

ANTIOXIDANT ACTIVITY, TOTAL POLYPHENOL CONTENT AND ANTHOCYANINS CONTENT OF *SAMBUCUS EBULUS* L. AQUEOUS AND AQUEOUS – ETHANOLIC EXTRACTS DEPEND ON THE TYPE AND CONCENTRATION OF EXTRAGENT

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ABSTRACT

Sambucus ebulus (SE) (Dwarf elder) is an herbaceous plant well known in the traditional medicine of the Balkans and Anatolia for its healing effects in many disorders. The present study aimed to compare SE aqueous (SEA) and aqueous – ethanolic (SEAE) (20%, 40%, 60% and 80%) fruit extracts in regard of their antioxidant activity (AOA), total polyphenol content (TPC) and anthocyanins content (AC). The ABTS cation radical decolorization assay was applied to determine *in vitro* AOA of the extracts. TPC was determined using Folin – Ciocalteu reagent. AC was evaluated by pH – differential method. SEA extract showed highest AOA (5.50 mM UAE) and TPC (52.19 mg QE/g dry weight), followed by 40% SEAE extract and 60% SEAE extract. Highest AC was measured for 40% SEAE (14.07 mg/g dry weight) and 60% SEAE (14.2mg/g dry weight). Using 80% ethanol as extragent resulted in 32% lower AOA as compared to SEA extract. Statistical analysis showed higher degree of correlation between AOA and TPC ($r=0.98$) than between AOA and AC ($r=0.81$). The results suggest that AOA and possible related to it beneficial effects of *Sambucus ebulus* extracts are primary due to its TPC.

Keywords: *Sambucus ebulus*, antioxidant, polyphenol content, anthocyanins

Introduction. *Sambucus ebulus* (SE) (Dwarf elder) is an herbaceous plant well known in the traditional medicine of the Balkans and Anatolia for its healing effects in many disorders (1, 8, 17, 19). Fruits are used fresh or as a tea, and are applied for immunostimulation and treatment of inflammatory gastrointestinal disorders (3, 8, 17). Jam prepared from fruits is used in case of tuberculosis and hemorrhoids (3). It has been reported that the herb shows insect repellent and antibacterial activity against *Helicobacter pylori*, that it is effective in treatment of infected wounds, burns, edema, eczema, urticaria, inflammation and rheumatism (4, 26, 28, 30). Scientific research has revealed a significant anti-inflammatory activity of hexan extract of aerial parts and rhizomes (5), and of methanol extract of SE fruits and leaves (4). Methanol extracts of fruits and flowers were established to exert high antioxidant activity (AOA) (7). Diethyl ether fraction of leaves was found to contain ursolic acid as anti-inflammatory agent (24). The content of flavanoids, steroids, tanins, glycosides, cardiac glycosides, caffeic acid derivatives, chlorogenic acid, volatile substances, and phenols was previously reported (5, 20, 22, 26, 31). Different preparations of extracts suggest presence of different active compounds, which exhibit different action.

By the moment a comprehensive study of SE fruit extracts in regard of their AOA, total polyphenol content (TPC) and anthocyanins content (AC) lacks and it is not clear how these parameters depend on the concentration of ethanol used as an extraction solvent. Therefore, the aim of our study was to compare SE aqueous (SEA) and aqueous–ethanolic (SEAE) (20%, 40%, 60% and 80%) fruit extracts in regard of their AOA, TPC and AC.

Material and Methods

Plant material. *Sambucus ebulus* fruits were collected in Northern Bulgaria from the end of July to the end of August. Plant material was dried at room temperature.

Extract preparation. Extracts were prepared as follows: 150mg crushed dry fruits were extracted three times with 3ml distilled water for SEA and with ethanol/distilled water (20%, 40%,

60% and 80%) for SEAE extracts. Supernatants from the extraction steps were combined and diluted to 15ml with the respective extragent.

