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Pharmata aims to contribute to the scientific literature by publishing manuscripts of the highest caliber. The journal accepts research articles, reviews, and short communications that adhere to ethical guidelines.

The scope of the journal encompasses various topics, including but not limited to:

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16. New analytical techniques and methods
17. Pharmaceutical chemistry
18. Synthesis and analysis of new drug molecules
19. Other areas: pharmaceutical solid materials (including biomaterials, polymers, and nanoparticles), biotechnology products (including genes, peptides, proteins, and vaccines), engineered cells.

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You can find the current version of the Instructions to Authors at https://pharmata-ataunipress.org/.

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Pharmata

CONTENTS

RESEARCH ARTICLES

1 **Total Phenolic Content and Antioxidant Activity of Different Extracts of *Aronia melanocarpa* L. Fruit**
Lale DUYSAK, Mehmet ŞEKEROĞULLARI, Nurcan Kılıç BAYGUTALP

7 **Development and Validation of a Rapid and Sensitive Ultrahigh-Performance Liquid Chromatography with Diode Array Detection Method for Quantification of Sertraline in Pharmaceutical Formulations**
Mahmut Can BEBEK, Tuğrul Çağrı AKMAN

14 **Evaluating the Antioxidant Capacity of Rheum Ribes via Cupric Reducing Antioxidant Capacity, Ferric Reducing Antioxidant Power and 2,2-Diphenyl-1-picrylhydrazyl Methods**
Semih ÖZLI, Onur ŞENOL, Esen SEZEN KARAOĞLAN

19 **Investigation of the Antibacterial Activity of Different *Pelargonium Graveolens* Essential Oils Sold in the Market**
Esen SEZEN KARAOĞLAN, Hayrunisa HANCI

REVIEW

22 **Rational Drug Use and Community Pharmacy**
Büşra BAYRAKTAR, Elif ULUTAŞ DENİZ

CASE REPORTS

27 **Impact of Coronavirus Disease Diagnosis on Maternal and Neonatal Health Outcomes in Pregnancy: Two Case Reports**
Leyla KAYA, Zahide KAYA

31 **Acknowledgement of Reviewers**

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Research Article

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Total Phenolic Content and Antioxidant Activity of Different Extracts of *Aronia melanocarpa* L. Fruit

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ABSTRACT

Objective: *Aronia melanocarpa* L. (aronia) fruit contains anthocyanins, flavonoids, phenolic acids, and compounds from the polyphenol group. The fruits of this plant are the most abundant natural source of anthocyanins. The aim of this study is to determine the total phenolic compound amount of methanol and ethanol extract in the fruit of the aronia plant and to investigate its antioxidant effects.

Methods: Ethanol and methanol extracts from aronia fruits were obtained. The Folin–Ciocalteu Reagent (FCR) was used to determine the total phenolic component levels in the extracts of aronia. By using the 1,1-diphenyl-2-picrylhydrazyl (DPPH), iron ion reducing antioxidant power (FRAP), and Cu^{2+} ion reducing (CUPRAC) techniques, antioxidant activities were assessed. To calculate the extracts' equivalent antioxidant capacity, different reference sample concentrations ranging from 125 to 500 g/mL were prepared.

Results: Both ethanol and methanol aronia extracts showed the highest phenolic component at a concentration of 500 μL/mL. Similary, both extracts FRAP and CUPRAC (Trolox Eq g/mL) activities and DPPH radical scavenging capacity (inhibition %) were highest at the concentration of 500 μL/mL.

Conclusion: Aronia stands out as an antioxidant fruit and a potential natural therapeutic agents to alleviate oxidative stress. More research is needed to elucidate the exact mechanisms of action, optimum extraction method, optimal dosage, and potential side effects of the extracts.

Keywords: Antioxidant, *Aronia melanocarpa* L., ethanol, extract, methanol.

