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To cite this article: Nurten Altanlar, Gülçin Saltan Çitoğlu & Betül Sever Yılmaz (2006) Antilisterial Activity of Some Plants Used in Folk Medicine, *Pharmaceutical Biology*, 44:2, 91-94, DOI: [10.1080/13880200600591907](https://doi.org/10.1080/13880200600591907)

To link to this article: <https://doi.org/10.1080/13880200600591907>



Published online: 07 Oct 2008.



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Antilisterial Activity of Some Plants Used in Folk Medicine

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Abstract

Ethanol extracts from six species representing six different families, used in traditional medicine in Turkey were evaluated for their antilisterial activities. The antilisterial activities of *Plantago major* L., *Ononis spinosa* L., *Lythrum salicaria* L., *Juglans regia* L., *Centranthus longiflorus* Stev., and *Teucrium polium* L. were tested against four different *Listeria* isolates (*L. monocytogenes*, *L. ivanovii*, *L. innocua*, and *L. murrayi*) by the agar diffusion method and the macrodilution method. All plants showed antilisterial activity against *L. monocytogenes*.

Keywords: *Centranthus longiflorus*, *Juglans regia*, *Listeria*, *Lythrum salicaria*, *Ononis spinosa*, *Plantago major*, *Teucrium polium*.

Introduction

Listeria is a Gram-positive rod, aerobic, non-spore-forming, and motile by means of flagella, food-borne bacilli. Similar to other Gram-positive bacteria, it produces an endotoxin (Lorber, 1997). The genus *Listeria* is composed of six species, named *L. monocytogenes*, *L. innocua*, *L. seeligeri*, *L. ivanovii*, *L. grayi*, *L. murrayi*, *L. monocytogenes*, and *L. ivanovii*. These species are pathogenic in mice and other animals. However *L. monocytogenes* is also commonly associated with human listeriosis (Jones & Seeliger, 1986).

Listeria monocytogenes is the most important *Listeria* species, causing a wide spectrum of clinical syndromes in humans, which are described as listeriosis. The manifestations of listeriosis include meningitis, septicemia, meningoencephalitis, and intrauterine or cervical infection in pregnant women, which may result in spontaneous abortion or stillbirth. *L. ivanovii* mainly causes abortion in sheep, but cases of listeriosis in cattle and

humans have also been reported (Stelma et al., 1987; Farber & Peterkin, 1991). *L. innocua* present in food-stuffs is the species that is closest to *L. monocytogenes*, but it is not pathogenic. Strains of nearly all *Listeria* species are consumed through food, drinks, (including pasteurized milk) and water, often in large amounts, and pathogenic properties have been found in “apathogenic” *Listeria* as well (Seeliger & Jones, 1986).

Listeria monocytogenes is resistant to different environmental conditions, including acid pH, high NaCl concentration, and refrigeration temperatures. *L. monocytogenes* can grow in many foods when stored at refrigeration temperatures (Embarek, 1993).

Food-borne listeriosis presents as systemic disease in humans, especially in the elderly, immunocompromised, pregnant, and as intestinal illness with fever (Schlech et al., 1983; Gellin & Broome, 1989; Harvey & Gilmour, 1993). *Listeria* species have been reported as susceptible to antibiotics active against Gram-positive bacteria, but more recently, reports of resistance in *Listeria* species have been published. Current therapy of choice for all forms of listeriosis is a combination ampicillin-gentamicin (Moellering et al., 1982).

Herbal medicine has been improved in developing countries as an alternative solution to health problems and costs of pharmaceutical products. The development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources, including plants. Plants used for traditional medicine contain a wide range of substances that are used to treat chronic as well as infectious diseases. Thus, as a continuation of our research on this plants (Çitoğlu & Altanlar, 2003; Çoban et al., 2003), the current study investigated the ability of these plant extracts to inhibit the growth of *Listeria* species by agar diffusion methods.

Accepted: December 1, 2005

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Table 1. The names and origins of the plants used in this study.

