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In vitro Antimicrobial Activity of *Desarmillaria tabescens*

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Abstract: Mushrooms are known to be nutritive and medicinal food stuff, which are good sources of some vitamins and essential minerals. They also contain some therapeutic agents, thus they have been used against several health problems for hundreds of years. The aim of this study is to determine the *in vitro* antimicrobial activity of *Desarmillaria tabescens* (Scop.) R.A. Koch & Aime 2017.

D. tabescens samples were air dried and extracted by using ethanol. Antimicrobial activity of *D. tabescens* 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) and *Streptococcus pneumoniae* (MDR) by using the disk diffusion method.

As a result, it was observed that ethanol extracts of *D. tabescens* has low to medium antimicrobial activity against several Gram positive and Gram negative microorganisms tested. The antimicrobial activity of *D. tabescens* especially observed against *K. pneumoniae* (MDR) and *S. pneumoniae* (MDR) is found to be remarkable.

Key words: *Desarmillaria tabescens*, antimicrobial activity, disk diffusion, multi drug resistant bacteria, MDR

Desarmillaria tabescens'in *in vitro* Antimikrobiyal Aktivitesi

Öz: Mantarların, bazı vitaminlerin ve temel minerallerin kaynağı olan besleyici ve tıbbi gıda maddeleri olduğu bilinmektedir. Ayrıca bazı terapötik maddeler içerirler, bu yüzden yüzlerce yıldır çeşitli sağlık problemlerine karşı kullanılmıştır. Bu çalışmanın amacı *Desarmillaria tabescens* (Scop.) R.A. Koch & Aime 2017'nin *in vitro* antimikrobiyal aktivitesini belirlemektir.

D. tabescens örnekleri kurutulmuş ve etanol kullanılarak ekstre edilmiştir. *D. tabescens* 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) 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 yöntemini ile araştırılmıştır.

Sonuç olarak, *D. tabescens*'in etanol ekstraktlarının, test edilen bazı Gram pozitif ve Gram negatif mikroorganizmaya karşı düşük ila orta antimikrobiyal aktiviteye sahip olduğu gözlenmiştir. *D. tabescens*'in özellikle *K. pneumoniae* (MDR) ve *S. pneumoniae*'ye (MDR) karşı gözlenen antimikrobiyal aktivitesinin dikkat çekici olduğu bulunmuştur.

Anahtar kelimeler: *Desarmillaria tabescens*, antimikrobiyal aktivite, disk difüzyon, çoklu ilaca dirençli bakteriler, MDR

Introduction

Mushrooms are known to be medicinal and nutritive food stuff, which are good sources of some vitamins, such as vitamin B and vitamin D, and some essential minerals, such as selenium (Bonatti et al., 2004; Agrahar-Murugkar and Subbulakshmi, 2005; Cheung and Cheung, 2005; Imtiaj and Lee, 2007; Falandysz, 2008; Watanabe et al., 2014; Cardwell et al., 2018). In addition, they contain some therapeutic agents, thus they have been used against several health problems for hundreds of years, as antibacterial and antifungal agents against several infectious diseases, anti-hypertensives, anti-arrhythmic agents, medications for asthma, anti-neoplastic drugs, analgesics and anti-inflammatory drugs (Clardy and Walsh, 2004; Webster et al., 2008; Canli et al., 2016a,b).

Antibiotics are known as the compounds, which are in use for preventing and treating bacterial diseases, but unfortunately bacteria have capability of changing their responses to antibiotics, which will led to antibiotic resistance (WHO, 2019). World Health Organization (WHO) (WHO, 2019) stated that the resistance to commonly used antibiotics is increasing tremendously all over the world and this causes a lack in treating common infections, since these antibiotics become less effective day by day. As a result of this, scientists are intensively working on determining new antibiotic candidates (Paudel et al., 2008; Altuner et al., 2014).

Starting with the discovery of penicillin by Fleming from a fungi, namely Penicillium, scientists interested in the antimicrobial potential of fungi in determining new antibiotic candidates (Bala et al., 2011). Until now, several compounds originating from fungi have been isolated and identified by researchers, which presented biological activities such as antimicrobial, antiviral, antidiabetic, anti-inflammatory, anti-fibrotic, liver protective and immune modulatory (Dülger et al., 1999; Gunde-Cimerman, 1999; Wasser and Weis, 1999a,b; Dulger et al., 2005; Ooi, 2010).

