Turk J Biol
30 (2006) 17-21

Antimicrobial Properties of *Silene multifida* (Adams) Rohrb. Plant Extracts

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Received: 02.03.2005

Abstract: Specimens of *Silene multifida* (Adams) Rohrb. were collected in the vicinity of Yomra, Trabzon, Turkey. The fresh plants were air-dried and chopped. This material was extracted with cold chloroform ($CHCl_3$). The chloroform extract was dissolved in dimethyl sulphoxide (DMSO), and, following column chromatography, 6 fractions were obtained. The serial dilutions (0.5, 1, 2, 4 and 10 mg/ml) of these fractions were prepared in 5% Tween 20. The fractions were tested for antimicrobial activity using the agar diffusion technique against 6 bacteria, *Bacillus subtilis, Staphylococcus aureus, Esherichia coli, Pseudomonas aeruginosa, Enterobacter cloacae* and *Proteus vulgaris*, and 1 fungus, *Candida albicans*. Fractions 1, 2, 3, 4, 5 and 6 of the extract of *S. multifida* showed activity against all tested bacteria. Fractions 1 and 5 showed higher antifungal activity than fractions 2 and 4. On the other hand, fractions 3 and 6 did not show any antifungal activity.

Key Words: *Silene multifida*, Plant extract, Antimicrobial activity

Silene multifida (Adams) Rohrb. Bitki Ekstraktlarının Antimikrobiyal Özellikleri

Özet: *Silene multifida* (Adams) Rohrb. bitkisine ait numuneler Trabzon'un Yomra ilçesi civarından toplandı. Taze bitkiler açık havada kurutuldu ve parçalandı. Bu materyaller soğuk kloroformla ekstre edildi. Kloroformun çeşitli fraksiyonları Dimetil sülfoksit (DMSO) ile çözüldü ve 6 fraksiyon elde edildi. Fraksiyonların seri dilisyonları (10, 4, 2, 1 ve 0,5 mg/ml) Tween 20 (%5)'de hazırlandı. Fraksiyonlar agar diffüzyon tekniği kullanılarak *Bacillus subtilis, Staphylococcus aureus, Esherichia coli, Pseudomonas aeruginosa, Enterobacter cloacae* ve *Proteus vulgaris* bakterilerine ve *Candida albicans* mantarına karşı test edildi. *S. multifida* bitkisinin ekstraktlarının 1, 2, 3, 4, 5 ve 6 numaralı fraksiyonları test edilen bakterilere karşı antimikrobiyal aktivite gösterdi. 2 ve 4 numaralı fraksiyonlarda 1 ve 5 numaralı fraksiyonlar daha yüksek antifungal etki gösterdi. Diğer taraftan, 3 ve 6 numaralı fraksiyonlar herhangi bir antifungal aktivite göstermediler.

Anahtar Sözcükler: *Silene multifida* (Adams) Rohrb., Bitki ekstraktı, Antimikrobiyal aktivite

Introduction

There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action because there has been an alarming increase in the incidence of new and re-emerging infectious diseases. Another big concern is the development of resistance to the antibiotics in current clinical use (1).

Higher plants produce hundreds to thousands of diverse chemical compounds with different biological activities (2). Thus, they have been used in the treatment of various human diseases for thousands of years all over the world. Similarly, some plants have been used by rural people in Turkey for the treatment of several diseases, including microbial infections for emetic and strengthening effects, and for increasing urine and decreasing blood pressure (3). Most of the plants used for medicinal purpose have been identified, and their uses are well documented and described by different authors (4-6), but the efficacy of many of these plants is yet to be verified. Moreover, natural plant extracts have been tested in the laboratory against bacteria and fungi. Natural plant products yield extracts with antineoplastic, antimicrobial, antifungal and antiviral activities (7).

The first compound with antimicrobial activity was found in the 1930s (8). Since that period the

development and use of these substances have increased because of the appearance of resistant strains (9). Attention has turned to natural antimicrobial agents in recent years (10). There have been many investigations on the antifungal (11), antibacterial (11), and antiviral (12) preparations and individual compounds isolated from natural sources.

Silene multifida (Adams) Rohrb. (Caryophyllaceae) is an ornamental plant. The antimicrobial effect of this plant has not been studied. In this study, we investigated the antimicrobial activity of serial dilutions of fractions prepared in Tween 20.

