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HPTLC PHENOLIC COMPOUNDS FINGERPRINT AND ANTIOXIDANT ACTIVITY OF *SAMBUCUS EBULUS* LEAVES AND FRUIT

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Abstract

Phenolic compounds fingerprint of the 50% (v/v) ethanol extracts obtained from leaves (LE) and fruits (FE) of Sambucus ebulus (dwarf elder), fam Adoxaceae, was obtained by high performance thin layer chromatography (HPTLC), in order to evaluated its qualitative chemical composition and antioxidant activity (by DPPH and TAC assays). Quantitative evaluation of total polyphenolic compounds was made by Folin Ciocalteu assay. The obtained fingerprints showed that both extracts are characterized by the presence of flavonoid glycosides and phenol carboxylic acids. The presence of anthocyanins was revealed only in fruit extract Total phenol content was 19.5 mg GAE/g dry material for LE and 52,5 mg GAE/g dry material for FE. Both extracts have important antioxidant activity in a concentration-dependent manner, fruits extract exhibiting a higher one.

Key words*: HPTLC profile, Sambucus ebulus, leaves, fruit, antioxidant activity.*

INTRODUCTION

Medicinal plants are important natural renewable resources. Superior valorification of natural resources is important in the context of the development of new and innovative products as food supplement and cosmetics that can contribute in a positive manner to the living standards of the population. Antioxidants compounds become a major research subject due to their important biological activity. They can protect the human body against damage produces by reactive oxygen species (ROS). Our bodies natural antioxidant system is composed of enzymes (SOD -superoxide dismutase and GPx – glutathione peroxidase) and low molecular weight antioxidants (lipid soluble antioxidants – tocopherol, carotenoids, quinones and some polyphenols and water soluble antioxidants (vitamin C, uric acid, and polyphenols). Results of the research studies suggest that polyphenols may protect cells against oxidative stress. Free radicals play important role in the pathogenesis of diseases as degenerative diseases such as atherosclerosis, diabetes, ischemia /reperfusion (l/R) injury, inflammatory diseases (rheumatoid arthritis, inflammatory bowel diseases and pancreatitis), cancer, neurological diseases, hypertension etc (Yoshihara, et al., 2010; Kumar, 2011; Opara, 2006).

Sambucus ebulus L. (dwarf elder, elderberry or danewort), is a perennial plant, belonging to *Adoxaceae* family. The species is widespread in most part of the Europe. Leaves, fruits, flowers and root are used for therapeutic purpose. In Romania, in south Transylvania, the fruits are used for wine and jam. In traditional medicine, the species is used for diuretic, laxative and purgative, diaphoretic, depurative properties. The species is also used as toothache analgesic, antispasmodic, anti-allergic and anti-inflammatory against insect bites (Parvu, 1997). Current pharmaceutical studies have revealed antioxidant, anti-inflammatory, antinociceptive, antimicrobial, antiarthritic effects of the species. According to recent studies, this species shows anticancer proprieties (Tasinov et al., 2013). Depending on the part of the plant used (leaves, fruits, flowers, aerial parts, leafy stems, roots, whole plant), the chemical composition is different. Thus, the leaves contain essential oil, polyphenolic compounds, proteins; flowers – essential oils, sugars, cyanogenic glycosides; fruits – anthocyanins, sugars, valerianic, malic and tartaric acids, tannins, pectin's, resin, vitamin C; roots - tannins, saponins, anthocyanins, terpenoids (Shokrzadeh and

Saeedi Saravi, 2010; Feizbakhsh et al., 2014; Pieri et al., 2009; Pribela et al., 1992; Tasinov et al., 2013; Popescu et al., 2014).

The present paper presents the polyphenolic compounds content analysed by qualitative determination (HPTLC – high-performance thin layer chromatography) and quantitative determination (Folin- Ciocalteu assay) and the antioxidant activity (DPPH and TAC assays) for 50% (v/v) ethanolic extracts of *Sambucus ebulus* leaves and fruits.

MATERIALS AND METHODS

Raw material – Sambucus ebulus L.- leaves and fruit. The fruit samples were harvested at the end of August 2015 at their optimum fruit maturity (Southern Romania). A voucher specimen is deposited in INCDCF-ICCF Plant Material Storing Room.

Sample preparation: Leaves (LE) and fruits (FE) samples were prepared by extraction with 50% (v/v) ethanol, 1/10 plant material/solvent ratio, at boiling temperature, for 30 minutes The solutions were filtered and kept frozen until analysis.

