Research Article

Antioxidant and antimicrobial activity of chloroform and methanol extracts of *Morchella conica* Pers

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Abstract

Purpose: The present study was conducted to evaluate the antioxidant and antimicrobial properties of chloroform and methanol extracts of the *Morchella conica*. **Methods:** Antioxidant activity was measured employing two methods namely, $ABTS^+$ radical cation scavenging activity and ferric reducing antioxidant/power activity, including total phenolic contents. In addition, the antimicrobial effects of *M. conica* chloroform and methanol extracts were tested against two species of Gram-positive bacteria, four species of Gram-negative bacteria and six species of yeast using the agar-well diffusion method. **Results:** Antioxidant studies suggested that methanol extract showed stronger $ABTS^+$ radical cation scavenging activity than chloroform extract. Also, chloroform extract exhibited higher ferric reducing antioxidant/power activity than methanol extract. The chloroform and methanol extracts of *M. conica* showed no antimicrobial activity against bacteria and yeasts used in this study at 2 mg/mL concentration. **Conclusion:** As a conclusion of this study, although both extracts obtained from *M. conica* showed lower activity than the standards, these extracts can be used as an antioxidant source after the toxicity studies of its are examined.

Keywords: *Morchella conica,* antioxidant, antimicrobial

Introduction

The scientific classification of *Morchella conica* is Ascomycota divison, Pezizales order, Morchellaceae family and *Morchella* genus. These mushrooms mostly and especially find in the pine forests in spring when the environment is suitable. *Morchella conica* harvested and sold predominantly at Kozak plateau of İzmir-Bergama and around Muğla, Kastamonu, Aydın, Denizli, Sinop, Çanakkale, Balıkesir in Turkey. Harvested mushrooms exported to other countries either airdried or fresh (Gucin, 1993).

Mushrooms contain a diversity of biomolecules with nutritional and/or bioactive properties. Due to these properties, they have been recognized as functional foods, and a source of natural medicines and nutraceuticals. Morel species can be used to find new antimicrobials overlapping the bacterial resistance to first choice antibiotics. Phenolic compounds, tocopherols and organic acids are considered to be the most responsible for antioxidant activity of mushrooms (Heleno et al., 2013). 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, immunosuppressor and antibiotic (Gucin, 1993; Turkoglu et al., 2006).

Morchella conica Pers. is a well known mushroom species found in Turkey. The head is distinctly conical in shape. 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 (Turkoglu et al., 2006).

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The antioxidant and antimicrobial activity of ethanol extract from *M. conica* have been reported before (Turkoglu et al., 2006). In that study, the antioxidant capacity of ethanol extract, which obtained stirring method were assayed using DPPH free radical scavenging and β-carotene/linoleic acid systems method. Also, the antimicrobial activity of the this extract were investigated against *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).

However, in our current study, the antioxidant activity of chloroform and methanol extracts, which obtained maceration method were assayed using $ABTS^+$ radical cation scavenging activity and ferric reducing antioxidant/power activity. In addition, in this study, the antimicrobial activity of the these extracts were investigated against *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 13315, *Klebsiella pneumoniae* ATCC 4352; and yeast strains: *Candida albicans* ATCC 90028, *Candida glabrata* ATCC 90030, *Candida guilliermondii* KUEN 998, *Candida tropicalis* KUEN 1021, *Candida parapsilosis* ATCC 90018, *Candida krusei* ATCC 6258.

It is also known that many mushrooms are used not only for daily diet but also for the medical purposes for people world. Therefore, this study aims to reveal the antioxidant and antimicrobial activities of the chloroform and methanol extracts from *M. conica.*

Materials and methods

Mushroom material

The *Morchella conica* which is used for this study is bought from one of the local supplier at Bucak, Burdur/Turkey and was identified by Prof. Dr. Mustafa Işıloğlu at the Muğla University, Pharmacy Faculty, Department of Pharmaceutic Botanic.