Materials and Methods

Plant collection and preparation of extracts. Specimens of *Silene multifida* (Adams) Rohrb. plants were collected in the vicinity of Yomra, Trabzon, northern Turkey, in June 1997. The specimens were identified using the Flora of Turkey (13).

Flash column chromatography was performed on silica gel 60 (230-400 mesh), and preparative TLC was performed with precoated silica gel F_{254} (20 x 20 cm, 0.2 mm) plates (14,15). A voucher specimen has been deposited in a deep-freeze at the Department of Chemistry, Karadeniz Technical University, Trabzon, Turkey. The fresh plants (1200 g) were air-dried and chopped. These materials (110 g) were extracted with cold chloroform ($CHCl_3$) (0.2 l, 7 days). The $CHCl_3$ extract was filtered and concentrated on a rotary evaporator in vacuo at 30 °C until dryness. The obtained crude mixture (2.1 g) was dissolved in chloroform-methanol ($CHCl_3$-CH_3OH) (2:1) and then chromatographed on a Kiesel gel 60 (60 g, 230-400 mesh, 2 x 30 cm column) using flash column chromatography. Elution with *n*-hexane (150 ml), then discontinuous gradient elution with *n*-hexane-$CHCl_3$ (4:1, 100 ml, 4:2, 100 ml, 2:2, 100 ml, 1:4 100 ml), $CHCl_3$ (100 ml), $CHCl_3$-CH_3OH (4:1, 50 ml) and finally with CH_3OH (100 ml) gave 10 fractions (ca. 40-50 ml each). The fractions were combined after the TLC analysis in *n*-hexane-$CHCl_3$ (0.5:0.5 ml each), with $CHCl_3$, $CHCl_3$-CH_3OH (0.9:0.1) and a CH_3OH solvent system to give 6 fractions.

Test organisms and culture media. The test organisms used were *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 25923), *Esherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 10145), *Enterobacter cloacae* (ATCC 13047), *Proteus vulgaris* (ATCC 13315) and *Candida albicans* (ATTC 60192). The bacterial cultures were maintained in Mueller Hinton agar (Merck), *Candida albicans* on Sabouraud Dextrose agar (Oxoid) and Sabouraud Dextrose broth (Difco). The concentrations of the bacterial suspensions were adjusted to 10^8 cells/ml, and fungal suspension to 10^7 cells/ml.

Antibacterial activity assay. Antibacterial activity was measured using the standard method of diffusion disc plates on agar (16,17). In order to test antibacterial activity, fractions 1, 2, 3, 4, 5 and 6 were dissolved in DMSO and diluted in Tween 20 (5%). A preliminary assay with aqueous solutions of Tween 20 up to 5% was performed to assure that no micro-organism growth inhibition occurred. Twenty millilitres of Mueller Hinton Agar medium (Merck) was poured into each 12 cm Petri dish. All bacterial strains were grown in Mueller Hinton Broth medium (Merck) for 24 h at 37 °C. Growth was adjusted to OD (600 nm) of 0.1 by dilution with Mueller Hinton Broth medium (Merck). One hundred microlitres of suspension with approximately 10^8 bacteria per millilitre was placed in Petri dishes and dispersed over agar. Then sterile paper discs (6 mm diameter) were placed on the agar to load 20 µl of each sample (10, 4, 2, 1 and 0.5 mg/ml). For bacteria, as a positive control, ampicillin (10 mg), streptomycin (10 mg) and kanamycin (30 mg) were used, and as negative control DMSO was used. Inhibition diameters were determined after incubation for 24 h at 37 °C. All tests were performed in triplicate.

Antifungal assay. *C. albicans* grown in Sabouraud Dextrose Broth (Difco) for 48 h at 27 °C and Sabouraud Dextrose Agar (Oxoid) were employed in the agar diffusion experiments. Fungal suspensions were adjusted to 10^7 cells/ml as explained above. One hundred units of nystain was used as a positive control and DMSO as a negative control. Inhibition zones were determined after incubation for 48 h at 27 °C. All tests were performed in triplicate.

Results

The results of the antibacterial and antifungal screening of 6 fractions obtained from *Silene multifida* plant are reported in the Table. Fractions obtained from this plant were more active against bacteria than against fungus.

Table. Antimicrobial effect of *Silene multifida* (Adams) Rohrb. plant extracts determined by the agar diffusion method (inhibition zone in mm).

