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Comparision of Antimicrobial Activity of *Silene laxa* Boiss. & Kotschy and *Silene caramanica* Boiss. & Heldr Different Extracts from Turkey

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The antimicrobial activities of the extracts of ethyl acetate, chloroform, methanol, ethanol and acetone *Silene laxa* Boiss. & Kotschy and *Silene caramanica* Boiss. & Heldr were studied by disc diffusion method. These extracts were tested against seven bacteria and one fungus, which revealed various levels of antimicrobial activity. The ethanol extracts of *Silene laxa* leaves showed the best antibacterial activity against *Pseudomonas aeruginosa, Enterobacter cloacae, Bacillus megaterium* (15mm 30 μl^{-1}). The chloroform extracts of *S. laxa* branches displayed the best antibacterial activity against *E.cloacae* (16mm 30 μl^{-1}). The ethyl acetate extracts of S.laxa fruits showed the best antibacterial activity against *B.megaterium* and *E.cloacae* (15mm 30 μl^{-1}). The methanol extracts of *S. laxa* leaves showed the best antibacterial activity against *Staphylococcus aureus* (18 mm 30 μl^{-1}). The methanol extracts of *S. laxa* fruits showed the best antibacterial activity against *B.megaterium* (14mm 30 μl^{-1}).

Key words: Antimicrobial activity; Plant extracts; *Silene laxa* Boiss. & Kotschy and *Silene caramanica* Boiss. & Heldr.

Despite the huge budget spent on development of antibiotics, successful treatment of bacterial infections are still a great challenge in medicine due to the indiscriminate use these conventional drugs which led to emergence of multi drug resistant strains[1]. Therefore, more natural antimicrobial subtances from plants are desired. It is known that a large number of herbs posses antimicrobial activity[2-7].

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Silene is one of the larger genera of flowering plants in the world, comprising ca. 750 species, of which approximately half occur in the Mediterranean area. The southern part of the Balkan Peninsual and SW Asia are two main centres of diversity [8]. *Silene* in Turkey was revised by Coode & Cullen[9]. Twelve new species from Turkey have been described and discovered and five species have been recorded as new for Turkey since the publication of the second volume of Flora of Turkey and the East Aegean Islands[8-14].

Silene (Caryophyllaceae) is an ornamental plant. The antimicrobial effect of some species of *Silene* has been studied in previous researchers[2,5]. As far as we know, this is the first study, the antimicrobial activity of *Silene laxa* Boiss. & Kotschy and *Silene caramanica* Boiss. & Heldr

five different extracts against to seven bacteria and one fungus were reported in this study from Turkey.

MATERIALS AND METHODS

Plant collection and preparation of extracts

Silene laxa was collected from C4 Karaman, Ermenek-Ba_yayla 4. km S. slopes, at 1260-1300 m altitude in 13.vii.2006 and *S. caramanica* was collected from C4 Karaman-Mut 24. km, at 1530-1570 m altitude, in 13.vii.2006. Voucher specimens of the plants are kept at the herbarium of Celal Bayar University, Faculty of Science and arts.

The plant parts used were dried and broken into small pieces under sterile conditions, and 20 g of each plant was extracted with 150 mL of ethyl acetate, chloroform, methanol, ethanol and acetone extracts (Merck, Darmstadt) for 24 h by Soxhlet appartaus[15]. Prepared extracts were dried at 30°C using a rotary evaporator until amount of each extracts was 1 mL.

Microorganisms and media

Seven bacteria (*Escherichia coli* ATCC 8739, *Staphylococcus aureus* Cowan 1, *Mycobacterium smegmatis* CCM 2067, *Pseudomonas aeruginosa* ATCC 27853, *Enterobacter cloacae* ATCC 13047, *Bacillus megaterium* DSM 32, *Micrococcus luteus* LA 2971 were obtained from the Biology Department of KSU, Science and Art Faculty. Cultures of these bacteria were grown in Nutrient Broth (NB) (Difco) at 37±0.1°C for 24 h. One fungus (*Rhodotorula rubra*). Cultures of these fungi were grown in Sabouraud Dextrose Broth (SDB) (Difco) at 25±0.1°C for 24 h.

