DE GRUYTER OPEN

ACTA FACULTATIS MEDICAE NAISSENSIS

UDC: 633.886

DOI: 10.2478/afmnai-2014-0009

Scientific Journal of the Faculty of Medicine in Niš 2014;31(1):81-85

Original article ■

Antimicrobial Activity of Selected Plant Species of Genera *Arbutus* L., *Bruckenthalia* Rchb., *Calluna* Salisb. and *Erica* L. (Ericaceae)

Dragana Pavlović[1], Branislava Lakušić[2], Dušanka Kitić[1], Milica Milutinović[1], Milica Kostić[1], Bojana Miladinović[1], Nada Kovačević[3]

[1]*University of Niš, Faculty of Medicine, Department of Pharmacy, Serbia*

[2]*University of Belgrade, Faculty of Pharmacy, Institute of Botany, Serbia*

[3]*University of Belgrade, Faculty of Pharmacy, Institute of Pharmacognosy, Serbia*

SUMMARY

***Uvae ursi folium* (*Arctostaphylos uva ursi*, Ericaceae) is the best known and most widely used herbal urinary antiseptic. In traditional medicine, other Ericaceae species are also used for the treatment of urinary tract infections. The present study investigates antimicrobial activity of five species of Ericaceae family native to the Balkan Peninsula: *Arbutus unedo*, *Bruckenthalia spiculifolia*, *Calluna vulgaris*, *Erica arborea* and *Erica carnea*.**

Ethanolic extracts were tested against 10 different gram-positive and gram-negative bacteria by the disc diffusion technique, where standard tetracycline, streptomycin and penicillin discs and discs containing crystal violet (1 mg/ml) and solvent (70.0% v/v ethanol) were used as controls.

The most prominent antibacterial effect was achieved on *Staphylococcus aureus* with extracts of *Calluna vulgaris* and *Erica carnea*. Tested samples showed no activity against the gram-negative strains *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae*. Inhibitory effects on the growth of gram-positive bacteria were more potent. The exception is *Arbutus unedo* ethanol extract which exhibited certain activity against a laboratory strain of wild *Escherichia coli*.

Antimicrobial activity of the ethanolic extracts against 10 tested strains of bacteria in disc diffusion assay was generally weak, even for sample in which HPLC determination confirmed the presence of arbutin (secondary metabolite responsible for most of the antibacterial activity of *Uvaeursi folium*).

Key words: **Ericaceae, antimicrobial activity, disc diffusion, arbutin**

Corresponding author:

Dušanka Kitić •

phone: 063 104 50 43 •

e-mail: duska@medfak.ni.ac.rs •

INTRODUCTION

Arctostaphylos uva ursi, the famous member of the Ericaceae family, is an ancient astringent and urinary antiseptic. Therapeutic indication for bearberry leaf, *Uvae ursi folium*, is uncomplicated infections of the lower urinary tract such as cystitis, when antibiotic treatment is not considered essential (1). The antimicrobial effect of *Uvae ursi folium* is associated with the aglycone hydroquinone released from arbutin or arbutin waste products in the alkaline urine (2). In traditional medicine, other Ericaceae species are also used for the treatment of urinary tract infections (3-5). Our research involved five species from Ericaceae family that grow wild in Serbia and Montenegro, in addition to *Arctostaphylos uva ursi*: *Arbutus unedo* L., *Bruckentalia spiculifolia* (Salisb.) Reichenb., *Calluna vulgaris* Salisb., *Erica arborea* L. and *Erica carnea* L. Despite the fact that these species are often used in popular medicine in their region of origin, only few of these selected species have been partly investigated.

