DOI: https://doi.org/10.36036/MR.31.2.2022.128268
***Mushroom Research* 31 (2) : 139-152, 2022**

In-vitro cultural, nutritional, antioxidant and antimicrobial potential of *Macrolepiota procera* and *Amanita caesaria*: a rare mushroom from Madhya Pradesh, India under submerged culture condition

Ashutosh Rajoriya*, Pushpanjali Dhok and Kevin Gawali

G H Raisoni University, Saikheda, Madhya Pradesh
**Corresponding author*, Email: ashutosh.rajoriya@ghru.edu.in

ABSTRACT

***Macrolepiota procera* and *Amanita caesaria* are the rarely accepted mushrooms among the society even if both are well known for their therapeutic properties. Present findings are focused on their *in-vitro* growth performance, colony characteristics, nutritional properties, antioxidant and antimicrobial activity under submerged culture conditions. The mushroom fruit bodies were collected from the different forest divisions of Madhya Pradesh and cultured. Malt extract broth was best suited medium for both *Macrolepiota procera* and *Amanita caesaria*. Biochemical studies in the mushroom mycelia grown in malt extract revealed a very good range of phyto-chemicals, which are considered as strong therapeutic agents. In addition, radical scavenging activity in the culture broth was assayed and highest activity was also recorded in malt extract media. *Macrolepiota procera* exhibited 55.74 % while *Amanita caesaria* exhibited 61.22 % of free radical scavenging activity. Malt extract medium thus proved to be the best medium for the culturing as well as for the production of antioxidants by both the mushroom species selected. In case of Antioxidant activity boiled water extract proven to be better than methanolic extract.**

Key words: Mushroom, Biomass, Antioxidant, *Macrolepiota procera*, *Amanita caesaria*

Mushrooms are well known functional foods which are continuously exploited for the formulations of therapeutic agents (Wasser *et al*., 2000; Rai *et al*., 2005; Barros *et al*., 2007). These properties have drawn upon the interest of many researchers to formulate the possible conditions for the enhanced production of mushrooms/mycelia. For the bioactive preparations in addition to mushrooms, their mycelia are also considered as an alternative or substitute source, which could also be used as food-flavouring agent as well as for the preparation of pharmaceutical products (Barros *et al*., 2007).

Cultivated mushroom mycelia under submerged culture conditions have potential to be used in the human diet (Yitzhak and Ephraim, 1986). Many workers have used various basal liquid media to grow and characterize some mushrooms (Johnsy and Kaviyarasan, 2013). Submerged culture, in contrast to cultivation on solid media, gives rise to potential advantages of higher mycelial production in a compact space and shorter time with lesser chances of contamination (Yang and Liau, 1998; Klomklung *et al*., 2014). To improve the production of fungal bioactive polysaccharides, investigators have worked to optimize the fermentation conditions and medium composition of solid state and submerged fermentation systems (Hwang and Yun, 2010). In Madhya Pradesh, cultivation of edible mushroom is at the level of commercialization but still many people depend mainly

Recieved on 29-09-2022; Accepted on 20-12-2022
https://epubs.icar.org.in/index.php/MR/article/view/128268

on wild indigenous species for the food and taste. The present study deals with media for the better proliferation of *Macrolepiota procera* and *Amanita caesaria,* cultural characteristics of their mycelia, antioxidant activity of mushroom mycelia using free radical scavenging activity and components related to reducing power of boiled and ethanolic extracts.

MATERIALS AND METHODS

Collection and identification of mushrooms

Healthy, fresh and fully succulent *Macrolepiota procera* and *Amanita caesaria* fruit bodies were collected from Chiraidongri, Mandla District of Madhya Pradesh. All morphological, macroscopic and microscopic parameters of various parts of mushrooms such as pileus, stipe, veil, ring, volva, lamellae and gills etc were taken into consideration for identifying species (Largent, 1973). The fruit bodies were cultured on Malt Extract Agar (MEA), maintained at 28 °C and sub-cultured in Petri plates for further use.

Mycelia and broth culture

Potato dextrose broth (PDB), Fungi Kimmig Broth (FKB), Malt extract broth (MEB), Corn meal broth (CMB), Sabouraud dextrose broth (SDB), Czapek dox broth (CZB), Soil extract broth (SEB), Wheat extract broth (WEB), Rye extract broth (REB), Maize extract broth (MaEB), Paddy straw extract broth (PEB), Leaf litter broth (LLB), Yeast extract broth (YEB), Glucose yeast peptone broth (GYP), Peptone Yeast extract broth (PYE) were used for stationary mycelial culture (30 ml/150 ml conical flask) by transferring mycelial disc from actively growing Petri plate culture under aseptic conditions. Inoculated broth cultures were incubated at 30°C for 10 days. The mycelium thus obtained was recovered and broth was centrifuged and used for antioxidant analysis.