Antibacterial activity

The disc assay described by Bauer *et al*. was used for antimicrobial activity[16]. All of the extracts individually were injected into empty sterlized antibiotic discs having a diameter of 6 mm (Schleicher & Schül No:2668, Germany) in the amount of 30 µL. Discs injected with pure ethyl acetate, chloroform, methanol, ethanol and acetone served as negative controls. The bacteria were incubated in Nutrient Broth (NB) (Difco) at 37±0.1°C for 24h, and then inoculated [10^6 mL^{-1} into petri dishes containing homogenously distributed 15 mL of streilized Muller-Hinton agar (MHA, Oxoid)[17-18]. After solidification the filter paper discs (6 mm in diameter) impregnated with the extracts were placed on test organism-seeded plates. The treated petri dishes were placed at 4°C for 1-2 h and then the antibacterial assay plates were incubated at 37°C for 18-24 h[18-21]. Vancomycin (30 µg/disc), Erythromycin (30µg/disc) discs were used as standard antibiotics (as positive control). After incubation, all plates were observed for zones of growth inhibition, and the diameters of these zones were measured in millimeters. The experiments were conducted three times.

Antifungal activity

Antifungal assay was performed by using Disc diffusion method[16]. The respective fungal cultures were inoculated [10^5 mL^{-1}] into petri dishes containing homogenously distributed sterilized Saboraud Dextrose Agar (SDA)[17-18]. The loaded discs were placed on the surface of medium and the extract was allowed to diffuse at least for 5 minutes. The treated petri dishes were placed at 4°C for 1-2 h and the plates were kept for incubation at 28°C for 24-48 hours. Nystatin 100 Units (10 µg/disc) discs were used as positive control. Different plant extracts were used to saturate the disc and placed on the seeded plates. Respective solvents act as a negative controls. After incubation period, the antifungal activity was evaluated by measuring the zone of inhibition against test organisms. The experiments were conducted three times.

RESULTS AND DISCUSSION

Antimicrobial activities of *S. laxa* Boiss. & Kotschy and *S. caramanica* Boiss. & Heldr leaf, branches and fruits extracts are presented in Table I and Table II. The ethyl acetate, chloroform, methanol, ethanol and acetone used as negative controls did not show antimicrobial activity against the all tested microorganisms.

The ethyl acetate extracts of *S.laxa* leaves (SLL) showed the best antibacterial activity against *B.megaterium* (11mm 30 μl^{-1}). The chloroform extracts of SLL presented the best antibacterial activity against *E.coli* (10mm 30 μl^{-1}). The methanol extracts of SLL displayed the best antibacterial activity against *E.cloacae* (13mm 30 μl^{-1}). The ethanol extracts of SLL showed the best antibacterial activity against *P.aeruginosa, E.cloacae, B.megaterium* (15mm 30 μl^{-1}). The ethanol extracts of SLL showed no inhibition zone against *R.rubra*. The acetone extracts of SLL

Table 1. Antimicrobial activity of five different solvent extracts of *Silene laxa* Boiss. & Kotschy leaves, branches and fruits.