INTRODUCTION

Free radicals are substances produced in the body by the contaminated air we breathe throughout the day, toxic substances in ruined meals, additives, unconscious nutrition, and inactivity. Oxygen atoms, broken off by these harmful effects from outside, circulate freely in the body, breaking off hydrogen atoms and causing tissue damage. Free radicals specifically attack cells and the immune system. Molecules that minimize and block the effects of free radicals in the body and prevent chain reactions that can cause many diseases and premature aging are called "antioxidant" substances.[1] It is known that the living organism has an antioxidative protection system to protect it from the effects of free radicals. In some cases, the antioxidative protective system is not adequate to maintain oxidant–antioxidant balance, and free radicals increase in the body. This causes some damage in the body; as the amount of free radicals increases, first aging accelerates, cell death, then tissue death, and then damage to the brain vessels occurs.[2,3] Some plants contain more antioxidants than others. The most important of these are grape-like fruits. These fruits play an important role in the prophylaxis and treatment of many diseases due to the high amount of antioxidants they contain. One of these fruits is aronia.[4,5]

Aronia is a plant belonging to the Rosaceae family with fruits rich in bioactive compounds with antioxidant properties. Ripe fruits are used in the pharmaceutical industry to make medicines.[6,7] Aronia fruit contains anthocyanins, flavonoids, phenolic acids, and compounds from the polyphenol group. These plant fruits are the richest natural source of anthocyanins.[8] Numerous investigations have revealed that these substances not only possess potent antioxidant, anti-inflammatory, hypotensive, antibacterial, and antitumor properties but also decrease the content of plasma lipids.[9-13] Studies have also found that anthocyanin is beneficial in cardiovascular system diseases.[14,15] In a review study, it was reported that aronia fruits can be considered a promising component of foods designed for their antioxidant potential.[16] Aronias, which are recommended and used as hypertensive and antiatherosclerotic drugs in Russia and Eastern European countries, are among the most important plants that have gained popularity in this geography to meet the need for herbal medicine.[12]

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Antioxidants used in in vivo and in vitro studies where antioxidant activity is investigated are generally synthetically prepared substances by manufacturers. Therefore, the use of naturally obtained antioxidants in studies is rare. In this study, we used the standard antioxidants gallic acid and Trolox.[17,18]

In this study, it was aimed to determine the total phenolic compounds and measure the antioxidant activity of ethanol and methanol extracts of aronia fruit by copper ion reducing antioxidant capacity (CUPRAC), 1,1-diphenyl-2-picrylhydrazyl (DPPH), and ferric reducing antioxidant power (FRAP) methods, and to compare the obtained data according to the extracts.

METHODS

Plant Material

Aronia fruit was obtained from the Tver Oblast region of Russia in September 2018 and dried under suitable conditions (in a dry environment, away from sunlight, at room temperature). After the plant was dried, it was pulverized using liquid nitrogen and a porcelain mortar. The powdered aronia fruit was stored under suitable conditions until the experiment began.

Preparation of Plant Extracts

The powdered aronia fruit was extracted with ethanol and methanol in a shaking water bath at 50°C for 72 hours and filtered every 24 hours. Filtrates were combined, and the filtrate was evaporated in the evaporator at 50°C and stored in the refrigerator at +4°C for determination.

Determination of total phenolic content

The amounts of total phenolic compounds in aronia ethanol and methanol extracts were determined using the modified version of the method developed by Slinkard and Singleton.[19]

First, 50 mL of 7.5% Na_2CO_3 was prepared. Then, after weighing 25 mg of gallic acid for the standard, it was completed with methanol to 25 mL in a test tube. Finally, the Folin and Ciocalteu reagent was taken into a beaker for phenolic compound determination. Stock solutions were prepared, and the necessary dilutions were made. First, 40 µL of sample and 200 µL of Folin and Ciocalteu reagent were added to the plates and incubated for 5 minutes. Finally, 160 µL of Na_2CO_3 was added and incubated again for 30 minutes. After incubation, absorbance was measured at 765 nm. Using the standard graph prepared using gallic acid, the results were given as mg gallic acid equivalent (GAE)/g.

