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OLGICA STEFANOVIC

LJILJANA COMIC

DRAGANA STANOJEVIC

SLAVICA SOLUJIC SUKDOLAK

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Antibacterial Activity of *Aegopodium podagraria* L. Extracts and Interaction Between Extracts and Antibiotics

Olgica STEFANOVIĆ¹, Ljiljana COMIĆ¹, Dragana STANOJEVIĆ¹, Slavica SOLUJIC-SUKDOLAK²

¹Department of Biology, Faculty of Science, University of Kragujevac, Radoja Domanovica 12, 34000 Kragujevac - SERBIA

²Department of Chemistry, Faculty of Science, University of Kragujevac, Radoja Domanovica 12, 34000 Kragujevac - SERBIA

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Abstract: Antibacterial activities of aqueous, ethanol, and ethyl acetate extracts of *Aegopodium podagraria* L. (Apiaceae) were tested in vitro against 6 bacteria species. Antibacterial properties were determined by disk diffusion and tube dilution method. Based on the minimal inhibitory concentrations (MIC), the ethanol extract showed the highest activity (1.25-5 mg/ml). Among the tested bacteria, the most sensitive species were *Enterobacter cloacae*, *Klebsiella pneumoniae*, and *Pseudomonas fluorescens*, while the most resistant was *Staphylococcus aureus*. Ethanol extract was chosen to investigate the effects of its combinations with commercial antibiotics by the checkerboard method. Results showed that the interactions between ethanol extract/streptomycin and ethanol extract/chloramphenicol were synergistic, additive, and indifferent against the tested human-pathogenic bacteria. Synergism was observed in relation to *Bacillus subtilis*. Synergy was confirmed at an ethanol extract concentration corresponding to 1/4 MIC and antibiotic concentrations corresponding to 1/4 MIC and lower.

Key Words: *Aegopodium podagraria*, antibacterial activity, herb-drug interactions, plant extracts

Aegopodium podagraria L. Ekstresinin Antibakteriyal Aktivitesi ve Ekstre ile Antibiyotik Etkileşim

Özet: *Aegopodium podagraria* L. (Apiaceae) bitkisinin sıvı, etanol, ve etil asetat ekstresinin altı bakteriye karşı in vitro antibakteriyal etkileri çalışılmıştır. Antibakteriyal özellikleri disk difüzyon ve tüp dilüsyon yöntemi ile çalışılmıştır. Minimal inhibisyon konsantrasyonlarına göre en yüksek aktivite (1.25-5 mg/ml) etanol ekstresinde gözlenmiştir. Denenen bakteriler içinde en hassas olanlar *Enterobacter cloacae*, *Klebsiella pneumoniae* ve *Pseudomonas fluorescens* bulunurken, *Staphylococcus aureus* bakterisi en dirençli bakteri olmuştur. Etanol ekstresi ticari antibiyotik ile mukayese edilmiştir. Sonuç olarak antibiyotik ekstre ilişkisi denenen insan patojen bakterilerine karşı etanol ekstresi/streptomisin ve etanol ekstresi/kloramfenikol sinerjistik, katkısız ve ilgisiz bulunmuştur. Sinerji *Bacillus subtilis* bakterisinde gözlenmiştir. Etanol ekstresinde konsantrasyon 1/4 MIC ve antibiyotik konsantrasyonunda 1/4 ve daha az bir MIC olarak hesaplanmıştır.

Anahtar Sözcükler: *Aegopodium podagraria*, antibakteriyal aktivite, bitki-ilaç ilişkisi, bitki ekstresi

Introduction

Aegopodium podagraria L. (ground elder or goutweed; fam. Apiaceae) is a perennial herbaceous plant widely distributed in Europe and Asia. The plant has been employed in traditional medicine since ancient times. It was used to treat gout and sciatica (1, 2) and as a diuretic, sedative, and vulnerary (3). Externally, it

was used as a poultice on burns, stings, wounds, and painful joints (4). In addition, leaves of young *A. podagraria* L. plants are consumed as flavouring or vegetable (5). The antimicrobial activity of the given species has been insufficiently investigated (6,7), and there are no data on synergy between extracts of this plant and antibiotics.

The species *A. podagraria* L. is a Eurasian floral element that, in Serbia, is widely distributed in damp places, among shrubbery, in meadows, and on the banks of creeks and rivers (8).

