Antimicrobial activity of three *Eryngium* L. species *(Apiaceae)*

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S u m m a r y

The antimicrobial activity of ethanolic extracts from leaves and roots of three *Eryngium* L. genera (*E. planum*, *E. campestre*, *E. maritimum*) native to Poland was tested by the method of series dilutions against different Gram-positive bacteria (two strains) and fungi (five species). The extracts were analyzed by TLC method to confirm phenolic acids, triterpenoid saponins, flavonoids and acetylenes presence. The antimicrobial activity of extracts compared with the reference substance were expressed by Minimal Inhibitory Concentration (MIC). The results have shown that the ethanolic extracts inhibit the growth of *Staphylococcus aureus* and all tested fungi.
Key words: *Eryngium planum, E. campestre, E. maritimum, antifungal activity, antibacterial activity, leaves and root extracts*

INTRODUCTION

Nowadays, many microorganisms have become resistant to commonly used antibiotics and fungicidal agents. Resistance to these medicines has led to research of new sources of bioactive substances against bacteria and fungi. Medicinal

plants may offer a natural source of antimicrobial bioactive compounds, alternative to antibiotics and fungicidal agents.

The genus *Eryngium* L., belonging to the subfamily *Saniculoideae* of the family *Apiaceae* is represented by 317 taxa, widespread in Central Asia, America, Central and Southeast Europe. The plants are perennial erect herbs with silvery-blue stems. Infusions of aerial and root parts of the investigated *Eryngium* species have been used in European folk medicine as antitussive, diuretic, appetizer, stimulant and aphrodisiac [1,2]. Earlier isolated constituents from these taxons are: triterpenoid saponins [3], flavonoids [4,5], phenolic acids – mainly rosmarinic acid [6], essential oil [7,8], coumarins [9] and polyacetylenes [10,7]. The above mentioned investigations proved that leaves and roots differ one from another by the saponins profile, flavonoids occurrence and level of active compounds. It suggested that the pharmacological properties of leaf extract could be different from those of root extract [2]. These bioactive secondary metabolites presence mostly in roots and herbs determines multidirectional pharmacological activities: diuretic, expectorant and spasmolytic. The pharmacological activity of *Eryngium* depends mainly on its high saponin content but presence of flavonoids, namely kaempferol and quercetin glycosides and phenolic acids (rosmarinic acid and chlorogenic acid) could play an important role. Saponins have been reported to have antifungal, antiyeast and antibacterial activity [11]. It was shown that many groups of flavonoids and phenolic acids, mainly rosmarinic acid, have antibacterial properties [12-14] and polyacetylenes posses antibacterial, antifungal and antimycobacterial activities [15]. The antimicrobial activities of *Eryngium* leaves and roots extracts have not been investigated yet, except *E. maritimum* [16].

In this study, the antibacterial and antifungal activities were tested and compared in different organs (leaves and roots) of three native to Poland sites species of *Eryngium*.

MATERIAL AND METHODS

Plant material

Plants of *Eryngium* L. – Eryngo (the family *Apiaceae*) were collected from its natural habitats in Poland: *E. planum* L. (Flat Sea Holly) from Kujawy region in August 2008, *E. campestre* L. (Field Eryngo) from steppe reserve Owczary near Kostrzyn nad Odrą in August 2009, and *E. maritimum* L. (Sea Holly, a rare and protected species in Europe) from Botanical Garden of Adam Mickiewicz University, Poznan (Poland) in September 2009. Three species were previously authenticated by Prof. J. Borysiak (The Botanical Garden of Adam Mickiewicz University, Poznan). The voucher specimens owning to Department of Pharmaceutical Botany and Plant Biotechnology, K. Marcinkowski University of Medical Sciences in Poznan are

deposited in the Herbarium of Medicinal Plants Garden in the Institute of Natural Fibers and Medicinal Plants in Poznan.

Preparation of *Eryngium* L. extracts

Air-dried leaves and roots were powdered and weighted, then extracted with 70% ethanol (3x) in a water bath under reflux and extracts were evaporated to dryness under reduced pressure. The ethanolic extracts from *E. planum* leaves (82.8 g) and roots (351.0 g) (yields 39.1%, 41.4%, respectively), from *E. campestre* leaves (99.8 g) and roots (100.0 g) (yields 24.6%, 33.0%, respectively) and from *E. maritimum* leaves (95.5 g) and roots (5.9 g) (yields 33.0%, 32.2%, respectively) were then used for phytochemical screening and assayed for antimicrobial activity.