Determination of Antioxidant Capacity

1,1-Diphenyl-2-picrylhydrazyl *Radical Scavenging Capacity Assay*

The DPPH radical scavenging capacities of ethanol and methanol extracts obtained from aronia were determined according to the Brand Williams method.[20] The inhibitory response of samples to DPPH radicals is measured spectrophotometrically to determine antioxidant capacity. The DPPH solution loses its color during the reduction reaction in the presence of an antioxidant, and the decrease in color intensity makes it easier to measure in the spectrophotometer. After preparing DPPH solution, 210 µL of extract sample was pipetted into the plate wells, and then 70 µL of DPPH solution was added to each well. The plate was mixed with a stirrer for 1 minute and incubated for 30 minutes in the dark. Trolox was used as the standard antioxidant for the control sample. Then absorbance was measured at 517 nm, and the results were calculated as percent inhibition.

The Ferric-Reducing Antioxidant Power Assay

The method of determination of the antioxidant capacity of extracts obtained from aronia based on electron transfer was applied by Huang et al.[21] First, 300 mmol/L acetate buffer (pH 3.6) was prepared. 10 mM TPTZ was taken into a 100 mL flask, 40 mM HCl was added, and the final volume was made up to 100 mL. Finally, a 20 mmol/L $FeCl_3$ solution was prepared. A total of 30 mL of FRAP solution was obtained by taking 2.5 mL of TPTZ, 2.5 mL of $FeCl_3$, and 25 mL of acetate buffer from these prepared solutions. Ten microliters of the extracted sample and 200 µL of FRAP solution were pipetted into the plate wells and allowed to incubate for 30 minutes, and then the absorbance was measured at 593 nm.

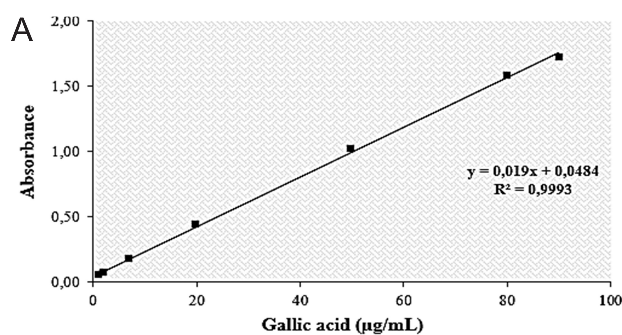
Cupric Ion (Cu^{2+}) Reducing Antioxidant Capacity Assay

This method used by Apak et al is based on the conversion of the Cu(II) neocuproin complex to Cu(I) neocuproin using antioxidant compounds in the environment and the measurement of the absorbance of this complex at 450 nm wavelength.[22] To prepare the CUPRAC reagent (10 mM), 0.4262 gram of $CuCl_2$•$2H_2O$ was weighed and dissolved in 250 mL of distilled water. To prepare the acetate buffer, 19.27 g of NH_4Ac was dissolved in 250 mL of water. 7.5 mM neocuproin solution was obtained by preparing 0.039 g neocuproin compound with 96% pure ethanol in a 25 mL flask. Afterward, solutions consisting of 60 µL $CuCl_2$, 60 µL acetate buffer, 60 µL neocuproin solution, and 66 µL extracts were mixed, and after 30 minutes of incubation, absorbances were measured at 450 nm wavelength. The standard antioxidant, Trolox, was used as a control sample. Calibration curves for the working range of 1-100 µg/mL, where the plot of absorbance versus concentration is linear, were derived.

RESULTS

Findings of Total Phenolic Compound Quantification

Total phenolic compound amounts of ethanol and methanol extracts prepared from aronia were determined by Folin–Ciocalteu Reagent (FCR). Gallic acid was used as the standard phenolic compound and was calculated as gallic acid equivalent from the equations obtained from the calibration curves of gallic acid (Figure 1).



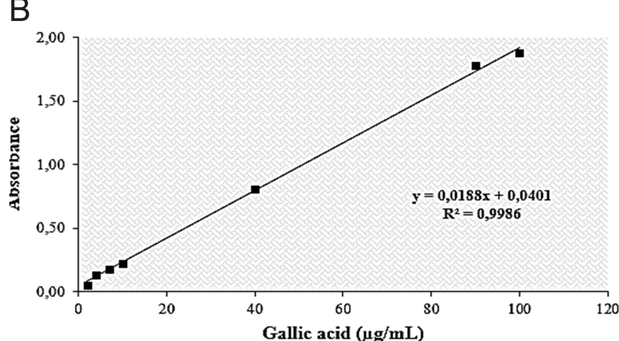


Figure 1. Calibration curves of gallic acid in different solvents. A. Ethanol. B. Methanol.

