

Antibacterial and Antifungal Activities of Turkish

Medicinal Plants

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Abstract

In this study, the antibacterial and antifungal activities of *Rhus coriaria* L. (Anacardiaceae), *Stachys annua* (L.) subsp. *annua* Ic., *Stachys pumilia* Banks & Sol., *Laurus nobilis* L. (Lauraceae), *Allium neapolitanum* Lyr. (Liliaceae), *Salvia viridis* L. (Lamiaceae), and *Nicotina rustica* (Solanaceae) species were investigated. The microbial effects of these plants were tested by a disk diffusion method using *Bacillus megaterium* DSM 32, *Bacillus brevis* FMC 3, *Bacillus subtilis* IMG 22, *Bacillus cereus* FMC 19, *Escherichia coli* DM, *Enterobacter aerogenes* CCM 2531, *Pseudomonas aeruginosa* DSM 50071, *Staphylococcus aureus* Cowan 1, *Listeria monocytogenes* Scott A and *Micrococcus luteus* LA 2971, *Candida tropicalis* and *Candida albicans* CCM 314. The results showed that the fruit extract of *R. coriaria* had the strongest antimicrobial effect with an inhibition zone of 35–51 mm against all the bacteria used, while *S. viridis* demonstrated the weakest antibacterial effect, inhibiting only the development of *S. aureus*, with an inhibition zone of 11 mm. *A. neapolitanum*, *L. nobilis* and *N. rustica* extracts were effective only with some yeasts. The growth of *S. aureus* was inhibited by all the plant extracts except for *S. annua* subsp. *annua*, having an inhibition zone ranging from 7–8 mm. With the exception of *B. subtilis* IMG 22, *L. monocytogenes* Scott A and *M. luteus* LA 2971, the growth of the other bacteria was inhibited by all the extracts. Except for the fruit extracts of *R. coriaria* and *A. neapolitanum*, all additional extracts of generated inhibition zones smaller than those generated by several reference antibiotics.

Keywords: Antimicrobial activities, *Rhus coriaria*, *Stachys annua* subsp. *annua*, *Stachys pumilia*, *Laurus nobilis*, *Allium neapolitanum*, *Salvia viridis*, *Nicotina rustica*.

Introduction

Although medicinal plant potential in Turkey is quite large, knowledge of this subject and studies on these plants are not sufficient. Most investigations have concerned folkloric uses (Öztürk & Özçelik, 1991; Yıldırım, 1994), and methods of how to use these plants have been investigated.

In the Far East countries, especially Japan, Korea, China and India, plant-based medicines have been used for treating various diseases (Sayar et al., 1995). More recently, with the development of natural antibacterial medicine, important studies have been performed (Diğrak et al., 1999a; İlçim et al., 1998; Taylor et al., 1996; Martinez et al., 1996; Olukoya et al., 1993).

In Turkey, the Anacardiaceae is represented by three genera. One of these genera is *R. coriaria* (so-called “sumac”), which is represented with only one species in Turkey. The plant was found in nearly all the regions of Turkey, Europe and USA. The leaves of this plant contain tannins, sugars, waxes and flavone derivatives (myricetine) which are yellow of color. They are used for the protection of leather against microorganisms.

Its wood, the so called “yellow root” and “yellow wood”, has been used for the painting of leather and textile for a long time (Eşberk & Hamamcıoğlu, 1953). Moreover, *R. coriaria* fruit extracts can be used in the form of an internal infusion (5%) as an antiseptic, protector of constipation, regulator of blood flow, and temperature reducer.

In the Kahramanmaraş region of Turkey, aqueous extracts obtained from the fruits of *R. coriaria* were used to produce a sour taste in food. Moreover, aqueous extracts of the plant have been used against viruses (Stomatitis aphthosa epizootica) that result in a typical disease (Aphthae epizootica) in the nail of sheep.

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On the other hand, in the leaves of *L. nobilis*, there are tannins, acidic materials, and volatile oil (Gökçe & Doğan, 1971). In the treatment of indigestion and chronic bronchitis, they are used in the form of infusion (5–10%). The oil obtained from the fruits of laurel has been used to kill parasites and decrease rheumatism pains, in the form of ointment [a mixture of laurel fruit oil (10 g) and suet (90 g)]. Also laurel oil has been used in veterinary medicine. They can be used to reduce external pain and protect animals against flies by rubbing them on the animal's skin.

