Istanbul J Pharm 52 (2): 148-155
DOI: 10.26650/IstanbulJPharm.2022.929762
Original Article

Determination of the chemical composition, antioxidant potential of *Sambucus ebulus* L. (dwarf elder) fruit extracts and investigation of antimicrobial activity on *Trichophyton rubrum* (Castell.) Sabour and some microorganisms

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Cite this article as: Demirci Kayiran, Serpil., Eroglu Ozkan, Esra., Mataraci Kara, Emel., Yazici Bektas, Nurdan., Tari, Ozden., & Nenni,Merve. (2022) Determination of the chemical composition, antioxidant potential of *Sambucus ebulus* L. (dwarf elder) fruit extracts and investigation of antimicrobial activity on *Trichophyton rubrum* (castell.) sabour and some microorganisms. *Istanbul Journal of Pharmacy, 52*(2), 148-155. DOI: 10.26650/IstanbulJPharm.2022.929762

ABSTRACT

Background and Aims: *Sambucus ebulus* L. is one of the medicinal plants well known in the traditional medicine of Anatolia since ancient times. The present study was aimed to investigate the antifungal potential of *S. ebulus* fruit extracts against *Trichophyton rubrum* (Castell.) Sabour as well as phytochemical composition, antibacterial, and antioxidant activities based on traditional usage.

Methods: Two extracts were prepared from *S. ebulus* fruits. The phytochemical composition of *S. ebulus* fruit extracts was identified by LC-MS/MS. The antimicrobial activity was examined by using the broth microdilution method against a panel of microorganisms. In addition to this, *S. ebulus* extracts were evaluated for their *in vitro* antifungal activity against three yeast and *T. rubrum* by disc diffusion method.

Results: The major compounds were determined in dried fruit methanol extract (DFM) as hederagenin (5.38±0.4949 µg/g) and fumaric acid (3.06±0.0275µg/g). The fumaric acid (3.97±0.0357µg/g) was detected as the abundant compound in the fresh fruit juice (FFJ). Acacetin, chrysin, eupatilin, hederagenin, isosakuranetin, myricitrin, and rhamnocitrin were detected in the extracts for the first time. DFM showed moderate activity against *E. coli* (MIC: 625 mg/L) and *Candida tropicalis* (MIC: 312.5 mg/L). Both extracts possessed weak activity against *Proteus mirabilis* and *Staphylococcus aureus*,which had the MIC values 1250 mg/L. *T. rubrum* was found resistant to both extracts. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical cleaning method was used to measure the antioxidant capacity of the extracts. DFM and FFJ exhibited strong antioxidant activity against DPPH radicals with IC50 value of 5.941±0.236 µg/mL and 7.893±0.939 µg/mL, respectively.

Conclusion: As a conclusion, although *S. ebulus* fruits are used to treat nail fungus (*onychomycosis*) by local folk, our results showed that it could not be useful to use in the antifungal topical formulations. In addition to this, the antibacterial activity result is parallel to the results of studies in this particular so far.

Keywords: *Sambucus ebulus*; LC-MS/MS; antifungal activity; *Trichophyton rubrum*; antioxidant activity

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Submitted: 29.04.2021
Revision Requested: 08.09.2021
Last Revision Received: 15.10.2021
Accepted: 02.12.2021
Published Online: 29.08.2022

INTRODUCTION

The genus *Sambucus* belongs to the Adoxaceae family, which comprises of 30 species all over the world, including out of which two, namely *Sambucus nigra* and *Sambucus ebulus*, have been recorded being from Turkey (Scopel et al., 2007; Senica, Stampar, & Mikulic-Petkovsek, 2019). *S. ebulus* L., whose common name is elderberry or dwarf elder, a kind of shrub, is widely distributed in southern and central Europe and southwest Asia (especially in Iran and Turkey) (Shokrzadeh & Saravi, 2010). The rhizomes, stem barks, aerial parts, leaves, flowers, and fruits of *S. ebulus* have long been used to treat different diseases such as colds and coughs, arthritis, edema, rheumatic diseases, constipation, infected wounds, eczema, burns, urticaria, hemorrhoids, and bee stingsin Turkish traditional medicine (Demirci & Özhatay, 2012; Kültür, 2007; Tuzlacı & Tolon, 2000; Yesilada, 1997). According to their extensive usage in treatment, *S. ebulus* is called "hekimana," which means "the mother of the physician" by Anatolian folk (Jabbari, Daneshfard, Emtiazy, Khiveh, & Hashempur, 2017; Yesilada, Gürbüz, & Toker, 2014). Traditionally, uses of *S. ebulus* fruits to treat nail fungus infections were reported for the first time in this study (Demirci & Özhatay, 2012). We were therefore inspired to organize the current study because of this remarkable knowledge obtained from the countryside in Kahramanmaras, Turkey.