Table 1. Total Phenolic Compound Amounts of *Aronia melanocarpa* L. extracts

Concentration (µg/mL)	Total Phenolic Compound (µg GAE/mg Extract)	
	Ethanol Extract	Methanol Extract
125	1.06	6.55
250	3.37	8.48
500	6.02	11.19

GAE, gallic acid equivalent.

Table 2. Comparison of DPPH Free Radical Scavenging Capacities of Extracts at Different Concentrations

Concentration (µg/mL)	% Inhibition [Trolox (Eq µg/mL)]	
	Ethanol Extract	Methanol Extract
125	21.57	11.82
250	57.69	29.94
500	84.19	52.55

The total phenolic contents of ethanol and methanol extracts of aronia fruit at different concentrations were determined. Accordingly, the highest total phenolic content was determined to be at a concentration of 500 µg/mL (Table 1).

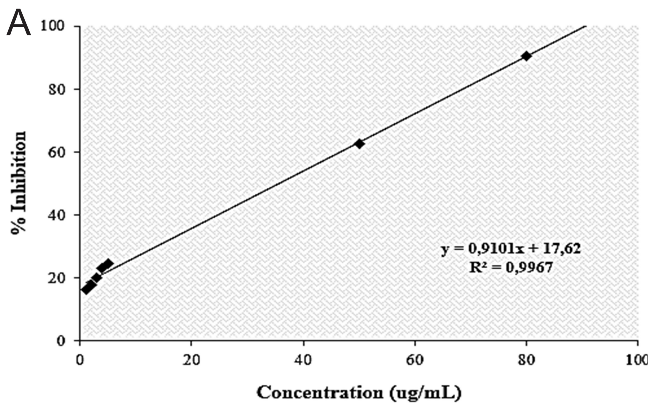
Antioxidant Capacity Findings

Findings from 1,1-Diphenyl-2-picrylhydrazyl radical scavenging studies

The DPPH radical scavenging activities of standard antioxidant compounds of ethanol and methanol extracts prepared from aronia were determined according to the Brand-Williams method.[20] The analyzed concentration range (1-100 µg/mL) was determined as a result of studies on standard antioxidant compounds. The DPPH radical scavenging activity of Trolox, a standard antioxidant, reached its highest value at a concentration of 90 µg/mL for both ethanol and methanol (Figure 2).

The DPPH radical scavenging capacities of methanol and ethanol extracts of aronia fruit in the range of (125-500 µg/mL) are shown in Table 2 as % inhibition.

Aronia fruit methanol extract showed the highest DPPH free radical scavenging effect at 500 µg/mL concentration.



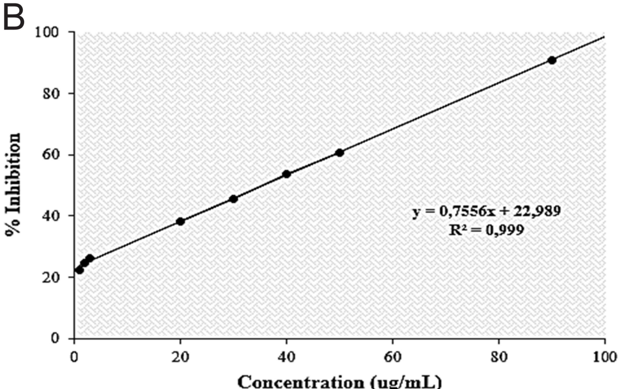


Figure 2. Concentration % inhibition graph of Trolox. A. Ethanol. B. Methanol.

