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HPLC ANALYSIS AND ANTIMICROBIAL POTENTIAL OF PLANT EXTRACTS

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Abstract

The use of plant and its products has a long history that began with folk medicine and through the years has been incorporated into traditional and allopathic medicine. The therapeutic effect of these plants is related to the content of many biologically active compounds, including flavonoids, phenols and tannins. Although there were several hundred thousand plant species around the globe, only a small proportion has been investigated both phytochemically and pharmacologically. Therefore, the aim of this study was to determine polyphenolic compounds and antimicrobial potential of acetone extract of *Echium italicum* and *Anchusa officinalis*. The plants belong to the family *Boraginaceae*, and have long been used in folk medicine. Determination of polyphenolic compounds in tested extracts was performed by HPLC analysis. The antimicrobial potential of acetone extracts was examined by the microdilution method. HPLC analysis of examined extracts of *A. officinalis* and *E. italicum* confirmed the presence of the following polyphenolic compounds: p-hydroxybenzoic acid, chlorogenic acid, p-coumaric acid, ferulic acid, sinapinic acid, rutin, lutein glycoside, apigenin glycoside, rosmarinic acid, quercetin, lutein, naringenin and kaempferol. Acetone extracts of the tested plants showed extremely good antimicrobial potential compared to the standard antibiotic amracin. The maximum antimicrobial activity showed by the acetone extract of *E. italicum* in the control of *S. enteritidis* (3.91 μg/ml) and *P. vulgaris* (7.81 μg/ml), and acetone extract of *A. officinalis* in the control of *E. aerogenes* and *L. ivanovii* (31.25 μg/ml). This study showed that acetone extracts had a significant amount of polyphenolic compounds with extremely good antimicrobial potential.

Keywords: *HPLC analysis, Antimicrobial potential, Plant, extract.*

Introduction

Before the advent of modern medicine, people depended essentially of the plants for the treatment of various diseases, and they still have been an important source for the development of new drugs (Kuete *et al.* 2009). The use of plant and its products has a long history that began with folk medicine and through the years has been incorporated into traditional and allopathic medicine (Dubey *et al.* 2011). According to the WHO, between 65% and 80% of the populations of developing countries currently use medicinal plants as remedies (WHO, 2011). The therapeutic effect of these plants is related to the content of many biologically active compounds, including naphthaquinones, flavonoids, terpenoids and phenols (Sharma *et al.* 2009). The advantage of herbal reparations over synthetic drugs is reflected in the fact that they are available to us in nature and are less toxic. To guarantee the quality of herbal medicines, certain steps established in the Pharmacopoeias must be followed, including correct identification of the plant species, analysis of the purity and confirmation of the presence and minimum concentration of the active ingredients (Brandao *et al.* 2013). The resistance of microorganisms to antimicrobial drugs has become a problem of global importance. Of the 2 million people who acquire bacterial infections in US hospitals each year, 70% of cases now involve strains that are resistant to at least one drug (IDSA, 2004). For this reason, there is a need to find new antimicrobial compounds from alternative sources, such as plants. According to Amenu (2014) medicinal plants possess immunomodulatory and antioxidant properties, leading to antibacterial activities. *Echium italicum* and *Anchusa officinalis* belong to the *Boraginaceae* family. Numerous studies have confirmed their use in folk medicine (Khare, 2007; Altundag and Ozturk, 2011). Many members of the *Boraginaceae* family produce secondary metabolites such as alkaloids, naphthoquinones, polyphenols, phytosterols and terpenoids (Gottschling et al, 2001; Zhou and Duan, 2005), which possess a wide range of pharmaceutical activities. Therefore, the aim of this research was to the determine polyphenolic compounds and antimicrobial activity of acetone extract of *Echium italicum* and *Anchusa officinalis*.

Matherial and methods

Chemicals

Muller–Hinton broth (MHB), resazurin, amracin, protocatechuic acid, caffeic acid, vanillic acid, chlorogenic acid, syringic acid, ferulic acid, rutin, rosmarinic acid, naringenin, luteolin, kaempferol and apigenin were purchased from Sigma- Aldrich GmbH (Sternheim, Germany). Methanol, acetone, quercetin and formic acid (standards for HPLC) were supplied by Merck KgaA (Darmstadt, Germany).

Plant material and extracts preparation

The plants were collected in the flowering stage of development in Serbia . Plant material was dried at room temperature. The above-ground part of the plants was grinded by cylindrical crusher and extracted with acetone in Soxhlet apparatus. The solutions were evaporated on a rotary-evaporator at a temperature of 40 °C.

HPLC screening

The HPLC analysis of acetone extracts were performed on the HPLC instrument Agilent 1200 Series with UV-Vis DAD for the detection of multi wavelengths. Separation of samples was performed using an Agilent column, Eclipse XDB-C18 (4.6 x 50 mm, 1.8 µm), which was thermostated at 25^0C. Two solvents were used for the gradient elution: A (H_2O+2% HCOOH) and B (80% ACN+2% HCOOH+H_2O). The identification of the phenolic compounds was done by comparing its retention time of the original standard.

