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Antimicrobial Activities of Various Medicinal and Commercial Plant Extracts

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Abstract: The antibacterial activities of the alcohol, ethyl acetate, acetone and chloroform extracts of 5 plant species were studied. The extracts of *Pimpinella anisum* (L.) (anise, aniseed) (seed), *Coriandrum sativum* (L.) (coriander, cilantro) (seed), *Glycyrrhiza glabra* (L.) (liquorice) (root), *Cinnamomum cassia* Blume (cassia bark, Chinese cinnamon) (bark), and *Juniperus oxycedrus* (L.) (juniper) (seed) were tested in vitro against 13 bacterial species and strains by the agar diffusion method. *Bacillus brevis* FMC 3, *Bacillus cereus* EU, *Bacillus megaterium* DSM 32, *Bacillus subtilis* IMG 22, *Bacillus subtilis* var. *niger* ATCC 10, *Enterococcus faecalis*, *Klebsiella pneumoniae* FMC 5, *Listeria monocytogenes* SCOTT A, *Micrococcus luteus* LA 2971, *Mycobacterium smegmatus* RUT, *Pseudomonas aeruginosa*, *Staphylococcus aureus* ATCC 25923 and *Yersinia enterocolitica* O:3 P 41797 were used in this investigation.

The results indicated that the alcohol extracts of *Pimpinella anisum* seeds showed antibacterial activity against *Micrococcus luteus* and *Mycobacterium smegmatus* 8 mm/20 µl, while the extracts of *Coriandrum sativum* seeds showed no inhibition zone against microorganisms that were tested. The extracts of *Glycyrrhiza glabra* roots showed various antibacterial activities (7-11 mm/20 µl inhibition zone) against the microorganisms tested. The alcohol extracts did not inhibit *B. subtilis* var. *niger*, *B. brevis*, *E. faecalis*, *L. monocytogenes*, *P. aeruginosa* and *Y. enterocolitica*. The ethyl acetate extracts did not inhibit *B. subtilis* or *Y. enterocolitica*, and the acetone extracts did not inhibit *E. faecalis*, *L. monocytogenes*, *P. aeruginosa* or *Y. enterocolitica*. The chloroform extracts showed no inhibition effect against *P. aeruginosa* or *Y. enterocolitica*. The extracts of *Cinnamomum cassia* bark showed antibacterial activity (7-29 mm/20 µl inhibition zone) to the microorganisms tested. The alcohol extracts showed a 7 mm/20 µl inhibition zone against *B. megaterium* and *E. faecalis*. The ethyl acetate extracts did not inhibit *B. subtilis* var. *niger*, *P. aeruginosa* or *Y. enterocolitica*, the acetone extracts showed no antibacterial activity against *B. subtilis* var. *niger* or *Y. enterocolitica*, and the chloroform extracts did not inhibit *B. subtilis* var. *niger* or *P. aeruginosa*. The *Juniperus oxycedrus* seed extracts showed a 7-15 mm/20 µl inhibition zone against the listed microorganisms, but the ethyl acetate extracts showed no inhibition zone against *E. faecalis*, while acetone and chloroform extracts did not inhibit *P. aeruginosa*.

Key Words: *Pimpinella anisum*, *Coriandrum sativum*, *Cinnamomum cassia*, *Juniperus oxycedrus*, *Glycyrrhiza glabra*, Antimicrobial activity.

Tıbbi ve Ticari Amaçlı Kullanılan Bazı Bitki Ekstraktlarının Antimikrobiyal Etkileri

Özet: Tıbbi ve ticari amaçlı kullanılan *Pimpinella anisum* (L.) (anise, aniseed) (tohum), *Coriandrum sativum* (L.) (coriander, cilantro) (tohum), *Glycyrrhiza glabra* (L.) (liquorice) (kök), *Cinnamomum cassia* Blume (cassia bark, Chinese cinnamon) (kabuk) ve *Juniperus oxycedrus* (L.) (juniper) (tohum)'un alkol, etil asetat, aseton ve kloroform ekstraktlarının antimikrobiyal etkileri in-vitro olarak *Bacillus brevis* FMC 3, *Bacillus cereus* EU, *Bacillus megaterium* DSM 32, *Bacillus subtilis* IMG 22, *Bacillus subtilis* var. *niger* ATCC 10, *Enterococcus faecalis*, *Klebsiella pneumoniae* FMC 5, *Listeria monocytogenes* SCOTT A, *Micrococcus luteus* LA 2971, *Mycobacterium smegmatus* RUT, Pseudomonas aeroginosa, *Staphylococcus aureus* ATCC 25923 ve *Yersinia enterocolitica* O:3 P 41797 'ya karşı agar difüzyon metodu ile araştırılmıştır.

