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ORIGINAL ARTICLE / ÖZGÜN MAKALE

ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITY OF DIFFERENT PARTS OF *SAMBUCUS EBULUS* L.

SAMBUCUS EBULUS L. BİTKİSİNİN FARKLI KISIMLARININ ANTİOKSİDAN VE ANTİ-ENFLAMATUVAR AKTİVİTELERİ

Büşra CUMHUR[1*], Sezen YILMAZ SARIALTIN[2], Safa GUMUSOK[1], Tülay COBAN[2], Ceyda Sibel KILIC[1]

[1]Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Botany, 06100 Ankara, Turkey

[2]Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, 06100 Ankara, Turkey

ABSTRACT

Objective: *Sambucus L. genus of Adoxaceae (Elderberry) family was investigated in previous studies for its antioxidant, anti-inflammatory, antiviral and antibacterial activities. Moreover, previous in vivo and in vitro studies performed on the leaves showed that the plant possesses anti-inflammatory activity. Our study aims to investigate the in vitro antioxidant and anti-inflammatory potential of the stem, fruit and leaf extracts of Sambucus ebulus L. plant.*

Material and Method: *The antioxidant activity was evaluated in a biological assay using Sambucus ebulus, whereas the radical scavenging activity was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ABTS methods.*

Result and Discussion: *The maximum anti-inflammatory effect was observed in stem extracts followed by leaf and fruit extracts, respectively. Stem extracts exhibited the highest ABTS and DPPH free radical scavenging activity (FRSA) similar to the results of anti-inflammatory activity. As a conclusion, stem extracts were the most potent extracts among the others regarding the FRSA and anti-inflammatory activities.In our study, the biological activity potential of the extracts was demonstrated, thus providing data supporting the traditional use of Sambucus ebulus L.*

Keywords: *Adoxaceae, anti-inflammatory activity, antioxidant activity, dwarf elder, Sambucus ebulus*

* **Corresponding Author / Sorumlu Yazar:** Büşra Cumhur
e-mail / e-posta: cmhr.busrra@gmail.com, **Phone / Tel.:** +05436988981

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ÖZ

Amaç: *Adoxaceae (Mürver) ailesinin Sambucus L. cinsi, önceki çalışmalarda antioksidan, anti-enflamatuar, antiviral ve antibakteriyel aktiviteleri nedeniyle araştırılmıştır. Ayrıca, yapraklarda yapılan önceki in vivo ve in vitro çalışmalar, bitkinin anti-enflamatuar aktiviteye sahip olduğunu göstermiştir. Çalışmamız, Sambucus ebulus L. bitkisinin kök, meyve ve yaprak ekstrelerinin in vitro antioksidan ve anti-enflamatuar potansiyelini araştırmayı amaçlamaktadır.*

Gereç ve Yöntem: *Antioksidan aktivite, Sambucus ebulus kullanılarak biyolojik bir tahlilde değerlendirilirken, radikal süpürücü aktivite 2,2-difenil-1-pikrilhidrazil (DPPH) ve ABTS + yöntemleri kullanılarak ölçülmüştür.*

Sonuç ve Tartışma: *Maksimum anti-enflamatuvar etki, sırasıyla yaprak ve meyve ekstrelerinin ardından kök ekstrelerinde gözlendi. Kök ekstreleri, anti-enflamatuvar aktivitenin sonuçlarına benzer şekilde en yüksek ABTS ve DPPH serbest radikal süpürme aktivitesini (FRSA) sergiledi. Sonuç olarak, kök ekstresi, FRSA ve antiinflamatuar aktivite açısından diğerleri arasında en güçlü ekstre olmuştur.*

Anahtar Kelimeler: *Adoxaceae, anti-enflamatuvar aktivite, antioksidan aktivite, cüce mürver, Sambucus ebulus*

INTRODUCTION

Sambucus L. genus, under the Dipsacales order, was previously considered to be a member of Caprifoliaceae (Honeysuckle) family, but recently was transferred to the Adoxaceae (Elderberry) family [1]. In Turkey, *Sambucus* species are represented by two tree-like herbaceous species having drupe type fruits [2]. Among these, *S. ebulus* L. is a perennial herbaceous plant growing up to 2 m having an unpleasant odor [3]. This species is mostly found in the southern and rarely in the northwestern parts of Turkey, and grows naturally in Europe, Syria, Iraq and Lebanon, as well [2]. The general appearance and flowers of the plant are given in Figure 1.