In this study the antimicrobial activity of *Desarmillaria tabescens* (Scop.) R.A. Koch & Aime 2017is investigated 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) ve *Streptococcus pneumoniae* (MDR) by using the disk diffusion method.

Material and Method

Macrofungi samples

The samples of *Desarmillaria tabescens* (Scop.) R.A. Koch & Aime 2017 in order to use in these experiments were supplied through a field study in Belgrad Forest, İstanbul, TURKEY. As a reference *D. tabescens* sample was kept in Biology Department of Ankara University.

Extraction of active compounds

Air dried macrofungi were ground and active compounds were extracted by using ethanol (Merck, Germany) through shaking. A filtration process was followed by using filter paper (Whatman No. 1) and ethanol was removed at low temperature (30°C) by a rotary evaporator (Heidolph Hei-Vap Value HL/HB-G1) (Altuner and Canli, 2012). The residue was used for preparing stock for extract (54.69 mg/mL).

Microorganism inocula

The list of microorganisms used is given in Table 1.

Table 1. The list of microorganisms used in the study

Microorganism	Strain/Isolate
Bacillus subtilis	DSMZ 1971
Candida albicans	DSMZ 1386
Enterobacter aerogenes	ATCC 13048
Enterococcus durans	Food isolate
Enterococcus faecalis	ATCC 29212
Enterococcus faecium	Food isolate
Escherichia coli	ATCC 25922
Escherichia coli (MDR)	Clinical isolate
Klebsiella pneumoniae	Food isolate
Klebsiella pneumoniae (MDR)	Clinical isolate
Listeria innocua	Food isolate
Listeria monocytogenes	ATCC 7644
Pseudomonas aeruginosa	DSMZ 5071
Pseudomonas fluorescence	P1
Salmonella enteritidis	ATCC 13075
Salmonella infantis	Food isolate
Salmonella kentucky	Food isolate
Salmonella typhimurium	SL 1344
Staphylococcus aureus	ATCC 25923
Staphylococcus aureus (MDR)	Clinical isolate
Staphylococcus epidermidis	DSMZ 20044
Staphylococcus pneumoniae (MDR)	Clinical isolate

The incubation conditions were 24 hours - 37 ˚C, and 48 hours - 27 ˚C for bacteria and *C. albicans* respectively. Inoculum for each microorganism was prepared in 0.9% sterile saline solution and the turbidity of all inocula were adjusted according to 0.5 McFarland standard (Hammer et al., 1999; Altuner et al., 2012; Canli et al., 2016c).

Antimicrobial activity test

In order to determine the antimicrobial activity of *D. tabescens*, a very commonly used test, namely disk diffusion test was chosen (Andrews, 2007). Three different volumes of extract (50, 100 and 200 µL) were loaded on 6 mm diameter sterile paper disks (Mahasneh and El-Oqlah,

1999; Silici and Koc, 2006). Ethanol in the extract was removed by leaving the disks in a sterile environment at a low temperature (30°C) for about 8 hours (Silici and Koc, 2006; Altuner et al., 2014). Right after the disks were completely dried out, inoculum of each microorganism was transferred on Müller Hinton Agar (MHA) plates and disks were placed on the plate surfaces. After incubation of MHA plates at suitable time and temperature combinations defined previously, the inhibition zones were measured by a ruler and recorded in millimeters (Onbasili et al., 2011)

Positive and negative controls

Ethanol loaded disks and empty sterile disks, and ciprofloxacin were used as negative and positive controls respectively.

Statistics

All tests were applied as triplicates and results were analyzed by the ANOVA test with p = 0.05 and Pearson's correlation coefficient was used to put forward any correlation 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

Table 2 clearly shows the disk diffusion test results for *D. tabescens* ethanol extract, which are the arithmetic means of triplicates with standard errors.

Ethanol and empty sterile disks, which are negative controls didn't present any activity. In addition, the ANOVA test showed that the difference between disk diffusion test results obtained from triplicates is not statistically significant ($p>0.05$). Pearson's correlation coefficient (0.0632) presented that there is a very weak correlation between the antimicrobial activity of the extract and increasing concentrations.

