COMPARATIVE STUDY ON THE POLYPHENOLS AND PECTIN OF THREE *ERYNGIUM* SPECIES AND THEIR ANTIMICROBIAL ACTIVITY

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The polyphenols and pectin from the aerial parts of *Eryngium planum*, *E. campestre* and *E. maritimum* were investigated. For screening the polyphenols, the HPLC-UV-MS method was employed, while pectin was quantified by the gravimetric method. Pectin isolation was performed using hot water extraction or by a selective extraction with 4 different solvents. Isoquercitrin, quercitrin, kaempferol, quercetol, chlorogenic acid, caffeic acid, p-coumaric acid and ferulic acid were the polyphenols detected in all *Eryngium* species. The pectin content yielded by hot water extraction varied substantially among the species, being the highest in *E. maritimum* (8.8%), medium in *E. planum* (4.65%) and the lowest in *E. campestre* (1.7%). The selective extraction method revealed the highest pectin content in *E. campestre* (26.74%), followed by *E. maritimum* (17.8%) and *E. planum* (12.21%). The screening of antimicrobial activity confirmed a moderate effect on *Staphyloccocus aureus* and *S. epidermidis*, as well as a high bacteriostatic effect on *Pseudomonas aeruginosa*. The effect of the *E. campestre* tincture was the most pronounced.

Keywords: *Eryngium*, polyphenols, phenolic acids, flavonoids, antimicrobial

INTRODUCTION

In the last few years, a number of studies have been conducted to prove the antimicrobial properties of several herb species.

The *Eryngium* L. genus, belonging to the *Apiaceae* family, consists of approximately 250 species spread in Eurasia, North Africa, North and South America, and Australia. In the wild flora of Romania, there are only three native species of *Eryngium*: *Eryngium planum* (plain eryngo), *Eryngium campestre* (field eryngo) and *Eryngium maritimum* (sea holly).[1]

In the past, *Eryngium* species were used as folk remedies in the treatment of a wide range of diseases due to expectorant, diuretic, anti-inflammatory, and anti-scorbutic properties.[1-6] The aerial parts of *Eryngium planum* are used as an old Romanian remedy for children with whooping-cough, *E. planum* being the only one from the three species introduced as a medicinal drug in modern Romanian phytotherapy.[1] In Romania, *E. campestre* and *E. maritimum* are rarely used nowadays and strictly for their diuretic properties, although in some European countries they are also known for the anti-inflammatory, antiepileptic and expectorant characteristics. Infusions of aerial or root parts of *E. maritimum* were reported to be used as diuretic, expectorant, diaphoretic, appetizer, cystotonic, stone inhibitor, aphrodisiac or antihelmintic.[5]

However, scientific papers on possible activities of *E. maritimum* highlight only the anti-inflammatory and antinociceptive properties.[7]

To date, *Eryngium* extracts or isolated compounds from various *Eryngium* species have demonstrated *in vitro* cytotoxicity against various human tumor cell lines, anti-inflammatory, anti-snake and scorpion venoms, antibacterial, antifungal, antimalarial, antioxidant and anti-hyperglycemic effects.[2-12] However, the chemical constituents and bioactivities of most species of this highly diversified genus have not been investigated.

Previous studies on the antimicrobial activity of ethanolic extracts from leaves and roots of *E. planum*, *E. campestre* and *E. maritimum* native to Poland concluded they all inhibited the growth of *Staphylococcus aureus* and some tested fungi.[3]

Other studies on some *Eryngium* species from the Balkan Peninsula showed that the essential oil of *E. campestre* inhibited the growth of methicillin-resistant *Staphylococcus aureus* strains.[2] Also, different extracts of *E. maritimum* roots inhibited the growth of *Listeria monocytogenes*, *Escherichia coli*, *Staphylococcus aureus* and *Bacillus cereus*.[5]

The active principles detected in these species up to now are: triterpene saponins, flavonoids, phenolic acids, essential oil, polyacetylenes and coumarins.[5,8,10,12-16] Among these compounds, saponins have been proved to be responsible for antibacterial, antifungal and anti-yeast activity.[16]

The folk use of these species and the relatively few papers on the chemical composition of the aerial parts of *Eryngium* species growing in Romania prompted us to perform a comparative study on the polyphenolic compounds by employing the HPLC-UV-MS method.