Microorganisms	Inhibition Zone (mm*)																		
	Leaves					Branches					Fruits					Antibiotics			Cont.
	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	V30	E15	N10	ABC DE
E.coli	8	10	11	14	10	9	8	10	8	10	9	8	11	7	8	11	10	NT	0
S.aureus	8	7	8	12	11	10	7	7	7	7	7	0	0	8	8	15	16	NT	0
M.smegmatis	8	8	12	14	9	14	11	7	8	9	7	0	0	0	0	22	27	NT	0
P.aeruginosa	9	8	11	15	10	12	8	0	0	0	11	7	0	0	0	17	35	NT	0
E.cloacae	7	7	13	15	10	15	16	15	14	8	15	12	10	7	9	27	28	NT	0
B.megaterium	11	8	12	15	11	12	12	8	7	9	15	12	8	9	10	16	25	NT	0
M.luteus	8	7	8	14	10	9	10	12	7	7	8	0	8	0	0	21	34	NT	0
R.rubra	8	7	7	0	7	8	7	8	7	8	10	8	0	0	8	NT	NT	18	0

Table 2. Antimicrobial activity of five different solvent extracts of *Silene caramanica* Boiss. & Heldr leaves, branches and fruits.

Microorganisms	Inhibition Zone (mm*)																		
	Leaves					Branches					Fruits					Antibiotics			Cont.
	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	V30	E15	N10	ABC DE
E.coli	7	0	9	7	8	0	0	0	7	0	7	7	7	7	7	11	10	NT	0
S.aureus	8	8	18	9	10	0	0	8	9	0	9	9	7	7	7	15	16	NT	0
M.smegmatis	9	0	15	10	9	7	0	0	9	0	9	9	8	10	12	22	27	NT	0
P.aeruginosa	8	0	9	10	7	0	0	7	8	0	7	9	7	9	7	17	35	NT	0
E.cloacae	9	12	16	16	12	7	7	7	8	8	10	10	10	11	9	27	28	NT	0
B.megaterium	9	10	14	8	10	0	0	0	7	7	10	12	14	9	10	16	25	NT	0
M.luteus	9	8	12	9	8	0	0	7	7	0	8	8	8	7	7	21	34	NT	0
R.rubra	8	8	9	10	8	8	8	8	8	8	8	8	7	8	7	NT	NT	18	0

A:Ethyl acetate B:Chloroform C: Methanol D:Ethanol E:Acetone V30: Vancomycin (30 ¼g/disc), E15: Erytromycin (15 ¼g/disc), N10: Nystatin 100 Units (10 ¼g/disc), NT: Not tested

showed the best antibacterial activity against *B.megaterium* and *S.aureus* (11mm 30 μl^{-1}).

When we compared to antimicrobial activity of *S.laxa* branches (SLB), the ethyl acetate extracts of SLB showed the best antibacterial activity against *E.cloacae* (15mm 30 μl^{-1}). While the chloroform extracts of SLB displayed the best antibacterial activity against *E.cloacae* (16mm 30 μl^{-1}). The methanol extracts of SLB showed the best antibacterial activity against *E.cloacae* (15mm 30 μl^{-1}). And also the ethanol extracts of SLB showed the best antibacterial activity against *E.cloacae* (14mm 30 μl^{-1}). The ethanol extracts of SLB showed no inhibition zone *P.aeruginosa.* While the acetone extracts of SLB showed the best antibacterial activity against *E.coli* (10mm 30 μl^{-1}). The acetone extracts of SLB showed no inhibition zone *P.aeruginosa.*

When it comes to the antimicrobial activity of *S.laxa* fruits (SLF), the ethyl acetate extracts of SLF showed the best antibacterial activity against *B.megaterium* and *E.cloacae* (15mm 30 μl^{-1}). The chloroform extracts of SLF showed the best antibacterial activity against *E.cloacae* and *B.megaterium* (12mm 30 μl^{-1}). The methanol extracts of SLF showed the best antibacterial activity against *E.coli* (11mm 30 μl^{-1}). The ethanol extracts of SLF showed the best antibacterial activity against *B.megaterium* (9mm 30 μl^{-1}). Similarly the acetone extracts of SLF showed the best antibacterial activity against *B.megaterium* (10mm 30 μl^{-1}).

