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Antibiotic Action of Seed Lipids from Five Tree Species Grown in Turkey

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Abstract

In this study, the seed lipids of Austrian pine (*Pinus nigra* Arnold.), Ehrami pine (*Pinus nigra* var. *pyramidalis*), Caucasian fir [*Abies nordmanniana* (Stev.) Spach], Eastern red (*Thuja orientalis* L.) and Scots pine (*Pinus sylvestris* L.) grown in Turkey were extracted with a mixture of methanol and chloroform. The bacterial and antifungal activities of the seed lipids were tested against various pathogenic bacteria and fungi by applying the disc-diffusion method. The seed lipids of *A. nordmanniana* were found to be most effective against the tested microorganisms, except for *Pseudomonas aeruginosa* and *Micrococcus luteus* bacteria, followed by *T. orientalis* and *P. sylvestris*, respectively. However, the seed lipids of *P. nigra* var. *pyramidalis* were not effective on the growth of any microorganism. *Bacillus subtilis* and *Listeria monocytogenes* bacteria were found as the most resistant bacteria in the study. Furthermore, the seed lipids of *A. nordmanniana* and, somewhat, *T. orientalis*, were generally comparable to two reference antibiotics.

Keywords: Seed lipids, antifungal activity, antibacterial activity, tree species, Turkey, disc-diffusion method.

Introduction

A number of extracts of plants have been used as traditional medicine because they contain a variety of substances useful for the treatment of infectious and chronic diseases (Arrabal & Cortija, 1994; Bağcı & Dığrak, 1996a; Dığrak et al., 1999; İlçim et al., 1998; Himejama et. al., 1992). Many plant genera such *as Betula*, *Fagus*, *Fraxinus*, *Juniperus*, *Picea*, *Pinus*, etc., are among the medicinal plants recorded in some Codex and Farmacopies (Ceylan, 1995).

Constant lipids are storage substances of plants and animals. They are generally found in seeds of the plants (Endospermae and Cotyledoneae) and, rarely, in mesocarp. It is known that the unsaturated lipids of Arecaceae, Asteraceae, Betulaceae and Euphorbiaceae families play an important role in medicine and economy. Constant lipids, esters made up of high fatty acids and the glycerol, are mainly collected in the seeds and fruits. They are used for medicinal purposes, margarine or industry (Baytop, 1983).

Seventy-eight percent of seeds, 80–58% of fruit pulps, 60–90% of animal tissues and 10–20% of fish contain fats or lipids. Lipids are fatty acids, and their derivatives and substances related, biosynthetically or functionally, to these compounds in higher plants, usually exhibit a more limited chain-length distribution. It was also reported that the fatty acids that occur in the seed lipids might be used as criteria in classification of species. For example, Δ5-C_{18} and Δ5-C_{20} polyenoic fatty acids are typical identification parameters in all conifers (Tagaki & Itabishi, 1982).

It is obvious that there are some compounds in plant seeds used for medicinal and therapeutic purposes. For example, γ-linolenic acid is available in seed lipids of a variety of plants. However, they are restricted to certain genera and families, e.g., Boraginaceae family (Tsevegsuren & Aietzetmuller, 1983). It is also well known that these fatty acids have a positive effect against the heart, multiplesclerosis and Parkinson diseases. Moreover, *Pinus pinea* seeds are collected and used for its oil (Sharma et al., 1990).

The aim of this study was to obtain various lipids from the seeds of five tree species grown in Turkey and to test them against some typical bacteria and fungi. Furthermore, the

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antibacterial and antifungal activities of the seed lipids were compared with those of known antibiotics.

Materials and methods

Materials

The seeds were obtained from several tree species grown in Turkish state forests. In this study, the seeds of *Pinus nigra* Arnold. (Pinaceae) (Ankara-Nallıhan-Inner Anatolia Region), *Thuja orientalis* L. (Cupressaceae) (Çankırı-Inner Anatolia Region), *Pinus sylvestris* L. (Pinaceae) (Kastamonu, Ilgaz-Gökdere, Middle Black Sea Region), *Pinus nigra* var. *pyramidalis* (Pinaceae) (Kütahya, Tavşanlı-İkizoluk, Inner West Aegean Region) and *Abies nordmanniana* (Stev.) Spach. (Pinaceae) (Artvin-Ortaköy, Eastern Black Sea Region) were collected in spring season. The collected seeds were stored in Fırat University Herbarium (FUH), Turkey. Chloroform, hexane, isopropanol and methanol (as solvents) were purchased from Merck.