Considering that *A. podagraria* L. has been insufficiently studied, the purpose of the present work was to determine the antibacterial activity of specimens originating from Southeast Europe and thus expand our knowledge of biotic properties of the species as a whole. Another aim was to establish synergy between extracts and commonly used antibiotics (streptomycin and chloramphenicol), thereby throwing light on the potential role of phytochemicals in increasing the effectiveness of antibiotics.

Materials and Methods

Plant material

The aerial parts of *A. podagraria* L. were collected during the summer of 2006 on Mt. Suvobor (Serbia). Identification and classification of the plant material was performed at the Faculty of Science, University of Kragujevac. The voucher specimen (PMFKg-H355) of the plant is deposited in the Herbarium of the Faculty of Science.

Preparation of samples for testing

Aqueous extract was obtained by boiling dry ground plant material (50 g for each extract) on a water bath for 2-3 h at 80 °C. Ethanol and ethyl acetate extracts were obtained by Soxhlet apparatus. Aqueous extract was concentrated to dryness on a water bath, while ethanol and ethyl acetate extracts were evaporated to dryness on a rotary evaporator at 40°C. Stock solutions were obtained by dissolving 1.5 g of extracts in 5 ml of 5% DMSO for disk diffusion method and 1.2 g of extracts in 10 ml of 5% DMSO for dilution and checkerboard method. The DMSO solvent was purchased from Merck (Germany). Solutions of streptomycin (Galenika, Belgrade) and chloramphenicol (Panpharma, France) were obtained by dissolving in a Mueller-Hinton broth (Torlak, Belgrade).

Test microorganisms

The following species of human-pathogenic bacteria were tested:

Gram-positive bacteria: *Bacillus mycoides* (PMFKg-B1), *Bacillus subtilis* (PMFKg-B2) and *Staphylococcus aureus* (PMFKg-B30)

Gram-negative bacteria: *Enterobacter cloacae* (PMFKg-B22), *Klebsiella pneumoniae* (PMFKg-B26), *Pseudomonas fluorescens* (PMFKg-B28).

All microorganisms were clinical isolates from the Institute of Public Health, Kragujevac and stored in microbiological collection at the Microbiology Laboratory (Faculty of Science, University of Kragujevac).

Disk diffusion method

The filter disk diffusion method (9) was used for primary screening of antibacterial activity of plant extracts. Overnight cultures of bacterial species were used for inoculum preparation. The inocula were prepared by suspending growth in a sterile saline and turbidity was adjusted to yield 0.5 McFarland standard (approximately 10^8 colony-forming units (CFU)/ml) and then diluted to 1:10 ratio. Petri dishes containing Mueller-Hinton agar (Torlak, Belgrade) were seeded with the prepared inocula. Then, 0.1 ml of the stock solution of plant extracts were applied to sterile filter paper disks (Whatman No.1, 10 mm diameter) to give the final concentrations of 5, 10 and 15 mg per disk and finally placed on the surface of the inoculated medium. The plates were incubated at 37 °C for 24 h. Antibacterial activity was determined by measuring the zone of growth inhibition (mm) around the disk. As positive control, we used a standard disk of the antibiotic streptomycin (30 µg per disk, Torlak, Belgrade). A disk soaked with the solvent (5% DMSO) was used as negative control. Tests were performed in 3 replications and the obtained results were statistically analysed.

Minimal inhibitory concentration

Minimal inhibitory concentrations (MICs) were determined by the tube dilution method (10). A solution of each extract was 2-fold serially diluted in Mueller-Hinton broth. The final concentrations of the extracts in the medium ranged from 20 mg/ml to 0.3125 mg/ml. Then, 50 µl of the prepared suspension was added into each tube. The test tubes were incubated at 37 °C for 24 h. The MIC was defined as the lowest concentration at which visible growth was inhibited.

The same method (10) was used to test MIC values in a range from 0.1 to 0.00078 mg/ml for streptomycin and from 1.0 to 0.0078 mg/ml for chloramphenicol.

Each test included 2 growth controls consisting of the medium with the solvent (5% DMSO) and medium with bacterial suspension as well as sterility control. All tests were performed in duplicates.