Phytochemical screening

The tested extracts were analyzed by TLC chromatography using specific spray reagents to check the presence of the main group of secondary metabolites [17]. The equal portions of each extract (0.1 g) were dissolved in 1 ml of 70% ethanol. Phenolic acids and flavonoids were analyzed by TLC in chambers on cellulose plates (Merck) or Silica gel HPTLC plates (Merck), eluted with ethyl acetate–acetic acid–water (8:1:1) mixture. Before and after spraying with NA (0.1% 2-aminoethanol diphenylborate in ethanol) the plates were viewed under UV_{254} nm light. The spots changing to yellow or orange fluorescence were considered as those of flavonoids, while blue spots changing to strong blue or blue-yellow fluorescence were considered as phenolic acids. For saponins, the analysis was performed by TLC on Silica gel DC–SiF plates (Merck) eluted with *n*-butanol–acetic acid–water (4:1:5) (organic phase) mixture. Spots of saponins were visualized (daylight) by spraying with vanillin-sulfuric acid or Libermann-Burchard reagent followed by heating for 5 min at 100°C. The spots of violet-pink colour in daylight were considered as those of saponins. The TLC analysis for polyacetylenes were carried out on Silica gel $60F_{254}$ (Merck), eluted with toluene-ethyl acetate (9:1) mixture. The chromatograms were sprayed with 0.38% $KMnO_4$ solution. The acetylenes were recognized as yellow spots against pink background in daylight.

Microorganism and media

Bacterial strains and fungi used in this study:

Gram-positive bacteria: *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633,

fungi: yeast *Candida albicans* ATCC 10231, *Candida glabrata* clinical strain, *Cryptococcus neoformans* clinical strain, molds *Aspergillus niger* ATCC 16404 and dermatophyte *Trichophyton mentagrophytes* clinical strain.

The strains were maintained and tested on culture media: Mueller-Hinton broth (bacteria) and Sabouraud broth (fungi). The media were purchased from Bio-Merieux (France). Twenty-hour cultures of the reference strains of bacteria and fungi were diluted (1:1) according to the McFarland scale 0.5 in a sterile solution of a normal saline (0.9%) at a concentration of 10^6 CFU ml^{-1} [18].

Antimicrobial activity

For antimicrobial testing the known amount of each extract was dissolved in the mixture of 1 ml ethanol and 9 ml of water to obtain stock solutions. One-milliliter suspension of the reference strains (bacteria and fungi) was added to 1 ml of each concentration of the extracts diluted with the sterile liquid medium to get final concentration ranging from 35 500 $\mu g \cdot ml^{-1}$ to 10 $\mu g \cdot ml^{-1}$ using serial dilution method [19]. The mixtures were incubated at 35–37°C for bacteria or 25°C (fungi) for 18 h with exception of *T. mentagrophytes* (3 days), then the medium was examined. A clear medium indicated inhibition of the microbial growth, while turbidity or precipitate developing in the medium confirmed its growth. Following this procedure, the lowest concentration of six extracts inhibiting the visible growth of each microorganism, denoted as Minimal Inhibitory Concentration (MIC) was determined.

Gentamycin for bacteria and nystatin for fungi was used as a reference positive control. Gentamycin showed MIC=2 $\mu g \cdot ml^{-1}$ against *S. aureus,* MIC=8.02 $\mu g \cdot ml^{-1}$ against *B. subtilis*. MIC for nystatin determined to be 4 $\mu g \cdot ml^{-1}$. All tests were performed in duplicate and the antibacterial activity was expressed as the mean values.

RESULTS AND DISSCUSION

Preliminary phytochemical analysis of *Eryngium* leaves and roots extracts showed that complex of saponins, flavonoids compounds, phenolic acids and acetylenes are present in studied plant materials. *Eryngium* leaves are characterized, in comparison to roots, by presence of flavonoids, different level of phenolic acids and acetylenes as well as different profile of saponin complex.

The ethanolic extracts from *Eryngium* demonstrated the moderate antibacterial activity against standard Gram-positive strain of *Staphylococcus aureus* and showed weak effect against *Bacillus subtilis,* whereas the studied extracts posses significantly antimycotic activity. The highest antifungal effect of studied extracts was obtained against standard *Candida albicans* and clinical strains: *Candida glabrata, Trichophyton mentagrophytes* and *Cryptococcus neoformans*.

The leaves and roots extracts from *Eryngium* showed the highest antifungal activity against dermatophyte strains of *Trichophyton mentagrophytes*. Fungal foot infection (*Tinea pedis*), the second one according to the frequency of occurrence of fungal skin diseases is caused by dermathophytes – especially *T. mentagrophytes*.

Long medical treatment is usually ineffective, thus, the demonstrated activity of *Eryngium* ethanolic extracts (40-100 μg.ml^{-1}) against this dermatophyte is promising. The obtained results were present in Table 1.