Findings of the Copper Ion Reducing Antioxidant Capacity Determination Method

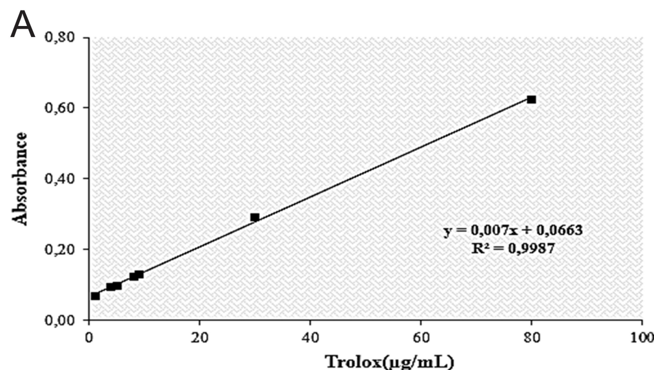
The conversion of ethanol and methanol extracts prepared from aronia and standard antioxidant compounds of Cu(II) neocuproin complex at 450 nm to Cu(I) neocuproin by means of compounds with antioxidant effects in the medium was done by measuring the absorbance at 450 nm. The concentration range to be analyzed (1-100 µg/mL) was determined as a result of studies on standard antioxidant compounds (Figure 3, Table 3)

Findings of Iron Ion Reducing Antioxidant Power

Spectrophotometric measurements of iron (III) reduction/antioxidant equivalent absorbances of aronia fruits, ethanol, methanol extract, and standard antioxidant compounds were made at 593 nm. Since the antioxidant power capacity of Trolox, one of the standard antioxidant compounds, reaches its maximum level at a concentration of 100 µg/mL in ethanol extract and 70 µg/mL in methanol extract, the study was carried out in the range of 1-100 µg/mL (Figure 4).

Comparison of the iron (III) reduction/antioxidant powers of aronia fruit ethanol and methanol extracts in terms of µg/mL Trolox equivalent antioxidant capacity (TEAC) using the spectrophotometric method at 593 nm are given in Table 4.

It was determined that the ethanol and methanol extracts prepared from aronia fruits had iron ion reducing antioxidant power



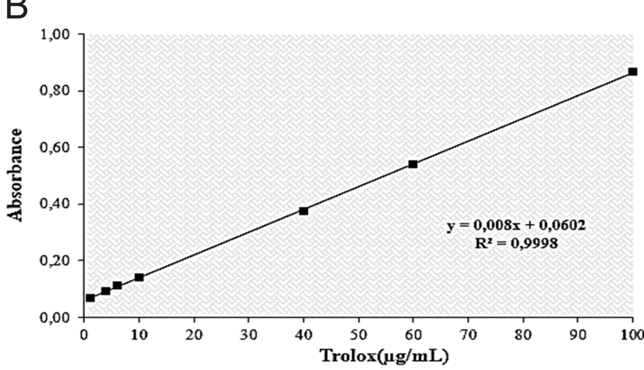


Figure 3. Trolox standard graph. A. Ethanol. B. Methanol.

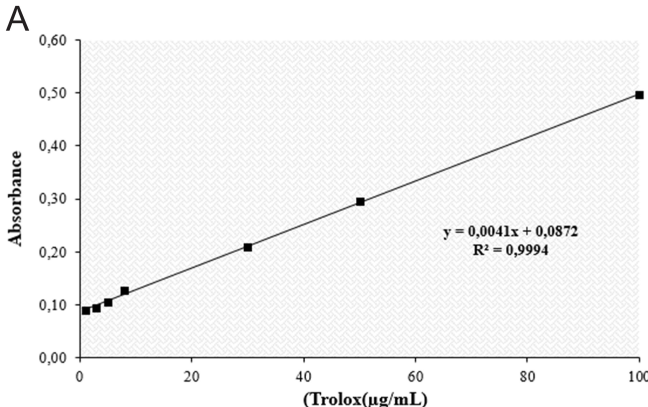
Table3. Comparison of the Conversion of the Extracts from Cu(II) Neocuproin Complex to Cu (I) Neocuproin at Different Concentrations in Terms of μg Trolox Equivalent Antioxidant Capacity

	Trolox Equivalent (μg/mL)	
Concentration (μg/mL)	Ethanol extract	Methanol extract
125	8.76	10.72
250	18.25	22.66
500	67.42	43.34

capacity; the concentration of 500 μg/mL was high in both extracts, and the results were close to each other.