Determination of biomass

Mycelial biomass was harvested from broth culture. The mycelia was filtered through filter paper (Whatman No. 1), washed thoroughly using double distilled water, dried to get a constant weight and weighed in the digital weighing balance (Kern KB 240-3N, Germany).

Mycelial characteristics

The morphological description of mycelial culture in the different solid media was performed weekly. The parameters recorded were: colony texture, colour, border appearance, reverse colour, medium coloration, aerial growth, exudates and rifts.

Proximate and antioxidant analysis

Protein content of the mycelia was estimated by Bradford (1976) while total carbohydrate content was quantified following phenol sulphuric acid method (Dubois *et al*. 1956; Hedge and Hofreiter 1962). Reducing sugar was estimated by DNS method (Miller, 1972). Non reducing sugar was calculated by subtracting reducing sugar content from total carbohydrate (Nazarudeen, 2010). DPPH free radical scavenging activity was assayed by following the method given by Chan *et al*. (2007). Estimation of total phenol content was carried out following Folin phenol method (Singleton and Rossi, 1965). Ferric Reducing Antioxidant Power (FRAP) assay was assayed following the method of Benzie and Strain (1996) and Athavale *et al*. (2012). The flavonoid content of the sample was estimated by using aluminium chloride colorimetric technique and flavonoid content was expressed in terms of mg quercetin equivalents per gram of extract (Chang *et al*., 2002).

Antioxidant activities in submerged culture conditions

Reagents for radical scavenging activity were prepared by taking dissolving 6 mg of 2, 2-diphenyl-1-picryl hydrazyl (DPPH) in 100 ml of methanol in an amber conical flask to avoid the direct contact of light with the reagent. Shaken well and allowed to stand for 15 minutes before the use.

The scavenging activity was estimated by means of colorimetric method (Chan *et al.*, 2007). One ml of culture filtrate was added with 2 ml of (DPPH) solution (1:2), mixed well and incubated in dark for 30 minutes. The optical density was measured at 517 nm and scavenging activity of each extract was calculated using following equation.

Scavenging activity (%) = (1 – Absorbance of sample/ Absorbance of Control) × 100.

Antimicrobial activity assay and MIC Assay

General infectious bacteria like *Staphylococcus aureus* (MTCC 1144), *Escherichia coli* (MTCC 835), *Klebsiella pneumoniae* (MTCC 4030), *Pseudomonas aeruginosa* (MTCC 1688) and *Enterococcus faecalis* (MTCC 9845) were procured from IMTECH Chandigarh, India. Their identification was confirmed at Agarkar Research Institute, Pune, India. The fungi used as test organisms were: *Aspergillus flavus* (MTCC 1783), *Aspergillus fumigatus* (MTCC 2550), *Candida albicans* (MTCC 3017), *Paecilomyces variotii* (MTCC 2205) (MTCC 22319) and *Penicillium purpurescens* (DBFS 418). Bacterial cultures were maintained on Muller-Hinton agar substrates. Fungal cultures were maintained on potato dextrose (PD) agar and Sabourad dextrose (SD) agar (Torlak, Belgrade). All cultures were stored at 4 °C.

The sensitivity of microorganisms to acetone and methanol extracts of the examined species of mushrooms was assayed by determining the minimal inhibitory concentration (MIC). Bacterial inoculums were obtained from bacterial cultures incubated for 24 h at 37 °C on Muller- Hinton agar substrate and brought up by dilution according to the 0.5 Mc Farland standards to approximately 10^8 CFU/mL. Suspensions of fungal spores were prepared from 3 to 7 day old cultures grown at 30 °C on a PD agar medium. Spores were rinsed with sterile distilled water, used to determine turbidity spectrophotometrically at 530 nm, and then further diluted to approximately 10^6 CFU/mL according to the procedure recommended by NCCLS (1998).