Given the practical importance of pectin in the pharmaceutical industry (gelling and emulsifying agents, viscosity enhancing additives for formulation of sustained-release drugs (SR formulations) and its pharmacological properties (bacteriostatic, haemostatic, hypocholesterolemic and hypoglycemic), as well as the lack of data regarding the presence of pectin in *Eryngium* species, we have aimed to get a deeper insight into these active principles.

Considering the antimicrobial potential of some other species belonging to the same *Apiaceae* family as *Eryngium* species, we also investigated the antimicrobial activities on several pathogenic strains.

EXPERIMENTAL

Plant material

The aerial parts of *E. planum* and *E. campestre* were collected in July from Jucu (Cluj, Romania), while the aerials parts of *E. maritimum* were collected from the seashore of Eforie Nord (Constanta, Romania). The plant materials were identified by Professor Mircea Tămaş, PhD, from the Department of Botany, University of Medicine and Pharmacy, Cluj-Napoca, Romania.

Sample preparation: 100 g air-dried plant material was macerated at room temperature in 500 mL 70^0 ethanol for 10 days. After filtering, 70^0 ethanol was added to afford a 20% tincture from each species.[17]

Study of polyphenols (glycosides, aglycons and polyphenolic acids)

The polyphenols from the three 20% tinctures of *Eryngium* were identified and quantified by reversed phase high-performance liquid chromatography (RPHPLC) with UV detection coupled with mass spectrometric detection (MS). 18 polyphenolic standards were used. Samples were analyzed before and after hydrolysis in order to identify the flavonoidic glycosides and the flavonoid aglycons that are released after acid hydrolysis.[18,19] Experiments were performed in triplicate.

The standards were the following: caffeic acid, chlorogenic acid, p-coumaric acid, kaempferol, apigenin, rutoside, quercetin, quercitrin, isoquercitrin, fisetin, hyperoside, myricetin (Sigma, Germany), ferulic acid, gentisic acid, sinapic acid, patuletin, luteolin (Roth, Germany), caftaric acid (Dalton, USA).

In order to study the flavonoid aglycons, the tinctures were treated with an equal volume of 2N HCl and heated for 40 minutes at 80 ^oC on a water bath. The hydrolyzed samples were properly diluted with distilled water.[18,19]

The HPLC equipment consisted in: an HP 1100 series liquid chromatograph with a degasser, a binary pump, an autosampler, a column thermostat and a UV detector. The HPLC system was coupled with an Agilent 1100 MSD Ion Trap VL mass detector.

Chromatographic conditions: Compounds were separated on a reversed-phased Zorbax SB-C18 analytical column (100 mm x 3.0 mm i.d., 3.5 μm particle) with methanol: acetic acid 0.1% (v/v) as mobile phase. The elution begun with a linear gradient started at 5% to 42% methanol for the first 35 minutes, followed by isocratic elution (with 42% methanol for the next 3 minutes). The flow rate was 1 mL/min and the injection volume was 5 μL. Detection was performed at 330 nm and 370 nm. The time required for one sample run was 35 min.

MS conditions: The MS was equipped with a Turbo-Ionspray (ESI – electrospray ionisation) interface, negative ion mode. ESI settings were: negative ionization, ion source temperature of 360 ^oC, gas: nitrogen, flow rate of 12 L/min, nebulizer: nitrogen at 70 psi pressure, capillary voltage of 3000 V. The analysis mode was multiple reaction monitoring (MRM) and single ion monitoring (SIM).[20-22]

Statistical analysis

All results were expressed as the mean ± standard deviation (SD). Statistical comparisons between two independent groups were performed using the Student's *t*-test. A p-value <0.05 was considered as statistically significant. Analyses were performed using SPSS 16.0 for Windows (SPSS Inc, USA).

Isolation of pectin

In order to isolate the pectin from the aerial parts of *Eryngium* species, the repeated hot water extraction

method, followed by the classic gravimetric method, was employed.

