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In vitro antioxidant, antimicrobial, anticholinesterase activities, and phenolic and heavy metal compositions of *Prunus avium* fruit extracts

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Abstract

Prunus avium L. (*P. avium*) is one of the most admired fruits for its organoleptic and nutritional value. This research examined cherry fruit chloroform and methanol extracts antioxidant, anticholinesterase, antibacterial, and chemical and elemental abilities. The extracts antioxidant capacity was assessed by evaluating total phenolic and flavonoid content and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. *P. avium* fruit chloroform (Chl) extract had the greatest phenolic and flavonoid content. However, the methanol (Me-OH) extract exhibited stronger radical-scavenging activity. The extracts antibacterial activity was determined using the MIC method, and the enzyme inhibition activity was ascertained using a spectroscopic technique. Both extracts inhibited *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), and *Candida albicans* (*C. albicans*) growth at low concentrations better than standard antibiotics. The phenolic chemicals that could be causing the biological activity in the extracts were identified using LC-MS/MS. Major components in methanol extract were cichoric acid, chlorogenic acid, and citric acid, while in chloroform extract vanillic acid, cichoric acid, and chlorogenic acid. Additionally, the presence of zinc, copper, mercury, iron, nickel, arsenic, cadmium, lead, and selenium in *P. avium* fruits was investigated by ICP-OES Optical Emission Spectrometry. Mercury, nickel, and selenium weren't detected in the sample, while arsenic was beyond permitted levels. The fruit of *P. avium* may have potential biological and industrial applications owing to its rich biochemical and elemental composition and its antioxidant, antibacterial, and anticholinesterase capabilities.

Keywords: Anticholinesterase activity, antimicrobial activity, heavy metal composition, phenolic composition, *prunus avium*

Introduction

Plants are essential in the identification of new pharmacological molecules that possess therapeutic effects. They serve as a distinct reservoir of secondary metabolites found in plants and are used as medical food additives, tastes, and other industrial applications. Because these secondary metabolites are important for the economy, there has been a lot of interest in finding ways to make them more plentiful and easier to make using tissue culture and other farming methods in recent years [1]. Oxidative stress is caused by a disparity between generating reactive oxygen species (ROS) and eliminating them, resulting in elevated ROS concentrations. Elevated levels of ROS result in a decline in cellular functionality. ROS induces cellular macromolecular damage by lipid peroxidation, alterations in nucleic acids, and protein modifications [2]. ROS formations are regarded as a pathobiochemical mechanism implicated in the initiation or advancement of several diseases, including atherosclerosis, ischemic heart disease, diabetes, carcinogenesis, or the onset of liver disease [3]. Secondary metabolites that plants generate have been the focus of a significant amount of study in recent years due to their antibacterial and antioxidant characteristics [4]. Plants are the source of most of the bioactive components found in food, and the chemicals derived from plants are referred to collectively as phytochemicals. As a result of the fact that the great majority of these phytochemicals are molecules that demonstrate redox activity, they are classified as antioxidants. Free radicals and

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other reactive forms of oxygen and nitrogen, which have been demonstrated to be the root cause of a wide variety of chronic illnesses, may be neutralized by antioxidants, which can reduce their harmful effects [3,5]. It is generally accepted that antioxidants obtained from food can function as antioxidants in vivo and provide favorable health benefits via a variety of pathways, including inducers of antioxidant defense, lifespan, cell care, and DNA repair-related processes [6]. Typically, plants either synthesize novel compounds or increase the levels of pre-existing molecules. Plants can produce several important bioactive chemicals, such as quercetin, camptothecin (CPT), morphine, epicatechin-3-gallate, catharanthine, resveratrol, and kaempferol. Plant secondary metabolites (PSM) are plant defense chemicals that are functionally anti-inflammatory and antioxidative. The majority of PSM that are generated under diverse abiotic stress situations are plant defense chemicals. Terpenoids are shown to be the most abundant category of PSM chemicals, followed by alkaloids and phenolic compounds [7]. Various methods can be employed to evaluate the quantity of polyphenols found in food and beverages. Each of these approaches has advantages and disadvantages. Antioxidants can be either naturally present or artificially produced. The food, cosmetics, and pharmaceutical industries have long been utilizing natural antioxidants. Exclusively natural sources are utilized to manufacture natural antioxidants. Nevertheless, synthetic antioxidants are substances generated through chemical processes [8]. For these reasons, researchers interest in plant sources that contain natural antioxidants has increased. One of the most widely studied plants is *Prunus avium L. (P.avium)* which is a tree. The subspecies *avium*, genus *Prunus*, is an Asian-born leafy tree belonging to the subfamilies *Prunoideae* and *Rosaceae* [9]. The fruit of *P. avium*, more known as sweet cherry, is one of the most popular temperate-climate fruits, although it is loved around the world. It is characterized by being rich in bioactive ingredients, high water content, and low-calorie levels. Cherries are nutritious foods that are abundant in phenolic compounds, which are known to have a role in the prevention of a variety of chronic illnesses [10]. About 60–74% of the phenolic substance ratio is made up of flavonoids, anthocyanins, hydroxycinnamic acids, and catechins (flavan-3-ol), which are the main parts. The phenolic compounds found in cherries have been proven to have a strong link with antioxidant activity, according to the findings of researchers [11,12].

The study demonstrated the presence of antioxidant, antimicrobial, and anticholinesterase properties in the crude methanol and chloroform extracts derived from *P. avium* fruits. In addition, the presence of phenolic in the structure of the plant as well as the concentration of metals that may display these effects were taken into consideration.

Material and Methods

Materials

Fruit samples of *P. avium* were taken from the Zınnar Valley (Mardin, Türkiye) between March and June 2022. The samples were prepared for analysis by the principles of the method to be used.

The plant material was dried at room temperature for 7 days. Dried plant material was pulverized in a porous mill with a 2 mm diameter. For the extraction process, 30 g of the dried and powdered plant sample was weighed and placed into a soxhlet cartridge. The solvent compartment was initially filled with 300 mL of chloroform to carry out polarity-dependent fractionation. The plant sample was processed in a Soxhlet device for extraction for 4 hours. After the resulting extract was filtered, the same processes were repeated using methanol. Thus, two different fractionated plant extracts were obtained. A rotary evaporator (Heidolph 4000 Rotavapor) separated the obtained plant extracts from their solvents. Crude extracts were prepared by removing the extracts from their solvents after keeping them at low temperatures and under vacuum. Stock solutions were prepared at concentrations of 1 mg/mL for DPPH radical scavenging activity and total phenolic content measurements and 5 mg/mL for total flavonoid content determination.

