

Medical Characteristics of Arid-Semi Arid Truffle (*Terfezia* and *Picoa*) in the Elazığ-Malatya region of Turkey

Türkiye'nin Elazığ-Malatya Çevresindeki Kurak-Yarı Kurak Trüflerin Tıbbi Özellikleri

Research Article

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ABSTRACT

In this study, the antimicrobial and antioxidant activity of *Terfezia boudieri*, *T. claveryi*, *T. olbiensis*, *Picoa lefebvrei* and *P. juniperi* were investigated. It was determined that the methyl alcohol extraction of *T. boudieri*, *T. claveryi*, *T. olbiensis*, *P. lefebvrei* and *P. juniperi* inhibit the development of *E. coli*, *B. subtilis*, *P. aeruginosa*, *S. aureus*, *S. mutans*, *P. vulgaris*, *S. typhi*, *C. tropicalis* and *Trichophyton* sp. at various levels (8 mm to 22 mm diameter) and their effect to remove the DPPH radical was more efficient at groups to which samples of 25 µL (1.22% to 2.58%), 50 µL (2.31% to 3.28%) and 100 µL (5.24% to 8.00%) were added. It was determined that methyl alcohol that was extracted from *Terfezia* species were more effective than *Picoa* species against test microorganisms. When the groups were compared in terms of concentration, the highest level of free radical scavenging effect at the group, to which we added a 100 µL sample, was seen at *T. boudieri* with 8.00%.

Key Words

Antimicrobial activity, antioxidant property, arid-semi arid truffle, edible mushroom, *Terfezia*, *Picoa*.

ÖZET

Bu çalışmada, *Terfezia boudieri*, *T. claveryi*, *T. olbiensis*, *Picoa lefebvrei* ve *P. juniperi*'nin antimikrobiyal ve antioksidant özellikleri araştırıldı. *T. boudieri*, *T. claveryi*, *T. olbiensis*, *P. lefebvrei* ve *P. juniperi*'nin metil alkol özütlerinin; *E. coli*, *B. subtilis*, *P. aeruginosa*, *S. aureus*, *S. mutans*, *P. vulgaris*, *S. typhi*, *C. tropicalis* ve *Trichophyton* sp.'nin gelişmelerini değişik oranlarda (8-22 mm çap) inhibe ettiği ve DPPH radikalini giderme etkisinin ise 25 µL (% 1.22-2.58), 50 µL (% 2.31-3.28) ve 100 µL (% 5.24-8.00) örnek eklenen gruplarda daha etkili olduğu tespit edilmiştir. *Terfezia* türlerinden elde edilen metil alkol özütlerinin, test mikroorganizmalarına karşı *Picoa* türlerinden daha etkili olduğu tespit edilmiştir. Derişime bağlı olarak gruplar karşılaştırıldığında ise 100 µL örnek eklediğimiz grupta en fazla serbest radikal giderme etkisi % 8.00 ile *T. boudieri*'de görülmektedir.

Anahtar Kelimeler

Antimikrobiyal aktivite, antioksidant özellik, kurak-yarı kurak trüfler, yenilen mantar, *Terfezia*, *Picoa*.

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INTRODUCTION

Throughout human history, fungi have been used for nourishment, as functional foods to maintain good health and prevent ailments, and as a source of medicine to treat diseases [1-3]. Desert truffles have been found so far in most of the geographical areas characterized by these climate types, although historically, some of them have received more attention than other mushrooms. In some of these areas, hypogeous fungal species adapted to dry environments (steppes, deserts) are popularly known as desert truffles [4-6].

Desert truffles are hypogeous Ascomycetes that grow in arid and semiarid areas on every continent other than Antarctica (7). The desert truffles with the longest recorded history of use are species of *Terfezia*, *Picoa* and *Tirmania*, which are popular mainly in the Middle East, Mediterranean basin, and Northern Africa [6-8]. These desert truffles form mycorrhizas on the roots of plants in the Cistaceae family [7].

