Clinical and Experimental Health Sciences

Cytotoxicity of *Sambucus nigra* L. on Cancer Cell Line and In Vitro Antioxidant Properties

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ABSTRACT

Objective: Essential oils, free fatty acids, flavonoids, glycosides, phenolic acids, carotenoids, vitamins, and minerals are found in significant quantities in the characteristic chemical composition of Sambucus nigra L. This study aimed to evaluate the antioxidant potential of Sambucus nigra L. Fructus and evaluate the cytotoxicity on the cancer cell line.

Methods: The Sambucus nigra L. fruits were collected from Yalova Atatürk Horticultural Central Research Institute in September 2021. The ethanol extract was prepared. Antioxidant property of Sambucus nigra L. fruit extracts was evaluated with 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) radical scavenging activity, Cupric Ion Reducing Antioxidant Capacity (CUPRAC). Also, total phenolic content, total flavonoid content, and total anthocyanin content were calculated. Liver hepatocellular carcinoma cell line (HepG2) was used for cytotoxicity assay and an 2,5-diphenyl-2H-tetrazolium bromide (MTT) assay was applied.

Results: The total phenolic, total flavonoid contents and total monomeric anthocyanins were 9.75±0.92 mg GAE/ mg fruit extract, 0.07437±0.004 mg quercetin/ mg fruit extract, respectively. 0.45±0.0014 mg catechin/gram of fruit extract and 2.08±0.025 mg Cyanidin-3 glucosides/g fruit extract. CUPRAC and DPPH results showed that Sambucus nigra L. extract has strong antioxidant activity. The results of the cytotoxicity assay indicated that while concentrations of the extract increased, the viability of HepG2 decreased.

Conclusion: Our findings suggest that the *Sambucus* fruit extract is particularly rich in antioxidant components that are possibly modulating their beneficial use for hepatocellular malignancies, significantly reducing the number of viable cancer cells and inducing cell death.
Keywords: *Sambucus nigra,* elderberry, cytotoxicity, antioxidants

1. INTRODUCTION

Many herbs have been extensively studied for their antioxidant activity in recent years. It is believed that increased intake of food rich in natural antioxidants is associated with lower risks of degenerative diseases. Sambucus nigra L. is one of them and belonging to the Caprifoliaceae family, is an extremely accessible plant native to the northern hemisphere. Its seeds are rapidly spread by birds and other animals along with forests and roadsides, leading to its spread to different habitats today such as the subtropical regions of Asia, North Africa, and North America (1).

Essential oils, free fatty acids, flavonoids, glycosides, phenolic acids, carotenoids, vitamins, and minerals are found in significant quantities in the characteristic chemical composition of Sambucus nigra. Together with anthocyanins, phenolic acids and flavonols form the main secondary metabolites of Sambucus nigra L. (2). These phenolic compounds have strong antioxidant activities both in vitro and in vivo due to their reducing properties. Phenolic compounds exhibit a variety of biological activities such as anti-inflammatory, antiviral, antiallergic, vasoprotective, and anti-carcinogenic activities (3). In recent years, Sambucus nigra L.'s antioxidant activity and effects have attracted considerable attention. Under normal physiological conditions, the endogenous defence system can scavenge reactive oxygen species (ROS). On the other hand, the endogenous system may be insufficient in an excessive increase in the amount of ROS. Reactive oxygen species emerging with uncontrolled free radical production can cause oxidative damage to biomacromolecules, amino acids, DNA, lipids, and proteins. For this reason, it is a matter of curiosity whether the place of Sambucus nigra L. in human life can be expanded or not (4, 5).

This study aimed to evaluate the antioxidant potential of Sambucus nigra L. fructus and evaluate the cytotoxicity on

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the liver cancer cell line. It is expected that this study will reveal the potential natural antioxidant sources of Sambucus nigra L. fruits and their anti-tumour activity.

2. METHODS

2.1. Materials

The Sambucus nigra L. fruits were collected from the Yalova Atatürk Horticultural Central Research Institute in September 2022.