S. ebulus berries are rich in several important secondary metabolites such as anthocyanins (cyanidin-3,5-diglucoside, cyanidin-3-sambubioside-5-glucoside, cyanidin-3-O-sambubioside, and cyanidin-3-O-glucoside), flavonoids (isorhamnetin-3-O-β-D-glucopyranoside, isorhamnetin-3-O-rutinoside, hyperoside, and isoquercitrin), iridoid glycosides (sambulin A, sambulin B), lectins (ebulin), phytosterols, phenols, triterpenes, tannins, cardiac glycosides, and phenolic acids (caffeic acid derivatives, chlorogenic acid, ursolic acid) (Atay, Kirmizibekmez, Gören, & Yeşilada, 2015; Cvetanović, 2020; Kaya, Haji, Arvas, & Aksoy, 2019; Shokrzadeh & Saravi, 2010).

Aiming to reveal the new pharmaceutical applications of these plants, numerous studies have been focused on investigating their biological activities. Among them, antiinflammatory (Ahmadiani, Fereidoni, Semnanian, Kamalinejad, & Saremi, 1998; M. Ebrahimzadeh, Mahmoudi, & Salimi, 2006; Yesilada, 1997), antinociceptive (Ahmadiani et al., 1998; M. Ebrahimzadeh, Mahmoudi, Saiednia, Pourmorad, & Salimi, 2006), antimicrobial (Rodino et al., 2015; Salehzadeh, Asadpour, Naeemi, & Houshmand, 2014), antiherpes simplex (Zahmanov et al., 2015), antiulcerogenic (Yesilada et al., 2014), antioxidant (Cvetanović, 2020; Hashemi, Ebrahimzadeh, & Khalili, 2019), antihypoxic(Kaveh, Mohamadyan, & Ebrahimzadeh, 2019), hypolipidemic (Ivanova, Tasinov, & Kiselova-Kaneva, 2014), and wound healing (Süntar et al., 2010) activities have been demonstrated.

Incidentally, besides the studies that investigated the chemical compositions and biological activities of *S. ebulus*, some ethnopharmacological studies were also carried out by scientists. One of them reported that the leaves and fruits of *S. ebulus* were traditionally used to handle stomach pain, snake bites, and coughs (Kültür, 2007). Another study conducted by Demirci and Ozhatay indicated that the fruits of *S. ebulus* are used for the treatment of hemorrhoids, rheumatism, and nail fungus in Kahramanmaraş, Southern Turkey (Demirci & Özhatay, 2012).

Traditional uses of *S. ebulus* fruits to treat nail fungus infections were reported for the first time in this study (Demirci & Özhatay, 2012). We were therefore inspired to organize the current study because of this remarkable knowledge obtained from the countryside in Kahramanmaras, Turkey.

Nail fungus, also called *onychomycosis* or *tinea unguium*, is a fungal infection of the nail caused mainly by *Trichophyton rubrum*. It may cause pain, discomfort, cosmetic problems, and daily and social life limitations, and, consequently, reducesquality of life (Lipner & Scher, 2019). In the study conducted by Demirci and Ozhatay (2012), it was reported that the locals had been applying the crushed fresh fruits on the infected nail for the treatment of onychomycosis (Demirci & Özhatay, 2012). They repeat the procedure every two or three days until the nail is fully recovered. Therefore, the goal of the current study is to identify the chemical composition, antibacterial, antifungal, and antioxidant activities of *S. ebulus* fruits collected from Kahramanmaras, Turkey based on traditional uses in that area.