Plant Extracts (mg/ml)		Inhibition Zones (mm)						
		Bacteria						Fungus
		B. subtilis	*E. coli*	*P.aeruginosa*	*S. aureus*	*E. cloacae*	*P.vulgaris*	*C. albicans*
	10	13.3	14.2	14.7	15.0	12.3	14.7	14.4
	4	12.5	12.5	11.2	13.3	11.8	12.5	12.3
	2	10.1	10.6	11.3	11.7	10.4	10.2	12.0
	1	9.7	10.5	9.5	11.0	9.3	9.3	9.0
	0.5	8.3	9.4	9.5	10.3	9.0	9.6	*
Fraction 1	10	14.6	15.7	14.4	13.9	13.4	13.6	9.4
	4	12.3	11.8	12.5	12.5	11.6	11.3	8.2
	2	12.2	13.2	10.3	12.1	11.4	11.7	7.5
	1	11.0	10.2	10.1	11.0	9.7	11.3	*
	0.5	9.1	10.0	10.3	11.3	9.3	9.1	*
Fraction 2	10	15.8	13.1	14.1	17.3	12.4	11.8	*
	4	11.4	12.3	11.5	14.6	10.3	10.5	*
	2	10.1	10.7	11.0	14.3	10.0	9.0	*
	1	9.5	10.3	10.3	13.2	9.7	9.1	*
	0.5	9.2	9.1	9.1	10.0	9.4	8.5	*
Fraction 3	10	14.7	13.6	24.5	24.6	14.6	13.6	7.6
	4	12.3	12.2	20.4	19.5	12.2	11.3	7.2
	2	10.4	10.0	19.2	17.5	10.5	10.0	*
	1	10.0	9.4	17.5	16.2	9.2	9.3	*
	0.5	9.5	9.2	17.3	15.0	9.0	9.1	*
Fraction 4	10	14.3	13.3	23.4	24.4	14.2	15.3	13.4
	4	13.5	11.7	22.0	21.5	12.1	12.4	10.3
	2	13.2	10.0	20.2	18.3	11.0	11.2	9.0
	1	12.0	9.3	15.7	15.5	9.2	10.2	8.3
	0.5	10.0	9.1	14.6	14.3	9.0	9.0	*
Fraction 5	10	14.6	12.3	23.0	23.5	12.6	13.7	*
	4	13.3	10.3	17.3	18.4	11.2	10.3	*
	2	10.0	10.0	16.1	16.5	10.1	10.0	*
	1	9.6	10.0	14.5	13.1	9.7	9.3	*
	0.5	9.5	10.0	12.3	10.1	9.3	9.1	*
Antibiotics	nys	-	-	-	-	-	-	16
	K	19	26	30	27	21	25	-
	AM	30	17	30	20	*	24	-
	S	21	24	21	20	*	24	-

Control= NYS: 100 units Nystatin. K: Kanamycin 30 µg. AMP: Ampicillin 10 µg. S: Streptomycin 10 µg.
No inhibition. The numbers are means of 3 experiments by measuring the inhibition zone.

The fractions (1-5 and 6) were not completely dissolved in the water. Thus none of water dissolved fractions showed antibacterial and antifungal activity against any of the strains tested. Fractions dissolved in DMSO inhibited the growth of bacteria and fungus. All fractions at concentrations higher than 0.5 mg/ml showed antibacterial and antifungal activity. It is important to note that fractions 4, 5 and 6 showed better antibacterial activity on *P. aeruginosa* and *S. aureus* than fractions 1, 2 and 3 (Table). *E. cloacae* was highly resistant to ampicillin and streptomycin with 10 µg, whereas fractions 1-5 and 6 (at all tested concentrations) showed activity against *E. cloacae*. The results of the antimicrobial tests indicate that the extract components from *S. multifida* with larger inhibition zones at the same microgram quantities as the antibiotics tested are generally more effective than those reference antimicrobial agents. When the multicomponent compositions of the plant extract fractions are taken into consideration, much better antimicrobial potentials of the components of *S. multifida* species become apparent.

Fractions 1, 2, 4 and 5 showed antifungal activity against *Candida albicans* at various concentrations, while fractions 3 and 6 were ineffective at all concentrations.