Methods

Antioxidant Assay

Quantification of the total phenolic content

The solutions made from gallic acid and extracts were mixed with 40 µL and 1160 µL of pure water and 200 µL of 2.0 N FCP reagent, respectively. Afterward, 600 µL of a 20% solution of sodium carbonates was added to the mixture and shaken for 2 hours. UV-Vis spectrophotometry was used to measure the absorbance values at a wavelength of 765 nm [13].

Evaluation of the quantity of the total flavonoid component

The modified Moreno method determined the total amount of flavonoid components in cherry fruit extracts [13,14]. Quercetin was used as the standard flavonoid compound. A 5 mg/mL stock solution was prepared from cherry fruit chloroform and methanol crude extracts. For total flavonoid quantification, 0.1 mL of 10% aluminum nitrate, 1 M potassium acetate, and 3.8 mL of ethanol were added to the reaction medium. Finally, 1 mL of quercetin/plant solution was added to the 25°C water bath for 40 minutes, and the absorbance values for the color change were measured at 415 nm.

Assessment of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

Briefly, 3 mL of plant extracts prepared for this test at different concentrations (5-500 g/mL) and 1 mL of DPPH solution (0.1 mM) were added to the tubes. The mixture was kept at room temperature in darkness for 30 minutes and the absorbance values of samples at different concentrations were measured at 517 nm [13].

Antimicrobial Assay

Preparation of microorganism cultures and application of the minimum inhibitory concentration (MIC) test to cultures

Gram-positive *Staphylococcus aureus (S. aureus)*, gram-negative *Escherichia coli (E. coli)*, and *Candida albicans (C. albicans)*

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yeast were used for the antimicrobial assay. Yeast cultures were permitted to develop on RPMI broth, whereas bacterial cultures were allowed to grow on Muller-Hinton broth (MHB). After being incubated for a while, the organisms were put into a physiological saline solution that was 10 milliliters in size. The optical density readings were then compared to a McFarland standard of 0.5. MIC was determined by using bacterial solutions that contained 5x105 colony-forming units (CFU) per milliliter [15].

Assessment of antimicrobial activity

Antimicrobial activity minimum inhibition concentration was determined using the microdilution technique [15]. Muller Hinton liquid media were used for bacteria and RPMI liquid media were used for fungi (yeast). In this method, 100 µL of medium was added to the 96-well microplate wells (leaving the 11th well empty). 100 µL of plant solution was added to the first well. The homogeneity of the medium and plant extract was ensured with the help of a pipette. 100 µL was taken from the 1st well to the 2nd well and mixed. This process was performed for the remaining 8 wells and a series of microdilution were performed. 100 µL was taken from the 10th well and discarded. Mixtures with a volume of 100 µL were obtained in all wells except the 11th and 12th wells. 11. The well was left empty for sterilization control. The microorganism suspension prepared according to the McFarland standard 0.5 turbidity value was added to all wells, leaving well 11 empty. In the 12th well, only the medium and microorganism cultures were left for growth control. To assess the antibacterial properties of plant extracts, stock solutions with a concentration of 128 mg/mL were made using antibiotics obtained commercially. Vancomycin was used for gram-positive bacteria, colistin for gram-negative bacteria, and fluconazole for *C. albicans* yeast. The method mentioned above was also used for antibiotic solutions. The microplates were placed in an incubator and kept (37°C) for 24 hours. The first well, where growth began, was identified as the minimal inhibitory concentration.

Determination of anticholinesterase activity

The Ellman (1961) method was used to determine the Acetylcholinesterase (AChE) inhibitor activities of extracts (chloroform and methanol) from *P. avium* fruits [16]. As an enzyme source, the commercial version of AChE was used (EC 3.1.1.7, Sigma). The substrate for cholinergic reactions was Acetylthiocholine iodide (AChI). To initiate the reactions, 50 µL of AChE enzyme solution ($5.32x10^{-3}$) was combined with 10 µL of Tris/HCl buffer (1.0 M, pH 8.0) and *P. avium* chloroform and methanol extract solutions (0.00–0.25 µg/mL). To create stock solutions, all materials were dissolved, and the concentration was adjusted to 4000 µg/mL. To initiate the reaction, 20 µL AChE, 10 µL extract sample, and 150 mL sodium phosphate buffer (100 mM; pH 8.0) solutions were mixed. After 15 minutes of incubation at 25°C, 10 µL of Ellman's Reagent (DTNB) was added. After that, 10 µL of AChI was added to start the reaction. The tested solutions had a final concentration of 200 µg/mL.

The substrate's hydrolysis was quantified at a wavelength of 412 nm using a Biotech Power Wave XS. The interaction of DTNB with thiocholine resulted in the creation of a yellow 5-tyo-2-nitrobenzoate anion (n=3). Tacrin was employed as a reference substance for comparison.

Phenolic Component Identification with LC-ESI-MS/MS

Materials and reactants

The phenolic standards, ammonium formate, acetonitrile (ACN), formic acid, filter (0.45 micrometers), and solvents were obtained commercially (Sigma-Aldrich, Germany).

To enhance the analytical procedure, all commercially available phenolic compounds were employed. The main stock solution was 1000 mg/L; solid standard compounds were made by dissolving them in methanol. Methanol and water were used to dilute the produced stock solution (1:1). An instrument called an LC-ESI-MS/MS was used to analyze the final solutions.

Mass spectrometry and chromatography conditions

The compound qualitative examination was carried out using high-performance liquid chromatography (HPLC), a tandem mass spectrometer, and the 1260 Infinity II LC System model. Chromatographic parameters have been adjusted to minimize pressing effects and provide the greatest possible separation for the chemical. An Agilent Poroshell 120 EC-C18 type analytical colon (100 mm x 3.0 mm, 2.7 um) was used for chromatographic separation. A temperature of 25°C was chosen for the column. The Elusion Graduate was set up with a solvent flow rate of 0.250 mL/min and a flow volume of 5 µL for Selective A (5 mM ammonium format in water) and Selective B (0.1% formic acid in acetonitrile). A typical mixture consisting of 31 different compounds was injected into the device, which was then used to record the release timings of each chemical.