It has been suggested that some cases of *onychomycosis* are associated with a periungual inflammation while others may be associated with low-grade systemic inflammation (Duhard, 2014; Shi et al., 2016; Sinikumpu et al., 2018). The inflammatory response is the mechanism of injury involving oxidative stress (Balea, Pârvu, Pop, Marín, & Pârvu, 2018). Reactive oxygen species (ROS) can circulate freely in the cell by passing through the membranes. Then, ROS damage DNA, RNA, proteins, and lipids. As a result, it causes local or systemic damage (Andreicuț et al., 2018). Moreover, untreated onychomycosis can lead to further infection (Gupta, Versteeg, & Shear, 2017). Given these circumstances, oxidative stress should also be considered as a secondary target in onychomycosis treatments (Pârvu et al., 2019).

The therapeutic properties of plants are derived from chemical compounds isolated from extracts and essential oils that can scavenge free radicals (Granato et al., 2018; Morais et al., 2013). Extracts and essential oils are frequently used as antimicrobial agents in traditional medicine due to their antiseptic and antioxidant effects (Bakkali, Averbeck, Averbeck, & Idaomar, 2008; Nogueira et al., 2020).

MATERIAL AND METHODS

Plant material

S. ebulus fruits were collected from Andırın, Kahramanmaraş in June 2018. The voucher specimen was deposited at the Faculty of Pharmacy of Cukurova University Herbarium (CUEF 1671).

Extraction and fractionation

The fresh fruits were divided into two parts, then the first part was dried at room temperature in shade, and then the dried material was extracted with methanol: water (50:50; v/v) by using a shaker at 25 °C for 24 hours. The procedure was repeated 4 times until the samples were exhausted. After filtration, the solvent was removed by rotary, and the water was removed by lyophilization. The extract (DFM) was stored at -20 °C until the analysis. The other part (fresh fruits) was squeezed.

LC-MS/MS analysis

A Thermo Orbitrap Q-Exactive instrument in ESI Source was used for LC-HRMS (Liquid Chromatography High-Resolution Mass Spectrometry) measurements. The validated method was used for the analysis, and validation parameters of the method were reported by Gülçin et al. 2010 (Gülçin, Bursal, Şehitoğlu, Bilsel, & Gören, 2010; Han, Yilmaz, & Gulcin, 2018).

Determination of Minimum Inhibitory Concentrations (MIC)

Antimicrobial activities against standard microorganisms are listed in Table 1. Antimicrobial activity was identified by the broth microdilution technique using the Clinical Laboratory Standards Institute (CLSI) recommendations (CLSI, 1997; CLSI, 2020). For antimicrobial activity, Mueller–Hinton broth (Oxoid) and for antifungal activity, RPMI-1640 (Applichem, Darmstadt, Germany) medium were used as the medium. The extract by making serial twofold dilutions ranging from 2500 mg/L to 1.2 mg/L was produced in the media. The inoculum was made to give a final concentration of 5×10^5 CFU/mL for bacteria and 0.5×10^3 to 2.5×10^3 CFU/mL for yeast in the 96 well plate. The 96 well plates were covered and placed in plastic bags to prevent evaporation. The microplates were incubated at 35ºC for 18–20 h for bacteria while the microplates were incubated at 35ºC for 46–50 h for yeast strains. The MIC was defined as the lowest concentration of compound giving complete inhibition of visible growth. As a control, the antimicrobial effects of the solvents were investigated against test microorganisms. The results were evaluated according to the values of the controls.

Table 1. The quality control strains used to test with the extracts of *Sambucus ebulus* fruits.

Tested microorganisms
Staphylococcus aureus ATCC 29213
Staphylococcus epidermidis ATCC 12228
Escherichia coli ATCC 25922
Klebsiella pneumoniae ATCC 4352
Pseudomonas aeruginosa ATCC 27853
Proteus mirabilis ATCC 14153
Enterococcus faecalis ATCC 29212
Candida albicans ATCC 10231
Candida parapsilosis ATCC 22019
Candida tropicalis ATCC 750

ATCC: American Type Culture Collection, 12301, Parklawn Drive, Rockville, MD 20852, USA.