Araştırma sonunda *Pimpinella anisum* tohumunun alkol ekstraktı *Micrococcus luteus* ve *Mycobacterium smegmatus*'u 8 mm/20 µl oranında etkilerken, *Coriandrum sativum* tohumu adı geçen bakterilere karşı inhibisyon zonu oluşturmamıştır. *Glycyrrhiza glabra* bitkisinin kökünden elde edilen ekstraktlar çeşitli oranlarda antimikrobiyal etki gösterirken (7-11mm/20ml inhibisyon zonu), alkol ekstraktı *B. subtilis* var. *niger*, *B. brevis*, *E. faecalis*, *L. monocytogenes*, *P. aeruginosa* ve *Y. enterocolitica* üzerine etkisiz kalmıştır. Ayrıca bitkinin etil asetat ekstraktı *B. subtilis* ve *Y. enterocolitica*, aseton ekstraktı *E. faecalis*, *L. monocytogenes*, *P. aeruginosa* ve *Y. enterocolitica*, kloroform ekstraktı *P. aeruginosa* ve *Y. enterocolitica*'ya antimikrobiyal etki göstermemiştir. *Cinnamomum cassia*'nın kabuk ekstraktları çalışmada kullanılan bakterilere karşı 7-29 mm/20 µl inhibisyon zonu oluşturmuştur. Bitkinin alkol ekstraktı *B. megaterium* ve *E. faecalis*'i 7 mm/20 µl oranında etkilerken, etil asetat ekstraktı *B. subtilis* var. *niger*, *P. aeruginosa* ve

Y. enterocolitica'yı, aseton ekstraktı *B. subtilis* var. *niger* ve *Y. enterocolitica*'yı etkilememiştir. Ayrıca kloroform ekstraktı *B. subtilis* var. *niger* ve *P. aeruginosa* üzerine de etkisiz kalmıştır. *Juniperus oxycedrus* bitkisinin ekstraktları 7-15 mm/20 µl oranında inhibisyon alanı oluştururken etil asetat ekstraktı *E. faecalis*'i, aseton ve kloroform ekstraktı *P. aeruginosa*'yı etkilememiştir.

Anahtar Sözcükler: *Pimpinella anisum*, *Coriandrum sativum*, *Cinnamomum cassia*, *Juniperus oxycedrus*, *Glycyrrhiza glabra*, Antimikrobiyal aktivite.

Introduction

Various medical plants have been used for years in daily life to treat disease all over the world. Turkey is an important floristic center internationally because of its geographic location, climate and the presence of nearly 10,000 natural plant species. According to a study performed by the WHO based on publications on pharmacopoeias and medical plants in 91 countries, the number of medicinal plants is nearly 20,000 (1). The characteristics of the plants that inhibit microorganisms and are important for human health have been researched in laboratories since 1926 (2-6). Traditional medical treatments in daily life are now being used with empiric methods.

Pimpinella anisum (L.) (Umbelliferae-Apiaceae) (anise, aniseed) was first cultivated as a spice by the ancient Egyptians and later by the Greeks, Romans and Arabs. Although widely grown commercially its cultivation has declined in recent years through competition with cheaper anise flavorings, such as *Illicium verum* and synthetic anethole. It is a sweet, warming, and stimulant herb that improves digestion, benefits the liver and circulation, and has expectorant and estrogenic effects. The parts of the plant used are its leaves, seeds and oil. Fresh leaves are added to salads, vegetables and various cooked dishes in various countries. The seeds are used to flavor confectionery (especially aniseed balls), dried figs, cakes, bread and curries. Seeds and oil form the basis of all anise-flavored drinks, such as Pernod, ouzo, rakı and arak, which turn milky when diluted with water. In addition, its oil is used commercially in perfumery, tobacco manufacture and pharmaceutical productior. As a medicinal, it is used internally for dry coughs, whooping cough, bronchitis, tracheitis, bronchial asthma, indigestion, wind, colic and insufficient lactation. It is used externally for lice scabies and as a chest rub for bronchial complaints. Anise combines well with *Menthax piperita* for colic, *Prunus serotina* for tracheitis, *Lactuca* spp. for dry coughs, and with *Marrubium vulgare*, *Tussilago farfara*, *Lobelia inflata* and *Symlocarpus foetidus* for bronchial complaints. The oil is often mixed with the oil of *Sassafras albidum* for skin parasites, and with that of *Eucalyptus globulus* as a chest rub. Traditionally, anise has been regarded as an aphrodisiac (7).