Figure 1. *S. ebulus*; a: general view, b: fruits (Photos by B. Cumhur).

Aerial regions of the plant are used to clear up mastitis in cows [4], gastrointestinal disorders caused by inflammation, in the treatment of kidney and lung diseases, in the treatment of pathological

conditions due to oxidative stress, and furthermore, the fruit is an immune system stimulant [5]. Local names and provinces in which the plant is being used traditionally are given in Table 1 in which ethnobotanical uses of the plant are also specified.

Table 1. Ethnobotanical usages of *S. ebulus*.

Province	Local name	Used part	Purpose	Reference
Kastamonu	Hekimana	Fruit and leaves	Against stomach pain	[6]
		Leaves	Wound healing	
Kocaeli	Buzka Yivdin	Aerial parts	Relief of rheumatic pain	[7, 8]
		Ripe fruits	Hemorrhoid treatment	
		Buds	Wool dyeing	
Manavgat	Ayı Otu Mürver	Leaves	Furuncle treatment	[9]
Yalova	Şahmelik Sultanotu Bazeotu	Leaves and roots	Against scorpion and bee stings	[10]
		Ripe fruits	Anti-hemorrhoid	
Edirne	Sultan otu	Aerial parts	Against ticks	[11]
		Seeds	Against constipation	
Şile (İstanbul)	Şahmelik	Aerial parts	Cold	[12]
		Leaves	Urticaria	
			Knee pain	

It has been reported that a blue dye is obtained from the fruits and used in textile dyeing in Bosnia and Gorani [13]. In a study completed in the Kırklareli district of Turkey, it was reported that the plant was used in veterinary medicine, however both, *S. ebulus* and *Chelidonium majus* L. are known with the same local name [14].

Various biological effects of *Sambucus* species have been reported in prior research, such as anti-inflammatory [15-17], antibacterial [18], anti-Helicobacter pylori [19], antiviral [20] and radical scavenging (RS) [21]. Yeşilada et al. (1997) also documented the inhibitory effects of *S. ebulus* and *S. nigra* on interleukin (IL)-1α, 1β and tumor necrosis factor- α. Süntar et al. (2010) reported that the methanolic extract of the leaves collected from the Abant region (Bolu, Turkey) was demonstrated significant wound healing potential. Ahmadiani et al. (1998) reported that the anti-inflammatory effect of the plant might be due to its steroid or flavonoid content, however *n*-hexane extract of the leaves was reported to lack the aforementioned activity. Previous studies, both *in vitro* and *in vivo*, performed on the leaves showed that the plant had anti-inflammatory activity [16,17,22].

In this study, we aimed to examine *in vitro* antioxidant and anti-inflammatory potential of methanol extracts prepared from different parts of *S. ebulus*.

MATERIAL AND METHOD

Plant material

Plant materials were collected from Düzce, Akçakoca, Döngelli village, at landsides at an altitude of 96 m in June 2018, and fruit materials were collected from the same locality in October 2018 and voucher specimens are kept in AEF (Herbarium of Ankara University Faculty of Pharmacy) with herbarium numbers AEF 30759 and 30760, respectively.

Preparation of the plant extracts

Dried leaves (2.5 g), stems (5.5 g) and fruits (29.9 g) were grounded and macerated with methanol at room temperature (3 days). Extracts were filtered, evaporated with a rotary evaporator (Heidolph VV2000, Germany) and stored in a refrigerator until used.

Anti-inflammatory activity

Blood samples were collected from healthy individuals who were not administered any anti-inflammatory or steroidal drugs 15 days before the assay. The blood samples were centrifuged at 3000 rpm for 10 min and the packed cells were isolated. Then the cells were washed with isosaline at least three times (0.85%, pH 7.2) and the volume was determined. 10% v/v was made with isosaline. The anti-inflammatory effect of the samples was evaluated by measuring their membrane protection capacity against heat-induced hemolysis using the methods of Shinde et al. (1999) and Gunathilake et al. (2018). 10% cell suspension was mixed with the sample/standard and this mix was incubated at 56ºC for 30 min. Following the incubation period, the tubes were cooled to room temp. Then, centrifuge was applied at 2500 rpm for 5 min, and the absorbance was measured at 560 nm. Only the solvent was used as the control instead of the test sample, while ASA was the standard compound. Results were presented as stabilization percentage (%), defined by the formula: $[(A_o - A_s)/A_o] \times 100$. A_o: The absorbance of the control. A_s: The absorbance of the sample/standard. IC_{50} values were calculated by linear regression analysis for each sample. Each assay was run three times. The results were presented as mean IC_{50} values±standard deviation (SD).