MATERIAL AND METHODS

Plant material

Plant materials were collected from wild growing species of Ericaceae family: *Arbutus unedo* (Luštica, Montenegro) HFF (Herbarijum Farmaceutskog Fakulteta Univerziteta u Beogradu - Herbarium collection of the Faculty of Pharmacy, University of Belgrade) No. 1173; *Bruckentalia spiculifolia* (Kopaonik - Pančićev vrh, Serbia) HFF No. 1217; *Calluna vulgaris* (Loznica - Gučevo, Serbia) HFF No. 1272; *Erica arborea* (Luštica, Montenegro) HFF No. 1430; *Ericacarnea* (syn.: *Erica herbacea* L., *Erica saxatilis* Salisb.) (Mokra Gora, Serbia) HFF No. 1431. Authenticated voucher herbarium specimens have been deposited in the Herbarium collection of the Faculty of Pharmacy, University of Belgrade (HFF). Determination was done according to Flora of SR Srbija (6), Flora Europea (7) and Anatomy of the Dicotyledons (8).

Extraction procedure

Plant material, dried leaves of selected Ericaceae species, was reduced to a fine powder and extracted with ethanol (70.0%, v/v) by percolation, as described in European Pharmacopeia 6.0 (9). Ethanolic extracts of *A. unedo* (AE), *B. spiculifolia* (BE), *C. vulgaris* (VE), *E. arborea* (EE) and *E. carnea* (CE) were obtained after evaporation to the dryness in vacuo below 40ºC and extraction yields were 45.05, 32.35, 33.84, 38.98 and 38.61% (w/w), respectively.

Test Organisms

Both gram-positive and gram-negative bacterial strains used for the experiment were collected as pure cultures from ATCC (American Type of Culture Collection) and NCIMB (National Collection of Industrial Bacteria, Aberdeen). Gram-positive bacteria: *Staphylococcus aureus* (ATCC29213), *Staphylococcus epidermidis* (ATCC 12228), *Micrococcus flavus* (ATCC10240), *Bacillus subtilis* (ATCC10707), *Sarcinalutea* (ATCC9391). Gram-negative bacteria: *Pseudomonas aeruginosa* (ATCC9027), *Klebsiella pneumoniae* (NCIB9111) and *Escherichia coli* (*E. coli*) (ATCC25922). Two laboratory strains of *E. coli* were also included in the study: SY252 (strain with weakened wall) and LPCA (the wild laboratory strain). The bacterial inoculates were made up from overnight broth cultures. Suspensions with microorganisms were adjusted to 0.5 McFarland standard turbidity according to consensus standard of the National Committee for Clinical Laboratory Standards (10). Suspensions were spread on Muller-Hinton agar (MHA, Torlak) in sterilized Petri dished (90 mm in diameter).

Experimental Procedure

The antimicrobial study was carried out by disc diffusion technique (11). All tested extracts were weighed under aseptic conditions in sterile volumetric flasks, and dissolved with 70.0% sterile ethanol to obtain different extract concentrations (concentrations 1, 2, 3 and 4 contained 40.0, 20.0, 10.0 and 5.0% of crude extract, respectively). Standard tetracycline, streptomycin and penicillin discs and discs containing crystal violet (1 mg/ml) and solvent (70.0% v/v ethanol) were used as controls. All probes were applied in 20 μl volumen per disk. According to this method, the antimicrobial potency of the test samples was measured by determining the diameter of the zones of inhibition in millimetres. All determinations were made in duplicate.

RESULTS

The antimicrobial activity of tested samples against selected microorganisms is showed in Table 1. Diameter of sterile paper disk was 13 mm. Thus, diameters of the inhibition zones larger than 13 mm were presented as positive results. Applied extract concentrations (40.0, 20.0, 10.0 and 5.0% of crude extract) were labelled as concentrations 1, 2, 3 and 4, respectively. As referent antimicrobial drugs, tetracycline, streptomycin and penicillin alongside discs containing crystal violet exhibited obviously higher antimicrobial activity than tested ethanolic extracts of selected Ericaceae species. Extract solvent, ethanol, did not affect any of tested bacterial strains.