Antifungal susceptibility testing by disc diffusion method

A standard *Trichophyton rubrum* (ATCC 28188) isolate was applied for *in vitro* antifungal evaluation by using the CLSI M38-A guidelines. RPMI 1640 medium (Applichem, Darmstadt, Germany) was buffered to pH 7.0 with morpholinopropanesulfonic acid (MOPS; Applichem, Darmstadt, Germany) and filtered with a membrane filter for sterilization. A 2% glucose and 2% agar were suspended in 200 mL distilled water and autoclaved for sterilization. The RPMI 1640 medium and the solution of agar were combined in a water bath at 45–50°C. The mixture was passed onto plastic petri dishes and cooled at room temperature. The inoculum was prepared according to the CLSI M38-A criteria *T. rubrum* suspension was diluted to reach conidium concentration as $1–5x10^6$. The inoculum was dispersed to the area of 2% glucose RMPI 1640 agar plates and desiccated for 15 min. A hole (diameter; 2 mm, height; 4 mm) was opened in the middle of the agar plates. The extracts by making serial twofold dilutions ranging from 10000 mg/L to 1.2 mg/L were put in this hole with a sterile spatula and forceps. Plates were kept at 26°C for 4–7 days and investigated for the proliferation of fungus and zone diameter of inhibition. Ketoconazole (10 µL Liofilchem) was used as a reference drug for evaluating the inhibition zone diameter according to CLSI criteria (CLSI, 1997; CLSI, 2020).

2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals scavenging method

The antioxidant activity of *S. ebulus* extracts was measured by the DPPH radicals scavenging method, which was established by Blois (1958) and Brand-Williams et al. (1995) and was adapted for 96-well plates (Blois, 1958; Brand-Williams, Cuvelier, & Berset, 1995). Extracts were dissolved in ultra pure water, and dilutions were made with methanol. Ascorbic acid (Aldrich, St. Louis MO, USA) was used as a positive control. The negative control was without any *S. ebulus* extracts. 50 µL of 0.1 mM DPPH (Aldrich, St. Louis MO, USA) radical solution, which was freshly made in methanol (Aldrich, St. Louis MO, USA), was added to 150 µL of extracts or standards in 96-well plates. The plates were shaken 1 min with the microplate reader (Thermo Scientific™, Multiskan™ Sky Microplate Spectrophotometer, Waltham, MA, USA) and were incubated 45 min in the dark at room temperature at 517 nm % of DPPH scavenging activity was calculated according to % DPPH Scav. Act. = [(AControl – ASample)/AControl] × 100 formula.

Statistical analysis

Data were expressed as mean ± standard deviation (mean ± SD) of experiments. The experiment was repeated four times (n=4). The data were analyzed by Microsoft Office Excel and analyzed by an analysis of variance ($p<0.05$). The statistical evaluation of data was performed using a one-way analysis of variance (ANOVA) and Tukey multiple comparisons.

RESULTS AND DISCUSSION

LC-MS/MS analysis

In the current study, phytochemical compositions of the dried fruits methanol extract (DFM) and fresh fruits juice (FFJ) of *S. ebulus* collected from Kahramanmaraş, Turkey were determined by LC-MS/MS. The results of the LC-MS/MS analysis are shown in Table 2. In the DFM extract, 10 compounds were present. The most abundant secondary metabolites in the DFM were hederagenin (5.38±0.4949 µg/g) and fumaric acid (3.06±0.0275 µg/g). Additionally, hederagenin was only found in the DFM extract. Other compounds that were detected in DFM were acacetin (0.13±0.0001 µg/g), chrysin (0.13±0.0001 µg/g), eupatilin (0.14±0.0001 µg/g), hyperoside (0.25±0.0075 µg/g), isosakuranetin (0.01±0.0001 µg/g), myricitrin (0.09±0.0027 µg/g), naringenin (0.11±0.0046 µg/g), and rutin (0.11±0.0004 µg/g). In the FFJ extract of *S. ebulus*, 9 compounds were determined. FFJ is the richest extract in terms of one of the dicarboxylic acid derivates, fumaric acid

Table 2. Quantitative determination (µg/g) of 23 phytochemicals in the extracts of *Sambucus ebulus* fruits.