Determination of heavy metal contents

Following the elimination of potentially hazardous substances, the plant samples underwent meticulous drying in a well-ventilated area, shielded from direct sunlight, before their preparation for utilization. With the use of a mill, the plant samples were completely ground. After that, around 10 g of the samples were weighed and put into porcelain crucibles. After this procedure, the samples were left on the surface for one night at 100°C until the smoke emission was finished. Before the collection of white ash, baking was conducted in a muffle furnace at a temperature of 525°C for three to five hours. Subsequently, the samples containing carbon residue were exposed to heating at a temperature of 525°C for one to two hours, after their combination with 0.5 to 3.0 milliliters of nitric acid. Finally, the ash was dissolved using 3 mL of nitric acid with a concentration of 65%, followed by 1 mL of hydrochloric acid with a concentration of 37%. The measurement reliability was evaluated using Certified Reference Material (CRM).

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In the samples of *P. avium* fruits, the ICP-OES Optical Emission Spectrometer was used to determine the concentrations of the elements zinc (Zn), copper (Cu), mercury (Hg), iron (Fe), nickel (Ni), arsenic (As), cadmium (Cd), lead (Pb), and selenium (Se).

Results

Quantities of Plant Extracts

P. avium fruits were extracted with chloroform and methanol, respectively, resulting in 1.251 g of fractioned crude extract from the phase of *P. avium* chloroform (polarity index: 4.4) and 2.283 g from the methanol phase (polarity index: 6.6).

Quantification of Total Phenolic and Flavonoid Content

Polyphenols are recognized as the predominant and most abundant category of secondary metabolites in the plant kingdom. These chemicals are intriguing because of their production pathways and exceptional characteristics. Fruits and vegetables found in nature are the primary sources of these chemicals [17]. A graph was created to display the absorbance values for the concentration range of gallic acid standards, ranging from 50 to 500 µg/mL (Figure 1). The following equation was obtained by drawing the standard curve (R^2=0.997).

Absorbans (A) = 0.002 x [GAE] – 0.035

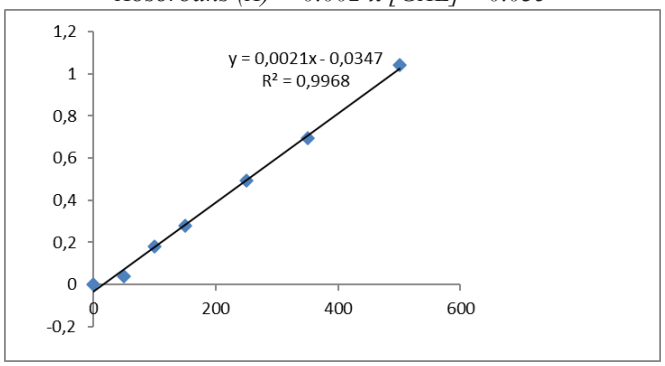

Figure 1. Measurement curve of total phenolic component quantity

Using these equations, the total amounts of phenolic components contained in chloroform and methanol extracts for *P. avium* were calculated as Gallic acid equivalent (GAE). In the study, the extracts obtained as a result of the fractioning process from a low-polar solvent to a polar solvent were determined according to the Folin-Ciocalteu method modifying the determination of total phenolic quantity [13]. Based on the results obtained, the raw extracts from the fruit parts of the plants used in the study were found to be higher in the chloroform extract with low polarity in the fractioning process in terms of quantities of phenolic and flavonoid components (Table 1).

Table 1. Total quantities of phenolic and flavonoid components in extracts obtained from *P. avium* fruits

Sample	The amount of total phenolic content (µg GAE/mg extract)		The amount of total flavonoid content (µg QE/mg extract)	
P. avium	Chl	Met-OH	Chl	Met-OH
	37.48±2.01	26.52±1.29	39.80±0.21	33.99±0.51

*1 mg per extract; the total phenolic component amount from the graph of the calibration curve drawn from the gallic acid equivalent (GAE) (A=0.002×[GAE]–0.035, R^2=0.999); and the total flavonoid component quantity per **5 mg per extract, using the calibrating curve from the quercetin-equivalent (QE) genre (A=0.015×[QE]-0.005, R^2=0.999), and were calculated by ±standard deviations (SD) (n=3)

500 µg/mL of quercetin in methanol were prepared in reserve solutions of 15 µg/mL, 30 µg/mL, 45 µg/ml, 60 µg/mL, and 75 µg/mL, and absorbance values measured at 415 nm wavelengths were translated into a graph (Figure 2). The following equation was obtained;

Absorbans (A) = 0.005 x [GAE] – 0.005

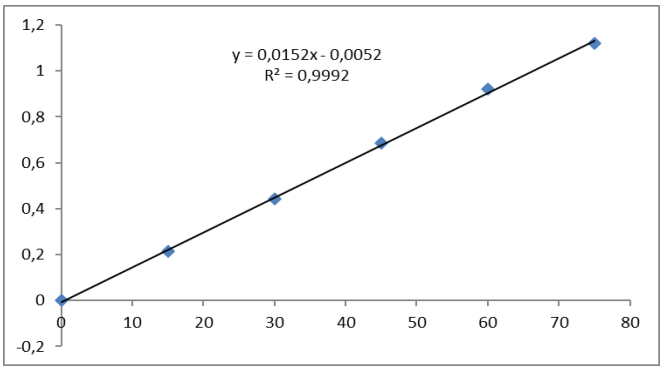

Figure 2. Total flavonoid component content measurement curve

The total amount of flavonoid components in the chloroform and methanol extracts of the *P. avium* plant used in the study was calculated as quercetin equivalent (QE). The quantified level of flavonoids, measured in terms of quercetin equivalence, was higher in the chloroform extracts of *P. avium* fruit (Table 1).