An amount of 2 g of dried powdered plant material (aerial parts), which had been previously degreased with chloroform in a Soxhlet apparatus and then was subjected to extraction with methanol, was extracted three times in a ration of 1:10 parts of water at reflux, on a boiling water bath for 30 minutes. The combined solutions were filtered and concentrated on a hot water bath to approx. 1: 5 volume. The resulting solution was poured under continuous stirring into ethanol to precipitate the pectin. After cooling, the precipitate containing the pectin was decanted and was separated by centrifugation. After drying in a desiccator over anhydrous CaCl2, the pectin was purified by dissolving it in a minimum volume of water, re-precipitated in ethanol following the same steps as above and weighed on an analytical balance. The content of pectin in *Eryngium* species was expressed in %.

Selective extraction and isolation of pectins

In order to improve the efficiency of pectin extraction, the technique of selective extraction with 4 different solvents was employed. Thus, solubilization of pectic substances is performed differently depending on their chemical affinities.[23]

The plant material (aerial parts) was subjected to successive extractions as follows:

1. water (cold) – fraction F1;
2. potassium oxalate solution (pH =4.5) – fraction F2;
3. 0.05 M HCl at 85 ^{o}C – fraction F3;
4. 0.05 M NaOH at 40 ^{o}C – fraction F4.

Evaluation of the antimicrobial activities

The evaluation of the antimicrobial activity of the tinctures was conducted according to the disk diffusion method.[23] The tinctures were tested at a concentration of 50 µl (10 mg plant material) per disc. The control sample was 70^{0} ethylic alcohol, representing the solvent used to prepare the tinctures of *Eryngium* species. Each microorganism, at a concentration of 10^{6} cells/mL was inoculated on the surface of Mueller-Hinton agar (MHA) plates. Filter paper discs (6 mm in diameter) saturated with each extract (50 µL) were placed on the surface of the inoculated plates.

Two antibiogram sets were performed in triplicate for each pathogenic strain, one on the control plates, with plain Mueller-Hinton agar and 50 µl of 70^{0} ethylic alcohol (control) per disc and another on the plates containing MHA plus 50 µl of tested *Eryngium* tinctures per disc. The diameters (mm) of the clear halos were recorded after incubation at 37 ^{o}C for 24 h. The data were presented as the difference between the growth inhibition zones of the *Eryngium* samples and of the control.

Statistical analysis

Each test was performed in triplicate and the results were subjected to the Student's *t*-test. Data were expressed as mean values ± standard deviation (SD). Results were considered significant when $p < 0.05$.

The antibacterial activity was ranked as no activity (inhibition diameter < 10 mm), low (inhibition diameter between 10 and 15 mm), moderate (inhibition diameter between 15 and 20 mm) and high activity (diameter inhibition ≥ 20 mm).[23, 24]

The antibacterial activities were tested in relation to the following Gram-positive and Gram-negative bacterial strains: *Escherichia coli ATCC 25922*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Salmonella enterititidis*, *Pseudomonas aeruginosa ATCC 27853*, *Staphylococcus aureus ATCC 29213* and *Staphylococcus epidermidis*.

All bacterial strains were provided from the stock cultures of the Microbiology Laboratory of the Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania.

Tinctures that revealed an important antimicrobial activity were later tested to determine the minimal inhibitory concentration (MIC) for each bacterial strain.

RESULTS AND DISCUSSION

Polyphenols analysis by UV detection

The high performance liquid chromatography method was employed for the separation of 18 polyphenolic compounds.[21] Each class of polyphenols was detected according to the wavelength corresponding to the maximum absorption of the UV spectra. Therefore, polyphenolic acids were detected at 300 nm, while flavonoids (glycosides and aglycons) were detected at 370 nm.

Calibration plots were obtained by using 18 calibration standards in the 0.5-50 µg/mL range; R^2 values were 0.9999. Calibration curves in the range of 0.5-50 mg mL^{-1} showed good linearity (R2>0.999) for a five point plot. Quantitative determinations were performed using an external standard method. Considering the incomplete separation of the two pairs of phenolic acids (caftaric acid-gentisic acid and caffeic acid-chlorogenic acid), only qualitative analysis based on the MS information was carried out for these compounds (Table 1).

The chromatograms of the tested samples of *Eryngium* (before and after hydrolysis) are presented in Figures 1-6.

