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Full Length Research Paper

Antioxidant and antimicrobial activities of *Morchella conica* Pers.

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Antioxidant capacity and antimicrobial activities of *Morchella conica* Pers. extracts obtained with ethanol were investigated in this study. Four complementary test systems; namely DPPH free radical scavenging, β-carotene/linoleic acid systems, total phenolic compounds and total flavonoid concentration were used. Inhibition values of *M. conica* ethanol extracts, buthylated hydroxyanisol (BHA) and α-tocopherol standards were found to be 96.9, 98.9 and 99.2%, respectively, at a concentration of 160 µg/ml. When compared the inhibition levels of methanol extract of *M. conica* and standards in linoleic acid system, it was observed that the higher the concentration of both *M. conica* ethanol extract and the standards the higher the inhibition effect. Total flavonoid amount was 9.17±0.56 µg mg^{-1} quercetin equivalent while the phenolic compound amount was 41.93±0.29 µg mg^{-1} pyrocatechol equivalent in the ethanolic extract. The antimicrobial effect of *M. conica* ethanol extract was tested against six species of Gram-positive bacteria, seven species of Gram-negative bacteria and one species of yeast. The *M. conica* ethanol extract had a narrow antibacterial spectrum against tested microorganisms. The most susceptible bacterium was *M. flavus*. The crude extract was found active on *S. aureus* ATCC 25923 and *S. aureus* Cowan I. The *M. conica* ethanol extract did not exhibit anticandidal activity against *C. albicans*.

Key words: *Morchella conica* Pers., mushroom, antioxidant and antimicrobial activity.

INTRODUCTION

Morchella conica Pers. is a well known and extraordinary mushroom species found in Turkey. The head is distinctly conical in shape. The surface of head comprises a honeycomb of sharp ridges and deep pits and is rich brown in colour. The texture is sponge-like. The head and stem is generally hollow. It grows generally on chalky soil in grassy woodlands, field margins ad roadside verges. *M. conica* is picked up every year if the weather condition is suitable for growth in Turkey. It is collected especially in April and May, and marketed in Turkey and abroad either fresh or dried.

Medicinal mushrooms have an established history of use in traditional oriental therapies. Modern clinical practice in Japan, China, Korea, and other Asian countries continue to rely on mushroom-derived preparations. Mushrooms have been used for many years in oriental culture as tea and nutritional food and because of their special fragrance and texture (Manzi et al., 1999). The scientific community, in searching for new therapeutic alternatives, has studied many kinds of mushrooms and has found variable therapeutic activity such as anticarcinogenic, anti-inflammatory, immuno-suppressor and antibiotic, among others (Asfors and Ley, 1993; Longvah and Deosthale, 1998). It has been known for many years that selected mushrooms of higher Basidiomycetes origin are effective against cancer.

Oxidation is essential to many living organisms for the

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production of energy to fuel biological processes. However, the uncontrolled production of oxygen derived free radicals is involved in the onset of many diseases such as cancer, rheumatoid arthritis, and atherosclerosis as well as in degenerative processes associated with aging (Halliwell and Gutteridge, 1984). Almost all organisms are well protected against free radical damage by enzymes such as superoxide dismutase and catalase, or compounds such as ascorbic acid, tocopherols and glutathione (Mau at al., 2002). When the mechanism of antioxidant protection becomes unbalanced by factors such as aging, deterioration of physiological functions may occur resulting in diseases and accelerated aging. However, the antioxidants present in human diet are of great interest as possible protective agents to help the human bodies reduce oxidative damage.

Researchers have reported the antimicrobial activity of several mushrooms (Lee at al., 1999; Kim and Fung, 2004; Gao et al., 2005). The chloroform and ethyl acetate extracts of some dried mushroom have antibacterial activity against *Streptococcus mutans* and *Prevotella intermedia* (Hirasawa at al, 1999). Both fruiting body and the mycelium contain compounds with wide-ranging antimicrobial activity (Jong and Birmingham, 1993).

In recent years, multiple drug resistance in human pathogenic microorganisms has developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. This situation has forced scientists to search for new antimicrobial substances from various sources as novel antimicrobial chemotherapeutic agents (Karaman et al., 2003).

