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In vitro Antibacterial and Antifungal Activity of Ethanolic *Allium tuncelianum* Extract

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Abstract: *Allium tuncelianum* is an endemic garlic species which is locally rising in Tunceli, Turkey. *A. tuncelianum* was extracted using ethanol and tested for their inhibitor activity against five Gram positive and five Gram negative bacteria and six fungal strains were determined by the broth dilution method according to CLSI standards. The ethanol *A. tuncelianum* extract demonstrated the highest antibacterial activity against *Bacillus cereus* (12.5mg/ml). However, the ethanol extract at 0.78 mg/ml had high antifungal activity against *Malassezia pachydermatis*. According to the results of this study, *A. tuncelianum* has significant potential as antibacterial and antifungal. But the antifungal activity is stronger than antibacterial activity.

Key words: *Allium tuncelianum* · Garlic · *In vitro* · Antibacterial Activity · Antifungal Activity

INTRODUCTION

It is indicated that nowadays, when the gaining of resistance of the bacteria that lead to infectious diseases against antibiotics has become a clinical problem, people have returned to natural antibiotic materials and there has been a significant increase in the researches on this subject [1-4]. It is known that many diseases have been cured with herbal medicines through the human history. Even today, it is expressed that the option of herbal treatment plays a significant part in many developing countries [5,6]. One of the oldest agricultural products used both as food and medicine is garlic [7-11]. *Allium sativum* is one of the types belonging to family *Liliaceae* among the widely known garlic types. There are historical records about the use of garlic and one of them dates back to BC 800 [8,12]. Today, garlic is commercially sold as garlic powder, garlic juice, garlic puree, garlic volatile oil and odourless garlic tablets as a result of its antibacterial and antimicotic effect [9]. Medical effects of *A. sativum* are based on the organo sulfur components it contains and that it especially has allicin antibacterial, antiprotozoal, antifungal and antiviral properties and antibacterial properties of *A. sativum* have been studied by many researchers [6,13-17]. Actually, it is reported that allicin and its garlic preparations exhibit antibacterial properties at a large spectrum and these bacteria include *Aeromonas* spp., *Bacillus* spp., *Clostridium* spp., *Cryptocaryon* spp., *Escherichia coli, Helicobacter pylori, Klebsiella pneumoniae, Mycobacterium* spp, *Photobacterium* spp., *Proteus* spp., *Pseudomonas* spp., *Salmonella* spp., *Staphylococcus* spp., *Streptococcus* spp., *Vibrio* spp., *Agrobacterium tumefaciens, Erwinia carotovora, Pseudomonas syringae, Xanthomonas campestris* [12]. They reported that *A. sativum* exhibits activity against many pathogenic bacteria and fungi at different rates [15, 18-21]. They determined that allicin and allicin substances that provide antimicrobial properties among 23 garlic examined exist in

A. tuncelianum at the highest rate following *Allium macrochaetum* [14]. In addition to their antibacterial properties, recent studies on Organosulfur compounds show that these compounds have protective effects against cancer, cardiovascular diseases, neurological disorders, liver diseases as allergies and arthritis [11]. They reported that the body weight of the hens of which feed is added garlic powder has increased and this significantly affected the increase in the length of their villi [22].

The original name of *A. tuncelianum* is *Allium macrochaetum* Boiss and Haussk subsp. *tuncelianum* Kollmann. It is peculiar to Tunceli region (Especially the

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plateau of munzur mountains in the ovacık region) and it naturally grows individually like an onion [23,24]. Thus, it is an endemic type peculiar to our country of which local name is Tunceli garlic, Munzur garlic or Ovacık garlic [23]. Although the antimicrobial effects of many garlic species are known, antimicrobial effects of *A. tuncelianum* grown naturally against many types of bacteria and fungi are not exactly known.

This study was carried out in order to determine the antibacterial and antifungal effect of *A. tuncelianum*, a different type of garlic, on certain standard bacteria and fungi strains.