Desert truffles have been shown to provide a rich source of fiber, protein, amino acids, fatty acids, minerals, vitamins, and carbohydrates and to contain considerably higher values of fat, potassium, and phosphate than many commonly consumed vegetables [9-14]. *Terfezia* and *Picoa* are two of the most common edible desert truffles in the world. These fungi constitute a popular food and are particularly appreciated for their flavour and texture profiles in countries of the Mediterranean Basin as a whole [13]. Desert truffles are traditionally gathered not just for nourishment, but also for their curative and aphrodisiac properties [15-16]. The juice of desert truffles, mainly of *T. nivea*, *T. claveryi*, and *T. boudieri*, has been used in the Middle East to treat eye and skin diseases [16-17]. More recently food scientists have focused on the bioactivities of truffles, including their antioxidant, antiviral, anticancer, antimicrobial, hepatoprotective, antimutagenic and antiinflammatory activities [9,12,18-27]. They have been popular as functional foods in traditional medicine have been found to contain substances with powerful antioxidant activities, including ascorbic acid, anthocyanins, esterified phenols, free phenolics, flavonoids, and carotenoids [9,12,17].

In this study, *T. boudieri*, *T. claveryi*, *T. olbiensis*, *P. lefebvrei* and *P. juniperi* are observed, which are well known and consumed in Elazığ-Malatya province of Turkey, and sold at the local bazaars. Villagers at rural areas pointed out that they consume these truffle types that they collect as daily source of nutrition, but they sell most of them to Malatya through merchants. It was also pointed out that the seasonal yield may not be high every year because of the unsuitable climatic conditions. It was observed that *T. boudieri* is the only product that has an economic value as it brings profit. *P. lefebvrei*, *T. olbiensis* and *P. juniperi*, which are used for daily consumption and are more popular amongs to the locals, don't have an economic value, as the first one is too small, the second one degrades quickly, and the third one can be found rarely and is very hard. It was observed that *T. claveryi* can be rarely found in the area and can be confused with *T. boudieri* by the local people.

Truffle types can grow in various exclusive habitats depending on the region's climate, altitude, vegetation and soil structure. Little is known about the truffle in Turkey [28], as truffle fungi have received less attention than epigeous fungi. Turkey is rich in mushroom diversity, as well as medicinal plants. Turkish people have a tradition of using various types of mushrooms for food, instead of using them for the treatment of infectious diseases and various ailments. Therefore, it is necessary to know the levels of antimicrobial and antioxidant activities in truffles before using them. The purpose of this study was to evaluate the potential antimicrobial and antioxidant activities of arid-semi arid truffles in the Elazığ-Malatya region of Turkey.

MATERIAL AND METHOD

Truffle materials

A wild samples of fresh *Picoa lefebvrei* (Pat.) Maire, *Picoa juniperi* Vittad., *Terfezia boudieri* Chatin, *Terfezia claveryi* Chatin and *Terfezia olbiensis* Tul. & C. Tul. were collected from Malatya (Central, Kale, Battalgazi, Arguvan Districts and theirs vicinity) and Elazığ (Baskil district and its vicinity), Turkey (N 38° 19'-43' E 038° 19'-51' with an altitude of 690-1375 m, the beginning of March

to the end of May (rarely continue until mid-July). They are usually collected near *Helianthemum salicifolium* (L.) Mill. and rarely *H. ledifolium* (L.) Mill. We identified the location of the truffles from crevices that appeared in the surface of the soil above the truffle. The samples were dried at room temperature for 15 days then placed in locked bags and stored at 25°C in lab. These samples were used in this study.