Minimum inhibitory concentration

The MIC was determined by the broth micro dilution method using 96-well micro- titer plates following Wiegand *et al.* (2008). A series of dilutions with concentrations ranging from 40 to 0.156 mg/ mL for extracts were used in experiment against every microorganism tested. The starting solutions of extracts were obtained by measuring off a certain quantity of extract and dissolving it in DMSO. Two-fold dilutions of extracts were prepared in Muller-Hinton broth for bacterial cultures and SD broth for fungal cultures. The MIC was determined by establishing visible growth of microorganisms. The boundary dilution without any visible growth was defined as the MIC for the tested microorganism at the given concentration. As a positive control of growth inhibition, streptomycin was used in case of bacteria, ketoconazole in case of fungi.

RESULTS AND DISCUSSION

Growth in culture mycelia and broth culture

Macrolepiota procera showed best radial growth in Malt extract and Glucose yeast peptone media. Sabouraud dextrose, Wheat extract and Potato dextrose broth were also proved to be a supportive media with comparatively less mycelial growth (Table 1). The optimal conditions reported for the mycelial growth of *M. procera* were 30 °C and pH 7 and PDA media (Shim *et al.,* 2005) while Singh *et al.* (1990) found Malt extract and Sabouraud dextrose as the best media for vegetative growth of *Lentinus connatus*. Peken and Kibar (2020) also studied the culture conditions for mycelia growth of *M. procera*. Cultural characteristic varied from media to media, a tuft mass of rhizoidous hyphae were seen in Potato dextrose, Malt extract and Glucose

Table 1. Culture characteristics of *Macrolepiota procera* under submerged culture condition

S.No	Growth media	Activity	Front Colour	Back Colour	Special Features
1	Wheat extract broth	+++	White	Off white to yellow	Continuous, flat mycelium, hairy filaments, Margin: Entire, O.D-transluscent
2	Rye extract broth	++	White	Yellowish brown	Radial growth with hairy mycelium, Margin- Uneven crennate, O.D- transluscent
3	Maize extract broth	++	White	White	Thin sparse mycelium, Convex and raised at the middle, Margin- Hairy, O.D- trasluscent
4	Paddy straw broth	+	Off White	Light brown	Very thin, radial, ContinuousO.D- Transluscent
5	Leaf litter broth	+	White	Brown	Filamentous, webby, Sparse mycelium and raised out growth, Margin- hairy, OD- Transluscent
6	Malt extract broth	++++	Pure white	White	Thick Matty growth Margin-Entire O.D-Opaque
7	Potato dextrose broth	+++	White	White	Radial growth, Flat, Continuous, Margin- Hairy, O.D-transluscent
8	Sabouraud dextrose broth	+++	White	Off white	Radial cottony growth, Margin-Hairy, O.D-Transluscent
9	Yeast extract broth	++	Brown	Dark brown	Undulate, Patchy, Discontinuous, Margin- Crennate, O.D- Opaque
10	Glucose yeast peptone broth	++++	Pale yellow	brown	Flat mycelium with distal convex rings, Margine- Entire, O.D-Transluscent
11	Soil extract broth	-	No growth	No growth	NO special features
12	Czapek dox broth	+	White	Off White	Very thin filamentous, radial growth, Continuous, Margin- Uneven, O.D- Transluscent
13	Peptone yeast extract broth	+	Brown	Brown	Very thin, Undulating and Sparse filaments, Margin-Crennate, OD-Transluscent
14	Fungi kimmig broth	+	White	white	Patchy, undulating, Convex, Margin- Hairy, OD- Transluscent.
15	Corn meal broth	+	White	White	Convex, Radial, filamentous patchy growth with sparse raised hairy out growth, Margin-Hairy, OD- Transluscent

yeast peptone broth, on the other hand Yeast extract, Fungi kimmig and Corn meal broth were poor supportive media which showed thin undulating growth of mycelia (Table 1).

Amanita caesaria proliferated well in wheat extract medium and other media like malt extract, potato dextrose and yeast extract medium also supported its growth (Table 2). Šašek - Musílek (1967) found the influence of composition of the media on the ability and intensity of growth of pure cultures of 17 ectomycorrhizal species (35 strains). A variety of culture media and methods of cultivation can be used for propagation, inoculum preparation, storage on agar, fruiting, and other experimental procedures (Cudlín *et al*., 1980; Hutchison, 1990; Harvey, 1991). Colonization of this mushroom mycelia was convex cottony/fuzzy being maximum growth at the centre, which gradually decreased with the advancement of hyphal colonization. Very poor growth was observed Leaf litter broth, Paddy straw broth, Czapek dox broth and Peptone yeast extract broth where as complete absence of growth was recorded in Soil extract and Glucose yeast peptone broth (Table 2).