Determination of polyphenol and anthocyanin content and antioxidant activity of the fruit extracts. Fruit extracts were analyzed immediately after preparation. Total polyphenol content (TPC) was determined using Folin – Ciocalteu reagent (25). For the calculation of TPC standard curve built of absorptions of standard quercetin solutions was used. Results were expressed as mg quercetin equivalents (QE)/g dry weight.

Anthocyanin content (AC) was evaluated by pH-differential method (10). Data are presented as mg anthocyanins/g dry weight.

ABTS^{•+} radical cation decolorization assay was performed to determine *in vitro* AOA of the extracts (23). Results were expressed as mM uric acid equivalent (UAE).

Statistical analysis. Results are presented as mean ±SEM. All measurements were performed in triplicate. GraphPad Prism 5.0 software was used to perform the statistical analysis. The values of $p < 0.05$ were considered as significant.

Results and Discussion

Measurement of the TPC in SEA and SEAE extracts showed that the increase of ethanol in the extraction solution (from 0% to 80%) resulted in a distinct decrease in TPC of the extracts (Figure 1, A). The TPC of the 80% SEAE extract (48.97 mg QE/g dry weight) was by 40% lower as compared to the TPC of SEA extract (52.19 mg QE/g dry weight) ($p < 0.001$) – the extract with highest TPC.

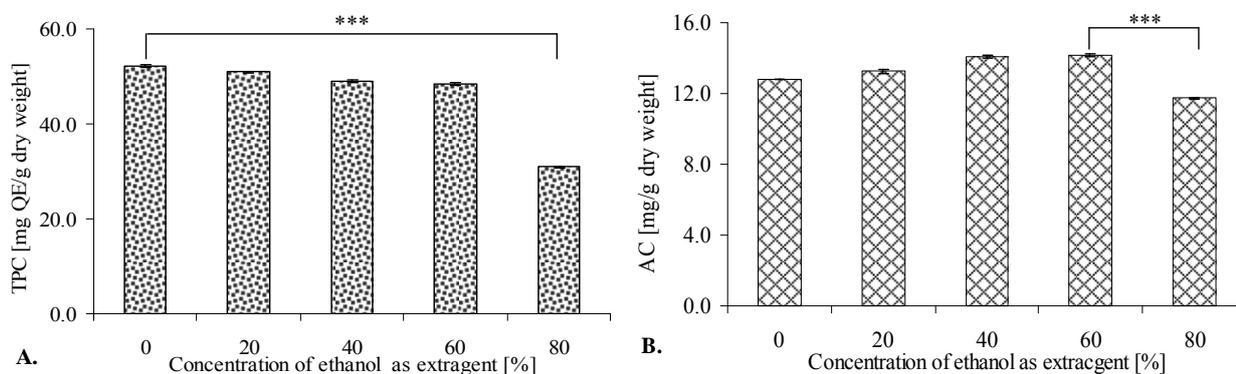


Figure 1. Total polyphenol content (A) and anthocyanin content (B) of *Sambucus ebulus* L. aqueous (0%) and aqueous–ethanolic (20, 40, 60 and 80%) extracts depend on the type and concentration of the extragent. Data are presented as mean ±SEM. *** $p < 0.001$

Our findings are in accordance with an earlier study (11), which establishes TPC of SEA extract significantly higher than that of the SEAE extracts. Another similar study (32) points out that 60% (v/v) ethanol is the optimal extraction solvent of plant polyphenols.

Highest AC was measured for 40% SEAE (14.07 mg/g dry weight) and 60% SEAE (14.2mg/g dry weight) extracts (Figure 1, B). Increasing of the concentration of ethanol in the extraction solution up to 60% resulted in an increase in AC the fruit extracts, followed by a significant decline at 80% solvent concentration. Using 80% of ethanol as extragent lead to 17% lower AC as compared to 60% SEAE extract (Figure 1, B) ($p < 0.001$). Previous research on red grape pomace has similarly established that solvents containing 70% and 50% ethanol in water leads to extracting of higher content of total anthocyanins, as compared to 10% and 30% ethanol, and to methanolic solutions (18).

