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In vitro Antimicrobial Activity of *Morchella esculenta* and *Trametes versicolor*

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Abstract: Fungi have a potential of using both as nutritive and medicinal food stuff. Because of containing several therapeutic agents, they are reported to be used for hundreds of years to treat several diseases caused by bacteria, fungi, viruses and parasites. The aim of this study is to determine the *in vitro* antimicrobial activity of *Morchella esculenta* (L.) Pers. 1801 and *Trametes versicolor* (L.) Lloyd 1921.

M. esculenta and *T. versicolor* samples were air dried and extracted by using ethanol. Antimicrobial activity of *M. esculenta* and *T. versicolor* ethanol extracts were investigated against several Gram positive and Gram negative bacteria strains, fungal strains, which are either standard or isolated from food and some multi drug resistant (MDR) clinical isolate bacteria namely, *Bacillus subtilis* DSMZ 1971, *Candida albicans* DSMZ 1386, *Enterobacter aerogenes* ATCC 13048, *Enterococcus durans*, *Enterococcus faecalis* ATCC 29212, *Enterococcus faecium*, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae*, *Listeria innocua*, *Listeria monocytogenes* ATCC 7644, *Pseudomonas aeruginosa* DSMZ 5071, *Pseudomonas fluorescence* P1, *Salmonella enteritidis* ATCC 13075, *Salmonella infantis*, *Salmonella kentucky*, *Salmonella typhimurium* SL 1344, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* DSMZ 20044, *Staphylococcus aureus* (MDR), *Escherichia coli* (MDR), *Klebsiella pneumoniae* (MDR), *Acinetobacter baumannii* (MDR), *Proteus vulgaris* (MDR), *Serratia odorifera* (MDR) and *Streptococcus pneumoniae* (MDR) by using the disk diffusion method.

As a result, it was observed that ethanol extracts of *M. esculenta* has medium to high antimicrobial activity against several Gram positive and Gram negative microorganisms tested, where *T. versicolor* presented low to high antimicrobial activity.

Key words: *Morchella esculenta*, *Trametes versicolor*, antimicrobial activity, disk diffusion, multi drug resistant bacteria, MDR

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Morchella esculenta ve *Trametes versicolor*'un *İn Vitro* Antimikrobiyal Aktivitesi

Öz: Mantarlar hem besleyici hem de tıbbi gıda maddesi olarak kullanma potansiyeline sahiptir. Terapötik ajanlar içerdiği için bakteri, mantar, virüs ve parazitlerin neden olduğu çeşitli hastalıkları tedavi etmek için yüzlerce yıl kullanıldıkları bildirilmektedir. Bu çalışmanın amacı *Morchella esculenta* (L.) Pers 1801 ve *Trametes versicolor* (L.) Lloyd 1921'in *in vitro* antimikrobiyal aktivitesini belirlemektir.

Çalışmada *M. esculenta* ve *T. versicolor* örnekleri kurutulmuş ve etanol kullanılarak ekstraktı elde edilmiştir. *M. esculenta* ve *T. versicolor* etanol ekstraktlarının antimikrobiyal aktivitesi, *Bacillus subtilis* DSMZ 1971, *Candida albicans* DSMZ 1386, *Enterobacter aerogenes* ATCC 13048, *Enterococcus durans*, *Enterococcus faecalis* ATCC 29212, *Enterococcus faecium*, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae*, *Listeria innocua*, *Listeria monocytogenes* ATCC 7644, *Pseudomonas aeruginosa* DSMZ 5071, *Pseudomonas fluorescence* P1, *Salmonella enteritidis* ATCC 13075, *Salmonella infantis*, *Salmonella kentucky*, *Salmonella typhimurium* SL 1344, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* DSMZ 20044, *Staphylococcus aureus* (MDR), *Escherichia coli* (MDR), *Klebsiella pneumoniae* (MDR), *Acinetobacter baumannii* (MDR), *Proteus vulgaris* (MDR), *Serratia odorifera* (MDR) ve *Streptococcus pneumoniae* (MDR) gibi Gram pozitif ve Gram negatif standart, gıdadan izole edilmiş bakteri suşları, mantar suşları ve klinik izole bazı çoklu ilaca dirençli (MDR) klinik izolat bakteriler kullanılarak disk difüzyon yöntemi ile araştırılmıştır.

