ISSN 2395-650X

International Journal of Life Sciences Biotechnology Pharma Sciences

E-mail: editorijlbps@gmail.com editor@ijlbps.org

International Journal of Life Sciences Biotechnology Pharma Sciences

ISSN 2395-650X
Volume9 Issue4Dec2021

Analysis of Sambucus ebulus L. (dwarf elder) fruit extracts for their chemical composition and antioxidant potential, as well as their antibacterial activity against Trichophyton rubrum (Castell.) Sabour and other microorganisms

K.Anand kumar, P.Chandrashekar, .G.Thanusha, Karthan Pragathi

ABSTRACT

Purpose and Background: Sambucus ebulus L. has a long history of use in Anatolian traditional medicine. Based on traditional use, this research aims to explore the phytochemical composition, antibacterial activities, and antioxidant activities of S. ebulus fruit extracts, as well as their antifungal capabilities against Trichophyton rubrum (Castell.) Sabour.

The fruits of S. ebulus were used to make two different extracts. We used LC-MS/MS to determine the phytochemical makeup of the fruit extracts of S. ebulus. We used the broth microdilution technique to test the antibacterial activity against a panel of microorganisms. Furthermore, the disc diffusion technique was used to assess the antifungal activity of S. ebulus extracts against three yeasts and T. rubrum in vitro.

The main components found in the dried fruit methanol extract (DFM) were identified as hederagenin (5.38±0.4949 µg/g) and fumaric acid (3.06±0.0275 µg/g). The most prevalent ingredient in the fresh fruit juice (FFJ) was found to be fumaric acid, with a concentration of 3.97±0.0357µg/g. The extracts included hitherto unseen compounds including acacetin, chrysin, eupatilin, hederagenin, isosakuranetin, myricitrin, and rhamnocitrin. Both E. coli and Candida tropicalis were moderately inhibited by DFM, with MIC values of 625 mg/L and 312.5 mg/L, respectively. Both extracts were ineffective against Staphylococcus aureus and Proteus mirabilis, with MIC values of 1250 mg/L; however, T. rubrum proved to be resistant to both extracts. The antioxidant capacity of the extracts was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical cleaning technique. With an IC50 value of 5.941±0.236 µg/mL and 7.893±0.939 µg/mL, respectively, DFM and FFJ demonstrated potent antioxidant activity against DPPH radicals.

In conclusion, our findings demonstrated that S. ebulus fruits could not be used in antifungal topical formulations, despite the fact that locals use them to cure nail fungus (onychomycosis). Furthermore, the antibacterial activity outcome is consistent with previous research in this area.

Antifungal activity, antioxidant activity, Sambucus ebulus, LC-MS/MS, and Trichophyton rubrum are all terms that relate to this study.

INTRODUCTION

Two species, Sambucus nigra and Sambucus ebulus, have been documented as being native to Turkey (Scopel et al., 2007; Senica, Stampar, & Mikulic-Petkovsek, 2019). The genus Sambucus is part of the Adoxaceae family and has thirty species worldwide. A shrub species, S. ebulus L. is found all throughout southwestern Asia (particularly in Iran and Turkey) and southern and central Europe (Shokrzadeh & Saravi, 2010). It goes by popular names like elderberry and dwarf elder.

In traditional Turkish medicine, the various parts of S. ebulus, including the roots, stems, barks, leaves, flowers, and fruits, have a long history of use in treating a variety of ailments, including common colds and coughs, arthritis, edema, rheumatic diseases, constipation, infected wounds, eczema, burns, urticaria, hemorrhoids, and bee stings (Demirci & Özhatay, 2012; Kültür, 2007; Tuzlacı & Tolon, 2000; Yesilada, 1997). As a result of its widespread medicinal use, S. ebulus is known as "hekimana" in Anatolian folklore (Jabbari, Daneshfard, Emtiazy, Khiveh, & Hashempur, 2017; Yesilada, Gürbüz, & Toker, 2014). The first report of using S. ebulus fruits to cure nail fungal infections was in this research (Demirci & Özhatay, 2012). This extraordinary information gathered from rural Kahramanmaras, Turkey, motivated us to conduct the present investigation.

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).

Many investigations on the biological activities of these plants have sought to uncover their novel medicinal uses. 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.

Interestingly, researchers have looked at the ethnopharmacological uses of S. ebulus in addition to its chemical makeup and biological activity.