Table 2. Culture characteristics of *Amanita ceasaria* under submerged culture condition

S.No	Growth media	Activity	Front Colour	Back Colour	Special Features
1	Wheat extract broth	++++	Off White	Off white to yellow	Thick mat web like Structure flat surface, Margin- Entire, O.D-translucent
2	Rye extract broth	+++	Off White	Off white	Thick mat, raised fluffy colony in between, Margin- Uneven, O.D translucent
3	Maize extract broth	+++	White to off white		WhiteCottony white growth, Margin-Umbonate, O.D- translucent
4	Paddy straw broth	+	White	Light brown	Very thin, undulating growth, Margin- Uneven, O.D- Translucent
5	Leaf litter broth	+	Off White	Off white	Sparse mycelium and raised growth, Margin-uneven, OD- Translucent
6	Malt extract broth	+++	white	White	Thick cottony growth, Margin- Entire, O.D- Opaque
7	Potato dextrose broth	+++	White	Off White	Thick raised growth of mycelium, Margin-Entire, O.D- Translucent
8	Sabouraud dextrose broth	++	white	Off white Brown	Flat, moderately thick, Margin-Uneven, OD- Opaque
9	Yeast extract broth	+++	White	Off white	Continuous pellicle, Margin- entire, OD- Opaque
10	Glucose yeast peptone broth	-	No growth	No growth	No special features
11	Soil extract broth	-	No growth	No growth	No special features
12	Czapek dox broth		White	White	Sparse flat mycelium, Continuous, Margin- Umbonate, OD- translucent
13	Peptone yeast extract broth	+	Pale white	Pale white	Flat mycelium, raised at the edges, Margin- uneven, OD- Translucent
14	Fungi kimmig broth	+	White	white	Flat, moderately thick, Margin- Uneven, OD- Opaque
15	Corn meal broth	+	White	White	Very thin, Sparse mycelium, Margin- Entire, OD-Translucent

Kadiri and Fasidi (1994) reported that the best utilizable carbon sources for *Lentinus subnudus* were fructose, maltose, dextrin and glucose. In this study, a similar kind of observation was made where Malt extract broth was best suited media for *Macrolepiota procera* cultivation in liquid culture condition in which it showed maximum biomass production i.e 1.17 g in fresh weight basis followed by Glucose yeast peptone, Sabouraud dextrose and Potato dextrose broth in which mycelia weighed out was 1.07, 0.96 and 0.65 g, respectively (Table 3).

It has been reported that maltose is a good carbon source for mycelium of some mushrooms (Amir, 1993; Amir *et al*., 1994). In case of *Amanita caesaria*, the maximum biomass was recorded in Malt extract broth during this study also. Fasidi and Olorunmaiye (1994) reported yeast extract (a complex nitrogen source) supported the maximum growth of *P. tuberregium.* Yeast extract broth also served as a good culture medium during the present study, followed by Wheat extract broth medium. Minimum amount of the growth was recorded in Capek dox and

Table 3. Growth performance of mycelium of *Macrolepiota procera* and *Amanita caesaria* under submerged culture conditions (fresh biomass in g) after the incubation period of 10 days and change in pH

S. No	Media	*M. procera*		*A. ceasaria*	
		Biomass(g)	pH Change	Biomass(g)	pH Change
1	Wheat extract broth	1.05±0.07	6.64±0.51	0.35±0.12	5.24±0.21
2	Rye extract broth	0.53±0.12	6.39±0.28	0.10±0.01	7.26±0.15
3	Maize extract broth	0.05±0.02	5.57±0.31	0.26±0.04	5.12±0.30
4	Paddy straw broth	0.04±0.01	4.67±0.14	0.02±0.01	4.53±0.17
5	Leaf litter broth	0.01±0.00	4.96±0.12	0.04±0.02	5.54±0.03
6	Malt extract broth	1.17±0.15	7.00±0.12	0.65±0.17	6.62±0.28
7	Potato dextrose broth	0.65±0.07	4.05±0.03	0.008±0.00	2.85±0.10
8	Sabouraud dextrose broth	0.96±0.08	5.00±0.26	0.24±0.10	5.24±0.34
9	Yeast extract broth	0.03±0.01	5.31±0.18	0.61±0.07	6.75±0.11
10	Glucose yeast peptone broth	1.07±0.25	6.79±0.13	No growth	5.92±0.56
11	Soil extract broth	No growth	6.59±0.13	No growth	6.13±0.22
12	Czapek dox broth	0.008±0.00	5.51±0.35	0.004±0.00	5.38±0.35
13	Peptone yeast extract broth	0.01±0.00	5.12±0.04	0.14±0.04	5.27±0.34
14	Fungi kimmig broth	0.07±0.02	7.04±0.16	0.12±0.01	5.94±0.09
15	Corn meal broth	0.03±0.00	5.47±0.00	0.09±0.01	5.70±0.25