2.2. Sample preparation and extraction

The fruit sample of Sambucus nigra L. plant was dried in an oven (37°C) and then 100 g of dried samples were weighed, and ethanol extracts were prepared by maceration method (48 hours). The collected ethanol filtrates were then filtered through filter paper. The solvents of the obtained filtrates were evaporated at low pressure and temperature using a rotary evaporator device, and crude extracts were obtained. The obtained crude extracts were kept in the refrigerator at +4 °C.

2.3. Total phenolic content (TPC)

4.5 mL of distilled water was added to the 0.1 mL extracts prepared at concentrations of 5 mg/mL. The mixture was then added with 0.1 mL Folin-Ciocalteu reagent (FCR) (diluted with 1:3 distilled water) and 0.3 mL 2 percent sodium carbonate solution, and the absorbance of mixed was measured at 760 nm 2 hours later against the reference. The total phenolic content was expressed as mg of gallic acid equivalents (GAE) per mg of extract (9).

2.4. Total flavonoid content (TFC)

For total flavonoid analysis, 1 mL of the extracted samples was taken into each tube, and 4 mL of distilled water and 0.3 mL of 5% sodium nitrite ($NaNO_2$) solution were added to it. After 5 minutes, 0.3 mL of 10% aluminium chloride ($AlCl_3$) solution was added, mixed and left for 6 minutes. Then, 2 mL of 1 M NaOH was added and the volume was made up to 10 mL with distilled water. The absorbance values of the samples were measured with a UV-VIS spectrophotometer at a wavelength of 510 nm. Results are given as catechin equivalents/mL.

2.5. Total anthocyanin content

Anthocyanin level was detected by using the pH differential method according to the procedure of Majki´c et al. (10). Total anthocyanin content (TAC) expressed as mg equivalent cyanidin-3-O-glucoside per g fruit extract.

2.6. DPPH radical scavenging activity

10 µL of extracts prepared at 0.5-5 mg/mL concentrations were taken, 240 µL of 0.1 mM DPPH solution was added, DPPH solution was added, and standard solutions were prepared for 1 min. After vortexing, they were left in room conditions and dark for 30 min. Their absorbance was measured against reference using a microplate reader at 517 nm. The control was prepared under the same conditions by using 10 µL methanol instead of the sample and standard material and the absorbance of the control was measured. The experiment was repeated three times and the average was calculated.

2.7. Cupric Reducing Antioxidant Capacity assay (CUPRAC)

In the Cupric Reducing Antioxidant Capacity (CUPRAC) assay, 60 µL each of copper (II) solution, neocuproin solution and ammonium acetate buffer (1 M) were mixed. 10 µL ethanol and 60 µL of extract were added and shake the solution. The solutions were kept in room conditions with their mouth closed for 60 minutes. At the end of this period, absorbance values at 450 nm were measured against the reference solution that does not contain a sample. CUPRAC values were given as mM TroloxE/mg extract (8)

2.8. Cell culture

The human liver hepatocellular carcinoma cell line (HepG2) was from American Type Culture Collection (ATCC), Manassas, VA, USA). All medium and solutions were heated to 37 °C before the process of cell cultivation. The cells were cultured in Eagle's Minimum Essential Medium (EMEM) supplemented with heat-inactivated fetal bovine serum (FBS) (a final concentration of 10%), L-alanyl-L-glutamine and 1% penicillin-streptomycin at 37 °C. It was maintained in 75 cm2 cell culture flasks in the incubator with the condition of 37 °C, 95% air and 5% CO2 for 24 h to confluence. After the incubation of 24 h, all waste medium was discarded and the monolayer cells were detached with trypsin-EDTA in the incubator for 3 min. The suspension was centrifuged in a refrigerated centrifuge at 125 x g for 5 min. The supernatant was discarded and the pellet was suspended in a fresh medium. Then, viable and dead cells were counted by the method of trypan blue (0.4%) staining with a haemocytometer. For the preparation of U-bottom 96-well microplates, 100 µl of the stock viable cell suspension (3 x 105 cell/ml) was seeded in each well (3 x 104 cell/well) and kept in the incubator for 24 h to confluence at least 90%.