Evaluation of the Radical-Scavenging Ability of *P. avium* Extracts

The DPPH radical-scavenging activity of the plant extracts was measured to assess their capacity to neutralize free radicals. Both extracts, along with BHA and BHT as positive controls, were made in a series of solutions with concentrations ranging from 5 to 500 µg/mL. The wavelength at which their absorbance was measured was 517 nm.

The *P. avium* plant's chloroform extract revealed inhibitory percentages between 4.55% and 51.11%, while the methanol extract showed percentages between 3.98% and 56.15%. BHT

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showed inhibition percentages ranging from 19.98% to 93.67% within the investigated dose range, whereas the positive control, BHA, displayed inhibition percentages ranging from 40.24% to 98.37% (Table 2).

Table 2. The DPPH radical-scavenging capabilities of extracts and positive controls derived from *P. avium* fruit

		DPPH radical scavenging activity* (µg/mL extract)						
		5	**25**	**50**	**100**	**250**	**350**	**500**
Controls	**BHA**	40.24±0.08	87.70±0.09	90.78±0.53	95.57±0.37	98.46±0.09	98.46±0.00	98.37±0.00
	BHT	19.98±0.59	40.51±0.59	66.00±1.57	81.92±2.04	91.59±1.44	93.58±0.08	93.67±0.45
P. avium	**Chl**	4.55±0.16	15.52±0.39	16.96±0.79	21.95±2.43	26.94±0.88	43.13±0.44	51.11±0.44
	Me OH	3.98±0.52	4.88±0.59	12.30±0.07	15.91±0.81	31.74±0.09	42.95±0.09	56.15±0.90

*Each value was repeated three times (n=3) and ±standard deviations (SD) were calculated; BHA: butylated hydroxyanisole, BHT: butylated hydroxytoluene, Chl: chloroform, Me OH: methanol

Antimicrobial Activity

Since drug-resistant illnesses are becoming more common, it is critical to use standardized modern analytical methods to discover and separate new bioactive compounds from medicinal plants. Compounds derived from medicinal plants have the potential to provide novel and uncomplicated approaches to combating pathogenic microorganisms [18,19].

The results of the minimum inhibition concentration (MIC) of *P. avium* fruit chloroform and methanol extracts and commercial antibiotics known to be effective against pathogenic microorganisms used in the study were evaluated. It was determined that the inhibition effect of all tested extracts on pathogenic microorganisms was quite high compared to commercial antibiotics (Table 3).

Table 3. Antimicrobial effects of extracts from *P. avium* fruit on pathogenic microorganisms

Tested microorganisms		***P. avium* extracts**		**Standard antibiotics**
		Chl [mg/mL]	**Met OH [mg/mL]**	**Antibiotics* [mg/mL]**
Gram (+) strains	***S. aureus***	0.125	0.0625	1
Gram (-) strains	***E. coli***	0.0312	0.0156	2
Yeast	***C. albicans***	0.0156	0.0312	2

*Antibiotics: colistin (gram-positive bacteria), vancomycin (gram-negative bacteria), and fluconazole (*C. albicans* yeast)

S. aureus is a bacterial species that can rapidly reproduce on food and induce intoxication as a result of the toxins it generates. This pathogen is capable of inducing diseases in humans, including meningitis, septicemia, and delayed wound healing [20]. The investigation found that the methanol extract utilized exhibited significant inhibition against *S. aureus*, as seen in Table 3. *E. coli* is a bacterium that often resides in the intestinal microbiota. However, as the environment undergoes changes and presents favorable conditions for reproduction, the virus can acquire new traits. At first, it might cause severe and hazardous medical diseases in individuals, such as meningitis and gram-negative phenomena, which include urinary tract infections [21].

Anticholinesterase Activity

Cholinergic acetylcholinesterase breaks down ACh, which is a neurotransmitter in the peripheral nervous system, into choline and acetate by hydrolysis. The levels of ACh decline as a person ages, leading to the development of some neurological illnesses, including Alzheimer's disease (AD). Research indicates that AChE activity exhibits an increase during the first phases of AD. Therefore, it is hypothesized that the utilization of AChE inhibitors to augment cholinergic deficiencies might have a substantial influence on mitigating the progression of illnesses [22,23]. Acetylcholinesterase inhibitors have been replaced among the preferred drugs in the treatment of neurodegenerative diseases, with few side effects and beneficial results [24,25]. The inhibitory effect of chloroform and methanol extracts obtained from *P. avium* fruit on the acetylcholinesterase (AChE) enzyme was tested according to the Ellman method. As a substrate of the AChI reaction, DTNB (5,5-dithio-bis (2-nitrobenzoic) acid) was also used to measure anticholinesterase activity. The reversible cholinesterase inhibitor Tacrin (9-amino-1, 2, 3, 4-tetrahydroacridine) was used as the reference for comparison of our results as it was the first medication used for the palliative treatment of AD's.

The AChE inhibition percentages and IC_{50} values of methanol and chloroform extracts of *P. avium* fruits were calculated (Figure 3,4). It was found that the chloroform extract in *P. avium* showed higher enzyme inhibition activity. The IC_{50} value for Tacrin, which was used as a standard to block cholinergic enzymes, was found to be

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98.4 µg/mL (R^2=0.9904). Phenolic substances are known to inhibit cholinergic enzymes. Especially vanillic, ferulic, and caffeic acids are known to be very effective compounds in retarding neurodegenerative diseases [26]. This leads us to conclude that the primary phenolic found in the fruit extracts of *P. avium* acts as an inhibitor of the enzyme ACh.