Sonuç olarak, *M. esculenta*'nın etanol ekstraktının, test edilen Gram pozitif ve Gram negatif mikroorganizmalara karşı orta ila yüksek antimikrobiyal aktiviteye sahip olduğu, *T. versicolor*'un ise düşük ila yüksek antimikrobiyal aktivite gösterdiği görülmüştür.

Anahtar kelimeler: *Morchella esculenta*, *Trametes versicolor*, antimikrobiyal aktivite, disk difüzyon, çoklu ilaca dirençli bakteriler, MDR

Introduction

After long-time overuse and misuse of antibiotics, drug resistance will occur as a result of a bacterial genome and gene mutations (Klein et al., 2007). Drug resistance of bacterial strains are threat to the effective infection treatment, so research of new antimicrobial candidates, like fungi extracts, became important for the treatment of numerous infections (Sullivan et al., 2006).

Fungi have a potential of using both as nutritive and medicinal food stuff. Because of containing several therapeutic agents, they are reported to be used for hundreds of years to treat several diseases caused by bacteria, fungi, viruses and parasites. A tremendous progress has been made in human medicine in the last decades, but bacterial, fungal and viral diseases are still threatening the public health in the developing countries (Atila et al., 2017).

Morchella esculenta (L.) Pers. 1801 is used for both medicinal and nutritional purposes thanks to containing many biological substances, such as proteins, polysaccharides and vitamins (Litchfteld et al., 1963). The anti-inflammatory, anticancer, antioxidant and antimicrobial effect of *M. esculenta* has been reported previously (Heleno et al., 2013).

Trametes versicolor (L.) Lloyd 1921, one of the white-rot fungi, is a well-researched species. It has intracellular and extracellular enzymes, such as laccases and peroxidases and the richness of biological material constitutes the potential to be used in various fields (Marco-Urrea et al., 2009).

The aim of this study is to determine the in vitro antimicrobial activity of *M. esculenta* and *T. versicolor* against a wide range of microorganisms.

Material and method

Fungi samples

Morchella esculenta (L.) Pers. 1801 and *Trametes versicolor* (L.) Lloyd 1921 samples were collected from Abant, Bolu, TURKEY.

Extraction process

Air dried fungi samples and extracted by using ethanol through shaking at room temperature according to the procedure given previously by Altuner and Canli (2012).

Inoculum preparation

The antimicrobial activity of *M. esculenta* and *T. versicolor* were tested against *Bacillus subtilis* DSMZ 1971, *Candida albicans* DSMZ 1386, *Enterobacter aerogenes* ATCC 13048, *Enterococcus durans*, *Enterococcus faecalis* ATCC 29212, *Enterococcus faecium*, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae*, *Listeria innocua*, *Listeria monocytogenes* ATCC 7644, *Pseudomonas aeruginosa* DSMZ 5071, *Pseudomonas fluorescence* P1, *Salmonella enteritidis* ATCC 13075, *Salmonella infantis*, *Salmonella kentucky*, *Salmonella typhimurium* SL 1344, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* DSMZ 20044, *Staphylococcus aureus* (MDR), *Escherichia coli* (MDR), *Klebsiella pneumoniae* (MDR), *Acinetobacter baumannii* (MDR), *Proteus vulgaris* (MDR), *Serratia odorifera* (MDR) and *Streptococcus pneumoniae* (MDR).

Inoculum of each microorganism was prepared according to the procedure, which was given previously by Hammer et al. (1999) and Canli et al. (2016).

Antimicrobial activity test

Extract stocks were prepared according to Canli et al (2015), which are 101 mg/mL for *M. esculenta* and 36.25 mg/mL for *T. versicolor*. Three different volumes of extract 40, 80 and 150 µL of *M. esculenta* and *T. versicolor* extracts were loaded on empty sterile antibiotic disks according to the procedure given in previous studies (Canli et al., 2015; Altuner et al, 2012). The antimicrobial activity of samples was performed by the disk diffusion test as mentioned previously by Canli et al. (2017).

Positive and negative controls

Extraction solvent (ethanol) and empty sterile disks, and ciprofloxacin were used as negative and positive controls respectively.

Statistics

All tests were applied as triplicates. The data obtained from experimental work were analyzed by ANOVA test with $p = 0.05$ in order to present the significance of the difference between triplicates and Pearson's correlation coefficient was used to show, whether a correlation exists between the antimicrobial activity of the extract and increasing concentrations. R Studio, version 3.3.2 was used for statistical analysis (Core R Team, 2019).