2.9. Cytotoxicity assay

The extract was 2-fold serially diluted with maintaining medium at the concentrations of 10, 5, 2.5, 1.25, 0.625 and 0.312 mg/ml. Then, 100 µl of each dilution was added to six-replicated wells of the 96-well microplate confluent with cells. 100 µl of maintaining medium was only added to cell control wells (medium + cell) and blank wells (only medium).

The microplate was incubated at 37 °C and 5% CO2 for 24 h. Inert microscopy (Olympus ix71, Tokyo, Japan) was used to observe the morphological changes of the cells. MTT assay (tetrazolium-based colorimetric assay) was used to determine the cytotoxic effects of the extract by spectrophotometry. MTT solution in PBS (5mg/ml) was prepared and filtered with 0.45 µm sterile syringe filters. MTT solution (10µl) was added to wells. The microplate was incubated for 4 hours at 37 °C in 5% CO2. After incubation, the supernatant was discarded and DMSO (100 µl) was added to the wells. The microplate was gently shaken to solubilize the formazan crystals. A microplate reader (Absorbance 96, Byonoy, Germany) was used to measure the absorbance at a wavelength of 570 nm. The percentage of cell viability and inhibition was calculated in the formula as follows,

Cell viability (%) = (Asample – Ablank)/(Acontrol – Ablank) x 100

Cell inhibition (%) = 100 – [(Asample – Ablank)/(Acontrol – Ablank) x 100]

Asample = absorbance value of test compound, Acontrol = absorbance value of control (cell), Ablank = absorbance value of blank (medium)

CC_{50} was expressed in mg/ml of the extract and calculated by the formula (y=mx+b) of linear regression analysis using the extract concentration-response curve (Fig 1).

2.10. Vitamin C equivalence

The assay was slightly adapted from the methods of Muraina et al. (6) and Liu and Nair (7). L-ascorbic acid (600 µmol) and the extract (1 mg/ml) were prepared in DMSO at two-fold concentrations. 380 µL of MTT solution (1 mg/ml in distilled water) and 20 µL of each dilution were vortexed in a 1.5 ml-eppendorf tube. Then, 400 µL of DMSO was added to all tubes to solve the formazan crystals and the reaction mixture was incubated at 37 °C for 4 h. 150 µL of each reaction mixture was pipetted to a 96-well microplate in quadruplicate. The microplate (Absorbance 96, Byonoy, Germany) was read to measure the absorbance at a wavelength of 570 nm. A standard curve was generated with the dilutions of L-ascorbic acid. Vitamin C equivalents of the extract were calculated with the curve (Fig 2).

3. RESULTS

In the study, the content of bioactive compounds in the extract of Sambucus nigra L. fruit was examined (Table 1). The total phenolic component content of the ethanol extracts of Sambucus nigra L. fruits was 9.75±0.92 mg GAE/mg dried extract, while the total flavonoid component content was 0.07437±0.004 mg quercetin/mg dried extract and 0.45±0.0014 mg catechin/gram dried extract. As for the content of total monomeric anthocyanins in Sambucus nigra L. fruit extracts, it was 2.08±0.025 mg Cyanidin 3 glucosides/g dried extract.

Table 1. *The content of total phenolics, flavonoids and anthocyanins in Sambucus nigra L. fruit extract*

Measure	Mean ± SD
TPCa (mg GAE per g extract)	9.75±0.92
TFC b (mg quercitin per mg extract)	0.07437±0.004
TFCc (mg catechin per g extract	0.45±0.0014
TACd (mg cyanidin 3-glucoside per g extract)	2.08±0.025

TPC: Total phenolic content; TFC: total flavonoid content; TAC: Total anthocyanins content

[a] mg gallic acid equivalent per mg of dry extract

[b] mg kersetin equivalent per mg of dry extract

[c] mg catechin equivalent per g of dry extract

[d] mg cyanidin 3-glucoside equivalent per g of dry extract

In this study, radical scavenging activity (DPPH) and reducing power (CUPRAC) of Sambucus nigra L. fruit extract were investigated (Table 2). As a result of all analyses applied to Sambucus nigra L. fruit extract, it was determined that it has strong antioxidant activity.