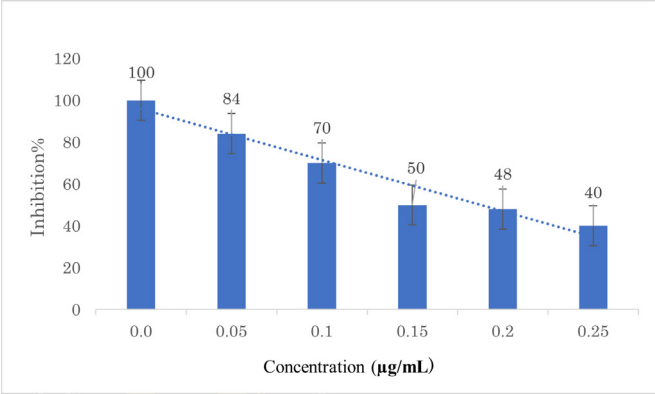

Figure 3. AChE inhibition % percentage of *P. avium* methanol extract

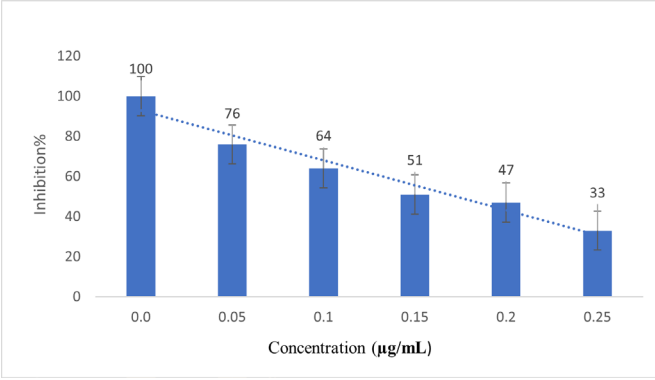

Figure 4. AChE inhibition % percentage of *P. avium* chloroform extract

Phenolic Component Analysis of *P. avium*, Fruit Methanol, And Chloroform Extracts with LC-ESI-MS/MS

P. avium fruit methanol and chloroform extracts were analyzed in terms of 31 standard phenolic compounds using the LC-ESI-MS/MS method (Table 4).

The main components of *P. avium* methanol extract were identified as cichoric acid (577.65 ng/ml), chlorogenic acid (160.89 mg/mL), citric acid (126.85) and vanillic acid, while the major components in chloroform extract are detected as vanillic acid (657.76 mg/mL), cichoric acid 563.08 mg/mL, chlorogenic acid (158.99 ng/mL) and 4-hydroxybenzoic acid (149.46 mg/mL). The % amounts of other components were given in Table 4.

Citric acid is a substance that may prevent certain nervous system degenerations, obesity, hepatoprotection, hyperlipidemia, and immunomodulation [27,28]. Additionally, the methanol and chloroform extracts of *P. avium* contained 4-hydroxybenzoic acid, which is one of the main catechins metabolites found naturally in coconut and in people who drink green tea, and epicatechin, which can stop the oxidative damage that comes with high blood pressure. Cichoric acid is a member of the phenylpropanoid family and is found in the roots of many plants. Many of these plants are widely used in traditional medicine practices and as a nutritional supplement [29]. Cichoric acid has crucial roles in terms of biological activity, especially due to its antioxidant, antiviral, and anticancer properties [29-31]. McDougall et al. (1998) reported that cichoric acid at different concentrations inhibited the entry and especially replication of human immunodeficiency virus [32]. Ferulic acid is an active compound against antioxidant, antimicrobial, anti-inflammatory, antidiabetic, and neurodegenerative disorders [33]. It is well known that the caffeic acid that is present in larger quantities in cherry chloroform extracts has effects that are comparable to those of other phenolic compounds, while at the same time exhibiting significant levels of anti-cancer and antibacterial activity [34-36]. Also, the vanillic acid found in cherry extracts is useful for treating pain, inflammatory illnesses, and neurological conditions. Also, phenolic acids have been shown to have several biological functions in addition to their beneficial antioxidant action. Caffeic acid is one of the most potent naturally occurring cinnamic acids. It can specifically halt the creation of leukotrienes, which are implicated in allergic responses, asthma, and immune system disorders. According to other research, several of caffeic acid's esters may have anticancer effects against the development of colon cancer [37].

Heavy Metal Composition Analysis

The amounts of some metals in the fruit samples of *P. avium* (cherry) were measured with ICP-OES Optical Emission Spectrometer. The amounts of As, Cu, Hg, Zn, Fe, Cd, Pb, Ni, and Se in the fruit samples of *P. avium* were measured. Measurement values, limits, and uncertainties were given in Table 5. The conformity of the measured values was assessed by the Turkish Food Code Infectious Persons Regulation [38].

In the *P. avium* sample that was measured, residues of lead, mercury, nickel, and selenium were not detected, while arsenic, cadmium, copper, and zinc were detected. The amount of arsenic (0.233 mg/kg) was determined to be above acceptable values. The non-constant weekly intake by FAO/WHO for Pb and Cd is 7 mg/kg/week. It is reported that Cd concentrations in herbal sources consumed for daily and medicinal purposes are between 0.731 and 1.121 mg/kg dry weight and the average value is 0.878 mg/kg [39]. Therefore, the concentrations of Cd found in the studied samples appear to be lower than the average permissible values in the literature. However, due to the constant use of this type of product, it is necessary to consume it carefully. All measurement values are given in detail in Table 5. The allowable concentration of Cu in edible crops by FAO/WHO is 3.0 mg/kg. However, there is no regulation for Cu levels in medicinal plants [40]. Cu concentration was found at 9.4 mg/kg.

Table 4. Results of *P. avium* methanol and chloroform extract quantification using LC-MS/MS

