POLYPHENOLIC PROFILE OF *SAMBUCUS EBULUS* ROOT, LEAF AND FRUIT EXTRACTS

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Abstract: *Sambucus ebulus* L. is a perennial herbaceous plant popular in folk medicine in Western Europe, Balkan and Middle East regions. Its preparations and extracts have shown wide range of biological activities against various disease and conditions. Isolation of the phenolic components was conducted using microwave-assisted extraction technique, while identification and quantification of isolated compounds were conducted using HPLC-DAD analysis. Fourteen compounds were detected and quantified in the extracts of the plant root, leaves and fruit, whereby rutin was predominant compound in all three samples. Minor compound in root sample was ferulic acid, in the leaves extract was chlorogenic acid, while in fruit sample was luteolin. Contrary, protocatehuic acid, caffeic acid, luteolin glycoside and apigenin glycoside were not detected in any of analyzed extracts.

Key words: *Sambucus ebulus*, microwave extraction, polyphenolic profile, HPLC analysis

Introduction

Sambucus ebulus L. is a perennial herbaceous plant widely distributed in the regions of Europe, Western Asia and North Africa. This plant, commonly known as dwarf elder (DE), belongs to the *Adoxaceae* plant family and is very popular in folk medicine in Western Europe, Balkan and Middle East regions (Zahmanov et al., 2015). Iranian people from Caspian Sea cost have used this plant as analgesic, anti-*Helicobacter pylori*, anti-hemorrhoid and anti-rheumatic drug (Fathi et al., 2015). Leaves, rhizomes and roots have been used for treatment of bites, burns, infectious wounds, edema, eczema, urticaria, arthritis and sore-throat (Shokrzadeh and Saeedi Saradi, 2010), while extracts of these parts of DE have been applied for the treatment of inflammatory diseases such as inflammatory joint disease and rheumatic pain (Hiremann, 2007). Fruits have been used for stimulation of the immune system against respiratory diseases (El Beyrouthy et al., 2008; Kultur, 2007; Nikolov, 2007; Petkov, 1982). Extract of the fruit has been known for its diuretic, antiseptic and laxative activity, while juice and jam made from fruits have found their application for amelioration of gastro-intestinal inflammatory disorders (Dimkov, 1977; Petkov, 1982). Raw fruits have also been used for wound healing (Süntar et al., 2010).

Recently conducted studies have confirmed the biological potential and pharmacological effects of DE. Preparations of this plant have shown anti-inflammatory (Schwaiger et al., 2011), anti-neoplastic activity in colon cancer (CT26 cell line) and

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hepatocellular carcinoma (HepG2 cell line) (Shokrzadeh et al., 2009), antimicrobial (Tosun et al., 2004), anti-*Helicobacter pylori* (Yesilada et al., 1999), antioxidant (Duymus et al., 2014), anti-ulcerogenic (Yesilada et al., 2014) activities as well as wound healing potential (Süntar et al., 2010), anti-inflammatory and antinociceptive effects (Ahmadiani et al., 1998). Ebrahimzadeh et al. (2006) have showed that hexane extracts of aerial parts of DE possess anti-inflammatory activity, while Yesilada et al. (1997) have reported the impact of leaves extracts on the cytokines concentration (interleukin-1α, interleukin-1β and TNF-α) in the blood samples. Fruit extracts have been established to exert high antioxidant (Ebrahimzadeh et al., 2008; Ebrahimzadeh et al., 2010; Kiselova et al., 2006; Tasinov et al.,2012; Zahmanov et al., 2015) and anti-herpes simplex activities (Zahmanov et al., 2015), to modulate expression of enzymes involved in glutathione metabolism in cell culture (Tasinov et al., 2013) and stimulate proliferation of 3T3-Ll pre-adipocyte cells (Ivanova et al., 2009).

Due to wide spectrum of biological activity of DE against various disease and conditions there is a strong need for isolation and characterization of biologically active compounds in this plant. The aim of this study was isolation of those compounds using microwave-assisted extraction method and their characterization and quantification performing HPLC-DAD method.

Material and methods

Plant material

Sambucus ebulus was collected in Southeast region of Serbia in August 2015. Separated parts of the plan were stacked in a crate with perforated bottom, in order to ensure air flow. Drying was performed naturally in the draft and dark until moisture content of 10 %. Dry plant parts were packed in glass jar and stored in the dark until use.

Extraction procedure

Microwave-assisted extraction (MAE) was performed in an open system by using a modified domestic microwave oven previously described in the literature (Švarc-Gajić et al., 2013). Stems were mixed with the hexane in ratio 1:30. The extraction procedure was performed at 480 W during 30 minutes. Extracts were filtered and kept in the refrigerator until the analysis.

HPLC-DAD analysis

The HPLC analyses of phenolic components were performed using the Agilent-1200 series with a diode array (DAD) for multi wavelength detection. The column was thermostated at 25 ^{0}C. After injecting 5 μL of sample, the separation was performed in an Agilent-Eclipse XDB C-18 4.6·150 mm column. Two solvents were used for the gradient elution: eluent A was water with 2% HCOOH and eluent B 80% ACN plus water with 2% of acetic acid. The elution program used was as follows: from 0 to 10 min 0% B, from 10 to 28 min,25% B, from 28 to 30 min 25% B, from 30 to 35

min,50% B, from 35 to 40 min,80% B, and finally for the last 5 min gradually decreases 80-0% B. Phenolic compounds in the samples were identified by comparing their retention times and spectra with retention time and spectrum of standards for each component. Quantitative data were calculated from the calibration curves.