In the Southern Anatolia region (the city of Antakya-Turkey), by mixing the extracts of laurel oil with prina oil, a pale yellow-colored soup (so-called "Har soup") is made. This is used against various skin diseases and loosing hair (Baytop, 1984).

Moreover, *N. rustica*, in the form of powder, is mixed with the ashes of oak and walnut wood. The plant is also used as an insecticide against mange, especially in sheep.

Furthermore, in this study, the microbial effects of *S. annua* subsp. *annua* var. *annua*, *S. pumilia*, *S. viridis*, and *A. neapolitanum* were investigated, as well as the three species mentioned above. The medicinal plants, which grow naturally in Turkey were studied, and medical and economic importance was investigated.

Materials and methods

Materials

S. annua subsp. *annua* var. *annua*, *S. pumilia*, *A. neapolitanum*, and *L. nobilis* were collected from the Tutis Tunnel in Samandağı district, Antakya-Turkey, at the altitude of 10–20 m. *S. viridis*, was collected from Kırıkhan district, Antakya-Turkey, at the altitude of 80 m, *N. rustica* (cultivated) was collected from Türkoğlu district, Kahramanmaraş-Turkey, at the altitude of 620 m, and *R. coriaria* was collected from Ahrır Mountain, Kahramanmaraş-Turkey, at the altitude of 700 m.

Microorganisms tested in this study were provided from culture collections of the Microbiology Laboratory of Science & Art Faculty of the University of Kahramanmaraş Sütçü Imam, in Turkey. *Bacillus megaterium* DSM 32, *Bacillus brevis* FMC 3, *Bacillus subtilis* IMG 22, *Bacillus cereus* FMC 19, *Escherichia coli* DM, *Enterobacter aerogenes* CCM 2531, *Pseudomonas aeruginosa* DSM 50071, *Staphylococcus aureus* Cowan 1, *Listeria monocytogenes* Scott A and *Micrococcus luteus* LA 2971 (bacteria), and *Candida tropicalis* and *Candida albicans* CCM 314 (yeasts) were used.

Methods

The collected species were identified and broken into pieces under sterilized conditions. The pieces (20 g) were extracted with chloroform (150 ml) (Merck, Darmstadt) for 24 h by using Soxhlet equipment (Dıǵrak et al., 1999b; Alkofahi et

al., 1996). The obtained extracts and chloroform (as reference) 15 µl were injected into empty sterilized antibiotic discs having a diameter of 6 mm (Schleicher & Shüll No: 2668, Germany) (Collins et al., 1989; Bradshaw, 1992). In addition, reference antibiotic discs such as streptomycin sulphate, tobramycin, and nystatin were used for comparison (provided by the Microbiology Division of Medicine Faculty of Firat University in Elazığ-Turkey).

Preparation of microorganism culture

The above-mentioned bacteria were incubated at $30 \pm 0.1^\circ\text{C}$ for 24 h by injection into Nutrient Broth (Difco), and the studied yeasts were incubated in Sabourand Dextrose Broth (Difco) for 24 h. Müeller Hinton Agar (MHA) (Oxoid) and Sabourand Dextrose Agar (SDA) sterilized in a flask and cooled to $45\text{--}50^\circ\text{C}$ were distributed to sterilized Petri dishes having a diameter of 9 cm (15 ml) after injecting cultures (0.1 ml) of bacteria and yeasts (10^6 bacteria per ml and 10^5 yeasts per ml), and distributing medium in Petri dishes homogeneously. Dishes injected with extracts were positioned on the solid agar medium by pressing slightly (Sundar, 1996). Petri dishes were placed at 4°C for 2 h, placks injected with yeasts were incubated at $25 \pm 0.1^\circ\text{C}$ and bacteria were incubated at 37°C for 24 h (Collins et al., 1989; Bradshaw, 1992). At the end of the period, inhibition zones formed on the MHA and SDA were evaluated in mm. Studies were performed in triplicate, and the developing inhibition zones were compared with those of reference disks.