Coriandrum sativum (L.) (Umbelliferae-Apiaceae) (coriander, cilantro) was introduced to Chinese cooking and medicine around AD 600, since when it has been known as *hu*, 'foreign'. In the Chinese Materia Medica (G.A. Stuart, 1911), it was recommended for certain types of non-pathogenic food poisoning caused by decaying matter. The parts of the coriander used are its leaves, seeds and oil. The fresh leaves and ripe seeds have quite different aromas and uses. Both the leaves and seeds are rich in volatile oils that act mainly on the digestive system, stimulating the appetite and relieving irritation. They also act as an expectorant. The oil is fungicidal and bactericidal. The, leaves are widely used to flavor food, especially in the Middle East, and southeast Asia. The seeds are also an ingredient of curries and pickling spices, dishes *a la grecque*, and bakery products. Medicinally, coriander is used internally for minor digestive problems, and externally for hemorrhoids and painful joints (seeds). Seeds reduce griping in laxative preparations based on *Rheum officinale* and *Cassia angustifolia*. The oil adds to the flavor of gin, vermouth and Chartreuse, and is also prized in perfumery (7).

Roots and stolons of *Glycyrrhiza glabra* (L.) (Leguminosae/Papilionaceae) (liquorice) are used. The main ingredient of *G. glabra* is glycyrrhizin, a substance 50 times sweeter than sucrose, with cortisone-like effects. It is a very sweet, moist, soothing herb that is anti-inflammatory and expectorant, controls coughing, and has hormonal effects. It detoxifies and protects the liver. Medicinally, it is used internally for Addison's disease, asthma, bronchitis, coughs, peptic ulcer, arthritis, allergic complaints and following steroid therapy. It is not given to pregnant women or patients with high blood pressure, kidney disease or those taking digoxin-based medication. Externally, liquorice is used for eczema, herpes, and shingles. Economically, the roots are boiled to extract the familiar black substance used in liquorice confectionery, and this is sold dried to eat.

Liquorice is also the basis for most proprietary laxatives and its extracts flavor tobacco, beer, soft drinks and pharmaceutical products, and are used as a foaming agent in beers and fire extinguishers (7).

Cinnamomum cassia Blume (syn. *C. Aromaticum*) (Lauraceae) (Chinese cinnamon, cassia bark) is grown in the southeast of China (8). Various parts such as the fruits, oil, inner bark and leafy twigs of cinnamon are used. The inner bark is a pungent, sweet, hot herb that stimulates the circulatory system, improves digestion, relieves spasms and vomiting, and controls infections. Twigs increase perspiration and lower fever. It is used in western Asia in curries, the USA for baked foods, and in China for meat dishes (especially with anise, star anise, cloves and fennel seeds as one of the Chinese '5 spices'). Medicinally, it is used internally in Western medicine, mainly in preparations for diarrhea, flatulent dyspepsia and colic and colds; in Chinese medicine it is used for diarrhea, poor appetite, low vitality, kidney weakness, rheumatism and coldness. It is also used for colds, influenza, fevers, arthritic and rheumatic complaints, angina, palpitations and digestive complaints related to cold and chills. Economically, the fruits (known as 'cassia buds') resemble cloves in appearance and are widely used for flavoring in the food industry. Cassia oil contains 80-90 % cinnamaldehyde, used mainly in medicine, foods and cosmetics (7).