According to one of them, S. ebulus was historically used to treat coughs, snake bites, and gastrointestinal issues (Kültür, 2007). In Kahramanmaraş, Southern Turkey, a different research by Demirci and Ozhatay (2012) found that S. ebulus fruits are used to cure rheumatism, nail fungus, hemorrhoids, and other similar conditions.

This research was the first to disclose the traditional use of S. ebulus fruits as a treatment for nail fungal infections (Demirci & Özhatay, 2012). Our motivation to conduct this research stems from the exceptional insights gained from rural Kahramanmaras, Turkey.

The fungus Trichophyton rubrum is the most common cause of nail fungus, which is also known as onychomycosis or tinea unguium.According to Lipner and Scher (2019), it may lower quality of life due to pain, discomfort, cosmetic issues, limits in everyday and social life, and so on. According to research by Demirci and Ozhatay (2012), inhabitants in the area have been using crushed fresh fruits as a remedy for onychomycosis by putting them to affected nails. They will do it again in two or three days after the nail has healed completely. In light of the traditional usage of S. ebulus fruits in Kahramanmaras, Turkey, this research aims to determine their chemical composition as well as their antioxidant, antifungal, and antibacterial activity.

Some studies have linked onychomycosis to inflammation around the nail bed, while others have shown it to be linked to low-grade systemic inflammation (Duhard, 2014; Shi et al., 2016; Sinikumpu et al., 2018). According to Balea, Pârvu, Pop, Marín, and Pârvu (2018), oxidative stress is a component of the inflammatory response, which is a wound healing process. The cell membranes allow reactive oxygen species (ROS) unrestricted circulation. Next, reactive oxygen species (ROS) harm lipids, proteins, DNA, and RNA.According to Andreicuț et al. (2018), this leads to harm either locally or across the body. Additionally, additional infections may result from untreated onychomycosis (Gupta, Versteeg, & Shear, 2017). In light of these facts, onychomycosis therapies should think about oxidative stress as an additional target (Pârvu et al., 2019).Plants' medicinal benefits stem from anti-freeze chemical components found in their essential oils and extracts (Granato et al., 2018; Morais et al., 2013). According to Bakkali, Averbeck, Averbeck, and Idaomar (2008) and Nogueira et al. (2020), traditional medicine often employs extracts and essential oils as antimicrobial agents because of their antiseptic and antioxidant properties.

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MATERIAL AND METHODS

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

Distillation and separation

We split the fresh fruit in half, dried half in the shade at room temperature, and then used a shaker set at 25 ºC for 24 hours to extract the other half with a mixture of 50:50 methanol and water. Until all of the samples were used up, the process was repeated four times. Following the filtering process, the solvent was extracted using rotary extraction, and the water was extracted using lyophilization. Until the analysis, the extract (DFM) was kept at -20 ºC. We pressed the other portion, which was the fresh fruit.

LC-MS/MS research

Measurements were taken using LC-HRMS (Liquid Chromatography High-Resolution Mass Spectrometry) instruments in ESI Source, namely a Thermo Orbitrap Q-Exactive. The validation parameters of the approach were provided by Gülçin et al. (2010) (Gülçin, Bursal, Şehitoğlu, Bilsel, & Gören, 2010; Han, Yilmaz, & Gulcin, 2018), and the method that had been verified was used for the analysis.

Table 1 shows the results of the minimal inhibitory concentration (MIC) tests conducted on various bacteria. Following the guidelines laid down by the Clinical Laboratory Standards Institute (CLSI) (CLSI, 1997; CLSI, 2020), the antimicrobial activity was determined using the broth microdilution method. One medium was RPMI-1640 (Applichem, Darmstadt, Germany) for antifungal activity and the other was Mueller-Hinton broth (Oxoid) for antimicrobial activity. The extract was prepared in the medium by creating a series of twofold dilutions, with concentrations ranging from 2500 mg/L to 1.2 mg/L. The ultimate concentration of bacteria in the 96-well plate was 5×105 CFU/mL, whereas yeast had a concentration ranging from 0.5×103 to 2.5×103 CFU/mL. To stop the 96-well plates from drying out, we covered them and put them in plastic bags. The bacteria were cultured at 35ºC for 18–20 hours, while the yeast strains were incubated at the same temperature for 46–50 hours. We defined the minimum inhibitory concentration (MIC) as the concentration of a chemical at which all observable growth was completely inhibited. We tested the solvents' antibacterial capabilities against test microbes as a control. The values of the controls were used to assess the findings.

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.