MATERIAL AND METHODS

Collection of Plant Material: Samples of *A. tuncelianum* used in the study were collected from rural areas in Ovacik district of Tunceli Province, Turkey in July 2013. The plants were dried in a room without receiving direct sun light.

Extraction of Garlic: The extraction procedure was performed by the method of Ozkan *et al*., (2013). Briefly, a sample of 1000 g minced garlic was allowed to stand in 5000 ml of ethanol at room temperature for 20 h and then the mixture was filtered. The liquid portion was taken and evaporated at 30°C in a rotary evaporator [25]. It was kept at -20 °C until the extract was used.

Test Microorganisms: Gram positive bacteria used in the study are; *Bacillus cereus* (ATCC 11778), *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 29213), *Staphylococcus epidermidis* (ATCC 12228) strains, Gram negative bacteria; *Escherichia coli* (ATCC 25922), *Klebsiella pneumonia* (ATCC 4352), *Proteus mirabilis* (CCM 1944), *Pseudomonas aeruginosa* (ATCC 27853), *Enterococcus faecalis* (ATCC 29232) ve *Salmonella* Enteritidis (KUEN 349), *Fungi strains; Candida albicans* (ATCC 90028), *Candida parapsilosis* (ATCC22019), *Candida krusei* (ATCC 6258), *M. pachydermatis, Microsporum canis and Trichophyton mentagrophytes* strains. Microorganisms were provided by Department of Microbiology Culture Collection, Faculty of Veterinary Medicine, Istanbul University.

Media: Nutrient agar (Oxoid) with the addition of 7% defibrinated sheep blood was used in order to ensure the continuation of the bacteria used in the research. Müller-Hinton Broth (OXOID CM 405) (CAMHB) media with the addition of Ca ++ and Mg ++ cations were used in macro-dilution tube method carried out with the aim of determining the antibacterial effect quantitatively [26].

SDA and dermatophyt that do not contain actidion were used by preparing SDA with actidion in order to ensure the continuity of *C. albicans*, *C. parapsilosis*, *C. krusei*, *M. pachydermatis* strains. The antimiotic effect of *A. tuncelianum* ethanolic extract was examined with its minimum inhibition concentration (MIC) [27]. RPMI 1640 broth medium was used in macro dilution method.

Detection of the Antibacterial Activity: The antibacterial effect of *A. tuncelianum* ethanolic extract was examined using the minimum inhibition concentration with (MIC) broth macro dilution method Clinical and Laboratory Standards Institute [26]. Two-fold serial dilutions of *A. tuncelianum* ethanolic extracts were prepared in CAMHB (500 µl CAMHB, 500 µl *A. tuncelianum* ethanolic extract) between 0.195-100 mg/ml. A suspension equal to the 0.5 McFarland turbidity of 18-hour colonies of the bacteria to be tested was prepared. 500 µl was transferred to all tubes from these suspensions. Positive (Which are not added *A. tuncelianum* ethanolic extract) and negative (Which are not added bacterial inoculum) controls were used at the end of each serial dilution tested. All of the tubes were incubated for 24 hours at 37 °C. The lowest *A. tuncelianum* ethanolic concentration MIC that can totally inhibit bacterial reproduction and can be detected with the naked eye was determined. Furthermore, Gentamicin sulphate was used as the reference antibiotic standard. The MIC interval was used as 0.000975 - 16 mg /ml [26].