The ethyl acetate extracts of *S. caramanica* leaves (SCL) showed the best antibacterial activity against *M.smegmatis*, *E.cloacae B.megaterium* and *M.luteus* (9mm 30 μl^{-1}). The chloroform extracts of SCL showed the best antibacterial activity against *E.cloacae* (12mm 30 μl^{-1}). The methanol extracts of SCL showed the best antibacterial activity against *S.aureus* (18 mm 30 μl^{-1}). The ethanol extracts of SCL displayed the best antibacterial activity against *E.cloacae* (16mm 30 μl^{-1}). Similarly the acetone extracts of SCL showed the best antibacterial activity against *E.cloacae* (12mm 30 μl^{-1}).

When we compared to antimicrobial activity of *S.caramanica* branches (SCB), the ethyl acetate extracts of SCB showed antimicrobial activity *M.smegmatis*, *E.cloacae* (7mm 30 μl^{-1}) and *R.rubra* (8mm 30 μl^{-1}). The chloroform extracts of SCB showed inhibition zone *E.cloacae* (7mm 30 μl^{-1}) and *R.rubra* (8mm 30 μl^{-1}). The methanol extracts of SCB showed no inhibition zone against *M.smegmatis*, *E.coli* and *B.megaterium*. The ethanol extracts of SCB showed antimicrobial activity all tested microorganisms. The acetone extracts of SCB displayed antimicrobial activity against *E.cloacae* (8mm 30 μl^{-1}), *B.megaterium* (7mm 30 μl^{-1}) and *R.rubra* (8mm 30 μl^{-1}).

When we compared to antimicrobial activity of *S.caramanica* fruits (SCF), the ethyl acetate extracts of SCF showed the best antibacterial activity against *B.megaterium* and *E.cloacae* (10mm 30 μl^{-1}). The chloroform extracts of SCF showed the best antibacterial activity against *B.megaterium* (12mm 30 μl^{-1}). And also, the methanol extracts of SCF showed the best antibacterial activity against *B.megaterium* (14mm 30 μl^{-1}). The ethanol extracts of SCF showed the best antibacterial activity against *E.cloacae* (11mm 30 μl^{-1}). The acetone extracts of SCF displayed the best antibacterial activity against *M.smegmatis* (12mm 30 μl^{-1}).

In recent years, interests have been generated in the development of safer antimicrobial compounds such as plant-based essential oils and extracts to control food-borne pathogens. Historically, many plant oils and extracts have been reported to have antimicrobial properties[22-24]. It is important to investigate scientifically those plants which have been used in traditional medicines as potential sources of antimicrobial compounds[25,26]. Recently our group and various publications have documented the antimicrobial activity of essential oils and plant extracts including clove,olive, spices, walnut[27-31]. Bajpai *et al*[5]. reported that the major components in the volatile oil of *Silene armeria* were butene and methylcyclopropane. In their study, it has become clear that both essential oil and leaf extracts of chloroform, ethyl acetate and methanol of *S.armeria* possesses great potential to strongly inhibit the growth of Botrytis cinerea along with other plant pathogenic fungi tested. Ertürk *et al*.[2] reported that *Silene multifida* of six different fractions of chloroform extracts dissolved in dimethyl sulphoxide(DMSO) were tested for antimicrobial activity using agar diffusion techique against six bacteria (*Bacillus subtilis, S.aureus, E.coli, P.aeruginosa, E.cloacae and Protes vulgaris* and one fungus (*Candida*

albicans). They reported that the antimicrobial activity of extracts of *Silene multifida* against bacteria was more effective than against fungus like our study.

The antimicrobial efects of plants are mostly due to the essential oils present in their composition. It is known that members of *Caryohylaceae* contain high phenolic compounds, flavonoids, aldehydes, ketones, saponins, and alcohols [32-36].

CONCLUSION

It can be suggested that type of extracts can be affected ratio of antimicrobial activity. Solubility of antimicrobial compounds based on chemical properties. In our study, all of the extracts showed antimicrobial activity against all tested microorganisms. But antimicrobial activity have a varying degree according to microrganisms and extracts.

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