The disc diffusion technique for assessing antifungal susceptibility

Following the CLSI M38-A protocols, an in vitro antifungal assessment was conducted using a standard Trichophyton rubrum (ATCC 28188) isolate. A membrane filter was used to sterilize the RPMI 1640 medium (Applichem, Darmstadt, Germany), which was then buffered to a pH of 7.0 using morpholinopropanesulfonic acid (MOPS; Applichem, Darmstadt, Germany). A solution of 200 mL of distilled water containing 2% glucose and 2% agar was autoclaved to ensure its sterility. The agar solution and RPMI 1640 medium were mixed in a water bath at temperatures ranging from 45 to 50°C. After pouring the liquid onto disposable petri dishes, it was allowed to cool to room temperature.

Following the CLSI M38-A guidelines, the inoculum was created. A conidium concentration of 1–5x106 was achieved by diluting the T. rubrum solution. Agar plates with 2% glucose RMPI 1640 were spread with the inoculum, and then they were dried for 15 minutes. In the center of the agar plates, a hole measuring 2 mm in diameter and 4 mm in height was punched. Using a sterile spatula and forceps, the extracts were carefully added to this hole in a series of twofold dilutions ranging from 10,000 mg/L to 1.2 mg/L. To measure fungal growth and zone width of inhibition, plates were incubated at 26°C for 4–7 days. The inhibitory zone width was evaluated using 10 µL of Liofilchem, a reference medication for ketoconazole, in accordance with CLSI guidelines (CLSI, 1997; CLSI, 2020). Technique for scavenging 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals

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ISSN 2395-650X
Volume9 Issue4Dec2021

The DPPH radicals scavenging technique, first developed for 96-well plates by Blois (1958) and updated by Brand-Williams et al. (1995), was used to assess the antioxidant activity of S. ebulus extracts. Methanol was used to make dilutions, and ultra clean water was used to dissolve the extracts. An ascorbic acid solution was used as a control group member (Aldrich, St. Louis MO, USA). There were no S. ebulus extracts in the control group. A 96-well plate was used to combine 150 µL of extracts or standards with 50 µL of a recently prepared 0.1 mM DPPH radical solution (Aldrich, St. Louis MO, USA) in methanol. After a minute of shaking using the microplate reader (a Multiskan™ Sky Microplate Spectrophotometer manufactured by Thermo Scientific, located in Waltham, MA, USA), the plates were left to incubate at room temperature in the dark for 45 minutes at a wavelength of 517 nm. The formula to determine the percentage of DPPH scavenging activity was as follows: % DPPH Scav. Act. = [(AControl − ASample)/AControl] × 100.

Data analysis using statistics

Experiment data were presented as means plus or minus standard deviations (mean ± SD). For a total of four iterations, the experiment was conducted. An analysis of variance ($p<0.05$) was used to evaluate the data in Microsoft Office Excel. The data was statistically evaluated using Tukey multiple comparisons and a one-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

LC-MS/MS analysis

Using LCMS/MS, the present research investigated the phytochemical contents of S. ebulus fresh fruit juice (FFJ) and dried fruits methanol extract (DFM) obtained from Kahramanmaraş, Turkey. Table 2 displays the outcomes of the LC-MS/MS investigation. The DFM extract included ten different chemicals. The secondary metabolites that were found in the DFM in the highest concentrations were fumaric acid (3.06±0.0275 µg/g) and hederagenin (5.38±0.4949 µg/g). Furthermore, the DFM extract was the only one that contained hederagenin.

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

The following chemicals were found in DFM: 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). Nine chemicals were identified in the FFJ extract of S. ebulus. Among the dicarboxylic acid derivatives, fumaric acid is most abundant in FFJ. 3.97±0.0357 µg/g. The extract also contained the following compounds: (-)-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).

The chemical makeup of S. ebulus fruits has been the subject of a small number of investigations. An LC-PDA-MS approach was used to examine the ripe fruits' acetone extract in an earlier investigation. In the research conducted by Vankova, Todorova, Kisselova-Kaneva, and Galunska (2019), it was shown that the extract included epicatechin (0.84 mg/100 g FW: fresh weight), quercetin (0.15 mg/100 g FW), and kaempferol (0.05 mg/100 g FW).