Table 2. *Biological activities of Sambucus nigra L. fruit extract*

	Value
DPPH (IC_{50} mg/mL)	0.13±0.057
DPPH (Ascorbic acid)	0.004± 0.007
CUPRAC Value[a] (mM troloks equivalent/mg extract)	45.20±1.530

[a] mM equivalent Trolox per mg of dry extract

DPPH: 2,2-diphenyl-1-picryl-hydrazyl-hydrate; CUPRAC: Cupric Ion Reducing Antioxidant Capacity

The results of the cytotoxicity assay indicated that while concentrations of the extract increased, the viability of HepG2 decreased. The highest percentage of cell viability was determined with the concentrations of 2.5, 1.25, 0.625 and 0.312 mg/ml as 58.8%, 71.2%, 84.2% and 100.9% respectively. The lowest was determined with the concentrations of 1 and 0.5 mg/ml as 39.7% and 30.4% respectively. The linear regression between the concentration of the extract and the cell viability was determined as R2=0.794 (Fig 1). CC50 of the extract was calculated as 5.49 mg/ml by the formula of y = –6.43x + 85.293 in Fig 1.

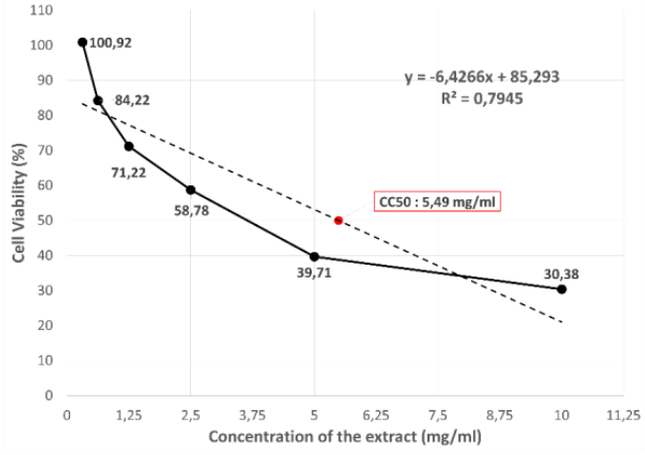

Figure 1. *Effect of elderberry fruit extract on cancer cell viability*

Vitamin C equivalents linearly increased by increasing the concentrations of the extract in dilutions (R2=0.978, Fig 2). Vitamin C equivalents were determined as 671.43, 420, 214.29, 130.95, 34.29 and 11.43 µmol of vitamin C for 1, 0.5, 0.25, 0.125, 0.0625 and 0.0312 respectively.

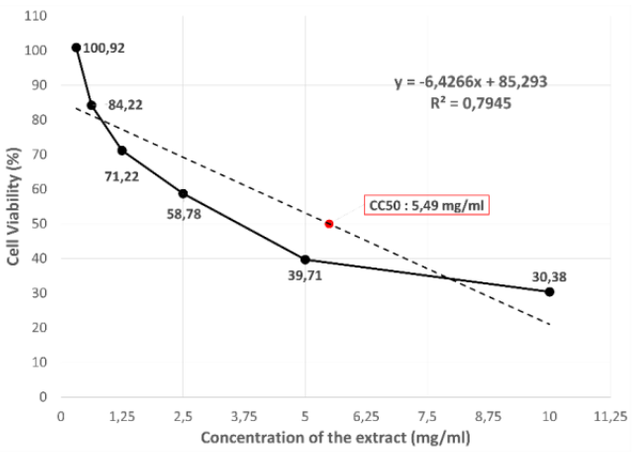

Figure 2. *Vitamin C equivilant of Sambucus nigra L. fruit extract*

4. DISCUSSION

There are many pharmacologically active compounds in polyphenols and flavonoids are among the most prominent. Flavonoids are mostly found in high amounts in plants and are involved in processes such as plant pigmentation, antioxidant capacity, protection from biotic and abiotic stresses, and plant-environment interactions. Anthocyanin gives the plant orange, red and blue colors. Berries are rich in antioxidant components, Sambucus nigra L. is one of these fruits (11).