	Content of the extracts (µg/g)		
Compounds	**DFM**	**FFJ**	**U % (k=2)**
(-)-Epicatechin	<LOD	<LOD	3.6
(-)-Epigallocatechin gallate	<LOD	0.01±0.0002	2.7
(+)-Trans Taxifolin	<LOD	<LOD	3.0
Acacetin	0.13±0.0001	0.13±0.0001	1.5
Caffeic acid	<LOD	<LOD	2.4
Chrysin	0.12±0.0001	0.13±0.0001	1.2
Dihydrokaempferol	<LOD	<LOD	3.8
Eupatilin	0.14±0.0001	0.11±0.0001	1.4
Fumaric Acid	3.06±0.0275	3.97±0.0357	0.9
Hederagenin	5.38±0.4949	<LOD	9.2
Herniarin	<LOD	<LOD	2.4
Hispidulin	<LOD	<LOD	1.7
Hyperoside	0.25±0.0075	0.63±0.0189	3.0
Isosakuranetin	0.01±0.0001	<LOD	1.2
Myricitrin	0.09±0.0027	0.46±0.0142	3.1
Naringenin	0.11±0.0046	<LOD	4.2
Nepetin-7-glucoside	<LOD	<LOD	4.4
Orientin	<LOD	<LOD	3.8
Quercitrin	<LOD	<LOD	4.8
Rhamnocitrin	<LOD	0.01±0.0003	3.2
Rutin	0.11±0.0004	0.34±0.0153	4.5

DFM: Dried fruit methanol extract; FFJ: Fresh fruit juice; LOD: Limit of detection

(3.97±0.0357µg/g). Also, (-)-Epigallocatechin gallate (0.01±0.0002 µg/g), acacetin (0.13±0.0001 µg/g), chrysin (0.13±0.0001 µg/g), eupatilin (0.11±0.0001 µg/g), hyperoside (0.63±0.0189 µg/g), myricitrin (0.46±0.0142 µg/g), rhamnocitrin (0.01±0.0003 µg/g), and rutin (0.34±0.0153 µg/g) were identified in the extract.

A few numbers of studies have been conducted on the chemical composition of *S. ebulus* fruits. In a previous study, acetone extract of the ripe fruits was analyzed using an LC-PDA-MS method. According to the results of the study, epicatechin (0.84 mg/100 g FW: fresh weight), quercetin (0.15 mg/100 g FW), and kaempferol (0.05 mg/100 g FW) were identified in the extract (Vankova, Todorova, Kisselova-Kaneva, & Galunska, 2019). Another study was also carried on to determine the secondary metabolites in water extract of *S. ebulus* fruits by HPLC. The results show that gallic acid (868.98 mg/mL), protocatechuic acid (39.16 mg/mL), chlorogenic acid (36.82 mg/mL), caffeic acid (17.21 mg/mL), ferulic acid (3.38 mg/mL), naringin (1.97 mg/mL), and rutin (6.53 mg/mL) were identified in the fruit water extract of *S. ebulus* (Cvetanović et al., 2018). In another study conducted by Mikulic-Petkovsek et al, *S. ebulus* fruit extract was analyzed using HPLC-DAD-MS; according to the result, quercetin-3-rutinoside (421.14±5.15 mg/kg FW) was the major compound in the extract. The other compounds were reported as quercetin-3-glucoside (42.30±1.37 mg/kg FW), quercetin-3-galactoside (79.54±2.75 mg/kg FW), quercetin-hexosidepentoside (77.08±1.83 mg/kg FW), kaempferol-3-rutinoside (77.22±1.19 mg/kg FW), isorhamnetin-3-rutinoside (145.75±1.49 mg/kg FW), and isorhamnetin-hexoside (29.05±0.79 mg/kg FW) (Mikulic-Petkovsek, Ivancic, Todorovic, Veberic, & Stampar, 2015). As reported by Cvetanovic et al. (2016), 13 compounds were detected in *S. ebulus* fruits by using HPLC-DAD (Cvetanovic et al., 2016). The study result showed that rutin (6.453 µg/mL), quercetin (1.407 µg/mL), and synaptic acid (1.291 µg/mL) were contained as a major compound in the fruits. The other compounds were determined as *p*-hydroxybenzoic acid (0.430 µg/mL), vanillic acid (0.506 µg/mL), syringic acid (0.378 µg/mL), *p*-coumaric acid (0.241 µg/mL), ferulic acid (0.212 µg/mL), rosmarinic acid (0.241 µg/mL), luteolin (0.134 µg/mL), naringenin (0.164 µg/mL), kaempferol (0.407 µg/mL), and apigenin (0.262 µg/mL).