Table 2. The disk diffusion test results for *D. tabescens* ethanol extract

Microorganism	50 µL	100 µL	200 µL	Ciprofloxacin
B. subtilis DSMZ 1971	-	-	-	36.00 ± 0.00
C. albicans DSMZ 1386	-	-	-	-
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	-	8.00 ± 0.71	9.00 ± 0.00	29.00 ± 0.00
E. coli ATCC 25922	-	-	-	-
E. coli (MDR)	-	-	-	-
K. pneumoniae	-	7.00 ± 0.00	7.00 ± 0.71	30.00 ± 0.00
K. pneumoniae (MDR)	9.00 ± 0.00	9.00 ± 0.00	9.00 ± 0.00	-
L. innocua	-	-	-	18.00 ± 0.00
L. monocytogenes ATCC 7644	-	-	-	20.00 ± 0.00
P. aeruginosa DSMZ 5071	-	7.00 ± 0.00	7.00 ± 0.71	28.00 ± 0.00
P. fluorescence P1	-	-	-	19.00 ± 0.00
S. enteritidis ATCC 13075	-	-	7.00 ± 0.00	36.00 ± 0.00
S. infantis	-	-	-	24.00 ± 0.00
S. kentucky	-	-	-	34.00 ± 0.00
S. typhimurium SL 1344	-	-	-	35.00 ± 0.00
S. aureus ATCC 25923	7.00 ± 0.00	7.00 ± 0.00	8.00 ± 0.00	22.00 ± 0.00
S. aureus (MDR)	-	-	9.00 ± 0.00	22.00 ± 0.00
S. epidermidis DSMZ 20044	-	-	-	34.00 ± 0.00
S. pneumoniae (MDR)	-	-	7.00 ± 0.00	-

"-": No activity

According to the data given in Table 2, 50 µL ethanol extract of *D. tabescens* presented activity against only *K. pneumoniae* (MDR) and *S. aureus* ATCC 25923 with inhibition zones of 9 mm and 7 mm respectively. 100 µL ethanol extract of *D. tabescens* showed antimicrobial activity against *E. faecium*, *K. pneumoniae*, *K. pneumoniae* (MDR), *P. aeruginosa* DSMZ 5071 and *S. aureus* ATCC 25923 with inhibition zones ranging between 7 mm and 9 mm. In addition, 100 µL ethanol extract of *D. tabescens* presented antimicrobial activity against *E. faecalis* ATCC 29212, *E. faecium*, *K. pneumoniae*, *K. pneumoniae* (MDR), *P. aeruginosa* DSMZ 5071, *S. enteritidis* ATCC 13075, *S. aureus* ATCC 25923, *S. aureus* (MDR) and *S. pneumoniae* (MDR) with inhibition zones ranging between 7 mm and 9 mm.

Discussion

According to the results, it was observed that ethanol extracts of *D. tabescens* has low to medium antimicrobial activity against several Gram positive and Gram negative microorganisms tested. There are limited studies in the literature about the antimicrobial activity of *Armillaria tabescens* (Scop.) Emel, which is the synonym of *D. tabescens*.

Previous studies showed that *A. tabescens* contains several protoilludane sesquiterpene aryl esters, which have some biological activities (Donnelly et al., 1997).

Dundar et al. (2015) tested the methanol extract of *A. tabescens* against *E. coli* ATCC 10536, *S. aureus* ATCC 6538, *B. subtilis* ATCC 6051, *Enterococcus hirae* ATCC 10541, *Micrococcus luteus* ATCC 9341 and *P. aeruginosa* ATCC 9027 by disk diffusion test and observed antimicrobial activity against *E. coli* ATCC 10536 with an inhibition zone of 5.00 ± 0.87 mm, *B. subtilis* ATCC 6051 with an inhibition zone of 3.00 ± 0.54 mm, *E. hirae* ATCC 10541 with an inhibition zone of 2.00 ± 0.72 mm and *M. luteus* ATCC 9341 with an inhibition zone of 4.00 ± 0.65 mm. In addition they didn't observe activity against *S. aureus* ATCC 6538 and *P. aeruginosa* ATCC 9027.

In our study, we observed an antimicrobial activity against *S. aureus* ATCC 6538 with inhibition zones of either 7 mm or 8 mm depending on the amount of extract used, in addition we observed 9.00 ± 0.00 mm inhibition zone against *S. aureus* (MDR).

In contrary to Dundar et al. (2015), we haven't observed any antimicrobial activity against *E. coli* ATCC 25922, *E. coli* (MDR); but as Dundar et al. (2015) observed, antimicrobial activity wasn't determined against *B. subtilis* and *P. aeruginosa* in our study.