Estimation of synergy between plant extracts and antibiotics

The plant extract that showed the greatest activity (the lowest MIC values) was selected for further testing. Synergy between the ethanol extract and streptomycin and between the ethanol extract and chloramphenicol was studied by the checkerboard assay method (11). Two-dimensional arrays of test tubes were constructed. From the first to seventh columns, the ethanol extract solution was diluted 2-fold in Mueller-Hinton broth in order to obtain the final concentration (from 2 MIC to 1/32 MIC). Twofold dilutions of the antibiotic (from MIC to 1/32 MIC) were then added to the test tubes, from the first to the sixth row. Each test tube contained unique combinations of concentrations of ethanol extract/streptomycin and ethanol extract/chloramphenicol. The examined tubes were inoculated with 50 µl of the prepared inoculum to obtain the final turbidity (approximately 5×10^5 CFU/tube) and incubated at 37 °C for 24 h. The MIC was defined as the lowest concentration of antimicrobial agents in combination at which visible bacterial growth was inhibited. Each test included 2 growth controls consisting of the medium with the solvent (5% DMSO) and medium with bacterial suspension as well as sterility control. All tests were performed in duplicates.

In vitro interactions between antimicrobial agents were determined and quantified by calculating the fractional inhibitory concentration (FIC) index using the following formula: FIC index = (MIC of drug A in combination/MIC of drug A alone) + (MIC of drug B in

combination/MIC of drug B alone). Interpretation of the FIC index (FICI) was as follows: FICI \leq 0.5 synergy; FICI > 0.5 - 1 additive; FICI 1 - 4 indifference, and FICI > 4 antagonism. The action of antimicrobial agents is considered to be:

- synergistic if their joint effect is stronger than the sum of effects of individual agents
- additive if their joint effect is equal to the sum of effects of individual agents
- indifferent if their joint effect is equal to the effect of either individual agent
- antagonistic if their joint effect is weaker than the sum of effects of the individual agents or weaker than the effect of either individual agent (11).

Results

Test results of the antibacterial activity of aqueous, ethanol, and ethyl acetate extracts of *A. podagraria* L. against 6 species of bacteria are presented in Table 1. The negative control (5% DMSO) did not inhibit growth in the tested bacteria. The investigated extracts showed different degrees of antibacterial activity in relation to the tested bacterial species. The intensity of antimicrobial activity depended both on the species of microorganism and on the type and concentration of extract. The aqueous extract acted most strongly against *Enterobacter cloacae* (16 mm for a concentration of 15 mg per disk). The ethanol extract was most active against *Bacillus subtilis* (12.66 mm for a concentration of 15 mg per

Table 1. Antibacterial activity of the extracts of *Aegopodium podagraria* L.

Species	Aqueous extract				Ethanol extract				Ethyl acetat extract				Streptomycin (positive control)	
	5 mg per disk	10 mg per disk	15 mg per disk	MIC mg/ml	5 per disk	10 per disk	15 per disk	MIC mg/ml	5 per disk	10 per disk	15 per disk	MIC mg/ml	30 µg per disk	MIC mg/ml
<i>Bacillus mycoides</i>	0.00	11.00	12.00	>20	10.66	11.00	12.00	2.5	11.00	10.66	11.32	>15	37	0.006
<i>Bacillus subtilis</i>	10.32	11.00	15.32	>20	12.00	12.66	12.66	2.5	10.66	11.66	11.32	>15	27	0.012
<i>Enterobacter cloacae</i>	10.66	12.66	16.00	>20	11.00	11.00	12.00	2.5	10.66	10.66	10.66	15	25	0.006
<i>Klebsiella pneumoniae</i>	13.66	12.66	12.66	>20	10.66	11.00	11.32	1.25	11.00	10.66	11.00	>15	27	0.006
<i>Pseudomonas fluorescens</i>	11.00	12.66	14.00	>20	10.66	11.00	11.00	1.25	10.66	11.00	11.00	15	27	0.006
<i>Staphylococcus aureus</i>	10.66	11.00	11.32	>20	0.00	10.32	11.00	5.0	0.00	0.00	0.00	>15	25	0.05

Diameter of the zone of inhibition (mm) including the size of disk (10 mm).

disk), and the ethyl acetate extract against *Klebsiella pneumoniae* and *Pseudomonas fluorescens* (11 mm for a concentration of 15 mg per disk). Of the tested bacteria, *Staphylococcus aureus* was the most resistant to all 3 extracts.

In the case of the aqueous extract, MIC values were greater than 20 mg/ml against all of the tested bacteria. Concerning the ethanol extract, it ranged from 1.25 to 5 mg/ml, depending on the species of bacteria. For the ethyl acetate extract, the MIC values were 15 mg/ml for 2 species of bacteria and >15 mg/ml for the remaining 4 species. The tested antibiotics had MIC values ranging from 0.05 to 0.006 mg/ml for streptomycin and from 0.25 to 0.125 mg/ml for chloramphenicol (Table 2).