Extraction procedure

The dried and powdered mushroom materials were dried at 55°C in the oven for 1 h. Then, 1 of these powdered materials were mixed with 10 mL methyl alcohol solvent in a beaker and then placed on a rotary shaker for 24 h. The aqueous solutions were then filtered using Whatman filter paper and then concentrated in vacuo for 15 min at 37°C using a Rotary evaporator. The concentration was then dissolved in 15 min of dimethylsulfoxide and stored at 4°C for further study. Then, 100 µL (100 µg) extracts were injected into an antibiotic disc (Antimicrobial susceptibility test disc, Oxoid) having a diameter of 6 mm (29).

Test microorganisms

A total of 7 bacteria (*Bacillus subtilis* IMG 22, *Pseudomonas aeruginosa* DMS 50071 SCOTTA, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* COWAN 1, *Streptococcus mutans*, *Proteus vulgaris* FMC 1, *Salmonella typhi*), yeast (*Candida tropicalis* ATCC 13803), and dermatofit species (*Trichophyton* sp.) were used in this study. Microorganisms were provided by the Microbiology Research Laboratory, Department of Biology, Faculty of Science and Arts, Firat University, Elazığ-Turkey.

Antimicrobial activity

The antimicrobial tests were carried out by the disc diffusion method [29] using 100 µL of suspension containing 10⁶ per/mL of bacteria, 10⁴ per/mL yeast, and 10⁴ per/mL dermatofit fungi inoculated into Mueller Hinton Agar (Difco), Malt Extract Agar (Difco), and Glukoz Sabouroud Agar (Difco), respectively. The discs (6 mm) were then impregnated with 100 µL of mushroom extract and then placed on the inoculated agar. Petri dishes were prepared at 4°C for 2 h. Then, the

inoculated plates were incubated at 37±0.1°C for 24 h for bacterial strains and also 25±0.1°C for 72 h for yeast and dermatofit fungi. At the end of the incubation period, the inhibition zones were measured [29]. Streptomycin sülfat (10 µg/disk) and Nystatin (30 µg/disk) were used as standard.

Free Radical Scavenging Activity Measurement (DPPH)

Truffle samples were extracted in 1:5 (g/mL) ratio with methanol. The free radical scavenging effect in samples was assessed by the discoloration of a methanol solution of DPPH according to the method of Brand-Williams et al. [30]. 25 mg/L, α-Diphenyl-bpicrylhydrazyl (DPPH) solution and methanol was prepared and 3.9 mL of this solution was mixed with 10, 25, 50 and 100 µg/µL concentrations of mushroom extracts. The reaction mixture was stored in darkness at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. The ability to scavenge DPPH free radical was calculated by the following equation: DPPH free radical scavenging activity (%) = [(Control_{ABS} - Sample_{ABS})/(Control_{ABS})] x 100 where Control_{ABS} is the absorbance of DPPH free radical + methanol, Sample_{ABS} is the absorbance of DPPH free radical + sample extract/standard.

RESULTS

Many edible mushrooms may be used as a response to specific health problems. As can be seen in Table 1, the extract of *Terfezia* and *Picoa* types showed activity on other test microorganisms (8-22 mm). The antimicrobial activity of *Terfezia* species was observed to be very high, but observed low activity against test microorganisms as compared with *Picoa* species.

Table 1 shows the antimicrobial activities of the methyl alcohol extracts of truffles (*T. boudieri*, *T. claveryi*, *T. olbiensis*, *P. lefebvrei* and *P. juniperi*) against certain bacteria (*E. coli*, *B. subtilis*, *P. aeruginosa*, *S. aureus*, *S. mutans*, *P. vulgaris*, *S. typhi*), yeast (*C. tropicalis*), and dermatofit (*Trichophyton* sp.) types, in terms of disc diffusion technique. It was observed that the *T. boudieri*, *T. claveryi*, *T. olbiensis*, *P. lefebvrei* and *P. juniperi* extracts affected the development of tested microorganisms at various levels

(8 - 22 mm inhibition zone) and were more effective than the control antibiotic (Table 1).