<i>Plantago major</i>	B4: Ankara: Kızılcahamam, 1000 m, 22.7.2002, AEF 22910
<i>Ononis spinosa</i>	B4: Ankara: Kızılcahamam, 1000 m, 22.7.2002, AEF 22911
<i>Lythrum salicaria</i>	B4: Ankara: Kızılcahamam, 1000 m, 22.7.2002, AEF 22912
<i>Juglans regia</i>	B4: Ankara: Kızılcahamam, 1500 m, 22.7.2002, AEF 22944
<i>Teucrium polium</i>	B4: Ankara: Kızılcahamam, 1000 m, 22.7.2002, AEF 22924
<i>Centranthus longiflorus</i>	B4: Ankara: Kızılcahamam, 1000 m, 22.7.2002, AEF 22925

Plantago major L. (Plantaginaceae) is used externally in the treatment of wounds and boils. It is used internally to suppress coughs associated with bronchitis and upper respiratory inflammation, to reduce skin inflammation, and as a laxative. The folk name of this plant is *büyük sinirli ot* (Baytop, 1999; Gomez-Flores et al., 2000; Gruenwald et al., 2001; Holetz et al., 2002).

Ononis spinosa L. (Leguminosae) is used for inflammatory disease of the lower urinary tract and also for the treatment of kidney stones. Externally, it is used for the healing of wounds, eczema, and other skin disorders, and is known as *kayışkiran* (Weiss, 1992; Baytop, 1999; Mahasneh and Oqlah, 1999).

Lythrum salicaria L. (Lythraceae) is used internally for diarrhea, chronic intestinal catarrh, hemorrhoids, and eczema. Externally it is used in the treatment of varicose veins, bleeding of the gums, hemorrhoids, and eczema. The whole flowering plant and flowering branch tips of this plant are used in folk medicine, and it is known as *tıbbi levhulma* (Baytop, 1999, Rauha et al., 2000; Gruenwald et al., 2001).

Juglans regia L. (Juglandaceae) is used medicinally for centuries. The plant is used as a topical remedy for dermal inflammation and excessive perspiration of the hands and feet. It is also a common home remedy for the treatment of chronic eczema, scrofula, and inflammation of the lids. The German Commission E has approved the use of *J. regia* leaves for mild and superficial inflammations of the skin. The leaves of this plant are known as *ceviz* and are used topically to treat scalp itching, peeling and dandruff, sunburn and superficial burns, and as an adjunctive emollient and itch-relieving treatment in skin disorders (Alkhawajah, 1997; Ali-Shtayeh & Abu Ghdeib, 1999; Baytop, 1999; Robbers & Tyler, 1999; Blumenthal, 2000; Gruenwald et al., 2001).

Teucrium polium L. (Lamiaceae) is used medicinally as a steam bath for stomach troubles, colds, and fevers. A liquid extract of the plant has been used in the treatment of fungal diseases. In Turkey, it is known as *tüylü kısamahmut* (Baytop, 1999).

Centranthus longiflorus Stev. (Valerianaceae) is known as *kırmızı kedi otu* in Turkey. The aerial part and roots of the plant are used as sedative and anxiolytic (Baytop, 1999).

In this study, We present data on the antilisterial activities of the ethanol extracts of six plants traditionally used in Turkey.

Materials and Methods

Plant samples were collected from the vicinities of Kızılcahamam-Ankara. Prof. Dr. Hayri Duman confirmed taxonomic identities of the plants. Designation of the individuals and their origin are given in Table 1.

Extraction of plant material

The dried and chopped aerial parts of *Plantago major*, *Ononis spinosa*, *Lythrum salicaria*, *Centranthus longiflorus*, *Teucrium polium*, and the leaves of *Juglans regia* (20 g of each) were extracted with ethanol (75% aqueous, 150 ml of each) for 24 h by using a Soxhlet apparatus, respectively (Khan et al., 1988).