DISCUSSION

In this study, ethanol and methanol extracts prepared from aronia fruits were evaluated in terms of antioxidant activity. It was determined that the ethanol extract was rich in CUPRAC and DPPH radical-scavenging activity. (Table 2,3) The methanol extract had high antioxidant activity in terms of FRAP and total phenolic compounds (Table 1,4). This feature may be due to the compounds contained in aronia fruits. It is thought that the various antioxidant activity differences observed in the extracts are due to the differences in the level and chemical structures of polyphenolic compounds transferred to the solvent used. For this reason, data obtained from in vitro studies on plant-derived natural antioxidants, which are preferred over synthetic antioxidants, will form the basis of in vivo studies.



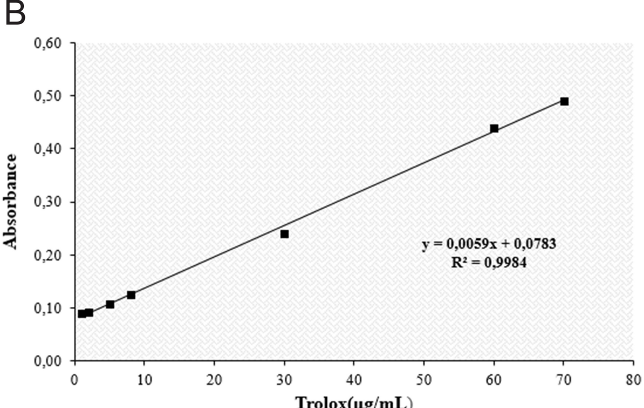


Figure 4. Trolox standard graph. A. Ethanol. B. Methanol.

Table 4. Comparison of İron (III) Reducing/Antioxidant Power of Extracts at Different Concentrations in μg Trolox Equivalent Antioxidant Capacity

	Trolox Equivalent (μg/mL)	
Concentration (μg/mL)	Ethanol Extract	Methanol Extract
125	4.44	4.72
250	7.67	10.03
500	18.65	18.74

In recent years, many studies have been conducted on ways to prevent, delay, and completely eliminate diseases. It has been proven as a result of research that many diseases are caused by the accumulation of free radicals in our bodies or as a result of environmental factors.[23-25] Preventing the formation of radicals or neutralizing the radicals before they damage the metabolism may delay the emergence of some diseases or positively affect the prognosis of the disease. One of the best ways to neutralize free radicals, which we ingest from external sources through various means, is to consume a diet rich in antioxidants.[23]

Since methods for determining antioxidant activity depend on various parameters and are affected by environmental factors, no single standard method is used to determine the antioxidant activity of a compound. Therefore, many methods are used to measure antioxidant activity. The different results in the literature may be due to the different methods and growing and drying conditions of the plants. For scientists working in the field of food, antioxidants are a very interesting topic due to their protective role against oxidative degradation in food products and pathological processes mediated by oxidative stress in the body. Effective investigation of natural antioxidant sources and the design of new antioxidant compounds require reliable methods for antioxidant activity assessment.[26]

There is still a need for traditional methods to quantify antioxidant activity, and certain methodological protocols are intricate and time-consuming to test. The pH is one of the most important selection factors in antioxidant testing. There are tests that function in alkaline (Folin–Ciocalteu), neutral (CUPRAC), or acidic (FRAP) environments. Another crucial element is the applicability of antioxidant testing to both lipophilic and hydrophilic antioxidants. Although both hydrophilic and lipophilic antioxidants can be measured using the ABTS and CUPRAC assays, certain techniques (FRAP and Folin–Ciocalteu) solely assess hydrophilic antioxidants, while others are limited to hydrophobic systems (DPPH).[27]

Aronia fruits are used in functional fruit juices and teas thanks to their high anthocyanin content, and in various products such as fruit juices, fruit juice concentrates, jams, and marmalade due to their high pectin content. It is also evaluated as a food supplement, in fresh or processed form, to improve the taste, color, and antioxidant properties of foods.[28-30]