An antimicrobial activity

Antimicrobial activity of the acetone extracts were performed by microdilution method (CLSI, 2012). In the study were included pure cultures of the following bacteria: *Proteus vulgaris* ATCC 13315, *Salmonella enteritidis* ATCC 13076, *Enterobacter aerogenes* ATCC 13048, *Salmonella Typhimurium* ATCC 14028 and *Listeria ivanovii* ATCC 19119. The extract solutions were serially diluted (1:1) in Mueller–Hinton Broth in a series of 96-well microplate wells, and then bacterial culture was added at an approximate concentration of 1.5 $\times$ 10^8 CFU/ml (colony-forming units). And then the resazurin indicator was added to all the holes. The microplates were incubated for 24 h at 37 °C, and thereafter minimum inhibitory concentration (MIC) was determined.

Result and discussion

The most striking feature of natural products in connection to their long lasting importance in drug discovery is their structural diversity that is still largely untapped (Veeresham, 2012). Although there are several hundred thousand plant species around the globe, only a small proportion has been investigated both phytochemically and pharmacologically (Boligon and Athayde, 2014). To isolate and purify the plant based products like secondary metabolites and proteins HPLC is highly useful for researchers and industrialists those who focus on quality (Thirumal and Laavu, 2017). HPLC analysis of examined extracts of *A. officinalis* and *E. italicum* confirmed the presence of the following polyphenolic compounds: p-hydroxybenzoic acid, chlorogenic acid, p-coumaric acid, ferulic acid, sinapinic acid, rutin, lutein glycoside, apigenin glycoside, rosmarinic acid, quercetin, lutein, naringenin and kaempferol (Table 1). The most common compounds in the acetone extract of *Anchusa officinalis* were rosmarinic acid (58,961 mg/g), chlorogenic acid (4,646 mg/g), naringenin (3,142 mg/g), luteolin glycoside (6,775 mg/g) and rutin (2,073 mg/g) – fig.1, which is in accordance with the research of Zengin et al (2015). Numerous studies have confirmed the antioxidant, antimicrobial, antiviral, antidiabetic, and anticarcinogenic properties of rosmarinic acid and rutin (Petersen and Simmonds, 2003; Lacopini *et al.* 2008; Hooker *et al.* 2001; Huang and Zeng, 2006).

Table 1. HPLC data analysis of acetone extract of plant *E. italicum* and *A. officinalis* (mg/g)

Compound/plant (mg/g)	*Echium italicum*	*Anchusa officinalis*
Protocatehuic acid	n.d.	n.d.
p-Hydroxybenzoic acid	4.865	0.845
Caffeic acid	n.d.	n.d.
Vanillic acid	n.d.	n.d.
Chlorogenic acid	1.915	4.646
Syringic acid	n.d.	n.d.
p-Coumaric acid	n.d.	0.008
Ferulic acid	0.519	0.247
Synapic acid	n.d.	2.057
Rutin	4.926	2.073
Luteolin-glycine	0.748	6.775
Apigenin-glycine	n.d.	1.605
Rosmarinic acid	12.131	58.961
Quercetin	1.006	0.396
Luteolin	n.d.	n.d.
Naringenin	0.371	3.142
Caempherol	0.985	0.417
Apigenin	n.d.	n.d.
Σ	27.466	81.172

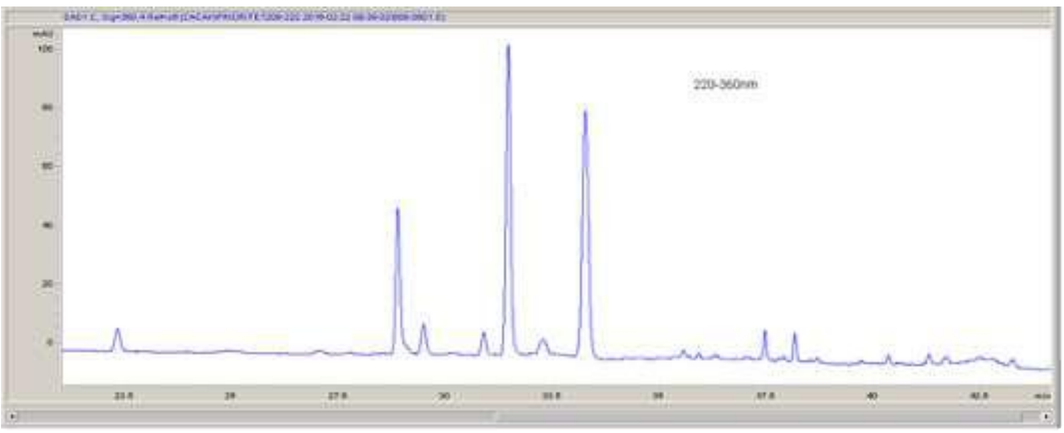

Fig. 1. HPLC chromatogram of acetone extract of *Anchusa officinalis* L.