Table 1
Parameters of the calibration line equation for the standards in UV detection

No.	Phenolic compound	Calibration line equation
1	Caftaric acid	Qualitatively
2	Gentisic acid	Qualitatively
3	Caffeic acid	Qualitatively
4	Chlorogenic acid	Qualitatively
5	p-Coumaric acid	A = -0.325 + 33.23 x
6	Ferulic acid	A = -1.016 + 39.55 x
7	Sinapic acid	A = -0.236 + 37.10 x
8	Hyperoside	A = 0.107 + 19.29 x
9	Isoquercitrin	A = -0.273 + 12.97 x
10	Rutoside	A = 0.226 + 13.47 x
11	Myricetin	A = -0.544 + 26.45 x
12	Fisetin	A = 0.241 + 19.19 x
13	Quercitrin	A = 0.047 + 10.69 x
14	Quercetin	A = -1.152 + 36.32 x
15	Patuletin	A= -0.429 + 31.44 x
16	Luteolin	A = -0.760 + 28.97 x
17	Kaempferol	A = -1.270 + 30.15 x
18	Apigenin	A = -0.908 + 20.40 x

A = peak area (mAU×s), x = concentration (µg mL^{-1}); Values are the mean ± SD (n = 3)

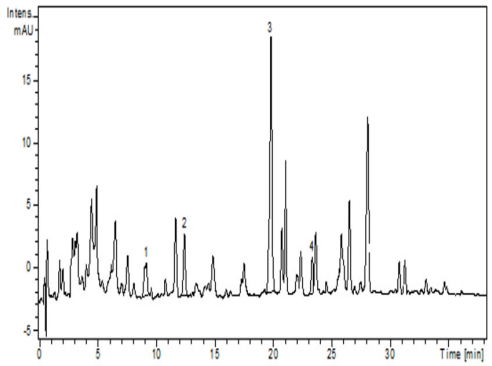

Figure 1: HPLC chromatogram of *Eryngium planum* tincture (unhydrolyzed)

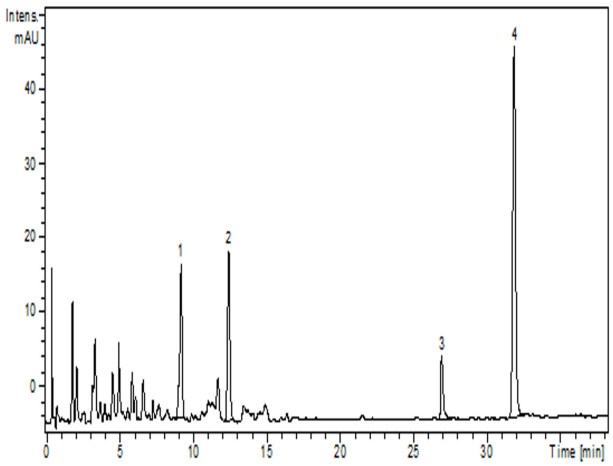

Figure 2: HPLC chromatogram of *Eryngium planum* tincture (hydrolyzed)

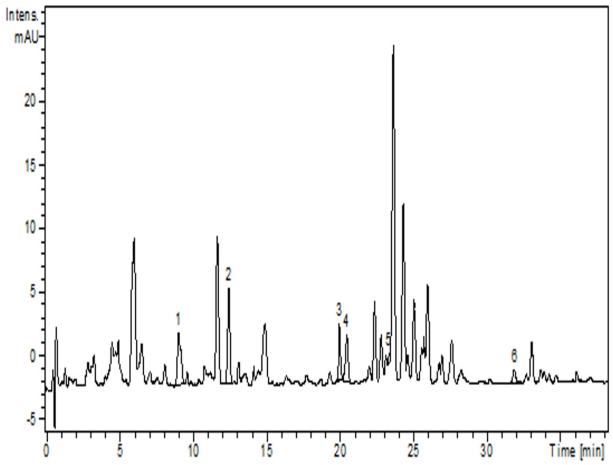

Figure 3: HPLC chromatogram of *Eryngium campestre* tincture (unhydrolyzed)