Results and discussion

The antimicrobial activities of the various plant extracts are given in Table 1. As can clearly be seen from this table, the extracts provided from the fruits of *Rhus coriaria* were found to be effective against *Bacillus megaterium* DSM 32, *M. luteus* LA 2971, *B. cereus* FMC 19, *E. aerogenes* CCM 2531, *P. aeruginosa* DSM 50071, *L. monocytogenes* Scoot A, and *S. aureus* Cowan 1 (bacteria), showing inhibition zones of 36–51 mm. However, *R. coriaria* was not effective against *E. coli* or *Candida albicans* CCM 314. Moreover, it was reported that the leaves of *R. coriaria* acted as an antibiotic medicine due to chemical compounds such as tannins and flavone derivatives (fisetine), and were not harmful in the treatment of constipation (Altinkurt & Heper, 1970; Schmaus, 1993; Nimri et al., 1999).

In comparison to standard antibiotics such as streptomycin sulphate, tobramycin and nystatin, shown in Table 2, the extracts in *R. coriaria* have much higher resistance against the studied bacteria. On the other hand, it is important to note from Tables 1 and 2, the standard antibiotics and the extracts of *R. coriaria*, have no effect against the fungi used.

It is also clear from Table 1 that extracts prepared from *S. annua* subsp. *annua* var. *annua* have an antibacterial effect

Table 1. Antibacterial and antifungal activities of various plant extracts.

Microorganisms	Inhibition zones (mm)							
	A	B	C	D	E	F	G	H
<i>B. megaterium</i> DSM 32	36	–	7	–	7	–	–	–
<i>B. brevis</i> FMC 3	37	–	8	7	16	–	–	–
<i>B. subtilis</i> IMG 22	35	–	–	–	–	–	–	–
<i>B. cereus</i> FMC 19	38	–	–	8	19	–	–	–
<i>E. coli</i> DM	–	–	–	–	23	–	–	–
<i>E. aerogenes</i> CCM 2531	39	8	8	8	8	–	–	–
<i>P. aeruginosa</i> DSM 50071	45	7	8	6	13	–	–	–
<i>S. aureus</i> Cowan 1	51	–	7	13	19	11	15	–
<i>L. monocytogenes</i> Scott A	46	–	7	–	–	–	–	–
<i>M. luteus</i> LA 2971	36	6	6	6	–	–	–	–
<i>C. albicans</i> CCM 314	–	–	–	–	24	–	13	–
<i>C. tropicalis</i>	–	–	–	32	–	–	–	–

A: *Rhus coriaria* B: *Stachys annua* subsp. *annua* C: *Stachys pumilia*
D: *Laurus nobilis* E: *Allium neapolitanum* F: *Salvia viridis*
G: *Nicotina rustica* H: Chloroform (Control)
Inactive (–) Includes diameter of disc (6 mm).

Table 2. Antimicrobial activities of some standard antibiotics.

Microorganisms	Inhibition zones (mm)		
	Streptomycin sulphate 10 µg/disc	Tobramycin 10 µg/disc	Nystatin 30 µg/disc
<i>B. megaterium</i> DSM 32	17	16	NT
<i>B. brevis</i> FMC 3	16	8	NT
<i>B. subtilis</i> IMG 22	19	16	NT
<i>B. cereus</i> FMC 19	–	–	NT
<i>E. coli</i> DM	–	10	NT
<i>E. aerogenes</i> CCM 2531	21	11	NT
<i>P. aeruginosa</i> DSM 50071	14	12	NT
<i>S. aureus</i> Cowan 1	17	13	NT
<i>L. monocytogenes</i> Scott A	19	7	NT
<i>M. luteus</i> LA 2971	–	–	NT
<i>C. albicans</i> CCM 314	–	–	18
<i>C. tropicalis</i>	–	–	15

Antibiotics	Resistant (mm)	Middle-resistant (mm)	Susceptible (mm)
Streptomycin sulphate	<11	12–14	>15
Tobramycin	<12	13–14	>15
Nystatin	<12	14–17	>18

NT: not tested.

against *P. aeruginosa*, *E. aerogenes* CCM 2531 and *M. luteus* LA 2971, showing an inhibition zones of 6–8 mm. However, they were not effective against the other bacteria and the fungi studied. As can be seen from Tables 1 and 2, the extracts from *S. annua* subsp. *annua* var. *annua* have much lower inhibition zones than the standard antibiotics. However, both of them have no resistance against the fungi.