Anthocyanins are one of the main groups of polyphenols presented in dark coloured fruits. So we have calculated the percentage portion of anthocyanins from the total polyphenol fraction (Table 1). Interestingly, in spite of the decrease of TPC of the extracts, the portion of anthocyanins increased gradually following the raise in ethanol concentration, peaking up in the 80% SEAE extract (38.1 ±0.251%). In average approximately 30% of TPC of the extracts was due to the anthocyanins (Table 1).

Table 1. Values of anthocyanin content as percentage of total polyphenol content for each extract. Data are presented as mean ±SEM.

Type of extract	SEA	20% SEAE	40% SEAE	60% SEAE	80% SEAE
AC/TPC [%]	24.6 ±0.17	26 ±0.38	28.8 ±0.3	29.2 ±0.11	38.1 ±0.25

SEA extract showed highest AOA (5.50 mM UAE), followed by the 40% SEAE extract (5.43 mM UAE) and the 60% SEAE extract (5.42 mM UAE) (Figure 2). Using 80% ethanol as an extragent resulted in 32% lower AOA as compared to the SEA extract (p<0.001).

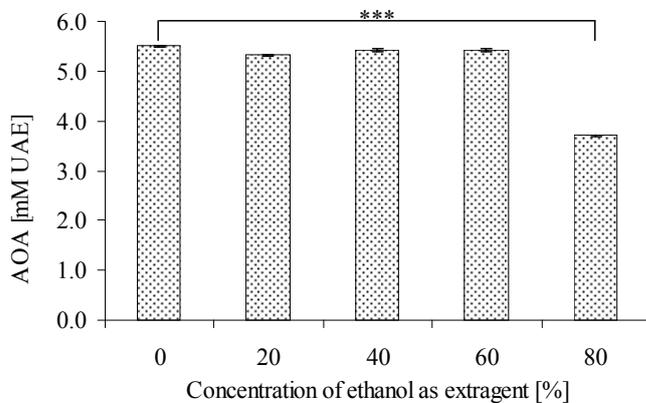


Figure 2. Antioxidant activity of *Sambucus ebulus* L. aqueous (0%) and aqueous–ethanolic (20, 40, 60 and 80%) extracts depend on the type and concentration of the extragent. Data are presented as mean ±SEM. ***p<0.001

Polyphenols are wide class of compounds including phenolic acids, catechins, flavonols and anthocyanins. It was shown that they exert strong AOA (9). There are reports revealing, that the AOA of complex fruit extracts is related rather to TPC than to AC (9). Polyphenol compounds are known for their free radical scavenging ability due to the hydroxyl groups in their structures (12). Therefore, the phenolic content of plants may contribute directly to their antioxidant action (27). We have established that the extracts with highest TPC had highest AOA (SEA and SEAE 40% and 60% extracts).

Statistical analysis showed higher degree of positive correlation between AOA and TPC (r=0.98) than between AOA and AC (r=0.81). Similar dependence between the *in vitro* AOA and TPC of herb extracts has also been reported by other researchers (13, 14, 15, 16, 21). Our study presents evidences for AOA of SEAE fruit extracts, similar to these shown for methanol extract of SE fruits (7).

Free radicals are implicated in many human diseases such as cancer, atherosclerosis, arthritis, neurodegenerative disorders and aging (2). Based on results of this study, SEA and SEAE fruit extracts could be considered as a good source of natural antioxidants and could be used as a part of daily diet in order to counteract the oxidative stress and related disorders.

Conclusion. The results suggest that AOA and the related beneficial effects of *Sambucus ebulus* extracts are primary due to their TPC. One possible explanation for the reported usage of

these extracts in traditional medicine could be the established high total polyphenol content of the SEA and anthocyanin content of the 40% and 60% SEAE extracts.

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