In a study, the total phenolic, flavonoid, and anthocyanin contents of fresh and dried fruits of *Aronia* were examined, and it was determined that there was a 28%-60% decrease in the phenolic substance content as a result of all drying methods applied.[31]

Previous studies have shown that aronia, which has a rich nutritional content, is especially rich in terms of phenolic compounds.[32]

Jurikova et al[13] have determined in their study on preventing chronic diseases of aronia fruit that aronia fruit has high antioxidant activity, its phenolic compounds are very valuable, and the importance of anthocyanins and procyanidins. They reported that the phenolic component content of aronia fruit varies depending on the maturity period of the fruit, variety, and ecology.

Benvenuti et al[14] investigated total polyphenols, total anthocyanins, and reduced ascorbic acid in fruits belonging to the *Rubus*, *Ribes*, and *Aronia* genera using spectrophotometric and high-performance liquid chromatography techniques. The 2,2-diphen yl-1-picrylhydrazyl radical scavenging activity of fruit extracts was tested, and the results showed that the tested fruits were good natural sources of antioxidants.[14]

Arancibia-Avila et al[33]extracted freeze-dried aronia fruits with water, acetone, and hexane twice at room temperature for 3 hours, and the resulting fruit extracts were found to have 57.4, 2.84, and 0.89 µM TE/g values, respectively, by the DPPH method. In the same study, as a result of antioxidant measurement with the FRAP and CUPRAC methods, water, acetone, and hexane extracts were found to have values of 87.12, 3.55, and 1.19 µM TE/g, respectively, with the FRAP method and 219.9, 7.16, and 3.12 µM TE/g, respectively, with the CUPRAC method. Based on the DPPH, FRAP, and CUPRAC techniques, it was shown that water extract produced the greatest results.[33]

Berries of the aronia plant, or chokeberries, are an extremely rich source of various compounds that have positive effects on health. These compounds, which include proanthocyanidins, anthocyanins, flavonoids, and phenolic acids, are primarily polyphenols, which have antiviral, antioxidative, anti-inflammatory, anticancer, antiatherosclerotic, hypotensive, antiplatelet, and antidiabetic qualities. In a study, it was emphasized that there are currently not enough studies on the effects of consuming different aronia products on the human body during occupational and environmental exposure to toxic compounds and on people poisoned by these substances. Aronia products and aronia-occurring polyphenolic compounds, however, are useful in counteracting the toxicity of various xenobiotics in experimental studies. This suggests that these compounds may also be effective in humans, and well-planned epidemiological studies are required to investigate this further. These studies should first look into subjects who have been exposed to the substances for which aronia effectiveness has been demonstrated in animal models.[34]

Our study revealed that the methanol and ethanol extracts obtained from the aronia fruit, which were analyzed in support of other studies, were rich in antioxidant activity and phenolic content. When the results obtained from the FCR, DPPH, FRAP, and CUPRAC methods of aronia fruit extract obtained from ethanol and methanol extracts were compared with each other, the effectiveness of different extracts in different methods was generally determined. The superiority of the extracts over each other could not be determined.

Methanol and ethanol extracts obtained from aronia fruit were rich in antioxidant activity and phenolic content. Therefore, the studied species can be considered an important source of natural antioxidants. As a result, it was determined that the ethanol and methanol extracts of the aronia plant were not superior to each other. It is thought that this study will contribute to other studies on aronia fruit.

Ethics Committee Approval: Ethical approval was not required as this study was conducted *in vitro*.

Peer-review: Externally peer-reviewed.

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Research Article

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Development and Validation of a Rapid and Sensitive Ultrahigh-Performance Liquid Chromatography with Diode Array Detection Method for Quantification of Sertraline in Pharmaceutical Formulations

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ABSTRACT

Objective: This study aimed to develop and validate a new, sensitive, fast, and simple ultrahigh-performance liquid chromatography (UHPLC) method for the quantification of sertraline in pharmaceutical preparations and then apply it to commercial pharmaceutical preparations.