The results of the checkerboard combination assays are presented in Table 2. FIC indices were calculated and the concentrations at which the highest level of activity was exhibited are listed. For both combinations of agents, synergism was recorded in relation to *Bacillus subtilis*. The strength of synergistic effects between the ethanol

extract and streptomycin and between this extract and chloramphenicol are indicated by the FICI values, which ranged from 0.28 to 0.5. Inhibition of bacterial growth was recorded at lower concentrations compared to the individually tested values. Synergy was confirmed at an ethanol extract concentration corresponding to 1/4 MIC and antibiotic concentrations corresponding to 1/4 MIC and lower.

Combinations of the ethanol extract and streptomycin gave the following kinds of effects against 6 human-pathogenic bacteria: synergistic against *Bacillus subtilis*, additive against *Bacillus mycoides*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*, and indifferent against *Pseudomonas fluorescens* while interactions between ethanol extract and chloramphenicol were: synergistic against *Bacillus subtilis*, additive against *Bacillus mycoides*, and indifferent against *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Pseudomonas fluorescens*, and *Staphylococcus aureus* (Table 3). No antagonistic effect was observed.

Table 2. Interaction between *Aegopodium podagraria* L extracts and antibiotics.

Species	Ethanol extract	Streptomycin	Ethanol extract + Streptomycin	FIC index	Ethanol extract	Chloramphenicol	Ethanol extract + Chloramphenicol	FIC index
	MIC (mg/ml)	MIC (mg/ml)	MIC (mg/ml)*		MIC (mg/ml)	MIC (mg/ml)*		
<i>B. mycoides</i>	2.5	0.006	1.25 + 0.00019	0.53	2.5	0.125	1.25 + 0.031	0.75
<i>B. subtilis</i>	2.5	0.012	0.625 + 0.00078	0.31	2.5	0.125	0.625 + 0.004	0.28
<i>Ent. cloacae</i>	2.5	0.006	1.25 + 0.00078	0.63	2.5	0.125	2.5 + 0.008	1.031
<i>Kl. pneumoniae</i>	1.25	0.006	0.625 + 0.00078	0.63	1.25	0.250	1.25 + 0.004	1.031
<i>Ps. fluorescens</i>	1.25	0.006	1.25 + 0.00019	1.031	1.25	0.250	1.25 + 0.008	1.031
<i>Staph. aureus</i>	5.0	0.05	2.5 + 0.00156	0.53	5.0	0.250	5.0 + 0.008	1.031

* MIC values of the most effective combinations

Table 3. FIC values and number of bacteria species showing synergy, additive effect, and indifference.

Combinations	ΣFIC		No. of bacteria species showing		
	Range	Median	Synergy (%)	Additive (%)	Indifference (%)
Ethanol extract + Streptomycin	0.31-3.00	0.61	1 (16.67)	4 (66.67)	1 (16.67)
Ethanol extract + Chloramphenicol	0.28-3.00	0.86	1 (16.67)	1 (16.67)	4 (66.67)
Total			6 (100)	6 (100)	6 (100)

Discussion

The species *A. podagraria* L. is a plant used in traditional medicine, but its antibacterial activity has been insufficiently investigated. Brković, Comić, Solujic-Sukdolak (7) tested inhibitory effects of *A. podagraria* extracts on phytopathogenic bacteria and showed broad-spectrum antibacterial activity. There have been no documents about the effect of *A. podagraria* on human-pathogenic bacteria. It is believed that plants of the family Apiaceae synthesize coumarins, which act as an inhibitor of microorganism growth (12). Ojala (13) studied the effect of a methanol extract of the leaves of *A. podagraria* and noticed weak antimicrobial activity and low total content of coumarins. Our results indicate that the intensity of antibacterial activity depends both on the species of microorganism and on the type and concentration of extract, and different MIC values are accordingly observed. In our case, the aqueous and the ethyl acetate extract showed weak inhibitory effects while the ethanol extract showed moderate inhibitory effect. Also, the sensitivity of microorganisms varies as a function of the type of extract. *Enterobacter cloacae* was most sensitive to the aqueous extract. The ethanol extract acted best in relation to *Klebsiella pneumoniae* and *Pseudomonas fluorescens*, and the ethyl acetate extract in relation to *Enterobacter cloacae* and *Pseudomonas fluorescens*. *Staphylococcus aureus* was the most resistant bacterial species in relation to all 3 extracts.