Evaluation of the *in vitro* antimicrobial activity

In the present study, four Gram-negative bacteria (*P. aeruginosa, E. coli, P. mirabilis,* and *K. pneumoniae*), three Gram-positive bacteria (*S. aureus, S. epidermidis,* and *E. faecalis*) for antibacterial activity, and three yeast (*C. albicans, C. tropicalis,* and *C. parapsilosis*) for antifungal activity were used to preliminarily screen their antibacterial and antifungal activity employing the standard drugs using the broth microdilutions method according to the Clinical Laboratory Standards Institute (CLSI) recommendations (CLSI, 1997; CLSI,

2020). The MIC values are summarized in Table 3. Taken together, with MIC values, the results identified that most of the tested compounds showed weak activity against the studied microorganisms. The test-cultures *P. aeruginosa, E. faecalis, K. pneumoniae,* and *S. epidermidis* in addition to *C. albicans* and *C. parapsilosis* appeared as resistant to all the studied extracts. However, all the studied extracts possessed activity against *P. mirabilis* and *S. aureus* which had the MIC values 1250 mg/L. As indicated in Table 3, among the extracts DFM showed moderate antimicrobial activity against *E. coli* (MIC: 625 mg/L) and *C. tropicalis* (MIC: 312.5 mg/L). Concerning the antifungal activity of DFM and FFJ, *T. rubrum* was found resistant to both extracts of all the tested concentrations.

Conforming to the literature survey, there are not many studies that covered the antimicrobial activity of *S. ebulus* fruits. In one of the studies among them, antimicrobial activities of chloroform, acetone, hexane, water, and methanol extracts obtained from *S. ebulus* fruits were tested against *Staphylococcus aureus, Bacillus subtilis, Pseudomanas aeruginosa, Escherichia coli, Salmonella typhimurium, Candida guillermondii,* and *Candida albicans* by using well diffusion method. The result showed that the extracts did not show activity against these strains compared to gentamicin and nystatin (Ginovyan & Trchounian, 2017). In another study, *S. ebulus* fruit extract was examined against *Staphylococcus aureus, S. epidermidis, E. coli, Klebsiella pneumoniae, P. aeruginosa, Proteus mirabilis,* and *C. albicans* to determine the antimicrobial activity by using the microbroth dilutions method. According to the result of the study, the extract had shown moderate antifungal activity against *C. albicans* and no activity against the other strains (Meriç, Bitiş, Birteksöz-Tan, Turan, & Akbuga, 2014). In the other study, antibacterial and antifungal activity of *S. ebulus* fruit extract were analyzed against *S. aureus, Bacillus cereus, B. subtilis, E. coli, Enterococcus faecalis, Pseudomonas fluorescens, Botrytis cinerea, Phytophthora infestans*, and *Rhizochtoniasolani* using the disc and the well diffusion methods. It was reported that the well diffusion method was better than the disc diffusion method to obtain the best results. In line with the results, it was observed that the extract had dose-dependent activity against *S. aureus, B. subtilis, E. fecalis,* and *P. fluores*, and it had resistance against *B. subtilis* and *E. coli* strains (Rodino et al., 2015). It seems that the antimicrobial activity results of the current study are in line with the results from literature.