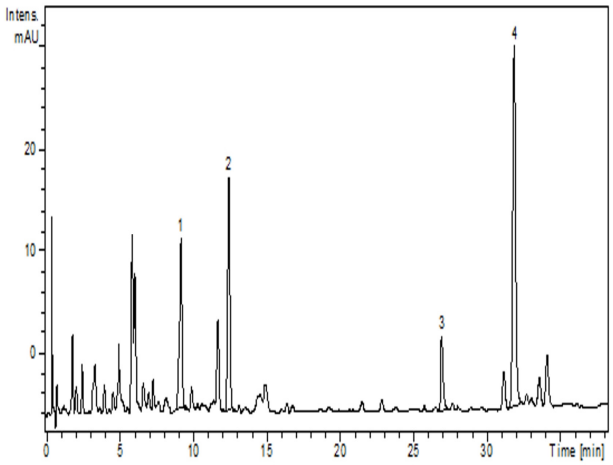

Figure 4: HPLC chromatogram of *Eryngium campestre* tincture (hydrolyzed)

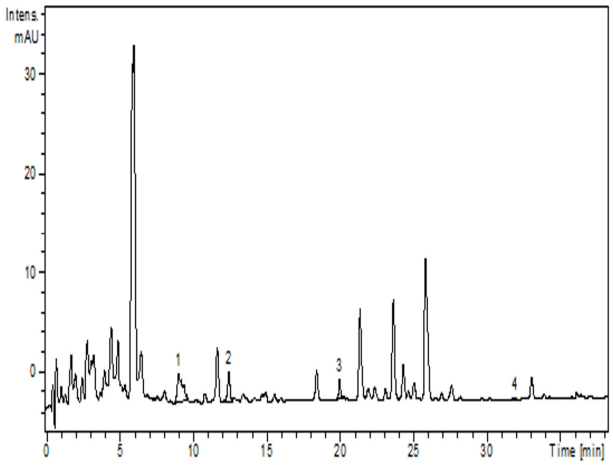

Figure 5: HPLC chromatogram of *Eryngium maritimum* tincture (unhydrolyzed)

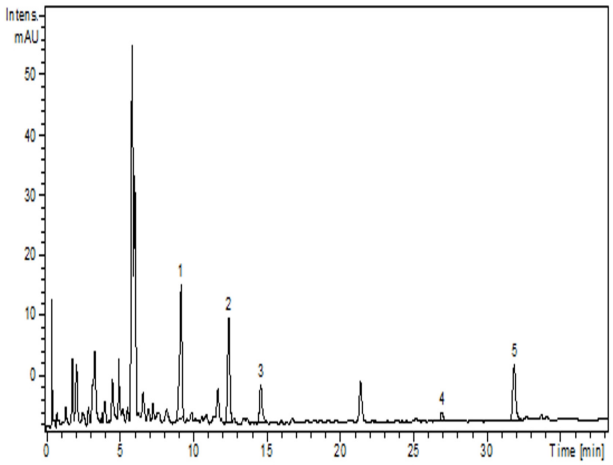

Figure 6: HPLC chromatogram of *Eryngium maritimum* tincture (hydrolyzed)

Polyphenols analysis by MS detection

The MS analysis mode and the specific ions from the mass spectra of the 18 standards of polyphenols are shown in Table 2.

The contents of polyphenolic compounds (mg/100 g plant material) in *Eryngium* tinctures analyzed before and after acid hydrolysis are presented in Tables 3-4. The results revealed the following polyphenolic compounds in all tested *Eryngium* tinctures: p-coumaric acid, ferulic acid, caffeic acid, isoquercitrin and quercitrin. Isoquercitrin was the major flavonoid glycoside detected in the *E. planum* tincture, while rutoside was the major flavonoid in the *E. campestre* tincture. Rutoside was detected only in the tincture of *E. campestre*, thus being a key compound that can be used for differentiation of *E. campestre* from *E. planum* and *E. maritimum*.

Among the phenolic acids, chlorogenic acid and caffeic acid were determined only qualitatively.

After hydrolysis, flavonoidic aglycons were released from the flavonoidic glycosides. Thus, isoquercitrin, quercitrin and rutoside could not be detected in the hydrolyzed samples, while the content of flavonoidic aglycons (quercetol and kaempferol) after hydrolysis rose, compared to the amounts of free aglycons from the unhydrolyzed samples.