The most dominant compounds in acetone extract of *Echium italicum* (Fig. 2) were rosmarinic acid (12,131 mg/), rutin (4,926 mg/g) and p-hydroxyenzoic acid (4,865 mg/g), which is partly in agreement with the results of Dresler *et al.* (2017). p-Hydroxybenzoic acid showed antimicrobial, antifungal, antisickling, and estrogenic activities (Pugazhendhi *et al.* 2005; Chong *et al.* 2009). According to Horvath *et al.* (2007) p-Hydroxy benzoic acid increases abiotic stress tolerance of plant and also increases the impermeability of the cell wall, leading to increased resistance against pathogen infection.

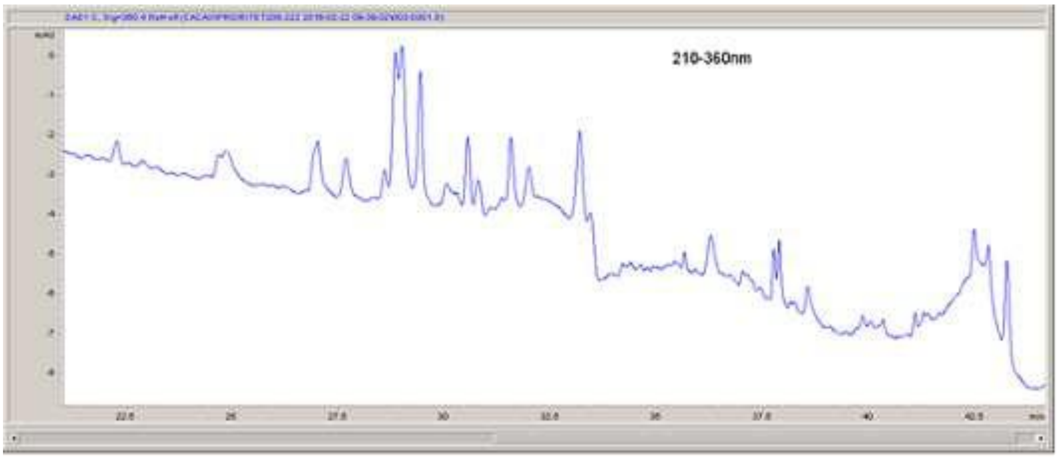

Fig. 2. HPLC chromatogram of acetone extract of *Echium italicum* L.

Plants have been used for centuries to treat infectious diseases and are considered as an important source of new antimicrobial agents (Cowan, 1999). The advantage of compounds of plant origin over synthetic drugs is that the plants have a larger pharmacological complex. Antimicrobial screening of plant extracts and phytochemicals, then, represents a starting point for antimicrobial drug discovery (Amenu, 2014). Table 2 shows the results of antimicrobial activity of acetone extracts of the plants *Anchusa officinalis* and *Echium italicum* against five bacteria. Acetone extracts showed a certain level of antimicrobial activity with MIC values from 3.91 μg/ml to 125 μg/ml, which was a good antimicrobial activity compared to standard antibiotics amracin. The maximum antimicrobial activity was shown by the acetone extract of *E. italicum* against *S. enteritidis* (3.91 μg/ml) and *P. vulgaris* (7.81 μg/ml), and the lowest sensitivity to *L. ivanovi*i, *E. aerogenes*, and *S. Typhimurium*. The antimicrobial potential of the plant extract was a consequence of the presence p-Hydroxybenzoic acid and Rosmarinic acid. The hydrophobicity of plants extract and their bioactive compounds contribute in the breaking down of the membrane cells lipid and make them more permeable for the penetration (Sikkema *et al.* 1995). Extensive leakage of critical molecules and ions from bacterial cells will lead to death (Santos and Novales, 2012).

Table 2. Minimum inhibitory concentrations (MIC) of acetone extracts of the plants *Anchusa officinalis* and *Echium italicum* (μg/ml)

plant/bacteria	*Proteus vulgaris*	*Salmonella enteritidis*	*Enterobacter aerogenes*	*Salmonella Typhimurium*	*Listeria ivanovi*
A. officinalis	62.5	62.5	31.25	62.5	31.25
E. italicum	7.81	3.91	125.00	125.00	62.5
Amracin	0.49	0.49	0.97	0.49	0.97

The acetone extract of *A. officinalis* showed the best antimicrobial activity against *E. aerogenes* and *L. ivanovii*, and the lowest sensitivity to *P. vulgaris*, *S. enteritidis* and *S. Typhimurium*. Manifested antimicrobial activity was a consequence of the presence of pharmacologically active compounds in plant extracts, which is in accordance with the research of Bošković (2017). Plants are characterized by a mixture of different active mechanisms with different pharmacological profiles, thanks to which they can affect several different diseases, unlike synthetic drugs, which are designed to inhibit or stimulate one of the pathways of pharmacological effects (Della Loggia, 2000).

Conclusion

The research confirmed that acetone extracts of the *A. officinalis* and *E. italicum* possess pharmacologically active substances and that they are justifiably used in folk medicine. Also, extracts showed good antimicrobial activity on the tested bacteria, and that it would be useful to further investigate their biological properties.

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