Discussion

In recent years, although technology and medicine have developed extensively, due to decreases in natural richness and drawbacks, some countries have made it obligatory to use natural products for many goals. Thus, like in other countries in the world, in Turkey too the plants known by people are picked and used for the treatment of various diseases.

In this study, the antimicrobial influence of the fractions extracted from *Silene multifida* against bacteria and fungus was determined.

The antimicrobial activity of extracts of *S. multifida* against bacteria was more effective than against fungus, which is similar to the results reported by Avato et al. (18) and Zavala et al. (19).

The use of ampicillin is no longer recommended because of the potency of the widespread resistance to it (20). Thus, fractions 4, 5 and 6 can be used instead of ampicillin.

As a consequence of this study, we will try to isolate the compounds causing the antimicrobial activity present in *S. multifida.* A further study is planned to examine in detail the properties of compounds of fractions.

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References

1. Rojas R, Bustamante B, Bauer J et al. Antimicrobial activity of selected Peruvian medicinal plants. J Ethnopharmacol 88: 199-204, 2003.
2. Hamburger M, Hostettmann, K. Bioactivity in plants: the link between phytochemistry and medicine. Phytochemistry 30: 3864–387, 1991.
3. Baytop T. Health treatment in Turkey Using Plant Extracts, Istanbul Univ., No. 3255, Sanal Matbaacılık, Istanbul, Turkey. 203-204, 1984.
4. Nadkarni KM. Indian Materia Medica. Bobbay Popular Prakashan, India. 1876.
5. Dastur RJ. Medicinal Plants of India and Pakistan. DB. Taraporevala, Bombay. 1985.
6. Saradamma L. All India Co-Ordinated Research Project on Ethnobiology, Final Techinical Report. RRI Drug Research, CCRAS, Goverment of India, Poojapura, Trivanndrum, Kerala, Indian. 1990.
7. Lau AF, Siedlecki J, Anleitner J et al. Inhibition of reverse transcriptase activity by extracts of cultured blue-green algae (Cyanophyta). Planta Med 59: 148-151, 1993.
8. Goodman GA, Rall TW, Nies AS, Taylor P. Las Bases Farmacológicas de la Terapéutica 8th ed., Editorial Médica Panamericana, Mexico D F 1991.
9. Zinhener H, Mear WK. Biology of Antibiotics. Springer-Verlag, New York. p.8, 1972.
10. Daferera DJ, Ziogas BN, Polissiou MG. The effectiveness of plant essential oils on the growth of *Botrytis cinerea*, *Fusarium* sp. and *Clavibacter michiganensis* ssp. *michigansis*. Crop Prot. 22: 39-44, 2003.

11. Elgayyar M, Draughon FA, Golden DA et al. Antimicrobial activity of essential oils from plants against selected pathogenic and saprophytic microorganisms. J Food Protect 64: 1019-1024, 2001.

12. Vander Berghe DA, Vietinck AJ. Screening methods for antibacterial and antiviral agents from higher plants. In: Dey PM, Harborne JB. (Eds.) Methods in Plant Biochemistry. Academic Press, London. 1991.

13. Davis PH. Flora of Turkey and the East Aegean Islands. Edinburgh University Press, Edinburgh. 1982.

14. Marston A, Hostettmann K. Modern separation methods. Nat Prod Rep 391-413, 1991.

15. Hostettmann K, Hostettmann M, Marston A. Preparative Chromatography Techniques Applications in Natural Product Isolation. Springer-Verlag, Berlin. 1986.

16. Ronald MA. Microbiología. Compañía Editorial Continental S.A. de C.V., Mexico, D. F. p. 505. 1990.

17. Demirbag Z, Belduz AO, Sezen K et al. Investigation of antibacterial effects of some plant extracts. KUKEM 20: 47-53, 1997.

18. Avato P, Vitali PM, Tava A. Antimicrobial activity of polyacetylenes from *Bellis perennis* and synthetic derivatives. Planta Med. 63: 503-507, 1997.

19. Zavala, SMA, Pérez GMS, Pérez GRM. Antimicrobial Screening of Some Medicinal Plants. Phytother. Res. 11: 368-371, 1997.

20. Meckes M, Torres J, Calzada F et al. Antibacterial properties of *Helianthemum glomeratum*, a plant used in Maya traditional medicine to treat diarrhoea. Phytother Res 11: 128-131, 1997.