Antimicrobial Activity of Some Species from Pinaceae and Cupressaceae

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The aim of this study was to determine the antimicrobial activities of ethereal extracts of some Pinaceae and Cupressaceae species collected from Turkey. The extracts from *Pinus nigra* Arn., *P. brutia* Ten., *P. halepensis* Mill., *Abies equi-trojani* (Asch. et Sint. ex Boiss.) Coode et Cullen, *A. bornmulleriana* Mattf., *A. cilicica* (Ant. et Kotschy) Carr., *A. nordmanniana* (Steven) Spach., *Cedrus libani* A. Rich. and *Picea orientalis* L. belong to Pinaceae family and *Juniperus oxycedrus* L. subsp. *oxycedrus*, *J. foetidissima* Willd., *J. excelsa* Bieb., *J. phoenicea* L., *Cupressus sempervirens* var. *pyramidalis* Nym. and *C. sempervirens* var. *horizontalis* (Mill.) Gord. belong to Cupressaceae family were examined against *Staphylococcus aureus* ATCC 25923, *S. aureus* ATCC 43300 (MRSA), *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* RSKK 574 and *Candida albicans* ATCC 10231. The disc diffusion method was used to determine the antimicrobial activities of these extracts. All the tested extracts, except *A. bornmulleriana*, *C. libani* and *P. halepensis* showed weak antibacterial activity against the various tested bacteria comparing with the standards. Nevertheless, no antifungal activity was observed against *C. albicans* in all extracts. It was concluded that the tested extracts did not show promising antimicrobial activity against tested microorganisms. However, this is the first comprehensive investigation on the evaluation of antimicrobial activities on ethereal extracts of these species.

Key words: Antimicrobial, Cupressaceae, Extract, Pinaceae

Pinaceae and Cupressaceae Familyalarına Ait Bazı Türlerin Antimikrobiyal Aktivitesi

Bu çalışmanın amacı, Türkiye'den toplanan Pinaceae ve Cupressaceae familyalarına ait bazı türlerin, eterli ekstrelerinin antimikrobiyal aktivitelerinin araştırılmasıdır. Pinaceae familyasına ait olan *Pinus nigra* Arn., *P. brutia* Ten., *P. halepensis* Mill., *Abies equi-trojani* (Asch. et Sint. ex Boiss.) Coode et Cullen, *A. bornmulleriana* Mattf., *A. cilicica* (Ant. et Kotschy) Carr., *A. nordmanniana* (Steven) Spach., *Cedrus libani* A. Rich. ve *Picea orientalis* L. türlerinin ve Cupressaceae familyasına ait olan *Juniperus oxycedrus* L. *subsp. oxycedrus*, *J. foetidissima* Willd., *J. excelsa* Bieb., *J. phoenicea* L., *Cupressus sempervirens* var. *pyramidalis* Nym. ve *C. sempervirens* var. *horizontalis* (Mill.) Gord. türlerinin ekstreleri, *Staphylococcus aureus* ATCC 25923, *S. aureus* ATCC 43300 (MRSA), *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* RSKK 574 ve *Candida albicans* ATCC 10231'a karşı disk difüzyon yöntemi ile araştırılmıştır. *A. bornmulleriana*, *C. libani* ve *P. halepensis*'in test mikroorganizmaları üzerinde antimikrobiyal aktivitesi saptanmazken; diğerlerinin çeşitli bakterilere karşı zayıf antibakteriyel etki gösterdiği ancak *C. albicans*'a karşı aktivite göstermediği belirlenmiştir. Bu çalışma, bu türlerin eterli ekstrelerinin antimikrobiyal aktivitelerinin değerlendirilmesi üzerine yapılan ilk çalışma olması nedeniyle önem taşımaktadır.