Although this research was focused on the chemical and volatile composition of this mushroom, no information is available about its antioxidant and antimicrobial activities in literature. Therefore, the aim of the present work is to evaluate the antioxidant and antimicrobial potential of the *M. conica* ethanol extract on several microorganisms of medicinal importance.

MATERIALS AND METHODS

Mushroom

Morchella conica Pers. samples were collected from Denizli, in the western part of Turkey. Identification and classification of macrofungus were carried out and all specimens were deposited at the laboratory of Department of Science Education, Pamukkale University, Denizli, Turkey. Specimens of *M. conica* representing a combination of young and old ascocarps, were collected in the area in the spring in 2004. Fresh mushroom were randomly selected into three samples, 150 g and air-dried in an oven at 40°C before analysis. Dried mushroom sample (20 g) was extracted by stirring with 200 ml of ethanol at 30°C at 150 rpm for 24 h and filtering through Whatman No. 4 filter paper. The residue was then extracted with two additional 200 ml of ethanol as described above. The combined ethanolic extract were then rotary evaporated at 40°C to dryness, redissolved in ethanol to a concentration of 10 mg ml^{-1} and stored at 4°C for further use.

Chemicals

β-carotene, linoleic acid, 1,1-Diphenly-2-picrylhydrazyl (DPPH), buthylated hydroxytoluene (BHT), buthylated hydroxyanisol (BHA) and α-tocopherol were purchased from Sigma (Sigma, Aldrich GmbH, Sternheim, Germany). Pyrocatechole, Tween-20, Folin-ciocalteu's phenol reagent (FCR), sodium carbonate, ethanol, chloroform and the other chemicals and reagents were purchased from Merck (Darmstat, Germany). All other chemicals and reagents were of analytical grade.

DPPH assay

The hydrogen atom or electron donation abilities of the corresponding extracts and some pure compounds were measured from the bleaching of the purple-coloured methanol solution of 1,1-Diphenly-2-picrylhydrazyl (DPPH). This spectrophotometric assay uses the stable radical DPPH as a reagent (Burits and Bucar, 2000; Cuendet at al., 1997). 1 ml of various concentrations of the extracts in ethanol was added to 4 ml of 0.004% methanol solution of DPPH. After a 30 min incubation period at room temperature, the absorbance was read against a blank at 517 nm. Inhibition of free radical by DPPH in percent (I%) was calculated in following way:

$$I\ (\%) = (A_{blank} - A_{sample} / A_{blank}) \times 100$$

where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the plot of inhibition (%) against extract concentration. Tests were carried out in triplicate.

Carotene-linoleic acid assay

In this assay, antioxidant capacity was determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation (Dapkevicius at al., 1998). A stock solution of β-carotene-linoleic acid mixture was prepared as follows: 0.5 mg β-carotene was dissolved in 1 ml of chloroform (HPLC grade) and 25 µl linoleic acid and 200 mg Tween 40 were added. Chloroform was completely evaporated using a vacuum evaporator. Then, 100 ml distilled water saturated with oxygen was added with vigorous shaking at a rate of 100 ml/min for 30 min. 4 ml of this reaction mixture were dispensed into test tubes and 200 µl portions of the extracts, prepared at 2 mg/l concentrations, were added and the emulsion system was incubated for 2 h at 50°C. The same procedure was repeated with synthetic antioxidant, BHT, BHA and α-tocoferol as positive control as well as a blank. After this incubation period, absorbances of the mixtures were measured at 490 nm. Antioxidative capacities of the extracts were compared with those of BHA, α-tocoferol and blank.