As also listed in Table 1, it was found that *S. pumilia* inhibits the development of *S. aureus* COWAN 1, *P. aeruginosa* DSM 50071, *B. brevis* FMC 3, *B. megaterium* DSM 32, *E. aerogenes* CCM 2531 *M. luteus* LA 2971, *B. cereus* FMC 19, and *L. monocytogenes* Scott A bacterial species (6–8 mm). As shown in Table 1, the extracts from both *S. annua* subsp. *annua* var. *annua* and *S. pumilia* have almost the same

inhibition zones for different bacteria. The extracts from *S. pumilia* were also found not to be effective against the fungi studied. The extracts from *S. pumilia* generated smaller inhibition zones than the standard antibiotics (see Table 2).

On the other hand, it was noticed that the antimicrobial activities and the chemical contents of the other species (e.g., *Stachys* genus, *Stachys lavandulifolice*, etc.), have not been studied in detail, however, they contain volatile oils and tannins used for discharging gas in the human body (Sezik & Sezer, 1983).

Extracts obtained from *L. nobilis* species were found to have inhibition zones against *S. aureus* Cowan 1, *P. aeruginosa* DSM 50071, *B. brevis* FMC *B. megaterium* DSM 32, *E. aerogenes* CCM 2531 *M. luteus* LA 2971, and *B. cereus* FMC 19 bacteria (6–13 mm). This extracts were also determined to be effective against one of the fungi used (*C. tropicalis*) with a sufficient inhibition zone of 32 mm. However, *Laurus nobilis* was not effective against the other bacteria and *C. albicans* CCM. Considering the inhibition of fungi, the extract was much more effective than standard antibiotics (Table 2). Akgül (1989) reported that Turkish laurel leaf oil had 35–50% cineol and was effective against various bacteria.

As also shown in Table 1, *A. neapolitanum* was found to be effective against all the bacteria studied with the exception of *L. monocytogenes* Scott A and *B. subtilis* IMG 22 bacteria, having inhibition zones of 7–19 mm, and on *C. albicans*, with inhibition zone of 24 mm. *B. megaterium* DSM 32 and *E. aerogenes* CCM 2531 bacterial species showed more resistance to the extracts of *A. neapolitanum* when compared to the reference antibiotics. However, other bacteria species, such as *B. brevis* FMC 3, *B. cereus* FMC 19, *E. coli* DM, *P. aeruginosa* DSM 50071, and *S. aureus* Cowan 1, were found to be susceptible. In comparison with the inhibition zones of reference antibiotics, those of plant extracts are somewhat higher.

It was reported that most *Allium* species had strong antibacterial effects due to their chemical compounds, e.g., alliin, alliin derivatives (metilalliin and propilalliin) and allil propyl disulphur (Baytop, 1984). For example, some researches pointed out that the extracts obtained from *Allium sativum* and *A. sativa* (Liliaceae), showed the same antimicrobial effect. This can be attributed to phytochemical contents in members of Liliaceae, dihydro-alliin (s-n-propyl-cystein-sulfoxide) and methyl-alliin (s-methyl-cystein-sulfoxide) (Virtanen, 1958).

As demonstrated in Table 1, the extracts of *S. viridis* and *N. rustica* inhibit only the development of *S. aureus* Cowan 1. As compared with the reference antibiotics studied, the extracts of both *S. viridis* and *N. rustica* were found to be comparable to each other in view of the inhibition zones shown in Tables 1 and 2. Furthermore, the antifungal effect of *S. viridis* was found to be much less in comparison to nystatin. Chemical constituents such as terpenes (30–50%), cineol (15%) and borneol (10%), the leaves of *Salvia triloba*

L. have antimicrobial activities and were recommended for use as antiseptics (Leiner, 1954).

Moreover, the studies performed with *Nicotina rustica* showed that most *Nicotina* species contained alkaloids (0.4–4.5%) such as nicotine (having very effective odor) and were found to be antiparasitic. Thereby, it should be used carefully (Apery, 1912).

In conclusion, the biologically active components in the tested plants are not well defined. It is very important to analyze the selective antimicrobial agents in the plants. According to the results found in this study, we will further study the plants which have antimicrobial activities in vivo to understand their potential as a source of antibiotic. The extracts of the plants could then be considered as disinfectants or antiseptics.

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