Determination of the Antifungal Activity: The antimycotic effect of the ethanolic extract of *A. tuncelianum* against yeast was examined with minimum inhibitor concentration using (MIC) broth macro dilution method CLSI [27]. A suspension equal to 0.5 McFarland turbidity in physiological salty water among 48-hour *C. albicans, C. parapsilosis, C. krusei, M. pachydermatis* strains in SDA medium was prepared in order to prepare the inoculum. RPMI-1640 was used as the medium [26]. The two-fold serial dilutions of 0.195-100 mg/ml garlic extract were prepared. 500 µl was transferred to the tubes from the suspensions prepared. A suspension equal to 0.5 McFarland turbidity was prepared from the 48-hour cultures of the yeast to be tested in SDA medium. 500 µl was transferred to all tubes from the suspension prepared. Positive (Which are not added *A. tuncelianum* ethanolic extract) and negative (Which are not added yeast inoculum) controls were added at the end of each serial dilution tested. Amphotericin B was used as the control antimicotic. The MIC interval 0.0023 – 128 µg/ml was used. The lowest *A. tuncelianum* ethanolic extract

concentration that completely inhibits the reproduction and can be determined with the naked eye was determined as the MIC value [26].

The *antimycotic* effect of *A. tuncelianum* ethanolic extract against fungus was examined with (MIC) broth macro dilution method with minimum inhibitor concentration [27]. The seven day *M. canis and T. mentagrophytes* colonies in SDA medium were collected with 5 ml 8.5% physiological salty water and transferred to a sterile tube in order to prepare the inoculum. The heavy particles were waited to deposit for 15 min. and the homogenous conidia suspension on top was transferred to another sterile tube and made homogenous by stirring for 15 seconds. A suspension equal to 0.5 McFarland turbidity was prepared. RPMI-1640 was used as the medium [27]. The ethanolic extract of *A. tuncelianum* was prepared in two-fold serial dilutions between the interval of 0.195-100 mg/ml. 500 µl of the fungi suspension prepared as equal to 0.5 McFarland turbidity was transferred to each of the tubes and left for incubation at 26 °C for seven days. Amphotericin B was used as control antimicotic. 0.0023 – 128 µg/ml was used as the MIC interval. Furthermore, control tubes which were added fungi cultures adjusted according to McFarland 0.5 tube to RPMI-1640 medium were used in order to control the reproduction. The results were compared with the control tube and determined with bare eye and using 0.5 McFarland Densitometry device (MIC 0-MIC4). They were assessed according to the criteria of MIC O: No reproduction, MIC 1: 75-80% decrease in the reproduction, MIC 2: 50% decrease in the reproduction, MIC 3: 25% decrease in the reproduction, MIC 4: No decrease in the reproduction.

RESULT

Values on the *in vitro* antibacterial and antifungal activity of *A. tuncelianum* ethanolic extract are shown in Table 1, 2 and 3, respectively. As is seen, it was determined that *A. tuncelianum* ethanolic extract are effective against all bacteria and fungi strains tested. When the results were examined, it was determined that the highest antibacterial activity is realized against *B. cereus* with a concentration of 12.5 mg/ml and the highest antifungal activity was formed against *M. pachydermatis* with a concentration of 0.78mg/ml. When the antibacterial and antifungal effect concentrations were compared (Dilution last point), it was observed that the antifungal activity is stronger than the antibacterial activities.

Table 1: *In vitro* antibacterial MIC values of *Allium tuncelianum* (mg/ml)

Bacterial Strains	*Allium tuncelianum* (mg/ml)	Gentamicin sulfate (mg/ml)
Bacillus cereus	12.5	0.0039
Bacillus subtilis	25	0.0039
Enterococcus faecalis	50	0.0156
Escherichia coli	25	0.0039
Klebsiella pneumonia	50	0.0156
Proteus mirabilis	50	0.0156
Pseudomonas aeruginosa	50	0.0156
Salmonella Enteritidis	25	0.0039
Staphylococcus aureus	50	0.0156
Staphylococcus epidermidis	50	0.0078

Table 2. *In vitro* antifungal MIC values of *Allium tuncelianum* (mg/ml)

Fungal Strains	*Allium tuncelianum* (mg/ml)	Amphotericin B (µg/ml)
Candida albicans	3.12	0.125
Candida krusei	3.12	1
Candida parapsilosis	3.12	1
Malassezia pachydermatis	0.78	0.125