Evaluation of the antioxidant activity

DPPH, a free radical cleaning method, was used in this study to measure antioxidant capacity. It is a colorimetric method that measures the conversion of the color of the radical solution from purple to yellow as a result of hydrogen or electron transfer (Braham et al., 2020; Do et al., 2014). This method has high sensitivity because it can detect antioxidant components in low concentrations. Besides this, it can analyze many samples simultaneously as a first step scanning strategy (Meza, Rojas, Cely-Veloza, Guerrero-Perilla, & Coy-Barrera, 2020). Many compounds are used as antioxidant standards. Ascorbic acid is most commonly used as the standard due to its strong scavenging activity (Al-Rifai, 2018). Table 4 shows the DPPH radical scavenging activity results of *S. ebulus* extracts compared to ascorbic acid.

Table 3.The MIC results (mg/L) of *Sambucus ebulus* fruits extracts.

	Microorganisms									
	***P. aeruginosa* ATCC 27853**	***E. coli* ATCC 25922**	***K. pneumoniae* ATCC 4352**	***P. mirabilis* ATCC 14153**	***E. faecalis* ATCC 29212**	***S. epidermidis* ATCC 12228**	***S. aureus* ATCC 29213**	***C. albicans* ATCC 10231**	***C. parapsilosis* ATCC 22019**	***C. tropicalis* ATCC 750**
FFJ	-	-	-	1250	-	-	1250	-	-	625
DFM	-	625	-	1250	-	-	1250	-	-	312.5
Reference antimicrobials	CAZ: 2.4	CFX: 4.9	CFX: 4.9	CFX: 2.4	AMK: 128	CFX: 9.8	CFX: 1.2	CL: 4.9	AMPHO: 0.5	AMPHO: 1

DFM: Dried fruit methanol extract; FFJ: Fresh fruit juice; CAZ: cefazidime; CFX: cefuroxime; AMK: amikacin; CL: clotrimazole; AMPHO: Amphotericin B.

Table 4. Antioxidant activities of *Sambucus ebulus* extracts.

	IC_{50} (µg/mL)	EC_{50} (mg/mg DPPH)	ARP Values	AEAC Values
DFM [a]	5.941±0.236	0.151	6.637	163075
FFJ [a,b]	24.784±0.873	0.629	1.591	39090
Ascorbic acid	9.688±1.025	0.246	4.070	-

IC_{50} values expressed are means ±S.D. of four measurements. The values having different superscript (Small alphabet and special character) letters within a column were significantly different ($p \leq 0.05$).
DFM: Dried fruit methanol extract, FFJ: Fresh fruit juice, EC_{50}: Effective concentration, ARP: Antiradical power, AEAC:Ascorbic acid equivalent antioxidant capacity.

Antioxidant parameters with IC_{50}, effective concentration (EC_{50}), antiradical power (ARP), and ascorbic acid equivalent antioxidant capacity (AEAC) values were calculated by drawing a logarithm graph with % radical scavenging capacity against the sample concentration. The AEAC value was calculated according to the formula 'AEAC=($IC_{50(AA)}$/$IC_{50(sample)}$) $\times 10^5$' after calculating the IC_{50} value. As can be seen from the formula, the AEAC value has no units. If low IC_{50} – EC_{50} and high ARP – AEAC values are the case, an antioxidant is reported to be stronger (Kedare & Singh, 2011). According to antioxidant parameters, the order of antioxidant potency for *S. ebulus* extract was as follow: DFM (IC_{50}: 5.941±0.236 µg/mL)> FFJ (IC_{50}: 7.893±0.939 µg/mL)> ascorbic acid (IC_{50}: 9.6880±1.02490 µg/mL) (Table 4). When the antioxidant activity of *S. ebulus* fruits was investigated by a DPPH experiment, different IC_{50} values were found in the literature. The study was conducted by Ebrahimzadeh et al. (2009) reported that the aqueous and methanol extracts of *S. ebulus* fruits collected from Mazandaran forest, Iran showed DPPH radical scavenging activity with IC_{50} value of 202.50 ± 1.38 µg/mL and 723.62 ± 3.36 µg/mL, respectively (M. A. Ebrahimzadeh, Ehsanifar, & Eslami, 2009). Another study, that was managed by Rodino et al. (2015), showed that the ethanol extract (70%) of *S. ebulus* fruits collected from Romania exhibited DPPH free radical scavenging potent with EC_{50} value of 68.45 ± 0.441µg/mL. The other study results indicated that aqueous and methanol extracts of *S. ebulus* fruits collected from Serbia demonstrated DPPH scavenging activity with IC_{50} value of 128.23 ± 0.65 µg/mL and 82.15 ± 0.33 µg/mL, respectively (Topuzović, Stanković, Jakovljević, & Bojović, 2016). The study carried on by Meric et al. (2014) expressed that the methanol extract of *S. ebulus* fruits collected from Istanbul, Turkey showed DPPH radical scavenging activity with IC_{50} value of 8.895 ± 1.391 mg/mL (Meriç et al., 2014).