Table 1.

Determination of MICs of *Eryngium* species ethanolic extracts against different microorganisms. MIC – Minimal Inhibitory Concentration

microorganism	tested extracts (MIC μg·ml^{-1})					
	E. planum		*E. campestre*		*E. maritimum*	
	leaves	roots	leaves	roots	leaves	roots
Staphylococcus aureus	400	1100	1900	900	700	700
Bacillus subtilis	2500	2200	15000	1900	5000	1300
Candida albicans	90	1100	7500	900	1300	1300
Candida glabrata	40	100	100	50	700	400
Cryptococcus neoformans	700	100	50	200	400	400
Trichophyton mentagrophytes	40	100	100	100	90	40
Aspergillus niger	700	100	200	1900	700	700

For further studies, raw materials should be extracted with solvents of different polarities. It has been reported that apolar (chloroformic) fractions were more active than polar (aqueous) phase. Thus, Sea Holly polar fraction inhibited the growth of only two bacteria, whereas its apolar phase was active against nine pathogens [16]. Ethanolic extracts of several herbal plants usually exhibit the moderate activity in comparison to ethyl ether tracts [20].

The activity of ethanolic extracts may result from the synergistic or additive interaction of active compounds of investigated *Eryngium* species. Our research was conducted on 70% ethanolic extracts of *Eryngium*, rich in various secondary metabolites which could possess antimicrobial activity. Rosmarinic acid, complex of saponins and falcarinol-type compounds could be responsible for antimicrobial activity of *Eryngium* extracts. Our results demonstrated antifungal activity of *Eryngium* extracts as those from other saponins-rich plants, like *Panax quinquefolium* L. [21] and *Hedera helix* L. [22].

The dichloromethane extract from the root of *Levisticum officinale* L. (*Apiaceae*) which contains polyacetylenes, exhibited significant antimycobacterial activity [15]. Polyacetylene compound, falcarinol, the main component found in the essential oil of *Eryngium planum* root [Kalemba, unpublished data], posses antibacterial, antifungal and antimycobacterial activities [15, 23].

In the screening study of herbal plants extracts Hołderna-Kędzia and Kędzia [20] reported that ethanolic extract (50%) from *Eryngium maritimum* roots indicates moderate activity against *Staphylococcus aureus* (MIC = 2 500 μg·ml^{-1}). However, Meot-Duros et al. [16] also reported antimicrobial activities of *E. maritimum*.

They found that the chloroformic fractions of the methanolic leaf extract inhibited the growth of nine microorganisms (MIC=100 μg·ml^{-1}), mainly *S. aureus* (MIC = 10 μg·ml^{-1}). Moreover, aqueous and chloroformic fractions presented a strong antibacterial activity against *Pseudomonas aeruginosa* and *P. fluorescens* (MIC=1 μg·ml^{-1} and 2 μg·ml^{-1}, respectively). In our study, ethanolic extracts (70%) from *Eryngium maritimum* showed moderate antibacterial activity against *S. aureus* (MIC=700 μg·ml^{-1}).

CONCLUSIONS

The crude ethanolic extracts of both leaves and roots of investigated three *Eryngium* species showed a significant antifungal activity and moderate antibacterial activity only against *Staphylococcus aureus*. The results confirm the capacity of this taxon to produce compounds responsible for antifungal properties of the extracts. For this reason, further studies are necessary to identify the main active constituents.

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PRZECIWDROBNOUSTROJOWA AKTYWNOŚĆ TRZECH GATUNKÓW *ERYNGIUM* L. *(APIACEAE)*

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S t r e s z c z e n i e

Przeciwdrobnoustrojową aktywność etanolowych ekstraktów z liści i korzeni trzech gatunków *Eryngium* L. (*E. planum*, *E. campestre*, *E. maritimum*) występujących w Polsce testowano metodą seryjnych rozcieńczeń w bulionie przeciw bakteriom Gram + (dwa szczepy) i grzybom (pięć gatunków). Ekstrakty analizowano metodą TLC w celu potwierdzenia obecności kwasów fenolowych, saponin triterpenowych, flawonoidów i acetylenów. Przeciwdrobnoustrojowa aktywność ekstraktów i związków referencyjnych wyrażona została przez MIC (*Minimal Inhibitory Concentration*, minimalna wartość hamująca). Wyniki badań wskazały, że ekstrakty etanolowe hamowały wzrost *Staphylococcus aureus* oraz wszystkich testowanych grzybów.

***Słowa kluczowe**: Eryngium planum, E. campestre, E. maritimum, aktywność przeciwgrzybicza, aktywność przeciwbakteryjna, ekstrakty z liści i korzeni*