Phenols HPTLC Analysis:

The densitometric analysis (HPTLC) was made according to TLC Atlas - Plant Drug Analyses (Wagner and Balt, 1997) and the characteristic fingerprint profile for phenolic compounds was determined. 3-3.5µl of the samples and 1-3µl of references substances (10^{-3}M rutin, hyperoside, chlorogenic acid, caffeic acid-Sigma-Aldrich) were loaded as 10mm band length in the 20 x 10 Silica gel 60F254 TLC plate using Hamilton- Bonaduz, Schweiz syringe and CAMAG LINOMAT 5 instrument. The mobile phase consisted in 100:11:11:27 (v/v/v/v) ethyl acetate-acetic acid-formic acid-water. The TLC twin chamber was pre-saturated with mobile phase for 30 min at ~20°C. The plate was developed in the mobile phase up to 90mm. After development, plates were dried and derivatized in Natural Product followed by PEG4000 reagent. The fingerprints were evaluated at UV with a WinCats and VideoScan software. Anthocyanins VIS detection was made without chemical treatment.

Total phenol content- Total phenol content was determined according to Folin – Ciocalteu method (European Pharmacopoeia 6,0). Briefly, 1ml of the extract was transferred to a 25ml volumetric flask, 10ml of water and 1ml of Folin Ciocalteu reagent was added. The volume was made to 25ml with 5% sodium carbonate (w/v). The blend was left at room temperature for 30 minutes. Then the absorbance of the samples was read at 760nm with a UV/VIS spectrophotometer (Helios λ, Thermo Electron Corporation). Distilled water was used as blank. Total phenol content was determined from the extrapolation of the calibration curve (*$y=0.0525x-0.020$, $R_2 = 0.992$)*, which was obtained for gallic acid (Sigma Chemical Co., St. Louis, USA) The results were expressed as milligrams of gallic acid equivalents (GAE) per gram of dried material.

Anthocyanins spectrophotometric determination was made according to European Pharmacopoeia 6.0. 0.1ml of extract was transferred to a 10ml volumetric flask. The volume was made to10 ml with 85:15 (v/v) ethanol: HCl 1.5M solution. The absorbance of the samples was determined at 546 nm with a UV/VIS spectrophotometer (Helios λ, Thermo Electron Corporation). The etanolic solution was used as blank. Anthocyanin content was determined from the extrapolation of the calibration curve (*$y=0.1976x-0.270$, $R_2 = 0.97$)*, which was obtained for cyanidin chloride (Sigma Chemical Co., St. Louis, USA) The results was expressed as miligrams of cyanidin chloride equivalents (CCE) per 100 grams of dried material.

*Free radical scavenging assay- w*as evaluated using the Sanchez-Moreno et al. (1998) assay. The extracts concentration was 0,1%, in methanol. 50µl aliquots of the extract were mixed with 2950µl of the DPPH methanolic solution (0.025g/l). The radical scavenging activity of the extracts against 2,2-diphenyl-1-picryl hydrazyl radical (Sigma-Aldrich) was determined by measuring UV absorbance at 517nm. A blank solution was prepared containing the same amount of methanol and DPPH, and measured after keeping at room temperature 30 minutes. The radical scavenging activity (RSA) was calculated using the following formula:

% inhibition = {(AB – AA)/AB} x 100.

Where AB is the absorption of blank sample and AA is the absorption of tested extract solution.

Total antioxidant capacity assay

Was assessed by phosphomolybdenum method, according to Prieto et al. 1999. To 0.3ml ethanolic solution of the sample (concentration 0.1mg/ml)was added 2.7ml of reagent solution (0.6M sulfuric acid, 28mM sodium molybdate, and 4mM ammonium phosphate). The mixtures were incubated at 95^0C for 90 minutes. After cooling the samples to room temperature, their extinction was measured at 695nm with UV-VIS spectrophotometer. Ethanol was used as negative control. The antioxidant capacity was expressed as ascorbic acid equivalent to 1mg of active substance. The calibration curve is linear for ascorbic acid in the range of 0.001 to 1mg/ml, n= 6, r^2=0.999.

RESULTS AND DISCUSSIONS

HPTLC analysis

Figure 1 shows the phenolic profiles of the *Sambucus ebulus* 50% (v/v) ethanolic fruit extract (FE) - track T1, T2, - duplicate sample, 50% (v/v) ethanolic leaves extract (LE) – track T7, T8- duplicate sample, comparatively with references substances caffeic acid T3, chlorogenic acid - T4, rutin - T5, hyperoside – T6.

Chromatographic profile of fruits extract (T2) have four proeminent yellow-orange (z1, z2, z5, z6) and one yellow – green flavonoid glycosides zones (z7), including rutin (z2 – Rf~0.42) and hyperoside (z6-Rf~0.66) accompanied by three blue florescent zones as phenol carboxylic acids with chlorogenic acid and caffeic acid as z3- Rf~0.49, respectively z8- Rf~0.96 spots. Track 1 reveals two clearly defined violet- blue pigment zones as anthocyanins.