Results

The diameters of inhibition zones, which were measured in millimeters, are given in Table 1 and Table 2 as the mean values of three replicates with standard errors. No activities were observed for the negative controls.

In addition, the ANOVA test showed that the difference between disk diffusion test results obtained from triplicates are not statistically significant ($p>0.05$). Pearson's correlation coefficient (0.2488) presented that there is a weak correlation between the antimicrobial activity of the extract and increasing concentrations.

As a result, it was observed that ethanol extracts of *M. esculenta* has medium to high antimicrobial activity against several Gram positive and Gram negative microorganisms tested, where *T. versicolor* presented low to high antimicrobial activity.

Table 1. Disk diffusion test results for *M. esculenta*

Microorganism	40 µL	80 µL	150 µL	Ciprofloxacin
B. subtilis DSMZ 1971	-	7.00 ± 0.00	8.00 ± 0.71	36.00 ± 0.00
C. albicans DSMZ 1386	-	-	8.00 ± 0.00	-
E. aerogenes ATCC 13048	-	7.00 ± 0.00	-	30.00 ± 0.00
E. durans	-	-	7.00 ± 0.00	24.00 ± 0.00
E. faecalis ATCC 29212	-	-	7.00 ± 0.00	19.00 ± 0.00
E. faecium	7.00 ± 0.00	10.00 ± 0.00	10.00 ± 0.71	29.00 ± 0.00
E. coli ATCC 25922	-	7.00 ± 0.00	-	-
E. coli (MDR)	7.00 ± 0.00	-	7.00 ± 0.00	-
K. pneumoniae	7.00 ± 0.00	7.00 ± 0.00	9.00 ± 0.71	30.00 ± 0.00
K. pneumoniae (MDR)	-	-	7.00 ± 0.00	-
L. innocua	-	7.00 ± 0.00	8.00 ± 0.00	18.00 ± 0.00
L. monocytogenes ATCC 7644	-	7.00 ± 0.00	8.00 ± 0.00	20.00 ± 0.00
P. aeruginosa DSMZ 5071	-	7.00 ± 0.00	-	28.00 ± 0.00
P. fluorescence P1	-	-	9.00 ± 0.00	19.00 ± 0.00
S. enteritidis ATCC 13075	-	7.00 ± 0.00	7.00 ± 0.00	36.00 ± 0.00
S. infantis	-	7.00 ± 0.00	7.00 ± 0.00	24.00 ± 0.00
S. kentucky	-	-	7.00 ± 0.00	34.00 ± 0.00
S. typhimurium SL 1344	-	-	7.00 ± 0.00	35.00 ± 0.00
S. aureus ATCC 25923	-	7.00 ± 0.00	8.00 ± 0.00	22.00 ± 0.00
S. aureus (MDR)	-	8.00 ± 0.00	8.00 ± 0.00	22.00 ± 0.00
S. epidermidis DSMZ 20044	-	10.00 ± 0.00	9.00 ± 0.71	34.00 ± 0.00
Acinetobacter baumannii (MDR)	-	-	-	-
Proteus vulgaris (MDR)	-	9.00 ± 0.00	13.00 ± 0.00	-
Serratia odorifera (MDR)	-	-	-	-
S. pneumoniae (MDR)	-	8.00 ± 0.00	10.00 ± 0.00	-