DISCUSSION

Historically, it was indicated that garlic was used in the treatment of many diseases using herbal medicines and garlic is considered as part of a healthy diet [11]. Chemical tests conducted on garlic show that garlic contains sulfur compounds in its concentration. While these compounds give garlic its peculiar taste and smell, they are also responsible for the medical effects of the plant. Allicin (Diallyl thiosulfate) is defined as the active substance among organosulfur compounds [10]. The chemical composition of the preparations obtained from the garlic through extraction depends on the extraction conditions (temperature, time, solvent parity, extraction method). The organosulfur compound content of garlic bulbs varies by growing areas, harvesting and storage conditions. Biological activities of these compounds depend on many factors such as the isolation methods to the country they originate in Yun e*t al.* [11].

Table 3: According to the reproduction, *In vitro* antifungal MIC values of *Allium tuncelianum* (mg/ml)

Fungal Strains	MIC 0	MIC 1	MIC 2	MIC 3	MIC 4
Microsporum canis	3.12	1.56	0.78	0.39	0.195
Trichophyton mentagrophytes	3.12	1.56	0.78	0.39	0.195

MIC O:No reproduction, MIC 1: 75-80% decrease in the reproduction, MIC2: 50% decrease in the reproduction, MIC 3: 25% decrease in the reproduction, MIC 4: No decrease in the reproduction.

A. tuncelianum (Tunceli garlic) used in this study is an endemic type peculiar to Turkey and has different characteristics than *A. sativum*. This difference also showed itself in the results of our study.

Sökmen *et al*. [6] tested the *in vitro* antimicrobial activities of various plants which also include *Allium scorodoprasum* on *B. cereus*, *E. coli*, *S. aureus*, *Branhamella catarrhalis*, *Clostridium perfringens* and *C. Albicans*. While *A. scorodoprasum* shows an inhibitor effect on only *C. albicans* among the test organisms, they reported that it does not have any effect on others.

Kallel *et al*. [13] examined the extracts of *A. sativum* obtained from different solvents in terms of *in vitro* antibacterial and antioxidant capacity. In the study where they used *B. subtilis, S. aureus, B. thuringiensis, P. aeruginosai, K. pneumoniae, E. coli* and *S*. typhimurium as test microorganisms, the ethanolic extract of *A. sativum* exhibited antimicrobial activities at varying levels. They reported that *A. sativum* extract has moderate level of activity on *B. subtilis* and *S. aureus,* low effect on *B. thuringiensis* and *P. aeruginosa* and no antibacterial effect on *K. pneumoniae E. coli* and *S*. typhimurium. In their study, Taþkın *et al.* [16] reported that they investigated the antimicrobial activity of 4 garlic types using the disc diffusion test, that the antimicrobial activity of two types of allium (*A. scabriflorum* and *A. viride)* are as low as they cannot be compared with *A. tuncelianum* and *A. sativum* has the highest level of activity among all garlic extracts. In the disk diffusion test of *A. tuncelianum* conducted against the reference strains of 10% ethanol extract used, they indicated that *S. aureus*, *B. subtilis*, *K. pneumoniae* exhibit a low level of activity except for *P. aeruginosa* and show higher activity against *C. albicans* reference strain tested from the fungi when compared to the bacteria. In an *in vitro* study conducted on guinea pigs, it was indicated that allicin with a concentration varying between 130-200 mg/ml has an inhibitor effect on the development of dermatophytes [28].

In this study, the *in vitro* antimicrobial activity of the ethanolic extract of *A. tuncelianum* was tested with ten reference bacteria and six reference fungi strains. When the MIC results detected were compared with the studies carried out by other researchers on other types of garlic, it is observed that *A. tuncelianum* clearly has a high level of antibacterial and antifungal activity. In the light of these data, it is obvious that *A. tuncelianum* can be used as a natural bioactive compound source. It was considered that the use of *A. tuncelianum*, an endemic type, in the area of health would be beneficial by comparatively assessing it with substances exhibiting antibacterial and antifungal properties with future studies.

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