Bandara Herath et al. (2013) tested the antimicrobial activity of ethyl acetate extract of *A. tabescens* against *C. albicans* ATCC 90028, *Candida glabrata* ATCC 90030, *Cryptococcus neoformans* ATCC 90113, *Aspergillus fumigatus* ATCC 204305, *S. aureus* ATCC 29213, methicillin-resistant *S. aureus* ATCC 33591 (MRSA), *E. coli* ATCC 35218, *P. aeruginosa* ATCC 27853 and *Mycobacterium intracellulare* ATCC 23068, and observed antimicrobial activity against *C. albicans* ATCC 90028, *C. neoformans* ATCC 90113, *E. coli* ATCC 35218 and *M. intracellulare* ATCC 23068, which are contrary to our observations.

The differences in these results can be explained as the microorganisms used in these two studies were not the same strains. Also since the extraction solvents used in previous two studies were not the same with the one used in our study; the extracted compounds, thus the results are different.

When the results are compared with the results obtained from positive control, ciprofloxacin, they can be clearly found to be lower than the inhibition zones obtained from ciprofloxacin. Isolation and purification of active compounds from *D. tabescens* and applying these compounds separately on microorganisms with higher concentrations could possibly increase the activity. Although the results are lower than ciprofloxacin, two results, which are the activity against *K. pneumoniae* (MDR) and *S. pneumoniae* (MDR) is found to be remarkable, because *D. tabescens* extract presented antimicrobial activity against these multi drug resistant strains, while ciprofloxacin didn't.

As a result, our study clearly presents that *D. tabescens* have antimicrobial activity, but further researches are needed in order to analyze active substances and their activity mechanisms in details.

References

Agrahar-Murugkar, D., and Subbulakshmi, G. (2005). Nutritional value of edible wild mushrooms collected from the Khasi hills of Meghalaya. *Food Chemistry*, *89*(4), 599-603.

Altuner, E. M., Akata, I., and Canlı, K. (2012). In vitro Antimicrobial Activity Screening of Bovista nigrescens Pers. *Kastamonu Üniversitesi Orman Fakültesi Dergisi*, *12*(1), 90-96.

Altuner, E. M., and Canli, K. (2012). In vitro antimicrobial screening of Hypnum andoi AJE Sm. *Kastamonu Üniversitesi Orman Fakültesi Dergisi*, *12*(1), 97-101.

Altuner, E. M., Canli, K., and Akata, I. (2014). Antimicrobial screening of Calliergonella cuspidata, Dicranum polysetum and Hypnum cupressiforme. *Journal of Pure and Applied Microbiology*, *8*(1), 539-545.

Andrews, J. M. (2007). BSAC standardized disc susceptibility testing method (version 6). *Journal of antimicrobial chemotherapy*, *60*(1), 20-41.

Bala, N., Aitken, E. A., Fechner, N., Cusack, A., and Steadman, K. J. (2011). Evaluation of antibacterial activity of Australian basidiomycetous macrofungi using a high-throughput 96-well plate assay. *Pharmaceutical biology*, *49*(5), 492-500.

Bonatti, M., Karnopp, P., Soares, H. M., and Furlan, S. A. (2004). Evaluation of Pleurotus ostreatus and Pleurotus sajor-caju nutritional characteristics when cultivated in different lignocellulosic wastes. *Food chemistry*, *88*(3), 425-428.

Canli, K., Akata, I., and Altuner, E. M. (2016b). In vitro antimicrobial activity screening of Xylaria hypoxylon. *African Journal of Traditional, Complementary and Alternative medicines*, *13*(4), 42-46.

Canli, K., Altuner, E. M., Akata, I., Turkmen, Y., and Uzek, U. (2016a). In vitro antimicrobial screening of Lycoperdon lividum and determination of the ethanol extract composition by gas chromatography/mass spectrometry. *Bangladesh Journal of Pharmacology*, *11*(2), 389-394.

Canli, K., Yetgin, A., Akata, I., and Altuner, E. M. (2016c). In vitro antimicrobial screening of Aquilaria agallocha roots. *African Journal of Traditional, Complementary and Alternative Medicines*, *13*(5), 178-181.

Cardwell, G., Bornman, J., James, A., and Black, L. (2018). A review of mushrooms as a potential source of dietary vitamin D. *Nutrients*, *10*(10), 1498.

Cheung, L. M., and Cheung, P. C. (2005). Mushroom extracts with antioxidant activity against lipid peroxidation. *Food Chemistry*, *89*(3), 403-409.

Clardy, J., and Walsh, C. (2004). Lessons from natural molecules. *Nature*, *432*(7019), 829-837.