The total phenolic component content of Sambucus nigra L. has been studied in many studies. TPC values have been determined in different ranges in the literature. It is thought that climatic and topographic differences may also affect berry quality and TPC values in Sambucus nigra L. which is determined by Atkinson and Atkinson (12). In a study examining the effectiveness of different solutions (ethanol and water), the TPC of Sambucus nigra L. extract powder, in which ethanol was used as a solvent, was measured as 339.68±1.47 mg GAE/g dried weight (13). Akbulut et al. (2009) studied the ethanol extract of Sambucus nigra L. and found that it had a high phenolic content (371-432 mg GAE/100 g fresh matter) (14). In the study of Özgen et al., the TPC was found between 2.898 and 5.006 µg GAE/g (15). Another study determined the TPC of different Sambucus nigra L. fruits as 17.115 mg GAE/g (16). Goud and Prasad measured the TPC value of 43±0.98 mg GAE/g dried weight in their study (17). In a review examining the antioxidant properties of elderberries, it was determined that the TPC value varied between 1.91-17.90 mg GAE/g fresh matter (18). In this study, the TPC value was calculated as 9.75±0.92 mg GAE/g extract. The findings of our study correspond to similar ranges with the data in the literature.

Total flavonoid component content (TFC) can be obtained using different standards. In a study the TFC of ethanol extract of Sambucus nigra L. was measured as 840.54±13.46 mg RE/g dried weight (13). In another study, the TFC of methanol extract of Sambucus nigra L. was measured as 15.00±1.12 mg rutin/g dry weight (17). In this study, the TFC value was calculated as 0,07±0,004 mg quercetin/mg extract and 0,45±0,0014 mg catechin/g extract similar to the literature. The flavonoid is one of the phenolic components, so it was an expected result that the phenolic content was higher than the flavonoid content. However, less TFC content was found in our study than the TFC estimates in the literature. Assays used for the estimation of TFC are only equivalent to relative quantities of selected standards or have limitations in analytical methods for quantification. Differences in results may be linked with differences between selected standards.

Berries are generally rich in anthocyanins, Sambucus nigra L. is one of these fruits (19). Özgen et al. (15) examined the antioxidant capacity of different Sambucus nigra L. accessions and found that they contained anthocyanins between 1.308 and 4.004 mg Cy3GE/g. In the study of Domínguez et al., the amount of TAC obtained from Sambucus nigra L. extracts varies between 287.8–645.7 mg/100 g (20). Csorba et al. in their study determined the total anthocyanin content as 854.57 mg GAE/100g (16). In another study, anthocyanin's content in Sambucus nigra L. fruits was found to vary between 660 and 800 mg/100 g (21). Parallel to the literature, TAC was determined at 2.08±0.025 mg Cy3GE/g in the present study.

Studies have established a strong relationship between the phenolic content of a fruit and its ability to scavenge free radicals (22). On the other hand, DPPH radical capture test and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical capture test is characterized by color change in the presence of antioxidants. Özgen et al.(15) evaluated the antioxidant capacity of Sambucus nigra L. by DPPH analysis and it was found to be between 5.4-16.9 µmol troloks equivalent/g(TE/g). In the study conducted by Jakobek et al. (2007), it was determined that Sambucus nigra L. fruits have strong antiradical activity (100.16 mmol TE 1 g-1) in the DPPH method (23). In another study evaluating the antioxidant properties of the methanol extract of Sambucus nigra L. fruits, the DPPH value was calculated as 104.35 ± 0.22 µmol TE/g (24). In the present study, it was determined as 0.004± 0.007 µmol ascorbic acid equivalent/g.