Anahtar kelimeler: Antimikrobiyal, Cupressaceae, Ekstre, Pinaceae

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INTRODUCTION

Turkey is a crossing Region of all climates, especially Mediterranean climates. Owing to its geographical position, it is very rich in forest area including coniferous species (1-3). Some of the conifer species belong to families of Cupressaceae and Pinaceae. The Cupressaceae species are native to northern America, Africa, south-eastern Europe and western Asia. Especially, the species are used for protection of the fields from the damage of the wind. In traditional medicine, cypress (Cupressaceae) species have been used for antiseptic, antipyretic, anthelminthic, astringent, antirheumatic, antihemorrhoidal, antidiarrheal, vasoconstrictive properties (4). In addition, the essential oils of the species are used in aromatherapy, food and perfume industries. On the other hand, various parts of some pine species have been used as a folk medicine for rheumatism or as anti-inflammatory, antioxidant and antiseptic (4, 5). Pinaceae family includes many of the conifer genuses which have commercial importance such as cedars, firs, hemlocks, larches, pines and spruces, mostly found in the Northern Hemisphere in temperate climates but ranging from sub-arctic to tropical (6).

The increasing prevalence of multi-drug resistant strains forced scientists to find new antimicrobial substances from various sources like medicinal plants (7, 8). The literature survey indicates that main constituents of the conifers are terpenoids and essential oils. It is well-known that these constituents have a potent antimicrobial activity (9, 10). The current study was carried out for the determination of *in vitro* antimicrobial activities of ethereal extracts from some Pinaceae and Cupressaceae species collected from Turkey. To our knowledge, this is the first comprehensive investigation on the evaluation of antimicrobial activities of ethereal extracts of these species.

EXPERIMENTAL

Plant materials

The species used in the study were selected from coniferous plants. *Pinus nigra* Arn., *P. brutia* Ten., *P. halepensis* Mill., *Abies equi trojani* (Asch. et Sint. ex Boiss.) Coode et Cullen, *A. bornmulleriana* Mattf., *A. cilicica* (Ant. et Kotschy) Carr., *A. nordmanniana* (Steven) Spach., *Cedrus libani* A. Rich., *Picea orientalis* L., *Juniperus oxycedrus* L. subsp. *oxycedrus*, *J. foetidissima* Willd., *J. excelsa* Bieb., *J. phoenicea* L., *Cupressus sempervirens* var. *pyramidalis* Nym. and *C. sempervirens* var. *horizontalis* (Mill.) Gord. were collected from different localities of Turkey and authenticated by Dr. Barbaros Yaman, from Faculty of Forestry at Bartin University, Bartin, Turkey. The species investigated against test microorganisms. Voucher specimens of these species were deposited at the Herbarium of Faculty of Forestry as BOF 350 (*P. nigra* Arn.), BOF 351 (*P. brutia* Ten.), BOF 352 (*P. halepensis* Mill.), BOF 353 (*A. equi trojani* (Asch. et Sint. ex Boiss.) Coode et Cullen), BOF 354 (*A. bornmulleriana* Mattf.), BOF 355 (*A. cilicica* (Ant. et Kotschy) Carr.). BOF 356 (*A. nordmanniana* (Steven) Spach.), BOF 357 (*C. libani* A. Rich.), BOF 358 (*P. orientalis* L.), BOF 359 (*J. oxycedrus* L. subsp. *oxycedrus*), BOF 360 (*J. foetidissima* Willd.), BOF361 (*J. excelsa* Bieb.), BOF 362 (*J. phoenicea* L.), BOF 363 (*C. sempervirens* var. *pyramidalis* Nym.) and BOF 364 (*C. sempervirens* var. *horizontalis* (Mill.) Gord.).

Between 500 and 2000 g of materials were collected for each species at their growth sites just at the time of maturity. Samples packed tightly in plastic bags and stored in -24°C until the laboratory studies (3, 4). Species names, plant parts, sampling site, climate zone, collection date and altitude of all specimens are listed in Table 1.

Table 1. The information about the tested species

Species	Plant parts	Sampling Site	Climate Zone	Collection Date (2011)	Altitude (m)
Pinus nigra	cone	Bartin	Temperate	September	765
Pinus brutia	cone	Izmir	Mediterranean	May	880
Pinus halepensis	cone	Mugla	Mediterranean	November	940
Abies equi-trojani	cone	Balikesir	Mediterranean	October	740
Abies bornmulleriana	cone	Bartin	Temperate	October	1.110
Abies cilicica	cone	Adana	Mediterranean	May	715
Abies nordmanniana	cone	Trabzon	Temperate	October	985
Cedrus libani	cone	Adana	Mediterranean	May	1.220
Picea orientalis	cone	Trabzon	Temperate	October	1.100
Juniperus oxycedrus* subsp. *oxycedrus	fruit	Karabük	Temperate	September	1.150
Juniperus foetidissima	fruit	Ankara	Temperate	October	250
Juniperus excels	fruit	Mersin	Mediterranean	October	230
Juniperus phoenicea	fruit	Mugla	Mediterranean	September	185
Cupressus sempervirens* var. *pyramidalis	cone	Antalya	Mediterranean	October	660
Cupressus sempervirens* var. *horizontalis	cone	Antalya	Mediterranean	October	685