Table 1. *Results of antimicrobial assay showed as diameters of the of inhibitory zones in millimetres*

Microorganism	AE	BE	VE	EE	CE	Ethanol 70% v/v	Crystal violet	Antibiotic
Staphylococcus aureus	/	1=14 2,3=13.5	1=14.5 2=14 3=13.5	/	1=16 2=15 3=14	/	17	18
Staphylococcus epidermidis	1,2=14.5 3=14	/	/	/	/	/	21	27
Micrococcusflavus	1=17 2,3=16 4=15	1=14.5 2=14	/	1,2=13.5	/	/	22	37
Bacillus subtilis	1=14.5 2,3=14	1,2,3=14	1,2=14 3=13.5	1=14	1-4=14	/	20	26
Sarcinalutea	1,2=14.5 3=14	1=15 2=14.5	/	1,2=13.5	1=13.5	/	20	47
Pseudomonas aeruginosa	/	/	/	/	/	/	/	22
Klebsiella pneumoniae	/	/	/	/	/	13.5	16	17
E. coli	/	/	/	/	/	/	/	15
E. coli SY252	/	/	/	/	/	/	14	18
E. coli LPCA	1=15 2=14 3=13.5	/	/	/	/	/	21	23

DISCUSSION

Ethanolic extracts of *A. unedo*, *B. spiculifolia*, *C. vulgaris*, *E. arborea* and *E. carnea* were compared between each other and with *A. uva ursi*, species of this family well known for its antimicrobial properties. The disc diffusion method was used to determine the inhibition zones of the five ethanolic extracts from different Ericaceae species. The five gram-positive and five gram-negative bacterial strains have been used. In general, phenolics are the predominant active chemicals in plants, with gram-positive bacteria being the most sensible germs (12). Our results for AE, BE, VE, EE and CE antimicrobial activity string along these findings. According to the results in Table 1, different ethanolic extracts showed certain antibacterial activity against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Micrococcus flavus*, *Bacillus subtillis* and *Sarcinalutea*. Antimicrobial activity against the *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *E. coli* was not observed. The only exception is mild impact of *A. unedo* extract (AE) on *E. coli* LPCA (the wild laboratory strain of *E. coli*). The most prominent antimicrobial effect was achieved on *Micrococcus flavus* with AE. Inhibition zones observed for concentration 1 (17 mm) were slightly larger than those observed for concentrations 2 and 3 (16 mm) and 4 (15 mm) of *A. unedo* ethanolic extracts.

The antimicrobial activity of *A. unedo* root extract against *E. coli* and *Staphylococcus aureus* (13) and antiprotosoal activity of *A. unedo* leaf extract against *Leishmania tropica* (14) and *Trichomonas vaginalis* (15) has already been reported. According to Kumarasamy et al. (2002) methanolic extract of *C. vulgaris* seed showed bacteriostatic activity against the gram-positive strains *Staphylococcus aureus* and *Staphylococcus hominis* (with minimal inhibitory concentration 0,1 μg/ml) (16). Our results for VE indicate very mild impact on *Staphylococcus aureus* and *Bacillus subtilis*.

The 30.0% ethanol extract of *Uvaeursi folium* inhibited the growth *in vitro* of *Bacillus subtilis*, *E. coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Serratia marcescens* and *Staphylococcus aureus*. Ethanol extract were also active *in vitro* against *E. coli*, *Proteus vulgaris*, *Staphylococcusfaecalis* and *Enterobacter aerogenes*. However, 95.0% ethanol extract had no antibacterial activity (17). Arbutin is generally considered to be respon-

sible for the antibacterial activity of the *Uvae ursi folium* extract. Preliminary chemical analysis showed the presence of hydroquinone and phenil propanoid derivates, flavonoids and tannins in *A. unedo*, *B. spiculifolia*, *C. vulgaris*, *E. arborea* and *E. carnea*. Our previous results, obtained using the HPLC method, also confirmed the presence of arbutin only in leaves of *Arbutus unedo* (1.21 ±0.03%) and absence of hydroquinone in all tested samples (18). The amount of arbutin in dry *A. uva ursi* leaves ranges from 5.0 to 15.0% (17), while according to the monograph of European Pharmacopoeia 6.0 (9), dried drug *Uvae ursi folium* should contain minimum 7.0% of anhydrous arbutin. Thus, the presence of arbutin in AE could be, at least partly, responsible for measured antibacterial activity. However, in this preliminary screening, it was observed that antimicrobial activity of all tested samples was generally weak.