Microbiology

Preparation of inoculum

In this study, four different isolates of *Listeria* (*L. ivanovii*, *L. monocytogenes*, *L. innocua*, and *L. murrayi*) were used. These isolates were isolated from meat or meat products. For isolation, Oxford Agar (Merck, Germany) was used (Jones & Seeliger, 1986). Plates were incubated at 37°C for 48 h for suspected *Listeria* colonies, which are small (1 mm diameter), black, and surrounded by black halos after 24 h. After 48 h, colonies 2–3 mm in diameter, black with black halo were selected for confirmation. They were streaked onto tryptone soya yeast extract agar (Biokar Diagnostic, France) plates and incubated at 37°C for 24 h. All isolates were tested by using standard methods (Jones & Seeliger, 1986; Van Netten et al., 1989).

Results was obtained as positive for Gram stain, catalase positive, urea negative, production of an acid slant and butt in TSI without production of H₂S, hydrolysis of sodium hippurate, esculin, and mannitol, motile at room temperature, β hemolysis; all species were given ++ reactions in MR-VP broth. *L. monocytogenes* and *L. ivanovii* produced hemolysis in sheep blood agar and were also positive in the CAMP test. *L. innocua* and *L. murrayi* were given negative reaction in the CAMP test (Seeliger & Jones, 1986). These results are given in Table 2.

Gram-positive, catalase-positive, motile cultures were tested with the API *Listeria* (bioMerieux, France) system. From the plates, single colonies were inoculated on 10 ml brain heart infusion broth (Oxoid) and incubated at 30°C for 24 h. The turbidity of the suspensions was adjusted to the McFarland I standard.

Table 2. Characteristics differentiating the species of the genus *Listeria*.

Characteristics	<i>L. monocytogenes</i>	<i>L. innocua</i>	<i>L. ivanovii</i>	<i>L. murrayi</i>
Gram stain	+	+	+	+
β -hemolysis	+	–	+	–
Mannitol	–	–	–	+
Hippurate	+	+	+	–
Voges proskauer	+	+	+	+
Esculin	+	+	+	+
CAMP-test	+	–	+	–
<i>S. aureus</i>				

Antilisterial activity assay

The Bauer-Kirby disk diffusion procedure according to NCCLS (National Committee for Clinical Laboratory Standards) regulations was used for determination of antilisterial activity of the extracts (NCCLS, 1998).

The disk diffusion method was performed on Mueller-Hinton agar (MHA) (Difco, Kansas City, MO, USA) supplemented with 5% defibrinated sheep blood and before *Listeria* isolates had been suspended in brain heart infusion broth (Oxoid) to the density of 1.0 McFarland standards, and this inoculum was inoculated onto the entire surface of a dried Mueller-Hinton agar plate by using a cotton swab. The plates were held at room temperature for 10 min to allow absorption of free surface liquid.

All extracts were dissolved in 75% aqueous ethanol to obtain 133 mg/ml extract concentration. The ethanol extracts were impregnated on sterile paper disks of 6 mm diameter, 0.02 ml capacity (Schleicher and Shül, no. 2668, Germany) and then filter paper disks impregnated with a solution were placed on the surface of each inoculated plate. Disks were impregnated with pure ethanol as negative control. Plates were incubated at 37°C for 48 h. After incubation, the diameter (mm) of the zone around each disk was measured. The solvent control

Table 3. Antilisterial activity of aqueous extracts of the plants (minimum inhibitory concentration, $\mu\text{g/ml}$).

Sample name	<i>L. monocytogenes</i>	<i>L. ivanovii</i>	<i>L. innocua</i>	<i>L. murrayi</i>
<i>Centranthus longiflorus</i>	25	NT	12.5	NT
<i>Juglans regia</i>	12.5	25	NT	12.5
<i>Lythrum selicaria</i>	50	NT	12.5	NT
<i>Ononis spinosa</i>	12.5	50	NT	NT
<i>Plantago major</i>	50	100	12.5	50
<i>Teucrium polium</i>	50	NT	12.5	50
Ofloxacin	3.125	6.25	6.25	6.25

NT, not tested. Because these extracts have no growth inhibition zone in the diffusion method, they were not tested here.

did not show any antimicrobial activity. Standard antibiotic disk ofloxacin (10 $\mu\text{g/disk}$, Oxoid) was used for positive control. All tests were performed under sterile conditions in duplicate and repeated three times.