Determination of total phenolic compounds

Total soluble phenolics in the mushroom ethanolic extracts were determined with Folin-Ciocalteu reagent according to the method of Slinkard (Slinkard and Singleton, 1977) using pyrocatechol as a standard. Briefly, 1 ml from extract solution (2000 ppm) was transferred into a volumetric flask of 50 ml, and made up to 46 ml with distilled water. Folin-Ciocalteu reagent (1 ml) was added and the contents of flask were mixed thoroughly. After 3 min, 3 ml of Na_2CO_3 (2%) was added, then the mixture was allowed to stand for 2 h with intermittent shaking. The absorbance was measured at 760

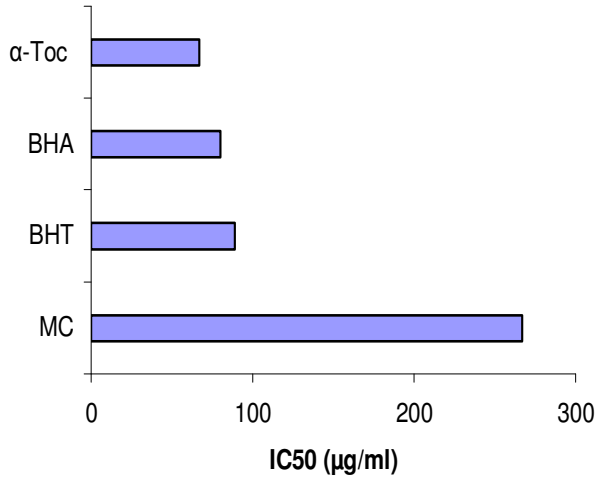

Figure 1. Free radical scavenging capacities of the *M. conica* ethanolic extracts measured in DPPH assay.

nm. The concentration of total phenolic compounds in the mushroom ethanolic extracts determined as microgram of pyrocatechol equivalent by using an equation that was obtained from standart pyrocatechol graph is given as:

Absorbance = 0.00246 µg pyrocatechol + 0.00325 (R^2: 0.9996)

Determination of total flavonoid concentration

Flavonoid concentration was determined as follows: mushroom ethanolic extracts solution (1 ml) was diluted with 4.3 ml of 80% aqueous ethanol and to the test tubes were added 0.1 ml of 10% aluminum nitrate and 0.1 ml of 1 M aqueous potassium acetate. After 40 min at room temperature, the absorbance was determined spectrophotometrically at 415 nm. Total flavonoid concentration was calculated using quercetin as standart (Park at al., 1997). Absorbance = 0.002108 µg quercetin – 0.01089 (R^2: 0.9999)

Microorganisms

The activities of ethyl alcohol extract of *M. conica* were measured against the following cultures: *Pseudomonas aeruginosa* (NRRL B-23), *Salmonella enteritidis* (RSKK 171), *Escherichia coli* (ATCC 35218), *Morganella morganii* (clinical isolate), *Yersinia enterecolitica* (RSKK 1501), *Klebsiella pneumoniae* (ATCC 27736), *Proteus vulgaris* (RSKK 96026), *Staphylococcus aureus* (ATCC 25923), *Staphylococcus aureus* Cowan I, *Micrococcus luteus* (NRRL B-4375), *Micrococcus flavus, Bacillus subtilis* (ATCC 6633), *Bacillus cereus* (RSKK 863), *Candida albicans* (clinical isolate). The bacteria were obtained from the culture collection of the Microbiology Department of Pamukkale University and Ankara University.

Screening of antimicrobial activity of mushroom samples

Antimicrobial activity of the *M. conica* ethanol extract was determined by the agar-well diffusion method. All the microorganisms mentioned above were incubated at 37±0.1°C (30±0.1°C for only *M. luteus* NRRL B-4375 and *M. flavus*) for 24 h by inoculation into Nutrient broth. *C. albicans* was incubated YPD broth at 28±0.1°C for 48 h. The culture suspensions were prepared and adjusted by comparing against 0.4-0.5 McFarland turbidity standard tubes. Nutrient Agar (NA) and YPD Agar (20 ml) were poured into each sterilized Petri dish (10x100 mm diameter) after injecting cultures (100 µl) of bacteria and yeast and distributing medium in Petri dishes homogeneously. For the investigation of the antibacterial and anticandidal activity, the dried mushroom extract were dissolved in dimethylsulfoxide (DMSO) to a final concentration of 20% and sterilized by filtration through a 0.22 µm membrane filter (Ali-Shtayeh at al., 1998; Tepe at al., 2005). Each sample (100 µl) was filled into the wells of agar plates directly. Plates injected with the yeast cultures were incubated at 28°C for 48 h, and the bacteria were incubated at 37°C (30°C for only *M. luteus* NRRL B-4375 and *M. flavus*) for 24 h. At the end of the incubated period, inhibition zones formed on the medium were evaluated in mm. Studies were performed in duplicate and the inhibition zones were compared with those of reference discs. Inhibitory activity of DMSO was also tested. Reference discs used for control are as follows: nystatin (100 U), ampicillin (10 µg), penicillin (10 U), Oxacillin (1 µg), tetracycline (30 µg) and gentamicin (10 µg). All determinations were done duplicate.