Antioxidant activity

The DPPH free radical scavenging activity

The antioxidant effect of the plant extracts was determined by their scavenging ability on DPPH free radical [23]. 100 μM DPPH solution was made and maintained in dark at room temperature. Several concentrations of the crude extracts were added to each well containing DPPH solution. The mix was incubated for 30 min at room temp. Then the absorbance was measured at 517 nm. BHT was served as the standard compound. Results were calculated as inhibition %, using the following formula: [(A_o

$-A_s)/A_o] \times 100$. A_o: The absorbance of the control. A_s: The absorbance of the sample/standard. IC_{50} values were calculated by linear regression analysis for each sample. Each assay was run three times. The results were expressed as mean IC_{50} values±SD.

The ABTS $^+$ free radical scavenging activity

The antioxidant effect of the plant extracts was determined measuring their ABTS free radical scavenging potential [24] 7mM ABTS $^+$ aqueous solution and 2.45 mM potassium persulfate was reacted to get $ABTS^{\bullet+}$ radical solution. This solution was maintained at at room temp overnight in the dark before usage. At the end of the time, intensely-colored $ABTS^{\bullet+}$ radical cation was obtained. This dark solution was diluted with ethanol until to get 0.700±0.02 at 734 nm (pH=7.4). Fresh radical solution was produced every 5 days due to avoid the self-degradation. Various concentrations of the crude extracts were added to each well containing radical solution and left to kept for 6 min. Then the absorbance was measured at 734 nm. Trolox was served as the reference compound. Results were calculated as inhibition %, using the following formula: $[(A_o - A_s)/A_o] \times 100$. A_o: The absorbance of the control. A_s: The absorbance of the sample/standard. IC_{50} values were calculated by linear regression analysis for each sample. Each assay was run three times. The results were presented as mean IC_{50} values±SD.

Statistical analysis

All tests were done three times and the results were presented as mean value±SD. Statistical analyses were performed with SPSS V.25.0 using one-way analysis of variance (ANOVA) following by post-hoc Tukey's test. A *p*-value less than 0.05 was accepted as statistically significant.

RESULT AND DISCUSSION

Anti-inflammatory activity of different parts of *S. ebulus* were analyzed by measuring their red blood cell membrane stability. Reference compound showed higher anti-inflammatory activity than the plant extracts ($p<0.05$). *S. ebulus* stem extracts displayed the most potent anti-inflammatory effect followed by leaf and fruit extracts, respectively (Table 2).

Table 2. *S. ebulus* plant parts' anti-inflammatory activity

Plant extract	IC_{50} (mg/mL)
Stem	1.0879±0.0856
Fruit	6.3439±0.7177
Leaves	2.400±0.0511
ASA	0.2915±0.001
Data were expressed as mean IC_{50} ± SD.	

Antioxidant activity of different regions of *S. ebulus* was investigated by measuring ABTS and DPPH FRSA and the results are presented in Table 3. The highest ABTS and DPPH FRSA were observed in stem extracts of *S. ebulus*. Fruit extracts of *S. ebulus* exhibited greater antioxidant potential than leaf extracts in both ABTS and DPPH FRSA methods. But still the reference compounds showed higher RS potentials than the plant extracts ($p<0.05$).

Table 3. DPPH and ABTS $^{+}$ FRSA

Plant extract	IC_{50} (mg/ml)	
	DPPH	**ABTS**
Stem	0.9145±0.0250	0.0188±0.001
Fruit	1.3801±0.0187	0.3969±0.035
Leaves	2.2345±0.1591	0.6681±0.064
BHT	0.0219±0.004	-
Trolox	-	0.0171±0.001
Data were expressed as mean IC_{50} ± SD.		

Medicinal plants have long been used in ethnobotany in the treatment of inflammatory conditions [25]. When we search the literature, we can see that extract of *S. australis* Cham. & Schltdl. That have been used in Brazilian traditional medicine to treat inflammatory ailments [25,26] exhibited antibacterial activity against *Klebsiella pneumoniae* (MIC 250 lg/mL) and *Salmonella typhimurium* (MIC 250 lg/mL). Chlorogenic acid and rutin were reported to be the main compounds as revealed by LC-MS/MS and this study also established that ethanolic extract of this plant possessed significant anti-inflammatory activity.