Table 2

MS analysis mode and the specific ions extracted from the mass spectra of the 18 standards of polyphenols

No.	Phenolic compound	MS analysis mode	Specific ions for identification Ion [M-H] > Ions from spectra
1	Caftaric acid	MRM	311>148.6, 178.6
2	Gentisic acid	MRM	153>108.7
3	Caffeic acid	MRM	179.4>134.7
4	Chlorogenic acid	MRM	353.5>178.7, 190.7
5	p-Coumaric acid	MRM	163> 118.7
6	Ferulic acid	MRM	193.2> 133.7, 148.7, 177.6
7	Sinapic acid	MRM	223.4>148.6, 163.6, 178.7, 207.7
8	Hyperoside	SIM	463.1
9	Isoquercitrin	SIM	463.1
10	Rutoside	SIM	609.1
11	Myricetin	SIM	317.1
12	Fisetin	SIM	285.1
13	Quercitrin	SIM	447.1
14	Quercetin	SIM	301.1
15	Patuletin	SIM	331.1
16	Luteolina	SIM	285.1
17	Kaempferol	SIM	285.1
18	Apigenin	SIM	269.2

Table 3
Polyphenolic compounds identified in the *Eryngium* tinctures (unhydrolyzed) (M ± S.D.)

Polyphenolic compound	mg/100 g plant material		
	E. planum	*E. campestre*	*E. maritimum*
p-Coumaric acid	1.304±0.065	1.815±0.090	4.052±0.202
Ferulic acid	1.315±0.039	1.846±0.055	1.619±0.048
Chlorogenic acid	Qualitatively	Qualitatively	Qualitatively
Caffeic acid	-	Qualitatively	Qualitatively
Isoquercitrin	20.979±0.839	3.565±0.106	3.433±0.240
Quercitrin	2.893±0.144	0.931±0.037	Qualitatively
Rutoside	-	29.520±1.476	-
Kaempferol	-	0.474±0.028	0.283±0.011

Note: Each value is the mean ± SD (n= 3)

Table 4
Polyphenolic compounds identified in the hydrolyzed samples of *Eryngium* tinctures

Polyphenolic compound	mg/100 g plant material		
	E. planum	*E. campestre*	*E. maritimum*
Ferulic acid	5.966±0.238	5.713±0.230	4.475±0.192
p-Coumaric acid	6.570±0.451	5.547±0.320	7.022±0.351
Caffeic acid	Qualitatively	Qualitatively	Qualitatively
Chlorogenic acid	Qualitatively	Qualitatively	Qualitatively
Sinapic acid	Qualitatively	Qualitatively	2.001±0.082
Gentisic acid	-	Qualitatively	Qualitatively
Quercetol	3.005±0.121	2.537±0.101	0.527±0.021
Kaempferol	20.969±0.82	14.668±0.732	4.022±0.199

Note: Each value is the mean ± SD (n= 3)

Table 5
Extraction yield of pectic fractions in four different solvents (%)

Pectic fraction	*E. planum*	*E. campestre*	*E. maritimum*
Water (F1)	1.366	3.8	2.9
Potassium oxalate (F2)	6.412	13.6	8.2
0.05 M HCl (F3)	1.152	2.54	2.73
0.05 M NaOH (F4)	3.282	6.8	3.96
Total pectin	12.212	26.74	17.8

Table 6
Antimicrobial activity of *Eryngium* tinctures (M± SD)

Samples	Diameter of the inhibition zone (mm)						
	E. coli	*P. vulgaris*	*K. pneumoniae*	*S. enteritidis*	*P. aeruginosa*	*S. aureus*	*S. epidermidis*
E. planum	-	-	-	-	20±1	15±0.45	15±0.30
E. campestre	-	-	-	-	25±1.5	-	-
E. maritimum	-	-	-	-	20±1.6	-	-

Note: Each value is the mean ± SD (n=3)

Flavonoidic aglycons, quercetol and kaempferol were identified and quantified in all hydrolyzed *Eryngium* samples. Kaempferol was found to be the major flavonoidic aglycon in all three *Eryngium* tinctures.

Phenolic acids like ferulic acid, caffeic acid, p-coumaric acid and chlorogenic acid were also identified in all hydrolyzed *Eryngium* samples. The amounts of p-coumaric and ferulic acid increased after hydrolysis, thus showing that *Eryngium* tinctures also contain derivatives of such phenolic acids.