"-": No activity

Table 2. Disk diffusion test results for *T. versicolor*

Microorganism	40 µL	80 µL	150 µL	Ciprofloxacin
B. subtilis DSMZ 1971	-	7.00 ± 0.00	7.00 ± 0.71	36.00 ± 0.00
C. albicans DSMZ 1386	-	-	7.00 ± 0.00	-
E. aerogenes ATCC 13048	-	-	-	30.00 ± 0.00
E. durans	-	-	-	24.00 ± 0.00
E. faecalis ATCC 29212	-	-	7.00 ± 0.00	19.00 ± 0.00
E. faecium	-	7.00 ± 0.00	14.00 ± 0.00	29.00 ± 0.00
E. coli ATCC 25922	-	7.00 ± 0.00	-	-
E. coli (MDR)	-	-	-	-
K. pneumoniae	-	7.00 ± 0.00	-	30.00 ± 0.00
K. pneumoniae (MDR)	-	-	7.00 ± 0.00	-
L. innocua	-	7.00 ± 0.00	8.00 ± 0.00	18.00 ± 0.00
L. monocytogenes ATCC 7644	-	7.00 ± 0.00	8.00 ± 0.00	20.00 ± 0.00
P. aeruginosa DSMZ 5071	-	9.00 ± 0.00	7.00 ± 0.00	28.00 ± 0.00
P. fluorescence P1	-	-	8.00 ± 0.00	19.00 ± 0.00
S. enteritidis ATCC 13075	-	-	7.00 ± 0.00	36.00 ± 0.00
S. infantis	-	-	-	24.00 ± 0.00
S. kentucky	-	-	7.00 ± 0.00	34.00 ± 0.00
S. typhimurium SL 1344	-	-	-	35.00 ± 0.00
S. aureus ATCC 25923	-	-	8.00 ± 0.00	22.00 ± 0.00
S. aureus (MDR)	-	-	8.00 ± 0.00	22.00 ± 0.00
S. epidermidis DSMZ 20044	-	9.00 ± 0.00	8.00 ± 0.00	34.00 ± 0.00
Acinetobacter baumannii (MDR)	-	-	-	-
Proteus vulgaris (MDR)	-	-	-	-
Serratia odorifera (MDR)	-	-	7.00 ± 0.00	-
S. pneumoniae (MDR)	-	-	7.00 ± 0.00	-

"-": No activity

Discussion

Kalyoncu et al. (2010) analyzed antimicrobial activity of *M. esculata* ethanol extract against 11 microorganisms and found low antimicrobial activity against *S. aureus*, *S. lutea*, *S. typhimurium* and *C. albicans*. As these results are compared to our study, it can be observed that more microorganism strains were affected in our study. The reason for the difference is thought to be that the samples were collected from different localities and the strains were different.

Although Venturini et al. (2008) studied the antibacterial activity of the different extracts of *M. esculata* against 5 strains, which are *E. coli*, *S. enteritidis*, *Shigella sonnei*, *Vibrio parahaemolyticus* and *Yersinia enterocolitica*, and they only found an activity against *Y. enterocolitica* with only the aqueous extract. The reason of the differences between the results of this study and our study is most probably due to the difference in the strains, the extraction solvents and the collection localities of fungi samples used in these two studies.

Yamaç and Bilgili (2006) analyzed antimicrobial activity of chloroform, ethyl acetate, acetone, dichloromethane, and ethanol extracts of *T. versicolor* against 9 strains (*E. coli*. ATCC 25922, *E. aerogenes*. NRRL-B-3567, *S. typhimurium*. NRRL-B-4440, *P. aeruginosa*. ATCC 27853, *S. aureus*. ATCC 25923, *S. epidermidis*. NRRL-B-4377, *B. subtilis*. NRRL-B-558, *C. albicans*. ATCC 10259 and *Saccharomyces cerevisiae*. NRRL-Y-2034). They observed activity against *E. aerogenes* with an inhibition zone between 10 and 15 mm, and *S. aureus* and *S. cerevisiae* with inhibition zones lower than 10 mm. The results about the activity against *S. aureus* are similar to our results, but although they have observed antimicrobial activity against *E. aerogenes*, in our study there wasn't any activity against the same strain. This difference is acceptable because the *E. aerogenes* strains used in these two studies were different.

It is known that Gram negative microorganisms are the dominant killers in the Intensive Care Units (ICU) (Villegas and Quinn, 2004). *Klebsiella* is one of these Gram negative bacteria that cause death in ICUs (Villegas and Quinn, 2004). Therefore, antimicrobial activity against *K. pneumoniae*, especially against multi drug resistant *K. pneumoniae* have great importance. In our study, we observed antimicrobial activity against both standard and MDR *K. pneumoniae* for *M. esculenta* and *T. versicolor*.

As a result, it can be concluded that the antimicrobial activity of *M. esculenta* especially observed against *E. faecium*, *S. epidermidis*, *K. pneumoniae* (MDR), *P. vulgaris* (MDR) and *S. pneumoniae* (MDR), and the antimicrobial activity of *T. versicolor* especially observed against *E. faecium*, *S. odorifera* (MDR) and *S. pneumoniae* (MDR) can be found to be remarkable.

It is possible to recommend that further researches are needed to purify and determine the active biological substances and their mechanism of actions.

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