The concentration required to scavenge 50% of the free radicals present is defined as the IC50 value. In a study, 50% scavenging capacity of Sambucus nigra L. was determined as 0.081 (mg/µl dry matter) (25). In a study carried out by preparing an acidified extract of Sambucus nigra L. fruits, lyophilization process was applied to the extracts and it was observed that DPPH free radicals were scavenged from 88.17% to 88.50% (26). In this study, the IC50 value was determined as 0.1311±0.0057 mg/mL.

The CUPRAC method is often preferred to measure the antioxidant capacity of plant materials. Haş et al. evaluated the antioxidant properties of Sambucus nigra L. fruit extract

and found it to be 52.3 ± 0.11 µmol TE/g dry weight (24). In a study, CUPRAC analysis of methanol extract of Sambucus nigra L. flower was performed and it was calculated as 0.42 mmol TR/g (27). In this study, it was calculated as 45.20±1.530 mM trolox equivalent/mg dry extract. In parallel with the literature, it has been shown that Sambucus nigra L. has a strong Copper (II) ion reduction potential.

The tumour is characterized by the establishment of the vessels feeding it as a result of the stimulation of pro-angiogenic factors and the provision of oxygenation. IC50 values are obtained as a result of measuring the cytotoxic effect. A lower IC50 value represents the higher ability of a cytotoxic compound to cause cell death or inhibit cell growth. Saeedi Saravi et. al (28) evaluated the cytotoxic effect of ethyl acetate extract of Sambucus ebulus on cancer (HepG2 and human colon carcinoma) and non-cancer (rat fibroblast and hamster ovary) cell lines. It was shown that the ethyl acetate extract of Sambucus ebulus had higher inhibition effects on cancer cell lines (0.097±0.152 mg/ml) compared to normal cell lines (0.312±0.346 mg/ml), which can act as an anticancer compound. In another study. In a study, HepG2 cells were exposed to different doses of Sambucus nigra L. fruit extracts and it was noted that the cell viability was still 100% at 500 µg/ml (29). However, the present study provides evidence that the ethanol extract of Sambucus nigra fruit acted as a cytotoxic agent on hepatic cancer cells (HepG2) with a CC50 of 5.49 mg/ml. These findings highlight that Sambucus nigra L. significantly reduces viable cancer cells and induces cell death.

The main findings of this study revealed that Sambucus nigra L. fruit extract is rich in antioxidant components. In addition, as expected as a result of the relationship between phenolic components and biological activities, it was observed that both the copper (II) ion reduction potential and the scavenging effect of DPPH radicals of Sambucus nigra L. fruit extract were high. On the other hand, the fact that the efficacy of the bioactive components in the structure of Sambucus fruit extract was not examined separately is a limitation of this study.

5. CONCLUSION

In light of our results and the literature's information on antioxidant and anticancer effects of bioactive components, we can conclude that Sambucus nigra L. with its high antioxidant capacity is a plausible candidate for the prevention of hepatic cancer. However, more research is needed to fully evaluate the safety and effectiveness of Sambucus nigra L.

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REFERENCES

[1] Młynarczyk K., Walkowiak-Tomczak D., Łysiak GP. Bioactive properties of Sambucus nigra L. As a functional ingredient for food and pharmaceutical industry. J Funct. Foods. 2018;40:377–390. DOI: 10.1016/j.jff.2017.11.025

[2] Lee J, Finn CE. Anthocyanins and other polyphenolics in American elderberry (Sambucus canadensis) and European elderberry (S. nigra) cultivars. J Sci Food Agric. 2007;87:2665–2675. DOI: 10.1002/jsfa.3029

[3] Paredes-López O, Cervantes-Ceja ML, Vigna-Pérez M, Hernández – Pérez T. Berries: improving human health and healthy aging, and promoting quality life – a review. Plant Foods Hum Nutr. 2010;65:299–308. DOI: 10.1007/s11130.010.0177-1