Potato dextrose broth, complete absence of growth was observed in Glucose yeast extract and Soil extract medium. Among chemosynthetic media Peptone yeast extract broth proved to be a better medium for the cultivation of this organism (Table 3).

Change in pH of medium during growth

In this experiment, decrease in pH towards acidity was recorded after incubation of 10 days in almost all the media in the broth culture of *Macrolepiota procera* except Fungi kimmig broth and Malt extract broth where neutral pH was recorded. Maximum acidity was observed in the Potato dextrose broth, Paddy straw broth and Leaf litter broth medium while comparatively high pH was recorded in other media ranging from 5.00-7.04. Mycelial culture of *Amanita caesaria* showed acidic pH in maximum media selected for the studies. Mainly pH ranged between 4.53-6.75, except Potato dextrose broth (2.85) and Rye extract broth (7.26), which showed very high acidity and neutral pH, respectively. Biomass obtained from moderate pH was better than the extreme pH conditions.

Antioxidant activity in submerged culture condition

Broth culture of *Macrolepiota procera* in Malt extract medium showed maximum scavenging of free radicals generated by DPPH i.e 55.74 % followed by Corn meal broth (54.76 %). However, Wheat extract (52.08 %), Maize extract (47.06 %) and Leaf litter extract medium (47.33 %) also showed an appreciable amount of scavenging activity. Complete absence of free radical scavenging activity was observed in Yeast extract and Soil extract broth. *Amanita caesaria* cultured on Malt extract as well Sabouraud dextrose medium showed good scavenging activity i.e. 61.22 % and 58.98 % respectively on the other hand

complete absence of scavenging effect was recorded in Soil extract, Wheat extract and Leaf litter extract medium (Table 4).

Table 4. DPPH scavenging activity (%) in the submerged culture conditions

Media	*Macrolepiota procera*	*Amanita caesaria*
Wheat extract	52.08±3.42	0.00
Rye extract	16.53±1.41	7.64±0.03
Maize extract	47.06±0.03	25.18±2.06
Paddy extract	25.00±7.06	10.66±3.10
Leaf litter extract	47.33±12.64	0.00
Malt extract	55.74±1.10	61.22±0.01
Potato dextrose	29.24±2.62	45.81±1.00
Sabouraud dextrose	43.20±0.24	58.98±0.24
Yeast extract	0.00	0.00
Glucose yeast peptone	8.54±0.67	4.32±1.70
Soil extract	0.00	0.00
Czapek dox	29.21±0.26	38.76±1.54
Peptone yeast extract	0.00	7.23±0.02
Fungi Kimmig	44.88±2.34	38.69±0.67
Corn meal	54.76±0.53	34.69±0.67

Proximate and Antioxidant analysis of mycelia

Protein content was found to be more in mycelium of *Macrolepiota procera* (9.23 mg/g) than *Amanita caesaria* (7.64 mg/g) while carbohydrate content was higher in *Amanita caesaria* than *Macrolepiota procera* (Table 5). Reducing sugars are found to be very less in the mushrooms as mainly the carbohydrates are utilized in other forms like chitin and polysaccharides. In the present study, *Amanita caesaria* mycelia showed 12 mg/g of reducing sugar content whereas *Macrolepiota procera* showed 4.18 mg/g. Similarly, non reducing sugar was higher in the *Amanita caesaria* mycelia compared to the mycelia obtained by *Macrolepiota procera*. FRAP assay showed highest value in the methanolic extract of *Macrolepiota procera* (0.58 mg AEAC/g) whereas relatively less activity was observed in case of *Amanita caesaria* (0.16 mg AEAC/g). In general it is considered that fungi don't concentrate flavonoids (Iwashina, 2000). Flavonoids are responsible for strong antioxidant behavior in the organisms, in the present study methanolic extracts of *Macrolepiota procera*

Table 5. Proximate component and antioxidant activity of mycelia of *Macrolepiota procera* and *Amanita caesaria* under submerged culture conditions