Core R Team, R: A language and environment for statistical computing. R Foundation for Statistical Computing, https://www.R-project.org/ (last accession: 15.08.2019).

Donnelly, D. M., Konishi, T., Dunne, O., and Cremin, P. (1997). Sesquiterpene aryl esters from Armillaria tabescens. *Phytochemistry*, *44*(8), 1473-1478.

Dülger, B., Fedai, Ş. E. N., and Gücin, F. (1999). Antimicrobial Activity of The Macrofungi Russula delica Fr. *Turkish Journal of Biology*, *23*(1), 127-134.

Dulger, B., Suerdem, T. B., Yesilyurt, D., Hacioglu, N., and Camdeviren, A. (2005). Evaluation of antimicrobial activity of the macrofungus Phellinus torulosus. *Journal of Biological Sciences*, *5*(4), 436-439.

Dundar, A., Okumus, V., Ozdemir, S., Celik, K. S., Boga, M., Ozcagli, E., Ozhan, G., and Yildiz, A. (2015). Antioxidant, antimicrobial, cytotoxic and anticholinesterase activities of seven mushroom species with their phenolic acid composition. *Journal of Horticulture*, *2*(4), 1000161.

Falandysz, J. (2008). Selenium in edible mushrooms. *Journal of Environmental Science and Health Part C*, *26*(3), 256-299.

Gunde-Cimerman, N. (1999). Medicinal value of the genus Pleurotus (Fr.) P. Karst.(agaricales sl, Basidiomycetes). *International Journal of Medicinal Mushrooms*, *1*(1), 69-80.

Hammer, K. A., Carson, C. F., and Riley, T. V. (1999). Antimicrobial activity of essential oils and other plant extracts. *Journal of applied microbiology*, *86*(6), 985-990.

Herath, H. B., Jacob, M., Wilson, A. D., Abbas, H. K., and Nanayakkara, N. D. (2013). New secondary metabolites from bioactive extracts of the fungus Armillaria tabescens. *Natural product research*, *27*(17), 1562-1568.

Imtiaj, A., and Lee, T. S. (2007). Screening of antibacterial and antifungal activities from Korean wild mushrooms. *World journal of agricultural sciences*, *3*(3), 316-321.

Mahasneh, A. M., and El-Oqlah, A. A. (1999). Antimicrobial activity of extracts of herbal plants used in the traditional medicine of Jordan. *Journal of Ethnopharmacology*, *64*(3), 271-276.

Onbasili, D., Altuner, E. M., and Çelik, G. Y. (2011). Mnium marginatum özütlerinin antimikrobiyal aktivitesi. *Kastamonu Üniversitesi Orman Fakültesi Dergisi*, *11*(2), 205-208.

Ooi, V.E.C. (2010) *Medicinally important fungi*. In: Van Griensven editor. Science and cultivation of edible fungi, Rotterdam: Balkema.

Paudel, B., Bhattarai, H. D., Lee, J. S., Hong, S. G., Shin, H. W., and Yim, J. H. (2008). Antibacterial potential of Antarctic lichens against human pathogenic Gram-positive bacteria. *Phytotherapy research*, *22*(9), 1269-1271.

Silici, S., and Koc, A. N. (2006). Comparative study of in vitro methods to analyse the antifungal activity of propolis against yeasts isolated from patients with superficial mycoses. *Letters in applied microbiology*, *43*(3), 318-324.

Wasser, S. P., and Weis, A. L. (1999a). Medicinal properties of substances occurring in higher basidiomycetes mushrooms: current perspectives. *International Journal of medicinal mushrooms*, *1*(1), 31-62.

Wasser, S. P., and Weis, A. L. (1999b). Therapeutic effects of substances occurring in higher Basidiomycetes mushrooms: a modern perspective. *Critical Reviews™ in Immunology*, *19*(1), 65-96.

Watanabe, F., Yabuta, Y., Bito, T. and Teng, F. (2014). Vitamin B12-containing plant food sources for vegetarians. *Nutrients*, *6*(5), 1861-1873.

Webster, D., Taschereau, P., Belland, R. J., Sand, C., and Rennie, R. P. (2008). Antifungal activity of medicinal plant extracts; preliminary screening studies. *Journal of Ethnopharmacology*, *115*(1), 140-146.

WHO, Antibiotic resistance, https://www.who.int/news-room/fact-sheets/detail/antibiotic-resistance (last accession: 15.08.2019).