Results and discussion

Obtained root (DER), leaf (DEL) and fruit (DEF) extracts of DE were examined for the presence of 18 different compounds of which 14 were detected and quantified (Table 1). The predominant compound in all parts of the plant was rutin with the concentration of 1.214 μg mL^{-1} in DER, 1.777 μg mL^{-1} in DEL and 6.453 in μg mL^{-1} in DEF, while quercetin was presented in significant amount in DEF sample (1.407 μg mL^{-1}). On the other hand, the minor compounds in extracts were chlorogenic acid (0.040 μg mL^{-1}) which was detected only in DEL sample, ferulic acid in DER sample with the concentration of 0.034 μg mL^{-1} and luteolin in DEF sample (0.134 μg mL^{-1}). Protocatehuic acid, caffeic acid, luteolin glycoside and apigenin glycoside were not detected in any of analyzed extracts. In the case of caffeic acid obtained result is inconsistent with the previous reported (Yesilada, 1992). This may be explained by the insolubility of this compound in the hexane, as is the case of glycosides. On the other hand, luteolin and apigenin were detected in all three DE extracts. Presence of rutin, apigenin and chlorogenic acid is in consistent with previous research (Ghannadi and Ghassemi-Dehkordi, 1997). Derivatives of *p*-coumaric acid, quercetin and kaempferol were previously detected in DE extracts by Mikulic-Petkovsek et al.(2015). These compounds were also detected in our extracts. Concretely, *p*-coumaric acid was detected in DEL and DEF samples (0.042 and 0.241 μg mL^{-1} respectively), while quercetin and kaempferol were detected in DER (0.317 and 0.373 μg mL^{-1} respectively), DEL (0.376 and 0.080 μg mL^{-1} respectively) and DEF (1.407 and 0.407 μg mL^{-1} respectively) samples. This is important, especially in the case of quercetin which glycoside was marked as one of the main compound responsible for wound healing potential (Süntar et al., 2010) and anti-ulcerogenic activity (Yesilada et al., 2014). Yesilada et al. (2014) were also isolated rutin and tested it for anti-ulcerogenic activity but it was found to be almost ineffective. Naringenin was also detected in our extracts, precisely in DEL and DEF samples. On the other hand Milkulic-Petkovsek et al. (2014) failed to isolate and detect hexoside of this compound in their extract. Zahmanov et al. (2015) also confirmed presence of quercetin glycosides in DE extract, and also detected *p*-hydrohybenzoic acid which was found only in DEF extracts. Beside mentioned compounds vanilic acid, syringic acid, ferulic acid, synapic acid and rosmarinic acid were detected. Vanilic acid was detected only in DEF sample (0.506 μg mL^{-1}), syringic acid and rosmarinic acid were detected in DEL (0.116 and 0.185 μg mL^{-1} respectively) and DEF (0.378 and 0.241 μg mL^{-1} respectively) samples, while sinapic and ferulic acid were detected in all three samples.

Tabela 1. Ispitivane i identifikovane komponente u ekstraktima korena, lista i ploda burijana

Table 1. List of investigated and identified compounds in the root, leaf and fruit extracts of dwarf elder

Jedinjenje *Compound*	Sadržaj u uzorku (μg ml^{-1}) *Sample content (μg mL^{-1})*		
	DER	DEL	DEF
Protokatehuinska kiselina *Protocatehuic acid*	ND	ND	ND
p-hidroksibenzoeva kiselina *p-Hydrohybenzoic acid*	ND	ND	0.430
Kafena kiselina *Caffeic acid*	ND	ND	ND
Vanilinska kiselina *Vanillic acid*	ND	ND	0.506
Hlorogena kiselina *Chlorogenic acid*	ND	0.040	ND
Siringinska kiselina *Syringic acid*	ND	0.116	0.378
p-kumarna kiselina *p-Coumaric acid*	ND	0.042	0.241
Ferulna kiselina *Ferulic acid*	0.034	0.061	0.212
Sinapinska kiselina *Synapic acid*	0.674	0.312	1.291
Rutin *Rutin*	1.214	1.777	6.453
Luteolin-glukozid *Luteolin glycoside*	ND	ND	ND
Apigenin-glukozid *Apigenin glycoside*	ND	ND	ND
Rozmarinska kiselina *Rosmarinic acid*	ND	0.185	0.241
Kvercetin *Quercetin*	0.317	0.376	1.407
Luteolin *Luteolin*	0.142	0.497	0.134
Naringenin *Naringenin*	ND	0.057	0.164
Kaempferol *Kaempferol*	0.373	0.080	0.407
Apigenin *Apigenin*	0.395	0.203	0.262
Ukupno *Summary*	3.149	3.746	12.126

ND-nije detektovano (*not detected*)

Conclusion

Extracts of DE root, leaves and fruit were prepared and analyzed in order to determine the composition of samples. Of 18 investigated compounds in root, leaves and fruit extracts of DE 14 were detected. The predominant compound in all three samples was rutin while the minor compound in extracts were chlorogenic acid in DEL sample, ferulic acid in DER sample and luteolin in DEF sample. Quercetin, which is marked as the one of the main bioactive component, was also detected. Due to previous obtained data about the biological activity of DE extracts, especially hexane extracts, and recognition of phenolic components as carriers of described activity, further investigation is necessary in order to expand our knowledge about chemical composition of the plant parts as well as to determine relationship between activity and the composition.

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