S. no	Parameters	*Macrolepiota procera*	*Amanita caesaria*
1	Protein (mg/g)	9.23±11	6.54±0.93
2	Carbohydrates (g/100g)	14.80±3.05	39.72±7.52
3	Reducing sugars (mg/g)	3.58±1.50	12.30±3.12
4	Non reducing sugars (g/100g)	12.64±0.35	38.68±7.32
5	DPPH scavenging (%)	95.24±0.17	95.24±0.79
6	AEAC value (mg/g)	0.43±0.00	0.42±0.008
7	Phenolics (mg/g)	1.73±0.45	0.90±0.16
8	Carotenoid (mg/g)	0.16±0.09	0.83±0.07
9	FRAP (mg AEAC/g)	0.58±0.05	0.16±0.02
10	Flavonoids (mg/g)	2.03±0.28	1.91±0.25
11	Tannins (mg/g)	3.31±0.20	4.95±0.53
12	Alkaloids (mg/g)	5.15±0.81	5.10±0.27
13	Beta carotene (mg/g)	0.085±0.035	0.036±0.00
14	Lycopene (mg/g)	0.040±0.00	0.006±0.00

and *Amanita caesaria* showed 2.03 mg/g and 1.91 mg/g of flavonoids respectively. Barros *et al*. (2008) reported that 16 species of mushrooms showed absence of flavonoid content.

Antioxidant properties of phenolic compounds also play a vital role in the stability of food products, as well as in the antioxidative defence mechanisms of biological systems (Macheix and Fleuriet, 1998). Methanolic extracts of mycelia of both the mushrooms showed low amount of phenols than reported by Wu and Hansen (2008) but when compared to the findings of Oke and Aslim (2011) almost similar quantity was observed in *Amanita caesaria*, while *Macrolepiota procera* showed much higher value in their methanolic extracts (Table 5). Carotenoids are known for their beneficial effects in preventing human diseases such as cardiovascular aliments including other chronic disorders (Paiva and Russell, 1999). They are very useful dietary sources of retinol, β-Carotene, α- carotene and β-cryptoxanthin, which could function as provitamin A (Liu, 2004). The results obtained here in regard of the level of β-carotene and lycopene was higher than the values obtained by Robaszkiewicz *et al*. (2010) where 0.085 mg/g and 0.036 mg/g of β-carotene was recorded in the mycelia of *Macrolepiota procera* and *Amanita caesaria* while lycopene was in relatively less quantity i.e. 0.040 mg/g and 0.006 mg/g, respectively. Alkaloids and Tannins are well known for their various biological activities (Ozçelik *et al*., 2011; Kumari and Jain, 2012) in the present case studies they were also matched with the results obtained by Edeoga *et al*. (2005) and Puttaraju *et al*. (2006).

Antioxidant activity and IC_{50} values of Boiled water and ethanolic extract

Radical Scavenging Activity (RSA) at 50 % inhibition (IC_{50}) was the parameter used to compare the RSA for different mushroom mycelial extracts. Lower IC_{50} value meant better RSA. Boiled water and ethanol extracts of the tested mushroom mycelia showed a good scavenging activity on DPPH radical. Significant values between extracts and control i.e. $P < 0.05$ was recorded during the studies. The IC_{50} values of all extracts ranged from 4.72 – 212.47µg/ml. Boiled water extract from *A. caesaria* showed largest DPPH radical scavenging activities (IC_{50} = 4.72 µg/ml) than that of *A. ceasaria* and greater than BHA and α-tocopherol. The scavenging activity was also good for the boiled water extracts from *M. procera* (IC_{50} = 8.63 µg/ml). Ethanol extracts from tested mushrooms showed weaker DPPH radical scavenging activities than Boiled water. IC_{50} for the ethanol extracts were 187.73 µg/ml for *M. procera* and 212.47 µg/ml for *A. caesaria* (Table 6).

Table 6. IC_{50} values of boiled water and ethanol extracts of *Macrolepiota procera* and *Amanita caesaria* by free radical scavenging method.

Samples	IC_{50} (µg/mL)	
	Macrolepiota procera	***Amanita caesaria***
Boiled water extracts	8.63	4.72
Ethanol extracts	187.73	212.47

DPPH radical scavenging, reducing power, determination of total phenolic compounds and determination of total flavonoid content of the hot water and methanol extracts of the mushrooms *Macrolepiota procera* and *Amanita caesaria* were assayed in this study (Table 7). High absorbance indicates high reducing power. Measured values of absorbance varied from 0.0014 to 0.0280. The reducing power of extracts increased concentration dependently. Among the tested extracts, hot water and methanol extracts of *Amanita caesaria* showed highest reducing power, followed by hot water extracts from *M. procera*.