Preparation of the extracts

Powdered plant materials were macerated with ether in a dark place by occasionally shaking during 3 weeks. After the extraction was completed, the solutions were filtrated and then evaporated not to exceed up to 30ºC. The extracts were kept in a refrigerator and weighed suitable amounts for the experiment.

Bacterial strains

The following reference strains were used for testing antimicrobial activity: **Gram (+) bacteria:** *Staphylococcus aureus* ATCC 25923, *S. aureus* ATCC 43300 (methicillin-resistant *S. aureus*), *Bacillus subtilis* ATCC 6633. **Gram (–) bacteria:** *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* RSKK 574. **Yeast:** *Candida albicans* ATCC 10231

Antimicrobial assay

Antimicrobial activities of the extracts were determined by the disc diffusion method. Prior to testing, all extracts were filter-sterilized through 0.45 µm membrane filters. The inoculums were suspended in sterile saline and diluted according to 0.5 Mc Farland standard and then spread on solid media plates. Empty paper discs (6 mm in diameter) were soaked with 20 µL of the extract (3000 µg/mL) and placed on the inoculated plates. These plates, after remaining 2 h at 4^{o}C, were incubated at 37^{o}C for 24 h. After incubation, the inhibition zones (mm) were measured. Ampicillin (10 µg), ciprofloxacin (5 µg) and fluconazole (25 µg) were used as standards (11, 12).

RESULTS

All the tested extracts, except *Abies bornmulleriana, Cedrus libani* and *Pinus halepensis* showed weak antibacterial activity against the various tested bacteria comparing with the

standards. No antifungal activity was observed against *C. albicans* for all the extracts. The results are listed in Table 2.

Table 2. Antimicrobial activity of the extracts against seven microorganisms

	Microorganisms						
Extracts	**A***	**B***	**C***	**D***	**E***	**F***	**G***
No 1*	-	8	8	-	-	-	-
No 2*	-	-	-	-	-	8	-
No 3*	-	-	-	-	-	-	-
No 4*	7	-	-	-	-	7	-
No 5*	-	-	-	-	-	-	-
No 6*	8	8	7	8	8	7	-
No 7*	-	-	-	7	-	7	-
No 8*	-	-	-	-	-	-	-
No 9*	-	7	7	7	8	7	-
No 10*	-	8	8	8	7	8	-
No 11*	-	7	7	8	-	8	-
No 12*	-	7	7	8	8	8	-
No 13*	10	-	7	8	8	-	-
No 14*	11	7	7	-	8	7	-
No 15*	9	7	7	7	8	8	-
Ciprofloxacin	22	-	-	20	22	22	-
Ampicillin	20	34	11	22	32	-	-
Fluconazole	-	-	-	-	-	-	28

(*) No 1*: *Pinus nigra*, **No 2:** *Pinus brutia*, **No 3:** *Pinus halepensis*, **No 4:** *Abies equi trojani*, **No 5:** *Abies bornmulleriana*, **No 6:** Abies *cilicica***, No 7:** *Abies nordmanniana*, **No 8:** *Cedrus libani*, **No 9:** *Picea orientalis*, **No 10:** *Juniperus oxycedrus* subsp. *oxycedrus*, **No 11:** *Juniperus foetidissima*, **No 12:** *Cupressus sempervirens* var. *pyramidalis*, **No 13:** *Juniperus excelsa*, **No 14:** *Juniperus phoenicea*, **No 15:** *Cupressus sempervirens* var. *horizontalis*
(*) A: *Bacillus subtilis*, **B:** *Staphylococcus aureus*, **C:** *Staphylococcus aureus* (MRSA), **D:** *Escherichia coli*, **E:** *Klebsiella pneumonia* **F:** *Pseudomonas aeruginosa* **G:** *Candida albicans*

DISCUSSION

The aim of this study is to determine the antimicrobial activities of the ethereal extracts from Pinaceae and Cupressaceae species due to terpenic compounds mostly migrated to ethereal extract which are generally known for their antimicrobial potency.