As seen in Table 1, the extract of *T. boudieri* inhibited the development of the test microorganisms at various rates (10-22 mm diameter). The antimicrobial activity of *Terfezia* species was observed to be very high. The methyl alcohol extract of *T. boudieri* was 22 mm against *S. mutans*, 20 mm against *E. coli*, 18 mm against *P. aeruginosa*, 16 mm against *C. tropicalis*, 13 mm against *Trichophyton* sp. and *B. subtilis*, 12 mm against *S. aureus*, 11 mm against *P. vulgaris* and 10 mm against *S. typhi* (Table 1). The extract of *T. claveryi* was 18 mm against *E. coli*, 20 mm against *P. aeruginosa* and *S. mutans*, 15 mm against *B. subtilis* and *Trichophyton* sp., 14 mm against *S. typhi*, but showed low inhibition zone against the other microorganisms. The extract of *T. olbiensis* present lower activity against *S. aureus*, *B. subtilis*, *S. mutans*, *P. vulgaris*, *S. typhi* and *C. tropicalis*, but showed high activity against *E. coli* (15 mm) when compared with the control group (Table 1). The extract of *P. lefebvrei* and *P. juniperi* have a lower antimicrobial activity as to comparison antibiotic (9-14 mm) (Table 1). As can be seen in Table 1, the *Picoa* extract showed varying degrees of antimicrobial activity against tested strains. The extract of *Picoa* showed low activity against *E. coli*, *B. subtilis*, *P. aeruginosa*, *S. aureus*, *P. vulgaris*, *S. typhi*, *C. tropicalis* and *Trichophyton* sp., but this was observed to be very high in *S. mutans* (14-20 mm) see Table 1. As a result, It was determined that methyl alcohol that was extracted from *Terfezia* species were more effective than *Picoa* species against test microorganisms (Table 1).

DPPH (α,α -Diphenyl-b-picrylhydrazyl) free radical scavenging effect could be seen at every group (Table 2), but this effect, which we evaluated in percentages, increased directly proportional with the increase in the amount of sample (Table 2). When the study groups were observed in general, it was seen that the DPPH effect was stronger at groups to which 25, 50 and 100 μ L samples were added (Table 2). When the groups were compared in terms of concentration, the highest level of free radical scavenging effect at the group, to which we added a 100 μ L sample,

was seen at *T. boudieri* with 8.00% (Table 2).

DISCUSSION

Desert truffles have been used for medicinal purposes for centuries by indigenous populations. Traditional medicinal uses of desert truffles prompted modern scientific research into their bioactive properties [16-17]. Studies have shown that the skin of desert truffles is particularly rich in nutrients and bioactive compounds [17].

The in vitro antimicrobial activities of semiarid, arid or desert truffles are shown in Table 1. The antimicrobial activities of truffles are changeable as seen in Table 1 (8-22 mm diam.). *T. boudieri* have higher antimicrobial activity as to comparison antibiotic (9-14 mm). The extract of *T. boudieri* showed the maximum activity against *S. mutans* (22 mm), *E. coli* (20 mm), *P. aeruginosa* (18 mm), *C. tropicalis* (16 mm), but minimum activity against *S. aureus*, *B. subtilis*, *P. vulgaris* and *S. typhi* see Table 1. The result of a previous study [31], methyl alcohol and ethyl acetate extract of *Terfezia* and *Tirmania* showed activity against *B. subtilis* and *S. aureus*. Methanolic extract of *Terfezia* truffles had antimicrobial activity against gram positive bacteria including *B. subtilis* and *S. aureus* [18]. Chloroform, acetone, methanol extracts of *T. boudieri* were observed to be more effective against bacteria and yeast (32-33). The extract of *T. boudieri* did not show any activity of *B. megaterium*, *E. coli*, *S. aureus*, *C. albicans*, *C. glabrata*, *Epidermophyton* spp. and *Trichophyton* sp., but was observed to be very high in *K. pneumoniae* (22). As previously discussed, indigenous populations have long used the extract of desert truffles as a remedy for eye diseases, including trachoma [16-17,25,34]. It seems that the antimicrobial activity of *T. boudieri* are changeable as reported by other researchers [18,22,31-33]. This may be indicative of the broad spectrum antibiotic compound in the truffles and due to the use of different solvent and test microorganism.