Quantitative analyis of pectin

Among the three *Eryngium* species, the content of pectin extracted by hot water varied considerably: 1.7% in *E. campestre*, 4.65% in *E. planum* and 8.8% in *E. maritimum*.

The isolation of pectin by the two extraction protocols (repeated extraction with hot water and selective extraction with four different solvents) showed that, using water as a single solvent for the extraction of pectin from *Eryngium* species is insufficient.

The selective extraction method of pectin afforded four different visible pectic fractions.

Among the four solvents used for the selective extraction of pectin, the potassium oxalate solution (pH 4.5) yielded the maximum production of pectin, between 46-52% from the total isolated pectin, followed by the fraction soluble in the 0.05 M NaOH solution (22-27%), the water soluble fraction (11-16%) and the 0.05M HCl soluble fraction (9.4-15.3%) (Table 5).

Antimicrobial activity

The premise for the study of the antimicrobial potential of the indigenous *Eryngium* species was the information that in the folk medicine extracts of these species were used in treating several respiratory, renal and dermatological diseases, thus a potential anti-infectious effect could be a benefit for therapy. Currently, there is an increasing interest in finding new sources of antimicrobial agents suitable for treating pathogenic bacteria resistant to common antibiotics. Strategies for extending the microbial spectrum rely on combined antibiotic therapy (2 antibiotics from different classes) or a newer generation antibiotic, which is often a more expensive approach for treatment.

Previous studies on different members of the *Apiaceae* family showed that several species possess a significant antimicrobial activity over some Gram-positive and Gram-negative strains.[16] The present results confirm that the *E. planum* tincture exhibits a moderate bacteriostatic activity on Gram-positive strains of *Staphyloccocus aureus* and *Staphyloccocus epidermidis*. A high bacteriostatic effect on *Pseudomonas aeruginosa* was highlighted for all tested *Eryngium* tinctures and no activity was remarked against the tested Gram-negative strains of *Escherichia coli*, *Proteus vulgaris*, *Klebsiella pneumoniae* and *Salmonella enteritidis* (Table 6).

Considering the high activity of *Eryngium* tinctures on *P. aeruginosa* the MIC value was also determined.

Minimum inhibitory concentrations (MIC) for *Pseudomonas aeruginosa*

The MIC is defined as the lowest antibiotic concentration that hinders a visible growth of a microorganism.[24]

The MIC of *Eryngium* tinctures was determined by the standardized dilution method.[24,25] The inoculum consisted in 10 µl of the suspension of a standard *Pseudomonas aeruginosa* strain, 10^6 cells/mL added in tubes containing 3 mL of agar medium. 2-fold dilutions of the tested tinctures were employed. Growth inhibition was observed following overnight incubation at 37 °C.

The lowest concentration of *E. campestre* tincture inhibiting a visible growth of *P. aeruginosa* was found to be 7.143±0.35 µg/mL. Results revealed that the antimicrobial activity against *P. aeruginosa* was 10 times higher for the *E. campestre* tincture than for the *E. planum* and *E. maritimum* tinctures (MIC value = 71.428±4 µg plant material/mL).

CONCLUSION

The present comparative phytochemical study revealed some similarities and differences among the three *Eryngium* species from Romania. The data could be important for therapeutically valorizing the species *E. campestre* and *E. maritimum*, which are rarely used in today's phytotherapy.

The polyphenolic compounds common in the tested *Eryngium* tinctures were p-coumaric acid, ferulic acid, caffeic acid, isoquercitrin and quercitrin.

The pectin content of the *Eryngium* species ranged between 1.7%-8.8%, its yield being maximum in *E. maritimum*. However, by employing a selective extraction with four different solvents, the pectin yield increased substantially to 12.21-26.74%, *E. campestre* showing the maximum pectin content.

Our study also demonstrated for the first time a high bacteriostatic effect of indigenous *Eryngium* species on *Pseudomonas aeruginosa*. As previous studies have concluded that several aromatic plants from the *Apiaceae* family show antimicrobial activities against different pathogenic bacteria, *Eryngium* species belonging to *Apiaceae*, especially *Eryngium campestre*, can be considered a possible remedy to control the antibiotic resistant pathogen *Pseudomonas aeruginosa*, which is often isolated from the hospital environment.[26]

Bacterial resistance to commonly used antibiotics requires a constant research for new compounds, while plant origin compounds have the advantage of being well-tolerated and have less adverse effects than most chemotherapeutics. The activity of *Eryngium* tinctures probably results from the synergistic effect of triterpene saponins, polyphenols, sterols, pectin and other active compounds.