Preparation of the extracts

The dried mushroom samples (38 g) were extracted with heptane, chloroform and methanol by maceration at room temperature until was achieved at a colourless solution, respectively. The extracts were filtered and evaporated to dryness under reduced pressure at 40 °C in a rotary evaporator. The crude extracts were then transferred to vials and kept at +4°C. Chloroform and methanol extracts were dissolved in solvents and used for the assessment of antioxidant and antimicrobial activity.

Determination of total phenolic compounds

The amount of total phenolic compounds in the *M. conica* extracts were determined according to the method of Taskin et al. (2016). 0.1 mL of extract solution was diluted with distilled water (4.6 mL). 0.1 mL of Folin-Ciocalteu reagent (diluted 1:3, v/v) was added. Then, 3 mL of Na_2CO_3 (2.0 %) were added and the mixture was left standing at ambient temperature for 2 hours. The absorbance value was read at 760 nm. Results were expressed as milligrams of total phenolics per gram extract (mg GAE/g extract).

$ABTS^+$ radical cation scavenging activity

The $ABTS^+$ assay was performed according to the method developed by Taskin et al.,(2017). This assay is based on the formation of the free radical cation $ABTS^+$ by reaction of ABTS aqueous solution (7mM) with $K_2S_2O_8$ (2.45 mM), at room temperature, under darkness, for 12–16 hours. According to the results of scanning the spectrum obtained in this study, $ABTS^+$ showed a strong absorption band (λmax) at 734 nm.

This stock solution was diluted with methanol to an absorbance of 0.700 ± 0.020 at 734 nm. The reaction mixture comprised 3.96 mL of $ABTS^+$ solution and 0.04 mL of the extracts at a variety of concentrations. After six minutes, the absorbance value was read off at 734 nm.

Ferric reducing antioxidant/power capacity (FRAP)

The ferric reducing power capacity assay was performed according to the method developed by Benzie & Strain (1996). An FRAP working solution was prepared afresh each time: 0.3 M acetate buffer (pH=3.6), 0.01 M TPTZ (2,4,6-tripyridyl-s-triazine) in 0.04 M HCl and 0.02 M $FeCl_3.6H_2O$ were mixed in 10:1:1 (v/v/v) and kept away from light. The mixture was incubated at 37 °C for 30 min. away from light. Then 0.2 mL of extract solution were added to 3.8 mL FRAP working solution. After 4 min., the absorbance was measured at 593 nm. A solution of $FeSO_4.7H_2O$ was used for calibration. The ferric reducing power activity of the extracts was calculated from the linear calibration curve. Results were expressed as mM $FeSO_4$ equivalents per milligram of extracts. The calibration equation for $FeSO_4$ was absorbance= 0.8226x -0.0337 (R^2= 0.9992).

In vitro antimicrobial activity

***Test microorganisms*:** In this study, bacteria and yeasts were obtained from the Standart ATCC strains collection of Department of Pharmaceutical Microbiology Laboratory,

Faculty of Pharmacy at Marmara University. The following bacteria strain: *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 13315, *Klebsiella pneumoniae* ATCC 4352; and yeast strains: *Candida albicans* ATCC 90028, *Candida glabrata* ATCC 90030, *Candida guilliermondii* KUEN 998, *Candida tropicalis* KUEN 1021, *Candida parapsilosis* ATCC 90018, *Candida krusei* ATCC 6258 were used.

Study Media: Mueller Hinton agar (Merck), Mueller Hinton broth (Merck) for the bacteria, Sabouraud dextrose agar (SDA)(Merck), Sabouraud dextrose broth (SDB)(Merck) for the yeasts are used as media.