The extract of *T. claveryi* was observed to be very high in *S. mutans* and *P. aeruginosa* (20 mm), *E. coli* (18 mm), *B. subtilis* and *Trichophyton* sp. (15 mm), *S. typhi* (14 mm), but lower in *C. tropicalis*

Table 1. Antimicrobial activity of arid-semi arid truffle in the Elazığ - Malatya region of Turkey (Inhibition zone (mm)).

Truffle Types	Bacteria						Yeast		Dermatofit <i>Trichophyton sp.</i>
	<i>E. coli</i>	<i>B. subtilis</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>S. mutans</i>	<i>P. vulgaris</i>	<i>S. typhi</i>	<i>C. tropicalis</i>	
<i>T. boudieri</i>	20	13	18	12	22	11	10	16	13
<i>T. clavaryi</i>	18	15	20	12	20	12	14	13	15
<i>T. olbiensis</i>	15	11	12	9	12	10	9	13	11
<i>P. juniperi</i>	10	9	11	9	14	9	8	9	9
<i>P. lefebvrei</i>	9	9	12	9	20	9	8	9	9
Standart	9*	13*	11*	14*	12*	10*	9*	13*	-

Comparison antibiotic: *streptomycin sulfate (10 µg/disc), **Nystatin (30 µg/disc)

Table 2. Antioxidant property of arid-semi arid truffle in the Elazığ - Malatya region of Turkey (DPPH, %).

Truffle Types	DPPH (%)		
	10 µL	25 µL	50 µL
<i>T. boudieri</i>	0.27	2.31	3.14
<i>T. clavaryi</i>	1.62	2.58	3.28
<i>T. olbiensis</i>	0.40	2.05	2.31
<i>P. juniperi</i>	0.13	1.49	3.00
<i>P. lefebvrei</i>	0.67	1.22	3.28

(13 mm) and *S. aureus* (12 mm) see Table 1. The extract of *T. olbiensis* show lower activity against *S. aureus*, *B. subtilis*, *S. mutans*, *P. vulgaris*, *S. typhi*, *C. tropicalis*, but observed to be very active against *E. coli* (15 mm) when compared with the control group (Table 1). The juice of desert truffles, and mainly of *T. boudieri*, *T. claveryi* and *T. nivea*, has been used to treat eye diseases and skin lesions for centuries [4,18,20]. Janakat et al. [19-20] investigated the efficacy of aqueous and methanolic extracts of *T. claveryi* as well as partially purified proteins from these extracts against *S. aureus* and *P. aeruginosa*. Aldebasei et al. [35] determined the potential antibacterial activity of *T. claveryi* aqueous extract against *S. aureus*, *S. epidermitis*, *S. faecalis*, *E. coli*, *P. aeruginosa*, *K. pneumonia*, *P. mirabilis* isolates causing corneal ulcer. Bekçi et al. [36] clearly indicated that the acetone extract of *T. claveryi* have significant antimicrobial activity against *B. cereus*, *E. coli*, *P. aeruginosa*, *S. enteritidis*, *S. aureus*, *C. glabrata* ve *C. albicans* at different ratios. Gouzi et al. [25] evaluated aqueous extracts of *T. claveryi*, *T. leonis*, and *T. nivea* against both *S. aureus* and *P. aeruginosa*, but *T. leonis* did not show any antibacterial activity. The antimicrobial activity of *T. claveryi* and *T. olbiensis* showed activity against test microorganism are similar to those reported by several researchers [19-20,25,35-36], but some values are variable. This study indicated that there are differences in the antimicrobial effects of mushroom groups, due to phytochemical differences among species.