The secondary metabolites in an HPLC-extracted water extract of S. ebulus fruits were the subject of an additional investigation. According to Cvetanović et al. (2018), the fruit water extract of S. ebulus contained the following compounds: 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). The main ingredient found in the S. ebulus fruit extract was identified as quercetin-3-rutinoside (421.14±5.15 mg/kg FW) in an additional investigation carried out by Mikulic-Petkovsek et al., which used HPLC-DAD-MS for analysis. 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). Cvetanovic et al. (2016) found that S. ebulus fruits contained thirteen different chemicals when they used HPLC-DAD. The fruits contained synthaptic acid (1.291 µg/mL), quercetin (1.407 µg/mL), and rutin (6.453 µg/mL), according to the research. The following compounds were identified: 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).

Assessment of the antibacterial efficacy in vitro

The present study utilized the broth microdilutions method in accordance with the recommendations of the Clinical Laboratory Standards Institute (CLSI) to preliminarily screen the antibacterial and antifungal activity of six different microorganisms. These included four Gram-negative bacteria (P. aeruginosa, E. coli, P. mirabilis, and K. pneumoniae), three Gram-positive bacteria (S. aureus, S. epidermidis, and E. faecalis), and three yeast species (C. albicans, C. tropicalis, and C. parapsilosis) for antifungal activity. The standard drugs were prepared according to the CLSI's recommendations (CLSI, 1997; CLSI, 2000).

The year 2020. Table 3 summarizes the MIC values. Combined with MIC values, the data revealed that the majority of the drugs tested had very little antimicrobial action against the bacteria under investigation.

All of the tested extracts proved ineffective against the test-cultures of Pseudomonas aeruginosa, Enterobacter faecalis, Klebsiella pneumoniae, Staphylococcus epidermidis, Candida albicans, and Candida parapsilosis. The MIC values for P. mirabilis and S. aureus were 1250 mg/L, however all of the extracts tested showed action against these bacteria. Extract DFM exhibited moderate antibacterial activity against E. coli (MIC: 625 mg/L) and C. tropicalis (MIC: 312.5 mg/L), as shown in Table 3. At every concentration examined, T. rubrum showed no antifungal activity against either DFM or FFJ extracts.

The literature review revealed a dearth of research on the antibacterial properties of S. ebulus fruits. Among these studies, one used the well diffusion method to examine the antimicrobial activities of chloroform, acetone, hexane, water, and methanol extracts of S. ebulus fruits against various bacteria and fungi. According to Ginovyan and Trchounian (2017), the extracts were ineffective against the strains tested, in contrast to gentamicin and nystatin. A different research used the microbroth dilutions technique to assess the antibacterial activity of S. ebulus fruit extract against Staphylococcus aureus, S. epidermidis, E. coli, Klebsiella pneumoniae, P. aeruginosa, Proteus mirabilis, and Candida albicans. Research by Meriç, Bitiş, Birteksöz-Tan, Turan, and Akbuga (2014) found that the extract had moderate antifungal activity against Candida albicans but no effect against the other strains. The other study used disc and well diffusion methods to examine the antibacterial and antifungal activity of S. ebulus fruit extract against various bacteria and fungi, including S. aureus, Bacillus cereus, B. subtilis, E. coli, Enterococcus faecalis, Pseudomonas fluorescens, Botrytis cinerea, Phytophthora infestans, and Rhizochtonia solani. It was shown that the well diffusion approach yielded superior outcomes compared to the disc diffusion method. Findings showed that the extract was effective against S. aureus, B. subtilis, E. fecalis, and P. fluores, with activity increasing with increasing dosage, and it was resistant to strains of B. subtilis and E. coli (Rodino et al., 2015). The present study's findings on antibacterial activity appear to be consistent with those found in the literature.

Assessment of the effectiveness of antioxidants

This research measured antioxidant capability using DPPH, a technique for clearing free radicals. This colorimetric approach quantifies the amount of hydrogen or electrons transferred from a radical solution, which causes its color to change from purple to yellow (Braham et al., 2020; Do et al., 2014).

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ISSN 2395-650X
Volume9 Issue4Dec2021

Because it can identify antioxidant components at low quantities, this approach is quite sensitive. Additionally, as a first-step scanning method, it may examine many samples concurrently (Meza, Rojas, Cely-Veloza, Guerrero-Perilla, & Coy-Barrera, 2020). Antioxidant standards are based on a wide variety of chemicals. Ascorbic acid's high scavenging action makes it the gold standard (Al-Rifai, 2018). When compared to ascorbic acid, Table 4 displays the findings of the DPPH radical scavenging activity of S. ebulus extracts.

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.

