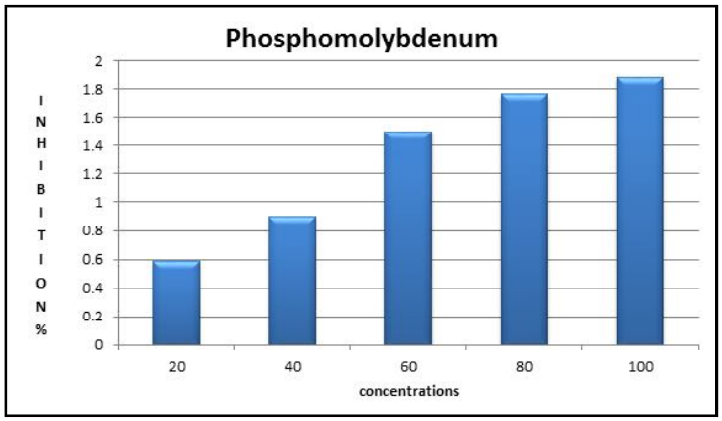

Figure 4 Phosphomolybdenum Reduction Activity

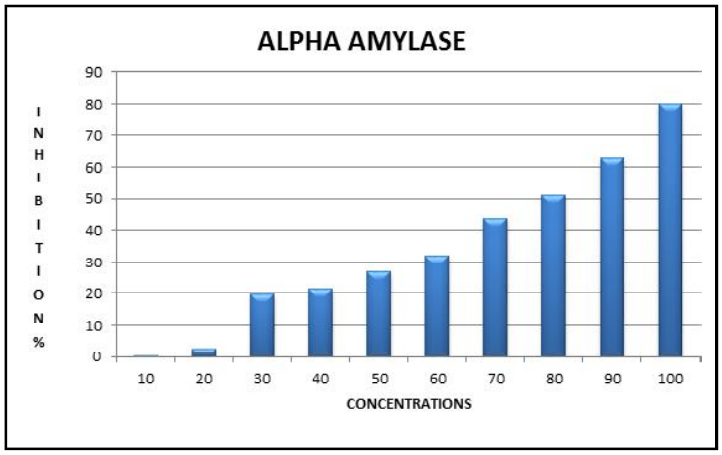

Figure 5 Alpha Amylase Assay

terpenoids and flavonoids were analysed. The ethanolic extract of *Macrocybe* sps showed the presence of five compounds such as alkaloids, carbohydrates, proteins, terpenoids and flavonoids. Acharya *et al.,*[1] reported that methanolic extract of *Macrocybe crassa* showed the presence of glycosides, carbohydrate, saponin, terpenoids, phenol and flavonoids while sterol and alkaloid were not detected.

Evaluation of antioxidant activity.

In the present study four in vitro methods of antioxidant were performed to evaluate the antioxidant activity of ethanolic extract of *Macrocybe* sps.

1. Determination of antioxidant by dpph method:

DPPH is a stable free radical used to determine the antioxidant activity of *Macrocybe* sps. The antioxidant effect of *Macrocybe* sps. was found with the IC_{50} value of 57.07µg/mL when compared to standard quercetin with the IC_{50} value of 6.1407µg/mL.

Gaur *et al.*,[6] reported that lyophilized methanolic extract of MA 2 strain of *Macrocybe gigantea* had EC 50 value of 96.03 µg/mL. fig 1.

2. Determination of antioxidant by ABTS method:

The ethanolic extract of *Macrocybe* sps. showed the IC_{50} value of 39.18µg/mL when compared to standard ascorbic acid with the IC 50 value of 41.11µg/mL. González-Palma, I.,*et al.,*[7] reported ABTS radical scavenging activity of methanolic extract of driedP.ostreatus with EC_{50} value of 217.24 ± 1.31mg/mL. The value reported was higher compared to the present study. fig. 2.

3. Determination of antioxidant by Ion chelating assay method:

The chelating activity of the ferrous ions was measured according to the method of Dinis *et al.*,[5]. The chelating ability of ethanolic extract of *Macrocybe* sps. showed the IC_{50} value of 64.87 µg/mL when compared to standard EDTA with the IC_{50} value of 41.87µg/mL. Gaur *et al.*,[6] reported that the chelating capacity was high in lyoplilized sample MA 2 strain of *Macrocybe gigantea* with EC_{50} value of 95µg/mL. fig. 3.

4. Phosphomolybdenum assay :

The antioxidant capacity is based on reduction of mo (VI) to mo(V) by the mushroom extract. The reducing assay was gradually increased from 0.583 to 1.885 at 20 to 100 µg/mL respectively when compared to standard ascorbic acid with OD value 39.13 to 95.65 at 20 to 100 µg/mL concentration. Loganathan *et al.*,[10] reported that *Termitomyces reticulatus* cap, stipe and entire mushroom showed the OD value for reducing power were 0.372, 0.586 and 0.420 respectively. fig 4.

α-amylase inhibitory assay :

The preliminary antidiabetic activity was carried out using the ethanolic extract of *Macrocybe* sps by following alpha amylase inhibitory assay. The IC_{50} value of ethanolic extract of *Macrocybe* sps was 78.1µg/mL when compared to standard acarbose with the IC_{50} value of µg/mL. The IC_{50} value of extract is high it is due to the presence of mixture compounds in the mushroom as compared to single compound. Stojkovic, *et al.*,[15] reported that methanolic extract of following mushroom species such as *Agaricus blazei* with IC_{50} value of 1719.20µg/mL,*Coprinus comatus* with IC_{50} value of 714.45µg/mL, *Inonotus obliquus* with IC_{50} value of 830.32µg/mL, *Phellinus linteus* with IC_{50} value of 1479µg/mL, *Morchella conica* and *Cordyceps militaris* does not showed any inhibition at that concentration. fig 5.

The present work can be concluded that ethanolic extract of the mushroom *Macrocybe* sps showed the presences of various phytoconstituents which are essentials for the normal physiological role to boost up the biological system. The invitro antioxidant study showed higher percentage of inhibition. Thus mushrooms are considered as natural antioxidant which strengthen the immune system and protect it from the oxidative damage. Mushrooms are rich in polysaccharides and vitamin D content. Thus based on the present result mushrooms have great potential

to develop as effective and safe antidiabetic drug.

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