Although the plant *Juniperus oxycedrus* (L.) (Cupressaceae) (juniper) is a Mediterranean plant, it is found in Thrace and many parts of Anatolia. A drug made from *J. oxycedrus* is called *Pix Juniperi* (T.K.) (Juniper Tarry, *Oleum cadinum*). *Pix Juniperi* shows a powerful antiseptic effect and is used externally in the field of dermatology for mange, eczema and curing oily hair. It is found in soaps and in pomades with the aim of curing alopecia (7). Various junipers are used medicinally for example, native North Americans use it to treat a wide range of conditions, from kidney complaints to dandruff and syphilis. Various parts such as the fruits, seeds and oil of the juniper are used (7).

In this study the antibacterial activities of alcohol, ethyl acetate, acetone and chloroform extracts of the 5 plant species were studied. The dried extracts of *Pimpinella anisum* (Anise seed), *Cinnamomum cassia* (Cinnamon bark), *Coriandrum sativum* (Coriander seed), *Juniperus oxycedrus* (Juniper seed) and *Glycyrrhiza glabra* (Liquorice root) were tested in vitro against 13 bacterial species and strains by the agar diffusion method. *Bacillus brevis* FMC 3, *Bacillus cereus* EU, *Bacillus megaterium* DSM 32, *Bacillus subtilis* IMG 22, *Bacillus subtilis* var. *niger* ATCC 10, *Enterococcus faecalis*, *Klebsiella pneumoniae* FMC 5, *Listeria monocytogenes* SCOTT A, *Micrococcus luteus* LA 2971, *Mycobacterium smegmatus* RUT, *Pseudomonas aeroginosa*, *Staphylococcus aureus* ATCC 25923 and *Yersinia enterocolitica* O:3 P 41797 were used in this investigation.

Materials and Methods

Glycyrrhiza glabra and *Juniperus oxycedrus* were collected from different locations in the Kahramanmaraş region, Turkey. *Pimpinella anisum*, *Coriandrum sativum* and *Cinnamomum cassia* were taken from different spicesellers in the Malatya region, Turkey. The taxonomic identities of these 5 plants were determined at the Biology Department of KSU's Science and Art Faculty. The anise seeds, cinnamon bark, coriander seeds, liquorice roots and juniper seeds were taken, dried and used in this study to determine their antibacterial activities against the microorganisms listed above.

The plants were dried and broken into small pieces under sterile conditions, and 20 g of each plant part were extracted with 150 ml of ethyl acetate, alcohol, chloroform and acetone solvent (Merck, Darmstadt) for 24 h by using Soxhlet equipment (9). All the extracts thus obtained were injected into empty sterilized antibiotic disks of 6 mm diameter (Schleicher & Schül No: 2668, Germany) in amounts of 20 µl. Disks injected with 20 µl of pure ethyl acetate, methanol, chloroform or acetone served as negative controls.

All the bacteria mentioned above were incubated at 30 ± 0.1 °C for 24 h by inoculation into nutrient broth (Difco). Sterilized petri dishes (9 cm diameter) were inoculated with 0.01 ml of one of the above culture media (10^5-10^6 bacteria per ml). Muller-Hinton agar (Oxoid) sterilized in a flask and cooled to 45-50 °C was distributed by pipette (15 ml) into each inoculated petri dish and swirled to distribute the medium homogeneously. Disks injected with extracts were applied on the solid agar medium by pressing slightly. The treated petri dishes were placed at 4 °C for 1-2 h and then incubated at 35 ± 0.1 °C for 18-24 h (9-11). At the end of the period, the inhibition zones formed on the media were measured with a transparent ruler in millimeters.

Results and Discussion

The in vitro antibacterial activities of the dried extracts of *Pimpinella anisum*, *Cinnamomum cassia*, *Coriandrum sativum*, *Juniperus oxycedrus* and *Glycyrrhiza glabra* are shown in Table 1. In addition, the inhibition zones formed by standard antibiotic disks, and those disks injected with only ethyl acetate, methanol, chloroform, and acetone (negative controls), are listed in Table 2.

As can be seen from Table 1, the extracts of *Pimpinella anisum* seed showed antibacterial activity to *Micrococcus luteus* and *Mycobacterium smegmatus* 8 mm/20 µl. Sökmen et al. (12,13) and Larhsiri et al. (14) observed that anise showed various inhibitory effects on the microorganisms they used.