Method: The antimicrobial activity of chloroform and methanol ectracts of *Morchella conica* has been investigated with using agar well diffusion test. Eighteen hours bacterium cultures were used and diluted with sterilized physiological saline to obtain 1-2 x 10^8 cfu/ml of microorganism equivalent to Mc Farland 0.5 standards of turbitidy. The yeast cultures were subcultured twice prior to use at SDB at 35 ^{0}C for 48 hours and were prepared in Saboraud Dextrose Broth up to 10^7 cfu/ml. 0.1 ml microorganism suspensions were cultivated on agar medium. After that, 6 mm in diameter were cut into the wells. Then, 0.05 ml was taken from the stock suspensions of extracts and put into the wells. The solvents of the extracts, meropenem (10 µg) and fluconazole (100 µg) were tested in the same manner as control. All the culture plates were incubated at 35 ^{0}C 18-24 hours for bacteria and 48 hours for *Candida* species. After the incubation, the diameter of zone inhibition was measured in milimeters. All tests were made in triplicate and the average of the results was taken (Perez et al., 1990; Magaldi et al., 2004).

Results and discussion

Determination of total phenolic compounds

Table 1 and Fig 1. summarize the total phenolic compounds in extracts expressed as gallic acid equivalents (GAE) varied between 2.50 ± 0.7 and 9.50 ± 0.7 mg/g extract. According to the results obtained, it was found that methanol extract from *M. conica* contained higher total phenolic compounds than chloroform extract.

According to Turkoglu et al.,(2006), the ethanol extract obtained by stirring of *M. conica* was found to contain 0.042 mg pyrocatechol /mg extract phenolic compounds. However, in our study we determined the amount of the total phenolic contents of chloroform and methanol extracts obtained by maceration. According to the results obtained, methanol extract (9.5 mg GAE/g extract) had higher phenolic content than chlorofom extract. Also, when we compare our work with their work, we found that the methanol extract had a higher phenolic content than the ethanol extract.

This difference in the studies may be due to the fact that the phenolic content determined by spectrophotometry is affected by various parameters (extraction method, location, harvest season, etc.)

Table 1. Total phenolic compounds of the extracts from *Morchella conica*

Extracts	Total phenolics (mgGAE/g extract)
Chloroform	2.50±0.7
Methanol	9.5 ±0.7

Values are mean of triplicate determination (n = 3) ± standard deviation GAE–Gallic acid equivalents.

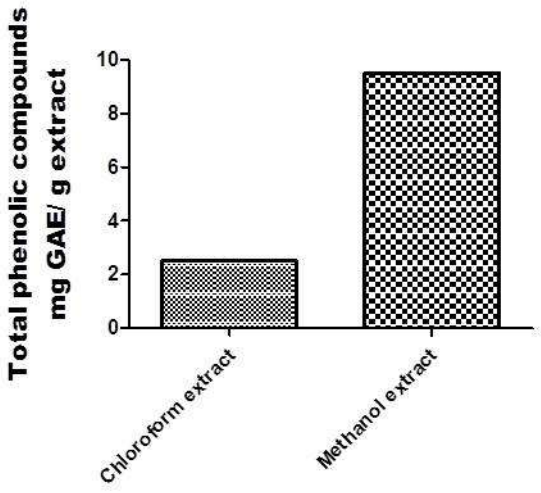

Figure 1. Total phenolic compounds of *M. conica*

Antioxidant activity of Morchella conica extracts

According to the results obtained from the ABTS test, the methanol extract of *Morchella conica* exhibited stronger ABTS radical cation scavenging activity than chloroform extract. BHT was used as a positive control. As shown in Table 2 and Fig 2, the radical scavenging ABTS activities of the chloroform and methanol extracts were lower than those of BHT.

Ferric reducing antioxidant/power (FRAP) activity of chloroform and methanol extracts have shown in Table 2 and Fig 3. The ferric reducing power effects of the extracts are in the following order: chloroform extract (3.45±0.03 mM$FeSO_4$/mg extract)>metanol extract (1.92±0.016 mM$FeSO_4$/mg extract). The chloroform extract of plant exhibited higher ferric reducing antioxidant power than methanol extract.