Total phenolic and flavonoid constituents of tested extracts are presented in Table 8. The amount of total phenolic compounds was determined as the pyrocatechol equivalent using an equation obtained from a standard pyrocatechol graph. Results of the

Table 7. Reducing power of boiled water and ethanol extracts of *Macrolepiota procera* and *Amanita caesaria*

Samples	Extracts	Absorbance (700 nm)		
		1000 g/ml	500 µg/ml	250 µg/ml
Macrolepiota procera	Boiled water	0.025	0.005	0.005
	Ethanol	0.028	0.006	0.003
Amanita caesaria	Boiled water	0.008	0.005	0.002
	Ethanol	0.009	0.007	0.003
Ascorbic acid		2.226	0.957	0.478
BHA		3.465	1.681	1.651
α-tocopherol		2.887	1.651	0.808

Table. 8 Total phenolics and flavonoid content of boiled water and ethanol extracts of *Macrolepiota procera* and *Amanita caesaria*

Samples	Extracts	Phenolics content (µg/mg of extract)	Flavonoid content (µg /mg of extract)
Macrolepiota procera	Boiled water	6.73 ± 1.065	3.20 ± 1.099
	Ethanol	4.91 ± 1.208	1.53 ± 1.105
Amanita caesaria	Boiled water	8.14 ± 1.211	4.93 ± 1.195
	Ethanol	4.64 ± 1.318	1.46 ± 1.128

study showed that the phenolic compound of the tested extracts varied from 4.64 to 8.14 ìg of pyrocatechol equivalent. Highest phenolic compounds was identified in hot water extract of *Amanita caesaria* at a 8.14 µg of pyrocatechol equivalent, followed by hot water extract of *Macrolepiota procera* with 6.73 µg of pyrocatechol equivalent.

The amount of total flavonoid compounds was determined as rutin equivalent using an equation obtained from a standard rutin graph. As shown in Table 8, good flavonoid content was found in the hot water extract of *Amanita caesaria* (4.93 µg of rutin equivalent) and hot water extract extract of *Macrolepiota procera* (3.20 µg of rutin equivalent).

The tested mushroom extract and their IC_{50} values was correlated with total phenolic and Flavonoid content. Notably negative correlation was established between the phenols and IC_{50} values of antioxidant activities (r = -0.93). Also, there is a good negative correlation between flavonoid compounds of the tested extracts and IC_{50} values of antioxidant activities (r = -0.83). These negative linear correlations prove that the sample with highest antioxidant contents show higher antioxidant activity with lowest IC_{50} values.

Antimicrobial activity

The antimicrobial activity of the tested mushrooms extracts against the selected microorganisms was shown in Table 9. The hot water and ethanol extracts of the tested mushrooms showed strong antimicrobial activity. The MIC for both extracts related to the tested bacteria and fungi were 1.8 - 12 mg/mL. Generally, the hot water extracts exerted stronger antimicrobial activity than ethanol extracts.

The maximum antimicrobial activity was found in the hot water extract of the mushrooms *M. procera* against *Enterococcus foecalis* (MIC = 1.8 mg/ml). The measured MIC values for *M. procera* against bacteria were 1.8-9 mg/ml for the hot water extract and 4.5-9 mg/ml for the ethanol extract. Both extracts of this mushroom inhibited the tested fungi in concentrations 4.5 mg/ml and 10 mg/ml.

The hot water extract and ethanol extract of *Macrolepiota procera* and *Amanita caesaria* had approximately equal antimicrobial activity. They inhibited the tested bacteria and fungi in concentrations 3.5 mg/ml, 5 mg/ml and 9 mg/ml.

The antimicrobial activities were compared to streptomycin (standard antibiotic) and ketoconazole (standard antimycotic). The results showed that streptomycin and ketoconazole had stronger activity than tested extracts as shown in Table 9. In a negative control, DMSO had no inhibitory effect on the tested organisms. The tested mushrooms extracts have a strong antioxidant activity against various oxidative systems *in-vitro*. The intensity of antioxidant activity depended on the tested mushroom species and the solvent which was used for extraction.

