EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 4
Issue: 4
XX-XX
Year: 2017

ANTIMICROBIAL ACTIVITY SCREENING OF *ALLIUM TUNCELIANUM*

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Article Received on 23/01/2017 **Article Revised on 13/02/2017** **Article Accepted on 05/03/2017**

ABSTRACT
Allium tuncelianum is locally cultivated in Tunceli, Turkey and it is an endemic garlic species. Antimicrobial activity of *A. tuncelianum* was tested against nine gram negative and eight gram positive bacteria (17 bacteria) including species of Bacillus, Enterobacter, Enterococcus, Escherichia, Klebsiella, Listeria Pseudomonas, Salmonella and Staphylococcus. By using *Candida albicans*, antifungal activity against also was investigated. Three different processes, namely chopping, freezing and silencing were applied before extraction and the antimicrobial activity of *A. tuncelianum* ethanol extract was analysed with disk diffusion method. The results were presented that *A. tuncelianum* has important antimicrobial potential against *C. albicans* DSMZ 1386, *B. subtilis* DSMZ 1971 and *E. faecium*, but its anti-candidal activity is greater than antibacterial activity.

KEYWORDS: *Allium tuncelianum*, garlic, antimicrobial activity, disk diffusion test, bacterial strains, fungal strains.

INTRODUCTION
Natural antibiotic materials research became an important subject because of developing resistance of the bacteria, which cause infective diseases, against commonly used antibiotics.[1] Since ancient times several diseases have been treated with natural products, such as plants. Today, this treatment has become critical for many developing countries.[2] Garlic is the most significant of them and it is used as medicine since ancient times. First experiment was applied by Pasteur, but antibacterial, antifungal, antiprotozoal and antiviral activity is determined at late numerous studies.[3,4] Allicin, which is diallil thiosulfate, one of the garlic components, however it is only released when garlic is chopped and allinase enzyme reacts with allinin. Antibacterial and antifungal activity is related to allicin – SH's (sulfhydryl group) specific linkage feature.[5]

Allium tuncelianum is an endemic species, which is generally produced at Munzur Mountains (Ovacık region) and it is used for cooking, but this increases the risk of extinction. For this reason, this endemic plant must be protected and related research should be supported. It has only one clove and it can be stored at 18-20^{0}C. Organosulfur compounds of *Allium tuncelianum* have inhibition activity against liver diseases, cardiovascular diseases and neurological disorders.[6]

Although the antimicrobial activity of many garlic species were determined until today, but the broad range antimicrobial activity of *A. tuncelianum* hasn't been analyzed before by disk diffusion method. Previously it was proved that chopped garlic clove has higher antimicrobial activity than frozen and sliced clove, because of allicin is released critically after chopping process. Therefore the effect of *A. tuncelianum* was firstly analyzed after these 3 different processes against 17 bacteria and 1 fungi.

MATERIALS AND METHODS
Obtaining of plant materials
Allium tuncelianum was obtained from Ovacık area of Tunceli (Turkey). Fresh garlic samples are used for experiment and it was free of any chemical treatment.

Ethanol extracts of garlic
Chopped, frozen and sliced garlics were prepared. Garlic samples were chopped into small pieces with grinder; were sliced just into two with knife; were frozen with ultra-freezer, then it was ground immediately by cold grinder (TF). In all processes 40 g of garlic was used and these samples were shaken in ethanol (Sigma-Aldrich) at 125 rpm for 2 days at room temperature.[7,8] After that, all of them were filtrated through Whatman No.1 filter paper into evaporation flasks. Filtrates were evaporated by a rotary evaporator (Buchi R3) at 45°C.[9] Finally,

remnants were collected and it is used to prepare to 303.75, 607.5 and 656.25 µg.