CONCLUSION

Based on the results of this *in vitro* tests on antimicrobial activity of leaf ethanolic extracts of *Arbutus unedo*, *Bruckentalia spiculifolia*, *Calluna vulgaris*, *Erica arborea* and *Erica carnea*, and bearing in mind that only leaves of *Arbutus unedo* contain arbutin (although in significantly smaller quantity than *Uvae ursi folium*), the traditional use of leaf of this species in the treatment of urinary tract infections cannot be considered plausible.

Acknowledgement

This research was supported by the Ministry of Education and Science of the Republic of Serbia (Grants No.III 41018 and III 46013).

References

1. ESCOP Monographs. 2nd edition. Thieme: European Scientific Cooperative On Phytotherapy; 2003.
2. Heinrich M, Barnes J, Gibbons S, Williamson EM. Fundamentals of Pharmacognosy and Phytotherapy. Churchill Livingstone, Edinburgh, 2004.
3. Tucakov J. Lečenje biljem. VII izdanje, Rad, Beograd, 1997.
4. Tasić S, Šavikin Fodulović K, Menković N. Vodič kroz svet lekovitog bilja. Valjevac, Valjevo, 2004.
5. Maleš Ž, Plazibat M, Bilušić-Vundać V, Žuntar I. Qualitative and quantitative analysis of flavonoids of the strawberry tree - *Arbutus unedo* L. (*Ericaceae*). Acta Pharm 2006;56:245–50.
6. Jovanović B. Ericaceae DC. In: Josifović, M. (Urednik): Flora SR Srbije, SANU, Beograd, 1972.
7. Weebb DA.Ericaceae L. InTutin TG. et al. (eds.) Flora Europea, Volume 3, Cambridge University Press, London, 1972.
8. Metcalfe CR, Chalk L. Anatomy of the Dicotyledons – Leaves, stem and wood in relation to taxonomy with notes on economic uses, Oxford University Press, Amen House, London, 1950.
9. European Pharmacopeia, 6th Edition. Council of Europe, Strasbourg, 2007.
10. National Committee for Clinical Laboratory Standards (NCCLS), Performance standards for anti-microbial susceptibility testing: eleventh informational supplement. Document M100-S11. National Committee for Clinical Laboratory Standard, Wayne, PA, USA, 2003.
11. Kitić D, Jovanović T, Ristić M, Palić R, Stojanović G. Chemical composition and antimicrobial activity of the essential oil of *Calaminthanepeta* (L.) Savissp *glandulosa* (Req.) P. W. Ball from Montenegro. J Essent Oil Res 2002; 14(2):150-2. http://dx.doi.org/10.1080/10412905.2002.9699802
12. Ríos JL, Recio MC. Medicinal plants and antimicrobial activity. J Ethno pharmacol 2005;100:80-4. http://dx.doi.org/10.1016/j.jep.2005.04.025
13. Dib MEA, Allali H, Bendiabdellah A, Meliani N, Tabti B. Antimicrobial activity and phytochemical screening of *Arbutus unedo* L. J Saudi ChemSoc 2011; http://dx.doi.org/10.1016/j.jscs.2011.05.001
14. Kivçak B, Mert T, Ertabaklar H, Balcioglu C, ÖzensoyTöz S. *In vitro* Activity of *Arbutus unedo* Against *Leishmaniatropica* Promastigotes. Türkiye Parazitol Derg 2009; 33(2):114-15.
15. Ertabaklar H, Kivçak B, Mert T, ÖzensoyTöz S. *In vitro* Activity of *Arbutus unedo*Leaf Extracts against *Trichomonas vaginalis* Trophozoites. Türkiye Parazitol Derg 2009; 33(4):263-5.
16. Kumarasamy Y, Cox PJ, Jaspars M, Nahar L, Sarker SD. Screening seeds of Scottish plants for antibacterial activity. J Ethnopharmacol 2002;83:73-7. http://dx.doi.org/10.1016/S0378-8741(02)00214-3
17. WHO monographs on selected medicinal plants, Vol 2. World Health Organization, Geneva, 2002.
18. Pavlović DR, Lakušić B, Došlov-Kokoruš Z, Kovačević N. Arbutin content and antioxidant activity of some species from Ericaceae family. Pharmazie2009; 64: 656-9.