Extraction of seed lipids

For the lipid extraction in the seed, the seeds (10 g) were homogenized with 100 ml of isopropanol to minimize artifactual degradation of lipids by tissue enzymes (Christie, 1989). The mixture was first filtered, the obtained insoluble residue was then re-extracted with fresh isopropanol and was finally left in isopropanol-chloroform solution (1 : 1; v/v) overnight. The filtrates were then combined, and most of the solvents were removed by a rotary evaporator. Subsequently, the remaining lipid residues were taken by using a chloroform-methanol solution (2 : 1; v/v), and nonlipid contaminants were removed by washing with 0.88% KCl. Total lipid extracts were determined (Christie, 1989).

Bioassay

Growth inhibitory activity was tested against 12 microorganisms: *Escherichia coli* ATCC 25922, *Bacillus megaterium* DSM 32, *Bacillus subtilis* IMG 22, *Bacillus brevis* FMC 3, *Pseudomonas aeruginosa* DSM 50071, *Listeria monocytogenes* A, *Klebsiella pneumoniae* FMC 5, *Enterobacter aerogenes* CCM 2531, *Staphylococcus aureus* Cowan 1, *Micrococcus luteus* LA 2971, *Pseudomonas fluorescens* and *Proteus vulgaris* FMC 1 bacteria, and *Candida albicans* CCM 314, *Kluvyeromyces fragilis* A and *Saccharomyces cerevisiae* WET 136 fungi. These microorganisms were provided by the Microbiology Laboratory Culture Collection, Department of Biology, Fırat University, Turkey.

The bacteria were incubated at 37 ± 0.1 °C for 24 h by injection into Nutrient Broth (Difco), and the fungi were incubated in Sabourand Dextrose Broth (Difco) at 25 ± 0.1 °C for 24 h. Mueller Hinton Agar (MHA) (oxoid) and Sabourand Dextrose Agar (SDA) sterilized in a flask and cooled to 45–50 °C was placed on the sterilized Petri dishes in the amount of 15 ml. Cultures of bacteria and fungi (0.1 ml; 10^6/ml for the bacteria and 10^5/ml for the fungi) were homogeneously injected. Dishes injected with extracts were located on the solid agar medium by pressing slightly (Sundar, 1996). The seed lipids from the species studied were dissolved in hexane and prepared as 5 and 10 ppm. Ten µl of seed lipid solutions and hexane (solvent) were dropped on to sterilized empty antibiotic discs of 6 mm diameter. After the Petri dishes so-obtained were left at 4 °C for 2 h. Plates with fungi were incubated at 25 ± 0.1 °C, for 24 h, however, and bacteria were incubated at 37 ± 0.1 °C for 24 h (Collins et al., 1989; Bradshaw, 1992).

The bioassay tests were performed in three parallel parts. Growth inhibition zones of the microorganisms for the seed lipids of five tree species and three reference antibiotics were measured in millimeters.

Results and discussion

The antimicrobial activities of lipids from five different tree species and streptomycin sulphate and ampicillin sodium antibiotics are listed in Tables 1 and 2, respectively. As depicted in Table 1, while the lipids of seeds of the *P. nigra* var. *pyramidalis* did not inhibit the growth of any microorganisms studied, the seed lipids of *P. nigra* inhibit the development of the *E. coli*, *P. aeruginosa*, *S. aureus* and *S. cerevisieae*. The seed lipids of *A. nordmanniana* were determined as the most effective agents against the whole microorganism tested, except for the *P. aeruginosa* and *M. luteus*, followed by *T. orientalis*, *P. nigra* and *P. sylvestris*, respectively (Table 1).

It is also clear from these data table that the lipids of the seeds of *T. orientalis* are quite effective against all the bacteria, with the exception of *E. coli*, *B. subtilis* and *L. monocytogenes*, showing an inhibition zone of 8–20 mm. Moreover, *P. sylvestris* seed lipids inhibit the growth of *B. brevis*, *P. aeruginosa*, *K. pneumoniae*, *S. aureus* and *M. luteus* resulting in inhibition zones of 7–18 mm (Table 1). Hexane (used as control solvent) did not have any effect against the microorganisms (Table 1).

On the other hand, the other three *Pinus* species, namely, *Pinus nigra* Arnold., *Pinus nigra* var. *pyramidalis* and *Pinus sylvestris* L., did not show inhibitory activity against *S. cerevisiae*, except for *P. nigra* (Table 1). Whereas all of the microorganisms are resistant against the seed lipids of *P. nigra* var. *pyramidalis*, *S. aureus* was found to be the most susceptible bacterium with the other four tested lipid samples. *B. subtilis* and *L. monocytogenes* bacteria were found to be the most resistant bacteria against tested lipids, except for seed lipids of *Abies nordmanniana*.