In the current study, we found that the ethereal extracts of Pinaceae species exhibited weak antibacterial activity against the various tested bacteria except for *Abies bornmulleriana*, *Cedrus libani* and *Pinus halepensis*. No antifungal activity was observed against *Candida albicans* in all extracts. On the other hand, ethereal extracts of the Cupressaceae species exhibited almost weak antimicrobial activity against the tested bacteria, but no antifungal activity was observed against *Candida* strain.

Dığrak et al. (13) investigated the antimicrobial activities of chloroform, acetone and methanol extracts of *Pinus brutia*, *Juniperus oxycedrus*, *Abies cilicica*, *Cedrus libani* and *Pinus nigra* against *Bacillus megaterium*, *B. subtilis*, *B. cereus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Staphylococcus aureus*, *Mycobacterium smegmatis*, *Proteus vulgaris*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Candida albicans*, *C. tropicalis* and *Penicillium italicum*. They indicated that none of the extracts had antifungal activity against the

tested fungi. Except the chloroform and acetone extracts of the leaves of *Abies cilicica*, none of them had antibacterial activity against *E. coli*. (13). In our study, *Abies* species similarly exhibited antimicrobial activity against the tested bacteria.

Karaman et al. (7) investigated the antimicrobial activities of the aqueous and methanol extracts of the leaves of *Juniperus oxycedrus*. They indicated that the aqueous extract of *J. oxycedrus* had no antimicrobial effect against the test microorganisms whereas the methanol extract had inhibitory effects on the growth of 57 strains of 24 bacterial species in the genera of *Acinetobacter*, *Bacillus*, *Brevundimonas*, *Brucella*, *Enterobacter*, *Escherichia*, *Micrococcus*, *Pseudomonas*, *Staphylococcus* and *Xanthomonas*. In addition, *Candida albicans* isolates were also inhibited by the extracts (7). However, in the present study, none of the extracts displayed antifungal activity.

Selim et al. (14) indicated that the methanol extract of *Cupressus sempervirens* had antibacterial activity against *Enterococcus faecalis*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Salmonella indica* but it did not have antifungal activity against *Candida albicans* (14). The results of this study are consistent with our study.

In another study, the essential oil of the leaves of *Cupressus horizontalis* had no effect against *Bacillus subtilis*, *Candida albicans*, *Escherichia coli* and *Staphylococcus aureus* while the essential oil of fruits of these species showed a weak antimicrobial activity against *B. subtilis*. The essential oil of *Cupressus sempervirens* leaves had a weak antimicrobial activity while the essential oil of fruits of this species exhibited stronger antimicrobial activity against all tested microorganisms (15). In most cases, the essential oils are well-known for their antimicrobial activity due to volatile components. The ethereal extracts have been considered mainly terpenoid components; probably volatiles are not present dominantly in order to emerging the antimicrobial activity in the extracts. Therefore, in this study, the extracts have been evaluated for their antimicrobial potency owing to their terpenic profile in ethereal extracts.

To our knowledge, this is the first comprehensive investigation on the evaluation of antimicrobial activities on ethereal extracts of these species.

CONCLUSION

The purpose of this study was to screen the antimicrobial potency of ethereal plant extracts from coniferous species against bacteria and a fungus. Therefore, we investigated the antimicrobial activity of the ethereal extract from the Pinaceae and Cupressaceae species which are mostly included the terpenic compounds well-known their antimicrobial potency. In this context, any notable activity has not been observed for the extracts of conifer species. Probably, the components in the ethereal extracts as much as whole extract do not lead to any activity. As a conclusion, the extracts cannot be consider as antimicrobial agents beside the terpenic compounds which are found in ethereal extracts of the tested species have no potency in antimicrobial activity. By the way, this is the first report from these conifer species which are tested in this assay for their possible antimicrobial activities.

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