Extracts exhibiting antilisterial activity by this method were further tested by the macro-broth dilution assay (NCCLS, 2000) to determine the minimum inhibitory concentrations (MICs) that are listed in Table 3. The extracts and the reference drug (ofloxacin) were dissolved in water at a concentration of 200 $\mu\text{g/ml}$. The concentration was adjusted to 100 $\mu\text{g/ml}$ fourfold dilution with media culture and bacteria solution at the first tube. Activity tests were performed with Mueller-Hinton broth (MHB) (Difco). The minimum inhibitory concentration was the concentration of the extract at which no visible growth of the bacteria was observed.

Results and Discussion

In our previous studies on ethanolic extracts of *Plantago major*, *Ononis spinosa*, *Lythrum salicaria*, *Juglans regia*, *Centranthus longiflorus*, and *Teucrium polium*, all used in folk medicine, we also observed their effects against Gram-positive (*S. aureus*, *B. subtilis*) and Gram-negative, (*P. aeruginosa*, *E. coli*) bacteria and yeast (*C. albicans*, *C. galabrata*, *C. krusei*). All plants showed higher antibacterial activity against *E. coli* and *S. aureus*. The highest activity against yeast-like fungi were obtained by the extracts of *O. spinosa* and *L. salicaria* (Citoglu and Altanlar, 2003).

In this study, all of the extracts were evaluated for antilisterial activity against *L. monocytogenes*, *L. ivanovii*, *L. innocua*, *L. murrayi*. The *in vitro* antilisterial activities of the aqueous extracts of the title plants are shown in Table 4. Table 4 shows the results of *in vitro* activity determination by disk diffusion method against *L. monocytogenes*, *L. ivanovii*, *L. innocua*, and *L. murrayi*. The inhibition zones formed by the standard antibiotic disks (positive control) and the disks injected with only ethanol (negative control) are also given in Table 4. When compared with the standard antibiotic, *Plantago major*

Table 4. The diameters (mm) of inhibition zones of free and ethanolic extracts of the plants.

Sample name	<i>L. monocytogenes</i>	<i>L. ivanovii</i>	<i>L. innocua</i>	<i>L. murrayi</i>
<i>Centranthus longiflorus</i>	10	0	14	0
<i>Juglans regia</i>	13	10	0	10
<i>Lythrum selicaria</i>	8	0	11	0
<i>Ononis spinosa</i>	15	9	0	0
<i>Plantago major</i>	12	9	15	10
<i>Teucrium polium</i>	11	0	11	10
Ofloxacin	22	20	20	20

exhibited activities against *L. monocytogenes*, *L. ivanovii*, *L. innocua*, and *L. murrayi*. However, *Centraranthus longiflorus* and *Lythrum salicaria* did not show any activity against *L. ivanovii*, *L. murrayi*, and *Juglans regia*. *Teucrium polium* did not show any activity against *L. innocua* and *L. ivanovii*.

As can clearly be seen in Table 4, *Plantago major* exhibited good activity against only *L. innocua*, and *Ononis spinosa* showed the best activity with 15-mm inhibition zone diameter against *L. monocytogenes*, similar to ofloxacin.

In addition, the tube dilution method was also employed for the active extracts in the agar diffusion method (NCCLS, 2000). Related extracts were dissolved in water with 200 µg/ml as the starting dose. The data (Table 3) show that most active extracts *Juglans regia* and *Ononis spinosa* exhibited as MIC value of 12.5 µg/ml against *L. monocytogenes*. *Centraranthus longiflorus*, *Lythrum salicaria*, *Plantago major*, and *Teucrium polium* showed good activity against *L. innocua* at 12.5 µg/ml (MIC value). On the other hand, most of the extracts were not active against *L. ivanovii* and *L. murrayi*, whereas *Juglans regia* showed activity against *L. ivanovii* and *L. murrayi*.

In conclusion, the current study shows that the extracts examined have variable antilisterial activities.

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