Test microorganisms

17 bacteria and 1 fungi species was used and these microorganism was sustained on Nutrient Agar (BD Difco, USA). There are 11 standard bacteria and 1 standard fungi. Five of them are standard gram positive bacteria, which are *Bacillus subtilis* DSMZ 1971, *Enterecoccus faecalis* ATCC 29212, *Listeria monocytogenes* ATCC 7644, *Staphylococcus aureus* ATCC 25923 and *Staphylococcus epidermidis* DSMZ 20044. The others are standard gram negative bacteria, which are *Enterobacter aerogenes* ATCC 13048, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* DSMZ 50071, *Pseudomonas fluorescens* P1, *Salmonella enteritidis* ATCC 13075 and *Salmonella typhimurium* SL1344. There is 1 standard fungi, which is *Candida albicans* DSMZ 1386. Besides, there are 6 non-standard bacteria, which are isolated from food at Ankara University microbiology laboratory. Three of them are gram positive bacteria, which are *Enterococcus durans*, *Enterecoccus faecium* and *Listeria innocua*. The others are gram negative bacteria, which are *Klebsiella pneumonia*, *Salmonella infantis* and *Salmonella kentucky*.

Preparation of Inocula

All bacterial strain were incubated at 37 °C for 24 hours, however *Candida albicans* DSMZ 1386 was incubated at 27°C for 48 hours.[10,11] Each bacteria and yeast were inoculated into 0.9% sterile saline solution and adjusted to 0.5 McFarland standard, in order to standardize inocula to contain about 10^8 cfu.mL^{-1} for bacteria and 10^7 cfu.mL^{-1} for *Candida albicans*.[12]

Antimicrobial activity test

The antimicrobial activity of garlic ethanol extract was performed by disk diffusion test, as described by Andrews (2003).[13] Firstly, Mueller Hinton Agar (BD Difco, USA) was poured into 90 mm sterile Petri dish in order to reach a meant depth of 4.0 mm ±0.5mm. 94.5, 189 and 262.5 µg of extracts were loaded on 6 mm Oxoid Antimicrobial Susceptibility Test Disks. Disks were left to dry overnight at 30°C in sterile conditions in order to prevent any remaining of solvent, which may interfere with the results. After that, prepared microorganisms, which were inoculated into saline solution were streaked on the surface of petri dishes. These plates were left to dry for 5 minutes at room temperature in aseptic conditions.[12] Next, disks were tightly applied to the surface of plates. Finally, these plates were incubated and inhibition zone diameters were observed.

Controls

Empty sterile disks and extraction solvent (ethanol) were used as negative controls.

Statistics

The statistical analysis was executed using a non-parametric method Kruskal-Wallis which is one-way analysis of variance with $p < 0.05$.

RESULTS AND DISCUSSION

Antimicrobial activity of *Allium tuncelianum* cloves ethanol extracts were analyzed. In order to load extracts, empty sterile disks were used, then these disks were applied on a Mueller Hinton Agar (culture medium), which was inoculated with microorganisms. Inhibition zone was observed, when the extracts had activity against these microorganisms. The diameter of these zones were measured as diameters in millimetres as given in Table 1. No activity for empty sterile disks and ethanol loaded on disks and evaporated before application, which are negative controls, were observed.

The diameter of inhibition zones for *A. tuncelianum* is given in Table 1. Tunceli- chopped garlic (*TC*) has antimicrobial activity against 5 bacteria, however Tunceli-frozen garlic (*TF*) has antimicrobial activity against 5 bacteria and Tunceli-sliced garlic (*TS*) has antimicrobial activity against 4 bacteria. According to table 1, *TC* and *TF* has moderate antimicrobial activity against *B. subtilis* DSMZ 1971 (14 and 12 mm respectively) and low antimicrobial activity against *E. faecium* (12 and 9 mm respectively at 656.25 µg). Furthermore, *TC* has great (17 mm), *TD* has moderate (13 mm) and *TS* less (10 mm) antifungal activity against *Candida albicans* DSMZ 1385. These results demonstrate that freezing slightly affected the antimicrobial activity of *A. tuncelianum*, however slicing negatively affected its activity.

Table 1. Disk diffusion test result for *Allium tuncelianum* (Inhibition zones in mm).