The seed lipids of the tree species are generally observed to be somewhat less effective against the studied bacteria and fungi in comparison with the reference antibiotics, i.e.,

Table 1. Inhibition zones of the seed lipids of five coniferous tree species by using various microorganisms.

	Inhibition zones of the seed lipids[a] (mm)					
Microorganisms	1[b]	2[c]	3[d]	4[e]	5[f]	6[g]
Escherichia coli	12	–	14	–	–	–
Bacillus megaterium	–	–	14	12	–	–
Bacillus subtilis	–	–	10	–	–	–
Bacillus brevis	–	–	12	9	7	–
Pseudomonas aeruginosa	6	–	–	14	7	–
Listeria monocytogenes	–	–	12	–	–	–
Klebsiella pneumoniae	–	–	13	9	7	–
Enterobacter aerogenes	–	–	15	7	–	–
Staphylococcus aureus	19	–	30	20	18	–
Micrococcus luteus	–	–	–	13	7	–
Pseudomonas fluorecnes	–	–	15	8	–	–
Proteus vulgaris	–	–	14	9	–	–
Saccharomyces cerevisiae	6	–	16	17	–	–
Candida albicans	–	–	13	12	–	–
Kluvyeromyce fragilis	–	–	14	16	–	–

[a] For each disk, 10 µl lipids were used. [b] 1, *Pinus nigra*; [c] 2, *Pinus nigra* var. *pyramidalis*; [d] 3, *Abies nordmanniana*; [e] 4, *Thuja orientalis*; [f] 5, *Pinus sylvestris*; [g] 6, Hexane (Control).

streptomycin sulphate and ampicillin sodium, shown in Table 2. However, as far as the sufficient limits of inhibition zone for *S. aureus* bacterium are concerned, the seed lipids of *T. orientalis*, *A. nordmanniana* and *P. sylvestris* are comparable to the reference antibiotics (Tables 1 and 2).

In addition, the antifungal activities of seed lipids of *A. nordmanniana* subsp. *nordmanniana* and *T. orientalis* were found as the most effective agents against *S. cerevisiae*, *C. albicans* and *K. fragilis* (Table 1). However, the other three *Pinus* species studied did not show any inhibitive activity against the fungi. In view of antifungal activity, it can be noted that all the seed lipids are much more effective than the reference antibiotics.

In addition, it was reported that the antimicrobial activities of the seed lipids of *T. orientalis* were found smaller than those of *Thuja plicata* and greater than those of *P. sylvestris*, studied elsewhere (Bağcı & Dığrak, 1996b). Furthermore, the antibacterial activities of the seed lipids of *A. nordmanniana* subsp. *nordmanniana* were generally greater than those of essential oils of *A. nordmanniana* (Bağcı & Dığrak, 1994; Bağcı & Dığrak, 1996a; Bağcı & Dığrak, 1997). Meanwhile, in comparison to the essential oils of *Abies nordmanniana* species, its seed lipids were found to have less antifungal activities.

Table 2. Inhibition zones of ampicillin sodium, streptomycin sulphate and antifungal nystatin by using various microorganisms.

	Inhibition zone (mm)	
Microorganisms	Ampicilin sodium (10 µg)	Streptomycine sulphate (10 µg)
Escherichia coli	10	–
Bacillus megaterium	15	17
Bacillus subtilis	15	19
Bacillus brevis	14	16
Pseudomonas aeruginosa	10	14
Listeria monocytogenes	12	19
Klebsiella pneumoniae	17	16
Enterobacter aerogenes	16	21
Staphylococcus aureus	16	17
Micrococcus luteus	34	–
Pseudomonas fluorecnes	15	15
Proteus vulgaris	14	17
	Antifungal Nystatin 30 µg	
Saccharomyces cerevisiae	20	
Candida albicans	18	
Kluvyeromyces fragilis	15	

Antibiotics	Resistant (mm)	Partially-resistant (mm)	Susceptible (mm)
Streptomycine sulphate	<11	12–14	>15
Ampicilline sodium	<11	12–15	>16
Nystatin	<12	14–17	>18

Conclusions

The seed lipids of *A. nordmanniana* were most effective against the microorganisms examined with the exception of *Pseudomonas aeruginosa* and *Micrococcus luteus*, followed by *T. orientalis* and *P. sylvestris*, respectively. The seed lipids of *P. nigra* var. *pyramidalis* were not effective on the growth of any microorganism. Among the studied bacteria, *Bacillus subtilis* and *Listeria monocytogenes* were the most resistant bacteria. Although the inhibition zones of reference antibiotics were generally higher than those of lipids, they could be considered to have sufficient resistant limits against the studied microorganisms.

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