REFERENCES

[1] B. Thiem, M. Kikowska, A. Kurowska and D. Kalemba, *Molecules*, **16**, 7115 (2011).
[2] L. Meot-Duros, G. Le Floch and C. Magne, *J. Ethnopharmacol.*, **116**, 258 (2008).
[3] B. Thiem, O. Goślińska, M. Kikowska and J. Budzianowski, *Herba Pol.*, **56**, 5 (2010).
[4] M. Kowalczyk, M. Masullo, B. Thiem, S. Piacente, A. Stochmal *et al.*, *Nat. Prod. Res.*, **28**, 653 (2014).
[5] W. Kholkhal, F. Ilias, C. Bekhechi and F. A. Bekkara, *Curr. Res. J. Biol. Sci.*, **4**, 437 (2012).
[6] S. Suciu and E. Bodoki, *TMJ*, **55**, 348 (2005).
[7] E. Kupeli, M. Kartal, S. Aslan and E. Yesilada, *J. Ethnopharmacol.*, **107**, 32 (2006).
[8] B. Osclik, S. Kusmenoglu, S. Turkoz and U. Abbasooglu, in "Antimicrobial Activities of Plants from the Apiaceae", Taylor & Francis, 2004, pp. 526-528.
[9] M. Strzelecka, M. Bzowska, J. Koziel, B. Szuba, O. Dubiel *et al.*, *J. Physiol. Pharmacol.*, **56**, 139 (2005).
[10] M. Kartal, A. C. Mitaine-Offer, T. Paululat, M. Abu-Asaker, H. Wagner *et al.*, *J. Nat. Prod.*, **69**, 1105 (2006).
[11] S. Suciu and A. E. Pârvu, *Annals of RSCB*, **17**, 86 (2012).
[12] W. Ping, S. Zushang, Y. Wei, D. Guangrui and L. Shiyou, *Pharm. Crops*, **3**, 99 (2012).
[13] E. Le Claire, S. Schwaiger, B. Banaigs, H. Stuppner and F. Gafner, *J. Agric. Food Chem.*, **53**, 4367 (2005).
[14] C. Tanase, C. I. Bara and V. I. Popa, *Cellulose Chem. Technol.*, **49**, 799 (2015).
[15] S. Conea, A. E. Pârvu, M. A. Taulescu and L. Vlase, *DJNB*, **10**, 693 (2015).
[16] K. Stalinska, A. Guzdek, M. Rokicki and A. Koj, *J. Physiol. Pharmacol.*, **56**, 157 (2005).
[17] "European Pharmacopoeia", 6th ed., Council of Europe, Strasbourg, EDQM, 2008, Suppl 6.1, 2371.
[18] L. Vlase, L. Radu, C. Fodorea, S. Leucuta and S. Gocan, *J. Chromatogr.*, **28**, 3109 (2005).
[19] A. I. Pag, D. G. Radu, D. Drăgănescu, M. I. Popa and C. Sîrghie, *Cellulose Chem. Technol.*, **48**, 265 (2014).
[20] I. Ignat, D. G. Radu, I. Volf, A. I. Pag and V. I. Popa, *Cellulose Chem. Technol.*, **47**, 387 (2013).
[21] S. Conea, O. Vostinaru and L. Vlase, *DJNB*, **9**, 1039 (2014).
[22] A. M. Anton, A. M. Pintea, D. O. Rugină, Z. M. Sconţa, D. Hanganu *et al.*, *DJNB*, **8**, 973 (2013).
[23] D. S. Moiceanu and M. Tămaş, *Farmacia*, **44**, 23 (1996).
[24] Clinical and Laboratory Standards Institute, Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, Approved Standard, M07-A8, CLSI, 2009.
[25] I. Boz, E. Gille, R. Necula, S. Dunca and M. Zamfirache, *Cellulose Chem. Technol.*, **49**, 169 (2015).
[26] B. L. Duško, L. Čomić and S. Solujić-Sukdolak, *Kragujevac J. Sci.*, **28**, 65 (2006).