Using a logarithmic graph that plotted the percentage of radical scavenging capacity against the sample concentration, antioxidant metrics such as IC50, EC50, ARP, and AEAC values were determined. After determining the IC50 value, the AEAC was computed using the following formula: 'AEAC=(IC50(AA)/IC50(sample)) × 105'. The calculation clearly shows that the AEAC value is not in units. According to Kedare and Singh (2011), an antioxidant is considered more potent when the IC50-EC50 value is low and the ARP-AEAC value is high. Here is the ranking of S. ebulus extract antioxidant potency according to antioxidant parameters: According to Table 4, DFM had an IC50 of 5.941±0.236 µg/mL, FFJ had an IC50 of 7.893±0.939 µg/mL, and ascorbic acid had an IC50 of 9.6880±1.02490 µg/mL. Research on the DPPH-based antioxidant activity of S. ebulus fruit has shown conflicting IC50 values. According to a study by Ebrahimzadeh et al. (2009), the DPPH radical scavenging activity was demonstrated by the aqueous and methanol extracts of S. ebulus fruits obtained from the Mazandaran forest in Iran. The IC50 values for the former were 202.50 ± 1.38 µg/mL and 723.62 ± 3.36 µg/mL, respectively (M. A. Ebrahimzadeh, Ehsanifar, & Eslami, 2009). The ethanol extract (70%) of S. ebulus fruits gathered in Romania shown strong DPPH free radical scavenging activity, according to another research conducted by Rodino et al. (2015). The EC50 value for this activity was 68.45 ± 0.441 µg/mL. Topuzović, Stanković, Jakovljević, and Bojović (2016) found that S. ebulus fruit extracts from Serbia showed DPPH scavenging activity with an IC50 value of 128.23 ± 0.65 µg/mL in the aqueous extract and 82.15 ± 0.33 µg/mL in the methanol extract. In their 2014 research, Meric et al. found that the methanol extract of S. ebulus fruits gathered in Istanbul, Turkey exhibited DPPH radical scavenging activity with an IC50 value of 8.895 ± 1.391 mg/mL.

Synopsis of the debate

Using different plant materials obtained from different places and different procedures for extracting the S. ebulus fruits might be the explanation for the found variations between the present research and the other investigations.

Additionally, the current study's findings suggest that S. ebulus extracts may have greater antioxidant activity than the reference ingredient, ascorbic acid. Glassman et al. (2003) created a topical formulation for the treatment of onychomycosis (nail fungus) that included urea and an antioxidant ingredient. According to the study's findings, nail fungus therapy is more effective when an antifungal drug and an antioxidant ingredient are used together. In 2003, Glassman et al. were granted a patent for their idea. The study shows that air pollutants, UV light, chemical oxidants, and aerobic microbes are all part of the oxidative environment that a nail is constantly exposed to, which may cause photooxidative damage. According to Glassman, Bhagwat, and Glassman (2004), the antioxidant component safeguards the stability and permeability of the nail cell membrane. The latest research backs up this claim and suggests that S. ebulus fruits may help cure nail fungal infections because of their high antioxidant activity.

Locals in Kahramanmaras, Turkey, have long relied on the fruits of the S. ebulus tree to alleviate the symptoms of onychomycosis, a kind of nail fungus.

Thus, the purpose of this research was to determine if S. ebulus fruits had any therapeutic potential against Trichophyton rubrum, a fungus that causes nail fungus. On the other hand, S. ebulus fruit extracts were ineffective against T. rubrum in terms of antifungal activity. However, findings showing antioxidant activity showed that the extracts are very effective in protecting against free radicals. The use of antioxidant chemicals in topical treatments for onychomycosis (nail fungus) has been reported in the literature.

Finally, although S. ebulus fruits did not exhibit antifungal action against T. rubrum, their antioxidant characteristics may be effective in treating onychomycosis, a fungal infection of the nails, and they might be a good addition to topical antifungal compositions. Furthermore, the results of the studied organisms' antibacterial activity are consistent with the findings of research conducted in this area so far. Part two of the investigation revealed that some chemicals, including acacetin, chrysin, eupatilin, hederagenin, isosakuranetin, myricitrin, and rhamnocitrin, were first identified in S. ebulus fruits when measured using LCMS/MS. Traditional medicinal plant uses should serve as an inspiration for pharmacological activity investigations, which we wholeheartedly support.

CONCLUSION

Research on the phytochemical components of the extract revealed that samples of S. ebulus had a strong antioxidant effect. These results provide credence to the traditional usage of S. ebulus for the treatment of onychomycosis and other dermatophyte diseases.

Finally, more research is needed to confirm the efficacy of S. ebulus extracts in human health and phytopathology.

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