The IC_{50} for DPPH radical scavenging activity of *S. ebulus* (SE) collected from Mazandaran Forest was found to be 202.50 ± 1.38 for water extract (SW) and 723.62 ± 3.36 μg ml^{-1} for methanol extract (SM). The tested extracts exhibited poor Fe^{2+} chelating ability. Both extracts possessed significant antioxidant activity. SW extracts exhibited a better activity pattern than BHA and Vitamin C at different incubation period. SE extracts had a good reducing ability for nitric oxide scavenging and anti-lipid peroxidation activity. The aqueous extract was found to have higher total phenol and flavonoid content than the methanol extract [27].

In another study that included 21 weel volunteers, aged 20 to 59 years, with a BMI of 23.12 ± 1.31, who consumed 200 ml of SE ripe fruit infusion per day for 30 days, blood samples were drawn before and at the end of the intervention. At the end of the study, significant decreases were found in triglycerides (14.92%), total cholesterol (15.04%) and LDL-C (24.67%). In addition, the HDL-C/LDL-C ratio was increased by 42.77% [28]. This study by Ivanova et al. (2014) is the first human intervention

study with SE fruit infusion, and the results demonstrated the plant's potential to enhance the lipid profile and serum antioxidant capacity in humans.

Total phenolic content of different extracts (petroleum ether, distilled water, ethyl acetate, acetone and methanol) obtained from stems, fruits, roots and leaves of SE (collected region of Šumarice, Kragujevac in central Serbia) were evaluated using Folin-Ciocalteu. Their total phenolic content ranged from 29.87 to 126.10 mg GAE/g, while the flavonoids ranged from 4.50 to 97.65 mg RUE/g. Their antioxidant activity was investigated by DPPH assay and IC_{50} values were ranged from 47.37 to 710.94 µg/ml. The ethyl acetate extracts of fruits and methanol extracts of leaves contain the highest level of phenolics and exhibited powerful antioxidant activity [29].

Total phenolic, proanthocyanidin, phenolic acid and flavonoid content analyses of SE (dwarf elder) fruit extracts were examined together with several antioxidant activity methods such as metal reduction and FRSA. High-performance thin-layer chromatography was performed to measure the quantity of chlorogenic acid, the bioactive metabolite of the plant sampling. It was shown that methanolic and aqueous extracts of SE fruits were affected by simulated human digestion, with changes in antioxidant activity consistent with changes in phenolics (collected Bolu province, Turkey) [30]. Table 4 shows collected region, used parts, ingredients and study methods of *Sambucus sp*.

Table 4. Studies on different *Sambucus sp*.; composition, used parts and collection localities.

Species	Collected location	Used part	Composition	Solvent	Study		References
					Method	Result	
S. australis	Brazil	aerial parts	-	methanol	Inhibition of (NF-kB) *in vitro*	anti-inflammatory activity (inhibition of of the transcription factor NF-kB activation and reducing levels of inflammatory cytokines and NO)	[25]
	South Brazil	leaf and bark	-	ethanol	FRAP DPPH NO radical scavenging assays	DPPH (IC50 43.5 and 66.2 lg/mL), FRAP (IC50 312.6 and 568.3 lg/mL) and NO radical scavenging assays (IC50 285.0 and 972.6 lg/mL) were observed	[27]
	Northeast Brazil	aerial parts	ursolic acid	ethanol	MIC	Antibacterial activity against *Escherichia coli*	[31]
					DPPH	significant activity.	

Table 4 (*continued*). Studies on different *Sambucus sp*.; composition, used parts and collection localities.