P. lefebvrei and *P. juniperi* extracts have a lower antimicrobial activity when compared to antibiotic (9-14 mm) and *Terfezia* species (Table 1). As can be seen in Table 1, *Picoa* extracts showed varying degrees of antimicrobial activity against tested strains. Table 1 show that the extract of *P. lefebvrei* and *P. juniperi* have antibacterial and antifungal activity with a 8-20 mm zone of inhibition at the microorganisms tested, and are also observed to be very high at *S. mutans* (14-20 mm), but showed low inhibition zone at other microorganisms. The antimicrobial activity of *P. lefebvrei* and *P. juniperi* showed activity against test microorganism, that are different from those reported by several researchers [18-20,22,25,31-33, 35-36], but some values were similar. They claimed that the sensitivity of microorganism to chemotherapeutic

compounds change even against different strains. The extracts of various mushrooms inhibited the growth of some microorganisms at different ratios. Different mushroom species possess different constituents and in different concentrations, which account for the differential antimicrobial effects, as suggested in the reports mentioned above. The broad spectrum of antimicrobial activity may be attributed to the presence of bioactive metabolites of various chemical types in mushroom compounds.

Antioxidant rich foods are currently valued by consumers for their positive impact on the detrimental effects of ROS induced oxidative stress which is theorised to play an important role in the development of many human degenerative diseases including inflammation, ageing, cancer and heart disease [26]. Truffles have high content of antioxidants such as vitamin A, C, β -carotene and phenolics and flavonoids compounds, besides many other bioactive compounds (anthocyanins, total esterified phenolics, total free phenolics and total flavonoids and total carotenoids etc.), which can scavenge peroxy radicals and chelate ferric ions, thus reducing lipid peroxidation etc. [9,12,17]. It is clearly indicated that *T. boudieri* have significant antioxidant activity against various antioxidant systems (β -carotene/linoleic systems, reducing power, DPPH, chelating effect and superoxide anion radical scavenging effect etc.) [37-38]. *T. claveryi* and *P. juniperi* exhibited a higher oxidative inhibition on the basis of lipid peroxidation, deoxyribose and peroxidase assays, in comparison with some common food antioxidants such as α -tocopherol, BHA, BHT, and propyl gallate [12]. The high correlation observed between the various assays employed and phenolic content is a strong indication that these phenolics are among the predominant source of antioxidant activity in desert truffles [9,23,38].

The effect to remove the DPPH radical was more efficient at groups to which samples of 25 μ L (1.22% to 2.58%), 50 μ L (2.31% to 3.28%) and 100 μ L (5.24% to 8.00%) were added. When the groups were compared in terms of concentration, the highest level of free radical scavenging effect at the group, to which we added a 100 μ L sample, was seen at *T. boudieri* with 8.00% (Table 2). It seems that the radical scavenging activity of *T. boudieri*

might be different than what was expressed in an earlier published report [9,12,17,23,37-38]. Bioactive compounds and antioxidant activity of mushrooms depend on its relation with the strain/type, the ascocarp size, harvest time, host plant, and the ecosystems. Thus, it is expected that truffles originating from different geographical origins can show some variations. Furthermore, it is also expected that the antioxidant attributes of the truffles may be affected by the nature and by the extent of association with its host root associate *Helianthemum* spp. as stated by Al-Laith [9]. Antioxidant activity of *Terfezia* and *Picoa* species may vary markedly among truffle species, in terms of their developmental stages, their habitats, and the analytical methods used. Our findings were supported by previous findings in the aforementioned studies.

At the end of the study, we have found that the extracts of *T. boudieri*, *T. claveryi*, *T. olbiensis*, *P. lefebvrei* and *P. juniperi* prepared with methyl alcohol revealed antimicrobial activities against some bacteria, yeasts, and dermatofit. It was observed that the biochemical components of truffles that have antimicrobial and antioxidant effects should be determined. In addition to this, it was established that different methods to determine antioxidant effects should be employed.

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