The leaves extract fingerprint is characterised by a six yellow-orange (z11, z12, z13, z16, z17, z19) and one green (z18) flavonoid glycosides zones, two blue fluorescence zones (z14, z15) including rutin, hyperoside, and chlorogenic acid.

Figure 2 presents the comparison between extracts LE and FE and references substances hyperoside and chlorogenic acid fingerprints.

Phenol content

Table 1 shows the total phenol and anthocyanins content of the extracts expressed as gallic (GAE) acid equivalents per g of dry material, respectively as cyanidin chloride equivalents (CCE) per 100 grams of dried material. The results obtained show that the fruits have a higher content in total phenolic compounds and also, contain anthocyanins.

Table 1 shows the total phenol and anthocyanins content of the extracts expressed as gallic (GAE) acid equivalents per g of dry material, respectively as cyanidin chloride equivalents (CCE) per 100 grams of dried material. The results obtained show that the fruits have a higher content in total phenolic compounds and also, contain anthocyanins.

Table 1. Phenol content of the extracts

No	Extract	mg (GAE)/g	mg (CCE)/100g
1	LE	19.25	-
2	FE	52.5	118

Our results are in agreement with the literature data, methanolic and water extracts obtained from fruits, were reported to have a content in total phenol compounds as 41.59 ± 0.25 and 27.37 ± 0.18 mg (GAE)/g of extract powder, respectively (Ebrahimzadeh et al., 2009).

Antioxidant activity

1,1-Diphenyl-2-picryl-hydrazyl (DPPH) is a stable free radical with an unpaired electron. The color change of the solution from violet to yellow was the measure of the ability to scavenge DPPH free radical. This action is considered radical scavenging properties (Brighente et al., 2007; Ionita, 2005).

TAC -total antioxidant capacity assay shows that the tested antioxidant has the ability to donate an electron, reducing the radicals. In the presence of extracts, Mo (VI) is reduced to Mo (V) and forms a green colored phosphomolybdenum V complex (Wan et al., 2011).

Table 2 presents the antioxidant activity of the extracts obtained by the two methods DPPH and TAC.

Table 2. Antioxidant activity

No	Extract	DPPH radical scavenging activity (%)	TAC (mg ascorbic acidequivalents)
1	FE	80.32 ±0.35	158 ±0.84
2	LE	72.14±1.21	123.03±2.35

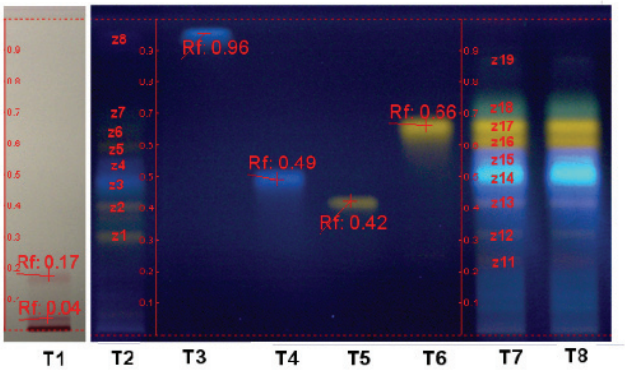

Figure 1. Phenolic profiles of fruit and leaves extracts of *Sambucus ebulus* comparative with references substances

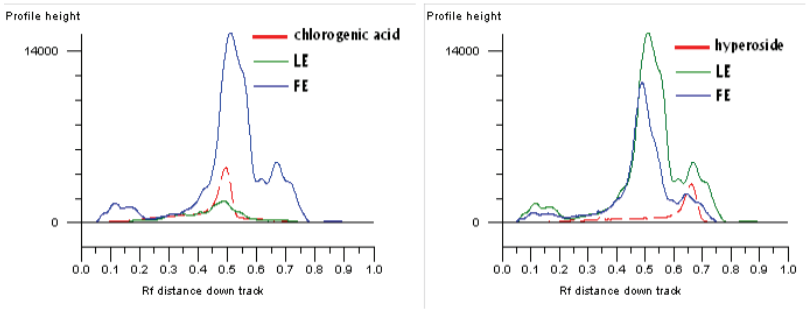

Figure 2. Profile comparison between *Sambucus ebulus* fruit and leaves extracts and references substances fingerprints

The results indicated that all the extracts have antioxidant activity in a concentration-dependent manner. According to both methods testing the antioxidant activity of the fruit extract is higher than the one of the leaves extract. Acidified methanol (0.3% v/v HCl) extract of the fruit have demonstrated neutralizing activity of DPPH – 83.17% (Anton AM et al., 2013).

CONCLUSIONS

This study shows that *Sambucus ebulus* is an important source for antioxidants compounds. Due to its antioxidant activity and to the scientific results regarding the therapeutic potential, the species is an important resource for bio-products with benefits for human health.

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