	TC			***TF***			***TS***		
	1.	**2.**	**3.**	**1.**	**2.**	**3.**	**1.**	**2.**	**3.**
B. subtilis DSMZ 1971	10	14	14	9	11	12	-	-	-
C. albicans DSMZ 1386	11	18	17	8	10	13	-	-	10
E. aerogenes ATCC 13048	-	-	-	-	-	-	-	-	7
E. durans	-	-	-	-	-	-	-	-	-
E. faecalis ATCC 29212	-	-	-	-	-	-	-	-	-
E. faecium	7	10	12	-	9	9	-	-	-
E. coli ATCC 25922	-	-	-	-	-	-	-	-	-
K. pneumonia	7	8	7	7	7	7	7	7	7
L. innocua	-	-	-	-	-	-	-	-	-

L. monocytogenes ATCC 7644	-	-	-	-	-	-	-	-	-
P. aeruginosa DSMZ 50071	-	-	-	-	-	-	-	-	-
P. fluorescens P1	-	-	-	-	-	-	-	-	-
S. enteritidis ATCC 13075	-	7	7	-	-	-	-	-	7
S. infantis	-	-	-	-	-	-	-	-	-
S. kentucky	-	-	-	-	-	-	-	-	-
S. typhimurium SL1344	7	7	7	-	-	7	7	7	7
S. aureus ATCC 25923	-	-	-	-	7	7	-	-	-
S. epidermidis DSMZ 20044	-	-	-	-	-	-	-	-	-

"-": No activity observed.

In the USA, Candida species are the fourth important nosocomial infection and it has 50% mortality rate.[14] Also, 75% of oral cavity population has *C. albicans*, which cause two critical human infection such as superficial infections and life-threatening systemic infections and it is called oral candidiasis.[15] According to our analyses, *A. tuncelianum* can be used specifically for oral cavity treatment.

According to Taşkın et al.[16], 10% ethanol extract of *A. tuncelianum* has moderate activity against *C. albicans* (12 mm); low level activity against *S. aureus* (10 mm), *B. subtilis* (6 mm) and *K. pneumoniae* (7 mm), but present no activity against *P. aeruginosa*. This research demonstrated that ethanol extract of *A. tuncelianum* has more antimicrobial activity than water and ether extracts, therefore ethanol extract is used in our analyses. Besides, Taşkın et al, determined that anticandidal activity is greater than antibacterial activity, which also supports our results.

Due to antimicrobial resistance (tolerance; intrinsic and acquired resistance), Enterococcus genus, especially *E. faecalis* and *E. faecium*, has significant clinical feature with colonization and infection. Penicillin and ampicillin activity critically diminished at the last decade on Enterococcus genus, so discovery of new antibiotics became critical.[17] *A. tuncelianum* has medium level activity against *E. faecium*, but *E. faecalis* was resistant to it at 656.25 µg. *E. faecalis* has resistance to quinupristin/dalfopristin (combination of two antibiotics), however *E. faecium* hasn't got specific resistance, so *E. faecalis* can be accepted as more resistant Enterococcus species.[18] This knowledge can be confirmed after our results and this garlic species can be used as *E. faecium* treatment.

Our analyses are important since the antimicrobial activity of *A. tuncelianum* was determined in a large scale of antibacterial strains by disk diffusion method.

CONCLUSION

Consequently, *A. tuncelianum* clove has clear antimicrobial activity against 6 of the tested strains at 656.25 mg. These analyses clearly presented that, *A. tuncelianum* clove has possible medicinal activity significantly against *C. albicans* DSMZ 1386, *B. subtilis* DSMZ 1971 and *E. faecium*. Also, freezing and slicing negatively affected the antimicrobial activity of *A. tuncelianum*. Motor force was prevented with freezing and slicing, because the transformation of alliin to allicin, which wasn't wanted.

In a previous antimicrobial research, enough amount of allicin activity was not obtained from frozen garlic clove. For this reason, *A. tuncelianum* frozen clove was used, but this allicin releasing inhibition was not affective because of their big clove. Therefore, chemical composition analyses is required for confirming our result. Besides, *C. albicans*, *B. subtilis* and *E. faecium* strain should be used for specific *A. tuncelianum* antimicrobial research. Also, further researches are needed in order to analyses the active substances and their activity mechanisms in details. Geographical differences can cause active compound differences, but this garlic species is endemic and only produced in similar places.