This study also exhibited that methanol extract had the higher total phenolic compound contents than chloroform extract. Hence, a linear relationship was found between ABTS radical cation scavenging activity and total phenolic content. However, a linear relationship wasn't found between ferric reducing antioxidant power and total phenolic content.

The antioxidant activity of ethanol extract from *M. conica* have been reported before (Turkoglu et al., 2006). In that study, the antioxidant capacity of ethanol extract were assayed using a different extraction technique (by stirring); it was reported that the free radical scavenging activity of ethanol extract (80 µg/mL) (77.9 %) is weaker than that of BHA (80 µg/mL) (96.4%).

However, in our current study, the antioxidant activity of extracts from *M. conica* were obtained by maceration method using chloroform and methanol solvents. In our study, methanol extract exhibited higher ABTS radical cation scavenging activity than chloroform extract. Also, chloroform extract showed stronger ferric reducing antioxidant activity than methanol extract.

Table 2. Antioxidant activity of the extracts from *Morchella conica*

Extracts/ Standard	ABTS (IC_{50}:mg/mL)	FRAP assay (mM Fe^{+2}/mg extract)
Chloroform	48.59±0.9[a]	3.45±0.03[a]
Methanol	14.72±0.6[b]	1.92±0.016[b]
BHT	0.32±0.02[c]	

Values are mean of triplicate determination (n = 3) ± standard deviation
Different superscript letters in each column exhibit significant differences in mean values at P< 0.05 according to Tukey's Multiple Comparison test.

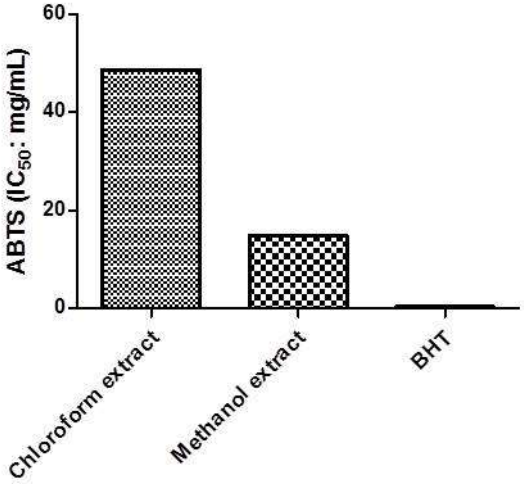

Figure 2. ABTS radical cation antioxidant activity of *M. conica*

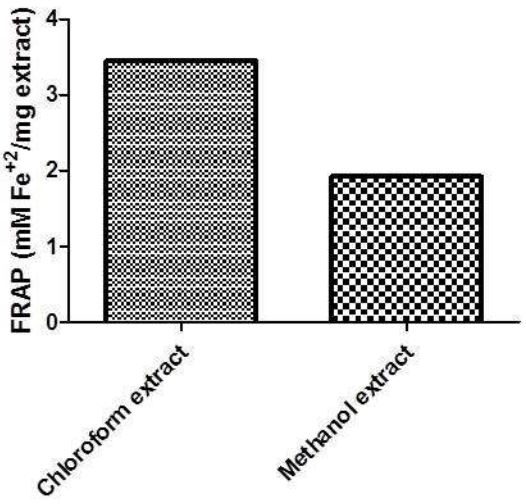

Figure 3. Ferric reducing antioxidant/power activity of *M. conica*

Antimicrobial activity

In this study, the antimicrobial activity of the chloroform and methanol extracts from *Morchella conica* were investigated against *Staphylococcus aureus*, *S. epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Candida albicans*, *C.glabrata*, *C.guilliermondii C.tropicalis*, *C.parapsilosis*, *C. krusei*.