ANTIMIKROBNA AKTIVNOST ODABRANIH BILJNIH VRSTA RODOVA *ARBUTUS* L., *BRUCKENTHALIA* RCHB., *CALLUNA* SALISB. I *ERICA* L. (ERICACEAE)

Dragana Pavlović[1], Branislava Lakušić[2], Dušanka Kitić[1], Milica Milutinović[1], Milica Kostić[1], Bojana Miladinović[1], Nada Kovačević[3]

[1]*Univerzitet u Nišu, Medicinski fakultet, Odsek za farmaciju, Srbija*
[2]*Univerzitet u Beogradu, Farmaceutski fakultet, Institut za botaniku, Srbija*
[3]*Univerzitet u Beogradu, Farmaceutski fakultet, Institut za farmakognoziju, Srbija*

Sažetak

Najpoznatiji i najviše korišćeni biljni uroantiseptik je list uve, *Uvaeursi folium* (*Arctostaphylos uva ursi*, Ericaceae). U tradicionalnoj medicini često se i druge vrste familije Ericaceae spominju u lečenju urinarnih infekcija. Provera antimikrobne aktivnosti izvršena je za sledeće biljne vrste ove familije koje samostalno rastu u flori Balkanskog poluostrva: *Arbutus unedo*, *Bruckentalia spiculifolia*, *Calluna vulgaris*, *Erica arborea* i *Erica carnea*.

Antimikrobna aktivnost etanolnih ekstrakata listova ispitivana je diskdifuzionom metodom, korišćenjem 10 sojeva gram pozitivnih i gram negativnih bakterija, pri čemu su kao kontrole korišćeni standardni diskovi tetraciklina, streptomicina i penicilina, kao i diskovi sa rastvorom kristalvioleta (1 mg/ml) i rastvarača (70.0% v/v etanol).

***Pseudomonas aeruginosa, Escherichia coli* i *Klebsiella pneumoniae* nisu osetljive ni na jedan od ispitivanih ekstrakata. Kao najbolji rezultati mogu se izdvojiti aktivnosti ekstrakta *Calluna vulgaris* i *Erica carnea* na bakterijski soj *Staphylococcus aureus*. Testirani uzorci uglavnom ispoljavaju antimikrobnu aktivnost prema gram pozitivnim sojevima. Izuzetak je etanolni ekstrakt vrste *Arbutus unedo,* koji ispoljava određenu antimikrobnu aktivnost prema laboratorijskom soju *Escherichia coli*.**

Antimikrobna aktivnost etanolnih ekstrakata prema 10 testiranih bakterijskih sojeva u diskdifuzionoj metodi je veoma slaba, čak i za uzorak za koji je HPLC analizom utvrđeno prisustvo arbutina (sekundarni metabolit odgovoran za uroantiseptično dejstvo lista uve).

Ključne reči: **Ericaceae, antimikrobna aktivnost, diskdifuziona metoda, arbutin**