ACKNOWLEDGEMENT

The authors would like to thank to Ali CAN for their support by supplying Garlic from Tunceli.

REFERENCES

1. Kumaraswamy MV, Kavitha HU and Satish S. Antibacterial potential of extracts of Woodferdia fruticosa kurz on human pathogens. World J. Med Sci., 2008; 3(2): 93-96.
2. Sokmen A, Jones BM and Erturk M. The in vitro antibacterial activity of Turkish plants. J. Ethnopharmacol, 1999; 67: 79-86.
3. Amer M, Tana M and Tosson Z. "Effect of aqueous garlic extract on growth of dermatophytes", Int. J. Dermatol, 1980; 19: 285-287.
4. Davis LE, Shen JK and Cai Y. "Antifungal activity in human cerebrospinal fluid and plasma after intravenous administration of Allium sativum", Antimicrob. Agents Chemother., 1990; 34(4): 651-653.
5. Mirelman D, Monheit D and Voron S. "Inhibition of growth of Entamoeba hystolytica by allicin, the active principle garlic extract (Allium sativum)", The Journal of Infectious Diseases, 1987; 156(1): 243-244.
6. Yun HM, Ban JO, Park KR, Lee CK, Jeong HS, Han B and Hong JT. Potential therapeutic effects of functionally active compounds isolated from garlic. Pharmacol Ther, 2014; 142(2): 183-195.

7. Altuner EM, Canli K and Akata I. Antimicrobial Screening of Calliergonella cuspidata, Dicranum polysetum and Hypnum cupressiforme. Journal of Pure and Applied Microbiology, 2013; 8(1): 539-545.
8. Altuner EM., Canli K. and Akata I. In vitro Antimicrobial Screening of Hedwigia ciliata Var. leucophaea and Determination of the Ethanol Extract Composition by Gas Chromatography/Mass Spectrometry (GC/MS). Journal of Pure and Applied Microbiology, 2014; 8(4): 2987-2998.
9. Canli K, Akata I and Altuner EM. In vitro Antimicrobial Activity Screening of Xylaria hypoxylon. African Journal of Traditional, Complementary and Alternative medicines, 2016; 13(4): 42-46.
10. Canli K, Altuner EM, Akata I. Antimicrobial screening of Mnium stellare. Bangladesh Journal of Pharmacology, 2015; 10: 321-325.
11. Canli K, Altuner EM, Akata I. Turkmen Y., Uzek U. In vitro antimicrobial screening of Lycoperdon lividum and determination of the ethanol extract composition by gas chromatography/mass spectrometry. Bangladesh Journal of Pharmacology, 2016; 11(2): 389-394.
12. Canli K, Yetgin A, Akata I, Altuner EM. In vitro Antimicrobial Screening of Aquilaria agallocha Roots. African Journal of Traditional, Complementary and Alternative medicines, 2016; 13(5): 178-181.
13. Andrews JM. BSAC standardized disc susceptibility testing method (version 6). Journal of Antimicrobial Chemotherapy, 2003; 60: 20-41.
14. Pfaller MA, Diekema DJ. Epidemiology of invasive mycoses in North America. Crit Rev Microbiol 2010; 36: 1-53.
15. Ruhnke M. Skin and mucous membrane infections. In: Calderone RA, ed. Candida and Candidiasis: ASM Press, Washington, DC, pp. 307-325., 2002.
16. Taskın R, Özgen U, Babacan M, Tuncel E and Koyuncu M. A Comparative Study on Antimicrobial Activities Of Garlic And Some Allium Species. J Fac Pharm Ankara, 1997; 26(2): 77-82.
17. Zervos MJ And Schaberg DR. Reversal of in vitro susceptibility of enterococci to trimethoprimsulfamethoxazole by folinic acid. Antimicrobial Agents and Chemotherapy, 1985; 28(3): 446–448.
18. Kristich CJ, Rice LB, Arias CA. Enterococcal Infection—Treatment and Antibiotic Resistance. From Commensals to Leading Causes of Drug Resistant Infection (2014).