According to the results obtained this study, methanol and chloroform extracts did not show antimicrobial activity at 2 mg/mL concentration. Solvents (chloroform, methanol) used in this experiment neither have affected the growth of any of the bacteria nor yeast species.

Immunolojik and anticancer effects of different kind of mushrooms are known. In addition the mushrooms have antioxidant, antihypertensive, antifibriotic, anti inflammatory, antidiabetic, antiviral and antimictobial effects as well as effective on cholesterol methabolism, protective on liver (Demirhan et al., 2007). Around 10000 macrofungus species only 5% of them have medical effects. Antimicrobial effects of these macrofungi is originated from some antogonistic substances like phenolic components, purines, pirimidines, quinones, terpenoides, phenil proponoides derivates which are highly specific for the fungus they synthetized (Bekçi et al., 2011). Reseachs design to investigate the antimicrobial activity of the macrofungi which include to prepare extracts in different solvent solutions showed that the extracts have different inactivatory effect on different type of microorganisms. In Turkey there are lots of studies about the flora of macrofungi but the studies related with the antimicrobial

activity of these macrofungi are lately started and there are just a few (Çalışkan, 2001).

However, in our study the antimicrobial activities of *M. conica* chloroform and methanol extracts have been investigated against *Stapyhlococcus aureus, Stapyhlococcus epidermidis, Escherichia coli*, *Pseudomonas aeruginosa, Proteus vulgaris Klebsiella pneumoniae and Candida* species. We have found that the chloroform and methanol extracts of *M. conica* did not not show any antimicrobial activity against the microorganisms mentioned above.

Bekçi et al., (2011), tested the antimicrobial effect of acetone extracts of *Morchella conica* and *Morchella elata* recieved from Kastamonu area, Turkey against some gram positive bacteria (*Bacillus cereus* ATCC 11778, *Staphylococcus aereus* ATCC 29213), gram negative bacteria (*Escherichia coli* ATCC 25922, *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella enteritidis* ATCC 13076) and yeast strains (*Candida glabrata* RSKK 04019, *Candida albicans* ATCC 90028) by using the disk diffusion method. Researchers suggested that the most inhibitory effect found agaist *E.coli* ATCC 25922 with *M. conica* extract and the smallest inhibitory effect was against *C. glabrata* RSKK 04019 with *M. elata* extract.

As far as we know, the antimicrobial feature of *Morchella conica* has been reported. The ethanol extract of *M. conica* was investigated for its antifungal and antibacterial activities. It was found that the ethanol extract of *M. conica* showed the strongest activity against *Micrococcus flavus* (29±1 mm in diameter). Also, the same extract showed moderate activity against *S. aureus* ATCC 25923 and *S. aureus* Cowan I (13 and 10 mm in diameter, respectively). Additionally, in the same study the ethanol extract of *M. conica* showed no antimicrobial activity against *Pseudomonas aeruginosa, E.coli, M.morgani* and *C.albicans* (Turkoglu et al., 2006).

In our study, we used chloroform and methanol extracts of *Morchella conica* but we didn't use ethyl acetate, acetone, or ethanol. Previos findings and our results showed that extracts prepared in different solvents have variety of antagonistic effects against diverse microorganisms. Researchers showed that extracts of some macrofungi have antagonistic effect against varied gram positive and gram negative bacteria as well as some yeast strains (Demirhan et al., 2007).

As a conclusion, it is important to continue our studies for discovering and classifying the macrofungi but we need to further investigate the pharmachologic, indrustrial and mediacal characteristics of the macrofungi for possible further usage. Also it is known that resistant bacteria and yeast strains against variety of antibiotics increase their numbers daily. Thus, it is important to investigate and invent new antibiotics. For this reason studies with different solvents and macrofungi against microorganisms required further investigation.

Conclusion

As a conclusion of this study, although both extracts obtained from *M. conica* showed lower activity than the standards, these extracts can be used as an antioxidant source after the toxicity studies of its are examined.

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