Coriandrum sativum seed extracts showed no inhibition zone to the microorganisms tested, but in 1998 Baratta et al. (15) and in 2001 Elgayyar et al. (16) and Larran et al. (17) studied *Coriandrum sativum* and observed that the essential oil of coriander inhibited microorganisms.

Glycyrrhiza glabra (liquorice) root extracts showed various antibacterial activities (7-11 mm/20 µl inhibition zone) against the microorganisms tested. The alcohol extracts did not inhibit *B. subtilis* var. *niger*, *B. brevis*, *E. faecalis*, *L. monocytogenes*, *P. aeruginosa* or *Y. enterocolitica*. The ethyl acetate extracts did not inhibit *B. subtilis* or *Y. enterocolitica*, and the acetone extracts did not inhibit *E. faecalis*, *L. monocytogenes*, *P. aeruginosa* or *Y. enterocolitica*. The chloroform extracts showed no inhibitory effects against *P. aeruginosa* and *Y. enterocolitica*.

Cinnamomum cassia bark extracts showed antibacterial activity (7-29 mm/20 µl inhibition zone) to the microorganisms tested. The alcohol extracts showed a 7 mm/20 µl inhibition zone against *B. megaterium* and *E. faecalis*. The ethyl acetate extracts did not inhibit *B. subtilis* var. *niger*, *P. aeruginosa* or *Y. enterocolitica*, the acetone extracts showed no antibacterial activity against *B. subtilis* var. *niger* or *Y. enterocolitica*, and the chloroform extracts did not inhibit *B. subtilis* var. *niger* or *P. aeruginosa*. In 1996 Biavati et al. (18) studied *Cinnamomum cassia* and they found that there was an antimicrobial effect of the essential oil of cinnamon.

Juniperus oxycedrus seed extracts showed a 7-15 mm/20 µl inhibition zone against the listed microorganisms, but the ethyl acetate extracts showed no inhibition zone against *E. faecalis*, while the acetone and chloroform extracts did not inhibit *P. aeruginosa*. In

Table 1. The antibacterial activities of alcohol, ethyl acetate, acetone and chloroform extracts of the dried extracts of *Pimpinella anisum* (anise seed), *Coriandrum sativum* (coriander seed), *Glycyrrhiza glabra* (liquorice root), *Cinnamomum cassia* (cinnamon bark), and *Juniperus oxycedrus* (juniper seed).

Microorganisms	Inhibition Zones (mm/20 µl)																			
	Pimpinella anisum				*Coriandrum sativum*				*Glycyrrhiza glabra*				*Cinnamomum cassia*				*Juniperus oxycedrus*			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
B. brevis	-	-	-	-	-	-	-	-	-	7	7	9	-	11	16	20	11	10	10	10
B. cereus	-	-	-	-	-	-	-	-	8	8	8	9	-	10	15	14	10	9	9	10
B. megaterium	-	-	-	-	-	-	-	-	7	9	7	8	7	9	15	14	10	9	9	10
B. subtilis	-	-	-	-	-	-	-	-	8	-	7	8	-	11	15	15	9	8	8	10
B. subtilis var. *niger*	-	-	-	-	-	-	-	-	-	7	10	11	-	-	-	-	9	10	9	9
E. fecalis	-	-	-	-	-	-	-	-	-	7	-	7	7	7	8	12	9	-	7	7
K. pneumoniae	-	-	-	-	-	-	-	-	8	8	7	8	-	9	15	14	9	8	8	9
L. monocytogenes	-	-	-	-	-	-	-	-	-	7	-	7	-	8	9	11	7	7	7	9
M. luteus	8	-	-	-	-	-	-	-	8	10	7	10	-	9	15	15	10	10	9	10
M. smegmatus	8	-	-	-	-	-	-	-	9	9	9	8	-	14	29	15	14	10	12	15
P. aeruginosa	-	-	-	-	-	-	-	-	-	7	-	-	-	-	7	-	9	7	-	-
S. aureus	-	-	-	-	-	-	-	-	7	7	7	7	-	7	9	12	8	9	9	10
Y. enterocolitica	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9	8	7	7	8

Extraction solvent (20 µl) A: alcohol, B: ethyl acetate, C: acetone, D: chloroform

Table 2. The inhibition zones formed by standard antibiotic disks, and the disks injected with only ethyl acetate, methanol, chloroform and acetone (negative controls).