[4] Neves D, Valentão P, Bernardo J, Oliveira MC, Ferreira JMG, Pereira DM, Andrade PB, Videira RA. A new insight on elderberry anthocyanins bioactivity: Modulation of mitochondrial redox chain functionality and cell redox state. J Funct Foods. 2019;56:145–155. DOI: 10.1016/j.jff.2019.03.019

[5] Duymuş HG, Göger F, Başer KHC. In vitro antioxidant properties and anthocyanin compositions of elderberry extracts. Food Chem. 2014;155:112–119. DOI: 10.1016/j.foodchem.2014.01.028

[6] Muraina IA, Suleiman MM, Eloff JN. Can MTT be used to quantify the antioxidant activity of plant extracts?. Phytomedicine. 2009;*16*(6-7):665-668. DOI: 10.1016/j.phymed.2008.11.005

[7] Liu Y, Nair MG. An efficient and economical MTT assay for determining the antioxidant activity of plant natural product extracts and pure compounds. J. Nat. Prod. 2010;*73*(7):1193-1195. DOI: 10.1021/np1000945

[8] Apak R, Güclü K, Ozyurek M, Karademir SE. Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC Method. J Agric Food Chem. 2004;52(26):7970–7981. DOI: 10.1021/jf048741x

[9] Taskin T, Balkan IA, Taskın D, Dogan A. Characterization of phenolic constituents and pharmacological activity of Achillea vermicularis. Indian J Pharm Sci. 2019;81(2):293–301. DOI: 10.36468/pharmaceutical-sciences.

[10] Majkic TM, Torovic LD, Lesjak MM, Cetojevic-Simin DD, Beara IN. Activity profiling of Serbian and some other European Merlot wines in inflammation and oxidation processes. Food Res Int. 2019;121: 51–160. DOI: 10.1016/j.foodres.2019.03.033

[11] Tungmunnithum D, Thongboonyou A, Pholboon A, Yangsabai A. Flavonoids and Other Phenolic Compounds from Medicinal Plants for Pharmaceutical and Medical Aspects: An Overview. Medicines. 2018;5(3): 93. DOI: 10.3390/medicines5030093

[12] Atkinson MD, Atkinson E. *Sambucus nigra* L. J. Ecol. 2002;90:895-923. DOI: 10.1046/j.1365-2745.2002.00698.x

[13] Yıldız Ö, Vahapoğlu B, Marangoz MA, Güven EÇ, Bayindirli A. Determination of Phenolic Compound Profiles and Antioxidant Effect of Plant Extracts on Late-Release Soft Lozenge. EAS J Nutr Food Sci. 2021;3(6):167-174. DOI: 10.36349/easjnfs.2021.v03i06.005.

[14] [14] Ercisli S, Tosun M, Akbulut M. Physico-chemical characteristics of some wild grown European Elderberry (*Sambucus nigra* L.) genotypes. Pharmacogn. Mag. 2009;5(20):320. DOI:10.4103/0973-1296.58153

[15] Özgen M, Scheerens JC, Reese RN, Miller RA. Total phenolic, anthocyanin contents and antioxidant capacity of selected elderberry (Sambucus canadensis L.) accessions. Pharmacogn Mag. 2010;6(23):198. DOI: 10.4103/0973-1296.66936

[16] Csorba V, Magdolna TÓTH., Laszlo AM, Kardos L, Kovacs S. Cultivar and year effects on the chemical composition of elderberry (Sambucus nigra L.) fruits. Not Bot Horti Agrobot Cluj Napoca. 2020;48(2):770-782. DOI:10.15835/nbha48211873

[17] Goud NS, Prasad G. Antioxidant, antimicrobial activity and total phenol and flavonoids analysis of Sambucus nigra (elderberry). Int J Curr Pharm Res. 2020;12(1):35-37. DOI: 10.22159/ijcpr.2020v12i1.36829.