Quantitative results										Met OH	Chl
Compounds	**R.T**	**M.I (m/z)**	**T (m/z)**	**I.M**	**R^2**	**L.R (µg//L)**	**LOD (µg/L)**	**LOQ (µg/L)**	**Rec (%)**	**Final cons**	**Final cons**
Tartaric acid	1.696	149	87	-	0.999	62.5-2000	12.31	37.3	98.91	34.30	ND
Citric acid	1.793	19.1	111	-	0.999	125-2000	6.28	18.9	98.35	126.85	91.80
Ascorbic acid	1.802	175.1	114.9	-	0.999	62.5-2000	7.75	23.5	96.70	51.06	51.06
Fumaric acid	1.821	115	71.1	-	0.999	31.25-2000	6.43	19.5	99.76	27.67	14.91
Maleic acid	1.821	115	71.2	-	0.999	62.5-2000	6	18.2	101.03	29.57	ND
Cichoric acid	1.989	472.8	310.5	-	0.999	250-2000	50.16	152	88.73	577.65	563.08
Gallic acid	2.605	169	125	-	0.998	62.5-2000	9	54.6	101.13	ND	ND
Chlorogenic acid	5.526	353	191	-	0.999	250-2000	64.68	196	88.83	160.89	158.99
4-Hydroxybenzoic acid	6.531	137	93.1	-	0.999	62.5-2000	2.38	7.2	94.67	44.56	149.46
Catechin	6.660	28.9	245.1	-	0.999	62.5-2000	2.57	7.8	98.03	33.00	31.80
Epicatechin	6.666	353	191	+	0.998	62.5-2000	2.90	8.8	93.70	45.32	46.10
Hesperidin	6.674	611.3	357	+	0.999	125-2000	32.67	99	102.77	18.08	1.75
Rutin	6.675	60.9	299.4	-	0.997	125-2000	28.5	85	101.33	ND	ND
Vanillic acid	6.687	167	151.8	-	0.998	62.5-2000	2.54	7.7	95.60	79.29	657.76
Syringic acid	6.703	197.1	181.8	-	0.999	62.5-2000	4.22	12.8	101.90	ND	ND
Caffeic acid	6.703	178.9	135.1	-	0.999	125-2000	25.74	78	100.70	0.78	3.52
Luteolin-7-glucoside	6.740	449	286.9	+	0.997	62.5-1000	16.5	50	104.33	ND	ND
Apigenin-7-o-glycoside	6.808	430.8	267.4	-	0.998	125-1000	18.24	55.3	96.53	ND	ND
Quercetin-3-xyloside	6.816	432.7	299.5	-	0.995	62.5-2000	9.87	29.9	103.77	ND	ND
Oleuropein	6.849	539.1	275.1	-	0.999	62.5-2000	17.35	52.6	102.37	ND	ND
Rosmarinic acid	6.875	35.9	160.7	-	0.998	62.5-2000	15.9	48.2	96.07	17.48	17.02
P-qumaric acid	6.919	163	119	-	0.999	62.5-2000	3	9.1	100.03	7.81	65.94
4-Hydroxybenzaldehyde	6.929	121	92	-	0.999	31.25-2000	1.91	5.7	99.83	8.93	33.12
Trans-ferulic acid	6.967	193.0	133.8	-	0.998	31.25-2000	7.26	22.3	95.90	22.35	97.99
Gentisic acid	7.243	153	109	-	0.999	250-2000	44.55	135	98.27	ND	ND
Protocatechuic acid	7.243	15.9	108.9	-	0.999	62.5-2000	15.44	46.8	96.85	26.87	31.05
Quercetin	7.306	300.7	150.9	-	0.997	62.5-1000	14.85	45	101.67	ND	1.14
Apigenin	7.555	269	117	-	0.999	125-2000	17.82	54	102.40	ND	12.59
Naringenin	7.588	270.9	119.1	-	0.999	125-2000	24.37	73.8	100.27	ND	85.83
Trans-cinnamic acid	7.592	148.9	104.9	-	0.999	62.5-2000	13.59	41.2	101.33	26.38	39.67
Kaempferol	7.613	284.9	116.9	-	0.998	62.5-2000	12.39	37.5	100.93	ND	22.26

R.T: retention time, M.I: main ion, T: transitions, I.M: ion mode, L.R: linearity range, LOQ: limit of quantification, Rec: recovery, LOD: limit of detection, N.D: not determined, Met OH: methanol, Chl: chloroform

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Table 5. ICP-OES analysis findings for heavy metal content in *P. avium* fruits

	Analyses	Results (mg/kg)	Acceptable detection limit (mg/kg)
	Arsenic (As)*	0.233±0.056	≤0.065
	Cooper (Cu)*	9.4±0.38	≤9.29
	Cadmium (Cd)*	0.04±0.006	≤0.0012
	Zinc (Zn)*	4.92±0.49	≤1.62
P. avium	**Iron (Fe)***	Not detected	≤6.05
	Mercury (Hg)*	Not detected	≤0.018
	Selenium (Se)*	Not detected	≤0.043
	Nickel (Ni)*	Not detected	≤5.25
	Lead (Pb)*	Not detected	≤0.009

The expanded measurement uncertainty is the value found by multiplying the coverage factor taken as k=2 and provides 95% reliability. Analysis methods marked with "*" are within the scope of accreditation

Discussion

The findings indicated that the total phenolic and total flavonoid contents of crude extracts from *P. avium* fruit portions were elevated in the chloroform extract. This may result from the polarity of the solvents used in the fractionation process. Research indicates that medicinal plants rich in phenolic and flavonoid compounds exhibit enhanced antioxidant and antimicrobial activities. The increasing demand for natural antimicrobials in the food and beverage sector, attributed to their effectiveness, environmental benefits, and absence of adverse effects, is a significant factor contributing to market growth [11,12]. The antioxidant defense system mitigates high levels of oxidant compounds generated by metabolic activities. Insufficiency leads to the occurrence of oxidative stress. Oxidative stress can lead to serious diseases in humans, including cancer, cardiovascular disorders, Alzheimer's disease, Parkinson's disease, and multiple sclerosis. Plants may serve as significant supplementary antioxidants to mitigate the potential consequences of oxidative stress [7]. DPPH radical scavenging activity test performed to measure antioxidant capacity showed that both extracts had moderate activity compared to standard antioxidants (Table 2). Microorganisms may develop resistance to conventional pharmaceuticals over time. This situation compels researchers to identify novel drug active ingredients for the advancement of new treatments. Medicinal plants serve as significant sources for the discovery and isolation of novel bioactive compounds. Recently isolated compounds from medicinal plants offer straightforward strategies to address resistant pathogenic microorganisms [18,19]. Our study yielded a promising outcome: all the extracts utilized in the research exhibited a significant inhibitory impact on the proliferation of *E. coli,* surpassing that of conventional antibiotics (Table 3). *C. albicans* is an opportunistic microorganism that lives in the human body. Infections, especially in patients with suppressed immunity, can lead to illness and even death. From this point of view, support is of considerable importance. The research also showed that the fruit extracts used in the study were better at inhibiting the growth of *C. albicans* at very low concentrations than fluconazole, which was used as a standard drug (Table 3). This suggests that these extracts may serve as a valuable source for the development of new antimicrobial agents. In the peripheral nervous system, acetylcholinesterase hydrolyzes ACh to generate choline and acetate. Aging lowers ACh levels, which may cause neurological illnesses like Alzheimer's. The only acknowledged hypothesis relates to inadequate levels of acetylcholine in the brain. The use of AChE inhibitors to treat cholinergic deficits may influence illness development. As a result, acetylcholinesterase inhibitors have been developed and employed for patient treatment [22-25].