	Diameter of Inhibition Zone (mm)								
	Amp 10 µg	Cef 30 µg	Cefu 30 µg	Cep 30 µg	Oxa 1 µg	Tob 10 µg	Oflo 10 µg	Van 30 µg	Cont A, B, C, D (20 µl)
Bacillus brevis	10	-	-	-	-	26	31	18	-
Bacillus cereus	10	-	-	-	-	24	29	18	-
Bacillus megaterium	20	-	-	-	-	22	30	20	-
Bacillus subtilis	10	-	13	-	-	24	35	20	-
B. subtilis var. *niger*	22	32	26	51	-	30	36	24	-
E. faecalis	15	13	-	-	-	15	23	17	-
K. pneumoniae	14	12	-	-	-	15	20	20	-
Listeria monocytogenes	20	16	15	16	-	24	35	24	-
Micrococcus luteus	15	-	-	-	-	15	25	20	-
Mycobacterium smegmatus	15	-	-	13	-	20	35	22	-
Pseudomonas aeroginosa	-	-	-	10	-	20	25	16	-
Staphylococcus aureus	14	12	-	45	-	25	34	20	-
Yersinia enterocolitica	-	-	12	35	-	10	32	23	-

Amp: ampicillin, cef: cefodizime, cefu: cefuroxime, cep: cephalothin, oxa: oxacillin, tob: tobramycin, oflo: ofloxacin, van: vancomycin, cont: control, A: alcohol, B: ethyl acetate, C: acetone, D: chloroform.

1998, Moreno et al. (19) studied the leaves and stems of *Juniperus oxycedrus* with methanol and dicloromethanol, and they showed that juniper had minor antibacterial activity in mice. When the results obtained with *Juniperus oxycedrus* were compared to those of antibiotics, it was determined that all of the bacteria used in this study are more resistant to oxacillin (1 µg). As can be seen from Table 2, *B. brevis*, *B. cereus*, *B. megaterium* and *M. luteus* were resistant to cefodizime (30 µg), cefuroxime (30 µg), cephalothin (30 µg) and oxacillin (1 µg). *B. subtilis* was resistant to cefodizime (30 µg), cephalothin (30 µg) and oxacillin (1 µg). *B. subtilis* var. *niger* was resistant to oxacillin (1 µg). *E. faecalis* and *K. pneumoniae* were resistant to cefuroxime (30 µg), cephalothin (30 µg) and oxacillin (1 µg). *L. monocytogenes* was resistant only to oxacillin (1 µg). *M. smegmatus* was resistant to cefodizime (30 µg), cefuroxime (30 µg) and oxacillin (1 µg). Ampicillin (10 µg), cefodizime (30 µg), cefuroxime (30 µg) and oxacillin (1 µg) had no effect on *P. aeruginosa*, while cefuroxime (30 µg) and oxacillin (1 µg) no effect on *S. aureus*. Ampicillin (10 µg), cefodizime (30 µg) and oxacillin (1 µg) had no effect on *Y. enterocolitica*.

This study indicated that alcohol extracts of the seed of *Pimpinella anisum*, and ethyl acetate, acetone and chloroform extracts of the bark of *Cinnamomum cassia*, the seed of *Juniperus oxycedrus* and the root of *Glycyrrhiza glabra* showed various antimicrobial effects on the microorganisms listed above, but that *Coriandrum sativum* extracts had no antimicrobial effect on the same microorganisms.

As shown in Table 1, the control disks injected with 20 µl of alcohol, ethyl acetate, acetone and chloroform showed no inhibitory effect against the microorganisms tested.

It is not surprising that there are differences in the antibacterial effects of plant groups, due to the phytochemical differences between species. To better evaluate the plants growing naturally in Turkey that are potentially useful resources, additional studies are necessary from both the medicinal and economic stand points.

According to our results, *P. anisum, C. cassia, G. glabra* and *J. oxycedrus* could be used as raw materials for phytotherapy because of their antibacterial activities.

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