[18] Moyer RA, Hummer KE, Finn CE, Frei B, Wrolstad RE. Anthocyanins, phenolics, and antioxidant capacity in diverse small fruits: vaccinium, rubus, and ribes. J Agric Food Chem. 2002;50(3):519-25. DOI: 10.1021/jf011062r.

[19] Veberic R, Jakopic J, Stampar F, Schmitzer V. European elderberry (Sambucus nigra L.) rich in sugars, organic acids, anthocyanins and selected polyphenols. Food Chem. 2009;114(2):511-515. DOI: 10.1016/j.foodchem.2008.09.080.

[20] Domínguez R, Zhang L, Rocchetti G, Lucini L, Pateiro M. Munekata PE, Lorenzo JM. Elderberry (Sambucus nigra L.) as potential source of antioxidants. Characterization, optimization of extraction parameters and bioactive properties. Food Chem. 2020;330: 127266. DOI: 10.1016/j.foodchem.2020.127266

[21] Walkowiak-Tomczak D, Czapski J, Młynarczyk K. Assessment of colour changes during storage of elderberry juice concentrate solutions using the optimization method. Acta Sci Pol Technol Aliment. 2016;15(3):299–309. DOI: 10.17306/J.AFS.2016.3.29

[22] Bahorun T, Luximon-Ramma A, Crozier A, Aruoma OI. Total phenol, flavonoid, proanthocyanidin and vitamin C levels and antioxidant activities of Mauritian vegetables. J Sci Food Agric. 2004;84(12):1553-1561. DOI: 10.1002/jsfa.1820.

[23] Jakobek L, Šeruga M, Novak I, Medvidović-Kosanović M, Šeruga B. DPPH radical inhibition kinetic and antiradical activity of polyphenols from chokeberry and elderberry fruits. Pomologia Croatica: Glasilo Hrvatskog agronomskog društva, 2008;14(2):101-118.

[24] Has IM, Teleky BE, Szabo K, Simon E, Ranga F, Diaconeasa ZM, Nițescu M. Bioactive Potential of Elderberry (Sambucus nigra L.): Antioxidant, Antimicrobial Activity, Bioaccessibility and Prebiotic Potential. Molecules, 2023;28(7):3099. DOI: 10.3390/molecules28073099

[25] Poráčová J, Sedlak V, Pošiváková T, Mirutenko V, Gruľová D, Mydlárová-Blaščáková M, Kotosova J. Measurement of antioxidant activity in chokeberry (Aronia melanocarpa WILD.) and black elderberry (Sambucus nigra L.) using the DPPH method. İkinci Uluslararası Bilimsel ve Pratik İnternet Konferansı "Tıbbi Bitki Yetiştiriciliği: Geçmiş Deneyimlerden Modern Teknolojilere" Bildirileri, 2013;161:131. DOI: 10.1055/s-0032.132.1041

[26] Pliszka B. Polyphenolic content, antiradical activity, stability and microbiological quality of elderberry (Sambucus nigra L.) extracts. Acta Sci. Pol. Technol. 2017;16(4):393-401. DOI: 10.17306/J.AFS.0523

[27] Esin Çelik S, Özyürek M, Güçlü K, Çapanoğlu E, Apak R. Identification and Anti-oxidant Capacity Determination of Phenolics and their Glycosides in Elderflower by On-line HPLC–CUPRAC Method. Phytochem. Anal. 2014;25(2):147-154. DOI: 10.1002/pca.2481

[28] Saravi SS, Shokrzadeh M, Shirazi FH. Cytotoxicity of Sambucus ebulus on cancer cell lines and protective effects of vitamins C and E against its cytotoxicity on normal cell lines. (2013). Afr J Biotechnol. 2013;12(21): 3360-3365. DOI: 10.5897/AJB09.1577

[29] Ferreira SS, Martins-Gomes C, Nunes FM, Silva AM. Elderberry (Sambucus nigra L.) extracts promote anti-inflammatory and cellular antioxidant activity. Food Chem. 2022;15:100437. DOI: 10.1016/j.fochx.2022.100437

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