Phenolic compounds vanillic, trans-ferulic and caffeic acids are known to be very effective compounds in delaying neurodegenerative diseases. The high presence of these compounds in both extracts (Table 4) indicates that P. avium fruit may be an important source of potential new AChE inhibitor drugs. The primary constituents of *P. avium* methanol and chloroform extract were determined to include cichoric acid, chlorogenic acid, citric acid, vanillic acid, and 4-hydroxybenzoic acid (Table 4). Each of these molecules is responsible for various biological functions, including antioxidant, antibacterial, anticholinesterase, and anti-inflammatory effects. The rich chemical composition of *P. avium* extracts may assist in identifying the origins of their biological actions. Further investigation is required to ascertain the mechanisms of action, in vivo effects, bioavailability, and bioefficacy of these active phenolic compounds. The main sources of micronutrients in human nutrition are vegetables and fruits, which include Cu, Zn, Ca, Fe, Mg, I, Na, K, antioxidants, vitamins, and other metabolites. Human growth and development need trace levels of heavy metals, including Cu, Zn, and Ni. Trace amounts of heavy metals, including Cu, Zn, and Ni, are essential micronutrients for human growth and development. Some toxic, non-essential heavy metals, including Pb, Hg, Cd and As, can be detrimental to health even at trace levels in individuals sensitive to heavy metal toxicity. Cd exhibits the highest potential for contamination within the

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food chain due to its superior mobility compared to other heavy metals in soil and plants. Plants have the capacity to absorb heavy metals from the soil and concentrate them in elevated levels [39,40]. Soil heavy metal pollution is the primary factor leading to the bioaccumulation of metals in plants. As the economy develops, the acceleration of industrialization, urbanization, and agricultural modernization heightens the risk of contaminated agricultural lands. The lack of mercury, lead, and nickel in *P. avium* fruit suggests that the soil in which the plant grows is likely uncontaminated. The study indicates that *P. avium* fruits are free from toxic metals that may negatively impact human health (Table 5).

Conclusion

Plants attract the interest of many researchers thanks to the natural metabolism products they produce. To protect plant life from changing environmental conditions, herbivores and pathogenic microorganisms, synthesize a wide range of secondary metabolites such as phenolic, flavonoids, terpenes, and proanthocyanidins. These products exhibit a multitude of biological actions. Antioxidant activity is considered to be one of the most crucial factors among them. Phenolic compounds are renowned for their exceptional antioxidant capabilities. Caffeic acid, chlorogenic acid, epicatechin, quercetin, and ferulic acid are the phenolic acids that are most often found in plants' chemical compositions. There are other roles that polyphenols perform, including the enhancement of the human immune system, the prevention of viral reproduction, the reduction of viral transition from the host target cell membrane, and the prevention of antimicrobial activity. The processes by which phenolic compounds exert their antibacterial effects have not yet been well described. Previous studies have described this action as increasing the permeability of cell membranes, binding hydrogen by phenolic compounds to various cellular enzymes, modifying numerous intracellular activities, and losing the microorganisms' cellular integrity. All of these mechanisms are responsible for antimicrobial activity. However, it is also well known that phenolic substances possess the ability to decrease the action of cholinergic enzymes. The high phenolic component content of the *P. avium* chloroform and methanol extract we used in our study is thought to be responsible for antioxidant, antimicrobial, and anticholinesterase activity. It also highlights the importance of our study that it does not contain toxic metals and can meet the levels of minerals such as copper and zinc needed for metabolism to meet the daily intake. The chemical-rich composition of *P. avium* fruit extracts makes them suitable for many scientific investigations and industrial applications.

Conflict of Interests
The authors declare that there is no conflict of interest in the study.

Financial Disclosure
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Ethical Approval
Our study does not require ethics committee approval.

References

1. Abdulhafiz F, Mohammed A, Reduan MFH, et al. Plant cell culture technologies: A promising alternative to produce high-value secondary metabolites. Arab J Chem. 2022;15:104161.
2. Jakubczyk K, Dec K, Kałduńska J, et al. Reactive oxygen species-sources, functions, oxidative damage. Pol Merkur Lekarski. 2020;48:124-7.
3. Adwas AA, Elsayed A, Azab AE, Quwaydir FA. Oxidative stress and antioxidant mechanisms in the human body. J Appl Biol Biotechnol. 2019;6:43-7.
4. Jain C, Khatana S, Vijayvergia R. Bioactivity of secondary metabolites of various plants: a review. Int J Pharm Sci Res. 2019;10:494-504.
5. Sarv V, Venskutonis PR, Bhat R. The Sorbus spp.-underutilised plants for foods and nutraceuticals: review on polyphenolic phytochemicals and antioxidant potential. Antioxidants (Basel) 2020;9:813.
6. Navarro-Hortal MD, Romero-Márquez JM, Jiménez-Trigo V, et al. Molecular bases for the use of functional foods in the management of healthy aging: berries, curcumin, virgin olive oil, and honey; three realities and a promise. Crit Rev Food Sci Nutr. 2023;63:11967-86.
7. Abeyrathne EDNS, Nam K, Huang X, Ahn DU. Plant and animal-based antioxidants' structure, efficacy, mechanisms, and applications: a review. Antioxidants (Basel). 2022;11:1025.
8. Augustyniak A, Bartosz G, Cipak A, et al. Natural and synthetic antioxidants: an updated overview. Free Radic Res. 2010;44:1216-62.
9. Gonçalves B, Moutinho-Pereira J, Santos A, et al. Scion–rootstock interaction affects the physiology and fruit quality of sweet cherries. Tree Physiol. 2006;26:93-104.
10. Ademović Z, Hodžić S, Halilić-Zahirović Z, et al. Phenolic compounds, antioxidant and antimicrobial properties of the wild cherry (Prunus avium L.) stem. Acta Period Technol. 2017;48:1-13.
11. Acero N, Gradillas A, Beltran M, et al. Comparison of phenolic compounds profile and antioxidant properties of different sweet cherry (Prunus avium L.) varieties. Food Chem. 2019;279:260-71.
12. Mousavi M, Zaiter A, Modarressi A, et al. The positive impact of a new parting process on antioxidant activity, malic acid, and phenolic content of Prunus avium L., Prunus persica L., and Prunus domestica subsp. insititia L. powders. Microchem J. 2019;149:103962.
13. Keskin C, Özen HÇ, Toker Z, et al. Determination of in vitro antioxidant and antimicrobial properties of shoot and root extracts of Astragalus diphtherites FENZL var. diphtherites and Astragalus gymnalopecias RECH. FIL. obtained by different solvents. KSU J Agric Nat. 2018;21:157-66.
14. Malešev D, Kuntić V. Investigation of metal-flavonoid chelates and the determination of flavonoids via metal-flavonoid complexing reactions. J Serb Chem Soc. 2007;72:921-39.
15. Keskin C, Baran A, Baran MF, et al. Green synthesis, characterization of gold nanomaterials using Gundelia tournefortii leaf extract, and determination of their nanomedicine (antibacterial, antifungal, and cytotoxic) potential. J Nanomater. 2022;2022:7211066.
16. Ellman GL, Courtney KD, Andres V Jr, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 1961;7:88-95.
17. Tanase C, Coşarca S, Muntean DL. A critical review of phenolic compounds extracted from the bark of woody vascular plants and their potential biological activity. Molecules. 2019;24:1182.
18. Mickymaray, S. Efficacy and mechanism of traditional medicinal plants and bioactive compounds against clinically important pathogens. Antibiotics. 2019;8:257.