The differences in the antioxidant activity of various solvents may be result of different capabilities to extract bioactive substances (Slinkard and Slingleton, 1997). When antioxidative capacities of the extracts are compared to their phenolic constituents, it could be concluded that antioxidative nature of the extracts might depend on their phenolics. We found that the tested extracts exhibited the highest radical scavenging activity with the greatest amount of phenolic content. Numerous researches found high correlations between antioxidative activities and phenolic content (Ferrreria *et al*., 2007; *Choi et al*., 2006; Alvarez *et al*., 2007). Antioxidant activities of mushroom extracts were studied by other researchers such as *Boletus edulis* (Vidovic *et al*., 2010; Shu *et al*., 2007; Sarikurcku *et al*., 2008.)**.** Ramesh and Manohar (2010) found strong antioxidant activity for methanol extracts from *Lycoperdon perlatum, Cantharellus cibarius, Clavaria vermiculris, Ramaria formosa, Marasmius oreades, Pleurotus pulmonarius*. Murcia *et al.* (2002) find an antioxidant activity for the *Lepista nuda, Lentinus edodes, Agrocybe cylindracea, Cantharellus lutescens,* and *Hydnum repandum.* Numerous mushrooms were screened for antimicrobial activity in search of the new antimicrobial agents (Ramesh and Manohar 2010; Gezer *et al*., 2006; Turkoglu *et al.,* 2007; Mercan *et al.,* 2006). It found that different

Table 9. Minimum inhibitory concentration (MIC) of boiled water (BW) and ethanol extracts of *Macrolepiota procera* and *Amanita caesaria.* Boiled wate and methanol has been replaced with BW and ethanol

Samples	***Macrolepiota procera***		***A. caesaria***		**S**	**K**
Test organisms	**BW**	**E**	**BW**	**E**		
E. fecalis	1.8	5.5	5.5	5.5	15.62	-
P. verrucosum	9	9	11	5.5	-	3.9
C. albicans	3.5	5	3.5	5.5	-	1.95
P. purpurescens	9	9	9	5.5	-	3.9
P. aeruginosa	5.5	5.5	3.5	5.5	15.62	-
S. aureus	4.5	4.5	3.5	5.5	31.25	-
K. pneumoniae	3.5	6	3.5	5	1.95	-
E. coli	6.5	9	5.5	12	31.25	-
A. flavus	6.5	9	11	12	-	3.9
A. fumigatus	9	10	11	12	-	3.9

[a] Minimum inhibitory concentration (MIC); values given as mg/ml for extracts and as ìg/ml for antibiotics. Values are the mean of three replicate.
Antibiotics: K – ketoconazole, S – streptomycin.

species of mushrooms exhibit different antimicrobial activity. These differences in antimicrobial activity of different species of mushrooms are probably a consequence of the presence of different components with antimicrobial activity. In our experiments, the tested mushroom extracts showed a relatively strong antimicrobial activity. The intensity of the antimicrobial effect depended on the species of mushroom, its concentration and the tested organism. The examined mushroom in the same concentrations showed a stronger antibacterial than antifungal activity. These results could be expected due to the fact that numerous tests proved that bacteria are more sensitive to the antibiotic compared to fungi (Hugo and Russel, 1983). The reason for different sensitivity between the fungi and bacteria can be found in different transparency of the cell wall (Yang and Anderson, 1999). The cell wall of the gram- positive bacteria consists of peptidoglycans (mureins) and teichoic acids, while the cell wall ofthegram-negativebacteriaconsists of lipo polysaccharides and lipopoliproteins, whereas, the cell wall of fungi consists of polysaccharides such as hitchin and glucan (Jean, 2001; Farkas, 2003).

CONCLUSION

The mushroom species used in the study has good nutritional and their extracts have shown a strong antioxidant and antimicrobial activity *in-vitro* against human/plant pathogenic bacteria and fungi. Based on these results, mushrooms appear to be good and safe natural sources of antioxidants and could be of significance in food and human therapy, animal and plant diseases. Further studies may be done on the isolation and characterization of new compounds from mushrooms, which are responsible for antioxidant and antimicrobial activity. On the basis of obtained results, natural substrates like extracts from grains can be used for the mass multiplication of edible mushrooms mycelia and their culture broth may be evaluated as an cheap and effective source of of natural antioxidants/secondary metabolites. Further work is needed on the isolation purification and characterization of the active components from crude extracts of mushroom, mushroom mycelia and their culture broth to ascertain their biological activities.

ACKNOWLEDGEMENTS

Authors are thankful to G H Raisoni University, Department of Plant Pathology for their financial assistance.

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