RESULTS AND DISCUSSION

Antioxidant activity of extracts

The ethanolic extract was subjected to screening for their possible antioxidant activity. Four complementary test systems, namely DPPH free radical scavenging, β-carotene/linoleic acid systems, total phenolic compounds, total flavonoid concentration were used for the analysis. DPPH, a stable free radical with a characteristic absorption at 517 nm, was used to study the radical scavenging effects of extracts. As antioxidants donate protons to these radicals, the absorption decreases. The decrease in absorption is taken as a measure of the extent of radical scavenging. Free radical scavenging capacities of the extracts, measured by DPPH assay, are shown in Figure 1. All concentration studied showed free radical scavenging activity. The 50% of inhibition value for *M. conica* ethanol extract seems to be fairly significant when compared to commonly used synthetic antioxidant BHA and α-tocopherol (IC_{50} = 267 µg/ml ethanolic extract was necessary to obtain 50% of DPPH degradation).

160 µg of *M. conica* ethanol extract has an equivalent inhibition value of 80 µg BHA. The inhibition value increases with concentration. Linoleic acid oxidation was compared for *M. conica* ethanol extract, α-tocopherol and BHA. It was found that inhibition values of both *M. conica* ethanol extract and the standards increased with concentration. For example, at 80 µg/ml concentration, *M. conica* extract, BHA and α-tocopherol showed 77.9, 96.4 and 98.6% inhibition, respectively, whereas at 160 µg/ml concentrations these were 96.9, 98.9 and 99.2% inhibition (Figure 2).

The total phenolic compound amount was calculated as quite high in *M. conica* ethanol extracts (41.93±0.29 µg mg^{-1} pyrocatechol equivalent). According to this, it is possible that the high inhibition value of *M. conica* extract is due to the high concentration of phenolic coumpounds. The key role of phenolic compounds as scavengers of

Table 1. Antimicrobial activity of the ethanol extracts of *M. conica* and antibiotic sensitivity of microorganisms (zone size, mm).

Test bacteria	*M. conica*	N	A	P	G	O	T
Pseudomonas aeruginosa NRRL B-23	-	NT	NT	NT	16	NT	8
Salmonella enteritidis RSKK 171	4 ± 0	NT	-	NT	NT	NT	12
Escherichia coli ATCC 35218	-	NT	10	11	NT	NT	8
Morganella morganii	-	NT	NT	NT	-	NT	-
Yersinia enterecolitica RSKK 1501	7 ± 1	NT	20	18	NT	NT	7
Klebsiella pneumoniae ATCC 27736	-	NT	-	NT	NT	NT	5
Proteus vulgaris RSKK 96026	4.5 ± 0.5	NT	-	NT	NT	NT	16
Staphylococcus aureus ATCC 25923	13 ± 0.5	NT	NT	31	NT	21	20
Staphylococcus aureus Cowan I	10 ± 1	NT	NT	28	NT	18	21
Micrococcus luteus NRRL B-4375	17 ± 1	NT	30	31	NT	22	19
Micrococcus flavus	29 ± 1	NT	29	31	NT	24	20
Bacillus subtilis ATCC 6633	6 ± 0	NT	NT	12	NT	8	17
Bacillus cereus RSKK 863	9 ± 1	NT	NT	22	NT	14	19
Candida albicans	-	19	NT	NT	NT	NT	NT

N: Nystatin (100 U), A: Ampicillin (10 µg), P: Penicillin (10 U), G: Gentamicin (10 µg), O: Oxacillin (1 µg), T: Tetracycline (30 µg), NT: Not tested, (-): No inhibition.