19. Vaou N, Stavropoulou E, Voidarou C, et al. Towards advances in medicinal plant antimicrobial activity: a review study on challenges and future perspectives. Microorganisms. 2021;9:2041.

20. Bashabsheh RH, AL-Fawares OL, Natsheh I, et al. Staphylococcus aureus epidemiology, pathophysiology, clinical manifestations and application of nano-therapeutics as a promising approach to combat methicillin resistant Staphylococcus aureus. Pathog Glob Health. 2024;118:209-231.

21. Abeni BA, Frank-Peterside N, Otokunefor K. Comparative analysis of virulence gene profiles of Escherichia coli from human and non-human sources in Rivers State, Nigeria. Access Microbiol. 2024;6:000776-v6.

22. Türkan F, Atalar MN, Aras A, et al. ICP-MS and HPLC analyses, enzyme inhibition and antioxidant potential of Achillea schischkinii Sosn. Bioorg Chem. 2020;94:103333.

23. Taslimi P, Gulçin İ. Antioxidant and anticholinergic properties of olivetol. J Food Biochem. 2018;42:e12516.

24. Pohanka M. Cholinesterases, a target of pharmacology and toxicology. Biomed Pap Med Fac Univ Palacky Olomouc. 2011;155:219-30.

25. Mukherjee PK, Kumar V, Mal M, Houghton PJ. Acetylcholinesterase inhibitors from plants. Phytomedicine. 2007;14:289-300.

26. Tohma H, Altay A, Köksal E, Gören AC, Gülçin İ. Measurement of anticancer, antidiabetic, and anticholinergic properties of sumac (Rhus coriaria): analysis of its phenolic compounds by LC-MS/MS. J Food Meas Charact. 2019;13:1607-19.

27. Brimson JM, Onlamoon N, Tencomnao T, Thitilertdecha P. Clerodendrum pesticides S. moore: the therapeutic potential of phytochemicals, hispidulin, vanillic acid, verbascoside, and apigenin. Biomed Pharmacother. 2019;118:109319.

28. Izquierdo-Vega JA, Arteaga-Badillo DA, Sánchez-Gutiérrez M, et al. Organic acids from Roselle (Hibiscus sabdariffa L.)- a brief review of its pharmacological effects. Biomedicines. 2020;8:100.

29. Peng Y, Sun Q, Park Y. The bioactive effects of cichoric acid as a functional food ingredient. J Med Food. 2019;22:645-52.

30. Lee J, Scagel CF. Chicoric acid: chemistry, distribution, and production. Front Chem. 2013;1:40.

31. Kuban-Jankowska A, Sahu KK, Gorska M, et al. Chicoric acid binds to two sites and decreases the activity of the YopH bacterial virulence factor. Oncotarget. 2016;7:2229-38.

32. McDougall B, King PJ, Wu BW, et al. Dicaffeoylquinic and dicaffeoyltartaric acids are selective inhibitors of human immunodeficiency virus type 1 integrase. Antimicrob Agents Chemother. 1998;42:140-6.

33. Di Giacomo S, Percaccio E, Gullì M, et al. Recent advances in the neuroprotective properties of ferulic acid in Alzheimer's disease: a narrative review. Nutrients. 2022;14:3709.

34. Kępa M, Miklasińska-Majdanik M, Wojtyczka RD, et al. Antimicrobial potential of caffeic acid against Staphylococcus aureus clinical strains. Biomed Res Int. 2018;2018:7413504.

35. Espíndola KMM, Ferreira RG, Narvaez LEM, et al. Chemical and pharmacological aspects of caffeic acid and its activity in hepatocarcinoma. Front Oncol. 2019;9:541.

36. Merlani M, Barbakadze V, Amiranashvili L, et al. New caffeic acid derivatives as antimicrobial Agents: Design, synthesis, evaluation and docking. Curr Top Med Chem. 2019;19:292-304.

37. Robbins RJ. Phenolic acids in foods: an overview of analytical methodology. J Agric Food Chem. 2003;51:2866-87.

38. Codex TF. Turkish food codex regulation on contaminants. Official Gazette. 28157, 29 December 2011.

39. Đurović D, Bulat Z, Buha A, Matović V. Cadmium, mercury, and lead in Hypericum perforatum L. collected in Western Serbia. Proceedings of the 16th International Conference on Heavy Metals in the Environment. 23-27, September 2012. Rome, Italy, 1, 15009.

40. Simon Y, Bone MK. The essential guide to herbal safety. St. Louis. MO, USA: Elsevier Health Sciences. 2004;111.