It is interesting that even ethanol extract showed moderate inhibitory activity; it exhibited synergistic and additive effects with streptomycin and chloramphenicol against bacteria. In the case of *Bacillus subtilis*, the activity of streptomycin and chloramphenicol was increased 8-fold and 10-fold, respectively. The ability of crude plant extracts to potentiate the activity of antibiotics has been researched by others scientists. Ahmad and Aquil (14) reported that crude extracts of Indian medicinal plants demonstrate synergistic interaction with tetracycline and

ciprofloxacin against ES L-producing multidrug-resistant enteric bacteria. Betoni et al. (15) also observed synergistic interactions between extracts of Brazilian medicinal plants and 8 antibiotics. Sibanda and Okoh (16) showed potentials of synergy between acetone extracts of *Garcinia kola* seeds and amoxicillin, ciprofloxacin, tetracycline and chloramphenicol against pathogenic organisms. It appears that phytochemicals with antibiotics are capable of increasing the total biological activity. The mechanism governing the joint action of plant extract components and antibiotics is still unknown. Biologically active components are believed to disturb permeability of the cytoplasmic membrane and thereby facilitate the influx of antibiotics (17).

Conclusions

Results of the present work showed that the ethanol extract of *A. podagraria* L. possesses antibacterial activity and show synergistic and additive effects with antibiotics. These findings could be helpful in resolving the problem of bacterial infection and bacterial resistance and thereby allow the use of antibiotics in smaller amounts.

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Corresponding Author:

Olgica STEFANOVIĆ
Department of Biology,
Faculty of Science,
University of Kragujevac,
Radoja Domanovica 12,
34000 Kragujevac - SERBIA
E-mail: olgicas@gmail.com

References

1. Launert E. The Hamlyn Guide to Edible and Medicinal Plants of Britain and Northern Europe. Hamlyn Publishing Group. London; 1981.
2. Grieve M. A Modern Herbal. Penguin, London; 1984.
3. Usher G. A Dictionary of Plants Used by Man. Constable and Company. London; 1974.
4. Stuart M. The Encyclopedia of Herbs and Herbalism. Orbis Publishing. London; 1979.
5. Facciola S. Cornucopia: A Source Book of Edible Plant. Kampong Publications. Vista, California, USA; 1990.
6. Garrod B, Lea E, Lewis GB. Studies on the mechanism of action of the antifungal compound faltarindiol. *New Phytol* 83: 463-471, 1979.

7. Brkovic DL, Comic LJ, Solujic-Sukdolak S. Antibacterial activity of some plants from family Apiaceae in relation to selected phytopathogenic bacteria. *Kragujevac J Sci* 28: 65-72, 2006.
8. Josifovic M. Flora of SR Serbia. Serbian Academy of Science and Arts. Belgrade; 1973.
9. NCCLS (National Committee for Clinical Laboratory Standards) (1997) Performance Standards for Antimicrobial Disk Susceptibility Test. Approved Standard M2-A6: Wayne PA.
10. NCCLS (National Committee for Clinical Laboratory Standards) (1997) Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically. Approved Standard M7-A4: Wayne PA.
11. Satish KP, Moellering RC, Eliopoulos GM. Antimicrobial Combinations. In: Lorian V, ed. *Antibiotics in Laboratory Medicine*. Lippincott Williams & Wilkins; 2005.
12. Ribeiro CC, Kaplan MC. Evolutionary tendency of coumarin-bearing families in angiospermae. *Quím Nova* 25: 533-538, 2002.
13. Ojala T. Biological Screening of Plant Coumarins, PhD, Faculty of Science, University of Helsinki, Helsinki, 2001.
14. Ahmad I, Aqil F. In vitro efficacy of bioactive extracts of 15 medicinal plants against ES L-producing multidrug-resistant enteric bacteria. *Micro Res* 162: 264-275, 2007.
15. Betoni JEC, Mantovani RP, Barbosa LN et al. Synergism between plant extract and antimicrobial drugs used on *Staphylococcus aureus* diseases. *Mem Inst Oswaldo Cruz* 101: 387-390, 2006.
16. Sibanda T, Okoh AI. In vitro evaluation of the interactions between acetone extracts of *Garcinia kola* seeds and some antibiotics. *Afr J Biotechnol* 7: 1672-1678, 2008.
17. Zhao WH, Hu ZQ, Okubo S et al. Mechanism of synergy between epigallocatechin gallate and -lactams against methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 45: 1737-1742, 2001.