Species	Collected location	Used part	Composition	Solvent	Study		References
					Method	Result	
					DPPH	significant activity.	
S. ebulus	Romania	fruits	phenolic acids and flavonoids (flavonols and anthocyanins) (LC-PDA-MS) hyperoside, quercetin-3-*O*-glucoside, 3-quercetin-3-O-rutinoside, 4- quercetin, 5-quercetin, 6-kaempherol (HPLC-MS); 5- Cyanidin-3-Osambubioside-5-glucoside; 6-Cyanidin-3,5-diglucoside, 7-Unknown; 8-Cyanidin-3-Osambubioside; 9- Cyanidin-3-O-glucoside, 10-Cyanidin-3-O-glucoside; 11-Pelargonidin; 12-cyanidin. (HPLC-DAD)	acidified methanol (0.3% HCl, v/v)	DPPH	antioxidant capacity	[32]
	Turkey	flowers, fruits and leaves	-	methanol	DPPH Folin–Ciocalteu	strongest antioxidant activity	[33]
					micro-broth dilution	moderate effect against *Candida albicans* by fruit extract	
					L929 (ATCC, CCL-1) mouse fibroblast cell line, HeLa (ATCC, CCl-2) human cervix adenocarcinoma cell line	highest anticarcinogenic activity with leaf extract (10µg/mL)	
	Bulgaria	fruits	-	methanol	-	anti-herpes simplex virus type 1 and antioxidant (in ORACFL and HORACFL) activities	[34]

Table 4 (*continued*). Studies on different *Sambucus sp*.; composition, used parts and collection localities.

S. ebulus	Southern Romania	fruit	-	ethanol 70%	spectrophotometric	antioxidant activity EC_{50} (μg/mL) 68.45 ± 0.441	[35]
					disc diffusion method and the well diffusion method	antimicrobial activity (significant results against *Pseudomonas fluorescens* and *Enterococcus fecalis*)	
	Kordkail Kola Sofla	Leaf and fruit	-	Hexane and methanol respectively	repellant activity	repellant activity (73.4% for leaf and 78% for fruit extracts)	[36]
S. nigra	Romania	fruits	phenolic acids and flavonoids (flavonols and anthocyanins) (LC-PDA-MS) quercetin-3-O-glucoside, 3-quercetin-3-O-rutinoside, 4- quercetin, 5-quercetin, 6-kaempherol, 7- p-coumaric acid (HPLC-MS); 5-Cyanidin-3-Osambubioside-5-glucoside; 6-Cyanidin-3,5-diglucoside, 7-Unknown; 8-Cyanidin-3-Osambubioside; 9- Cyanidin-3-O-glucoside, 10-Cyanidin-3-O-glucoside; 11-Pelargonidin; 12-Cyanidin.	acidified methanol (0.3% HCl, v/v)	DPPH	antioxidant capacity	[32]

Table 4 (*continued*). Studies on different *Sambucus sp*.; composition, used parts and collection localities.

S. nigra	-	fruits	Anthocyanins, Cyanidin, Pelargonidin 3-glucoside, Cyanidin 3-sambubioside-5-glucoside, Cyanidin 3,5-diglucoside, Cyanidin 3-sambubioside, Cyanidin 3-glucoside, Quercetin, Quercetin 3-O-rutinoside, Quercetin 3-O-glucoside, Proanthocyanidins	-	-	-	[37]
		flowers	Quercetin 3-O-rutinoside, Quercetin 3-O-glucoside, Kaempferol 3-O-glucoside, Isorhamnetin 3-O-rutinoside,				
	garden of the Urmia University	fruit, leaf and bark	-	methanol	DPPH iron chelating ability nitric oxide and hydrogen peroxide radical scavenging activities.	leaf extract showed highest activity (IC50 = 21.6 ± 1.1 µg/ml) and also a higher NO radical scavenging activity	[38]

When we searched the literature for antioxidant and anti-inflammatory studies performed on different *Sambucus* species along with the ones performed with the plant species that we have also examined, we have seen that similar results were obtained in previous studies. Our results indicate that stems of SE can be served as a source of natural anti-inflammatory components since this biological activity that we have observed is compatible with the studies found in the literature. Concurrently, our results also indicate that the extract prepared from the stems of *S. ebulus* can be used as a source of antioxidant components. New studies should be performed to examine the responsible compound(s) for these biological activity activities.

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AUTHOR CONTRIBUTIONS

Concept: *C.S.K., B.C.*; Design: *B.C.;* Control: *C.S.K., T.Ç.*; Sources: *B.C.;* Materials: *B.C., S.Y.S.*; Data Collection and/or processing: *B.C.*; Analysis and/or interpretation: *B.C., S.Y.S., S.G.*; Literature review: *B.C.*; Manuscript writing: *B.C.*; Critical review: *C.S.K.;* Other: *S.G.*

CONFLICT OF INTEREST

The authors declare that there is no real, potential or perceived conflict of interests for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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