An overview of the discussion

The reason for the identified differences between the results of the current study and the other studies might be correlated with using different plant materials collected from various areas and methods used for the extraction of the *S. ebulus* fruits. Furthermore, according to the present study results, it seems that *S. ebulus* extracts have more potential antioxidant activity than ascorbic acid used as a standard compound. In a study conducted by Glassman et al. (2003), a topical formulation containing urea and an antioxidant agent was developed to use in nail fungus (onychomycosis) treatment. The results of the study showed that a combination of the antifungal agent and antioxidant compound increases the efficacy of nail fungus treatment. The invention was patented by Glassman et al. in 2003. According to the research, a nail is continuously exposed to photooxidative damage and oxidative environment, including air pollutants, ultraviolet radiation, chemical oxidants, and aerobic microorganisms. The antioxidant compound protects the permeability and stability of the cell membrane of the nail (Glassman, Bhagwat, & Glassman, 2004). The current study results support this information and showed that due to having strong antioxidant potential, *S. ebulus* fruits might be beneficial for the treatment of nail fungus infections.

The fruits of *S. ebulus* have traditionally been used to treat nail fungus (onychomycosis) by local folk in Kahramanmaras, Turkey. Therefore, the current study focused on the identification of the potential of *S. ebulus* fruits for the treatment of nail fungus caused by *Trichophyton rubrum*. However, the antifungal activity results indicated that *S. ebulus* fruit extracts did not show activity against *T. rubrum*. On the other hand, antioxidant activity results demonstrated that the extracts have strong antioxidant potential. According to literature, antioxidant compounds contribute to the topical treatment of nail fungus (onychomycosis). As a conclusion, although *S. ebulus* fruits did not show antifungal activity against *T. rubrum*, it might help to treat nail fungus (onychomycosis) with its antioxidant properties and might be useful when used in the antifungal topical formulations for its antioxidant features. In addition to this, the antibacterial activity results for tested organisms are parallel to the results of studies in this particular so far. In the other part, the study showed that a few compounds, namely acacetin, chrysin, eupatilin, hederagenin, isosakuranetin, myricitrin, and rhamnocitrin, quantified by LC-MS/MS defined to *S. ebulus* fruits for the first time. We strongly encourage that pharmacological activity studies should be inspired by the traditional usage of medicinal plants.

CONCLUSION

S. ebulus samples were found to have a potent antioxidant effect on the phytochemical components of the extract. These findings support the use of *S. ebulus* in traditional medicine in the treatment of dermatophytes infections such as onychomycosis. In conclusion, the effectiveness of *S. ebulus* extracts in the field of human health and phytopathology should be complemented by further studies.

Peer-review: Externally peer-reviewed.

Informed Consent: Written consent was obtained from the participants.

Author Contributions: Conception/Design of Study- S.D.K.; Data Acquisition- E.E.O., E.M.K., N.Y.B., O.T., M.N.; Data Analysis/Interpretation- E.E.O., E.M.K., N.Y.B., O.T., M.N.; Drafting Manuscript- S.D.K., E.E.O., E.M.K., N.Y.B., O.T.; Critical Revision of Manuscript- S.D.K.; Final Approval and Accountability- S.D.K., E.E.O., E.M.K., N.Y.B., O.T., M.N

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: This study was supported by a Çukurova University Fund (TSA-2019-11424).).

Acknowledgement: The authors would like to thank Dr. Isil Gazioglu who supported the LC-MS/MS analysis in this study.

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