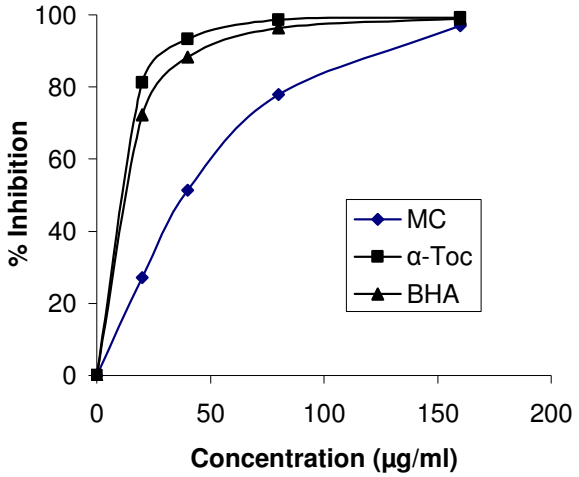

Figure 2. Total antioxidant activity of BHA, α-tocopherol and different doses of *M. conica* ethanolic extracts using linoleic acid oxidation.

free radicals is emphasised in several reports (Komali at al, 1999; Moller at al., 1999). Phenols are important components of plants. They were reported to eliminate radicals due to their hydroxyl groups (Hatano at al., 1989), and they contribute directly to antioxidant effect of system (Duh at al., 1999). Polyphenolic compounds have an important role in stabilizing lipid oxidation and are associated with antioxidant activity (Yen at al., 1993; Gülcin at al., 2003). The phenolic compounds may contribute directly to antioxidative action (Duh at al., 1999). It is suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans, when up to 1.0 g is ingested daily from a diet rich in fruits and vegetables (Tanaka at al., 1998). In contrast to this, the total flavonoid compound concentration was measured as 9.17±0.56 µg mg^{-1} quercetin equivalent. Like phenol compounds, the contribution of flavonoids to antioxidant activity is known. It has been reported that BHT I3, II8-biapigenin and hypericine which have the structure of biflavonoid have a very high antioxidant effect. This effect was proposed to stem from hydroxyl groups in the structure of the flavonoids (Cakir et al., 2003).

Therefore, this mushroom extract competes favorably with BHA and α-tocopherol in β-caroten-linoleic acid system used to determine the antioxidant capacity.

Antimicrobial activity of extracts

The antimicrobial effect of the *M. conica* ethanol extract was tested against six species of Gram-positive bacteria, seven species of Gram-negative bacteria and one species of yeast. As summarized in Table 1, *M. conica* ethanol extract had a narrow antibacterial spectrum against tested microorganisms. The most susceptible bacterium was *M. flavus* (29 ± 1 mm diameter). The crude extract was found active on *S. aureus* ATCC 25923 and *S. aureus* Cowan I (13 and 10 mm diameter, respectively). *S. aureus* is a pathogen which is known to cause infectious disorders of the skin (Jones et al., 2003; Rennie at al., 2003). Thus, *M. conica* maybe used as agent for the treatment of skin disorders. The *M. conica*

ethanol extract showed no antibacterial activity against *P. aeruginosa, E. coli, M. morganii* and *K. pneumoniae* at the concentration used. Previous studies revealed that extracts of some mushrooms were inactive against *K. pneumoniae, P. fluorescens, E. coli* (Dulger at al., 2002; Hatvani 2001). The culture fluid of *Lentinus edodes* showed poor activity against *C. albicans* (Hatvani, 2001). Dulger, Ergul, and Gucin (2002) reported that *C. albicans* and *Rhodotorula rubra* are resistant to the action of the methanolic extract of *Lepista nuda*. In the present study, the *M. conica* ethanol extract did not exhibit anticandidal activity against *C. albicans*.

In this study, the antibacterial properties of *M. conica* were not as effective as the commercial drugs. But, microorganisms do acquire resistance to the antibiotics after some time. Earlier, it was demonstrated that mushrooms show antimicrobial effects (Sheena at al., 2003; Hur et al., 2004; Ishikawa at al., 2001). Similarly in our survey, *M. conica* was found to inhibit the growth of microorganisms that cause infectious diseases. In summary, the observed activities with positive health benefits may provide a support for some of its uses in ethno-medicine.

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