Methods: Ultrahigh-performance liquid chromatography with diode array detection was used in the study. In the method, methanol and deionized water containing 0.05% TFA (70 : 30, v/v) were used as the mobile phase, and the C18 column was used as the stationary phase. The flow rate was 1.0 mL/min, the injection volume was 20 µL, and the column temperature was 40°C. The detector was set at 254 nm.

Results: The retention time of sertraline was 2.1 minutes, and the analysis time of the method was 4 minutes. The equation of the calibration curve was determined as $y = 0.1096x - 0.0156$ (R 0.9997). While the limit of detection and limit of quantitation values were 0.1 and 0.3 µg/mL, respectively, the method showed linearity between concentrations of 0.3 and 100 µg/mL. The analytical recovery of the method from the pharmaceutical preparation was within accepted limits (98%-102%). In addition, the relative error percentage and relative standard deviation values, which express the accuracy and precision parameters, respectively, were found to be less than 8%.

Conclusion: In the study, a UHPLC method with high sensitivity and a wide working range was developed for the quantification of sertraline in a short analysis time. As a result, the applicability of the developed method to pharmaceutical preparations has also been proven.

Keywords: Pharmaceutical dosage forms, sertraline, UHPLC-DAD, validation

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INTRODUCTION

Depression is a chronic or recurrent emotional disorder that negatively affects individual behavior. Major depression is a prevalent and debilitating mental illness that affects a significant number of individuals throughout their lifetime, with a prevalence rate of 15%-20%.[1] Antidepressants are the first line of treatment for major depressive disorder, which presents with psychotic and melancholic symptoms. Sertraline (SRT), an antidepressant drug, is a member of the selective serotonin reuptake inhibitors. The chemical name of SRT is (1S-cis)-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-N-methyl-1-naphthal enamine (Figure 1).[2] Sertraline is a potent and selective inhibitor of neuronal serotonin reuptake. It also has very weak effects on neuronal reuptake of norepinephrine and dopamine. For this reason, it is more preferred than tricyclic antidepressants.[3]

In determining the analytical method to be developed for drug analysis, parameters such as the facilities of the laboratory, the chemical properties of the active substance to be analyzed, and the matrix containing the drug should be taken into consideration. A thorough literature review provides an important guide in determining the analytical method to be used in drug analysis. Many studies have been reported in the literature for the determination of SRT in different matrices with different analytical methods. In these studies, spectroscopic,[4-7] high-performance liquid chromatography (HPLC) with

Figure 1. Molecular structure of sertraline.

fluorescence detection,[8] HPLC with ultraviolet detection,[6,7,9-11] gas chromatography (GC),[12-17] capillary electrophoresis-mass spectrometry,[18-20] and liquid chromatography–tandem mass spectrometry (LC-MS/MS)[14,21-23] techniques were used. The high cost of mass spectrometry detectors and the required derivatization step for GC, along with the low sensitivity in spectroscopy, lead to the preference for HPLC in drug quality control analysis.

In this study, a new ultrahigh-performance liquid chromatography with diode array detection (UHPLC-DAD) method was developed as an alternative to the literature that can be used for quality control and stability studies of commercial preparations containing SRT. To ensure the accuracy and reliability of the developed method, various parameters such as specificity, the limit of detection (LOD), the limit of quantitation (LOQ), linearity, precision, accuracy, and robustness were validated. Additionally, forced degradation studies were conducted on the drug product solution to demonstrate method stability and compliance with International Conference on Harmonization (ICH) guidelines.

METHODS

Reagents

Sertraline (99.7% purity, Merck, CAS number: 79559-97-0), a reference standard, was purchased from Sanovel (Istanbul, Turkey). Trifluoroacetic acid (TFA, >99.0% purity), formic acid (FA, >99.0% purity), HPLC-grade acetonitrile, and HPLC-grade methanol were obtained from Sigma-Aldrich (St. Louis, Mo, USA). Ultrapure water was generated by a Milli-Q Plus purification system from Millipore, Waters (Millipore, Bedford, Mass, USA). Three commercial tablets (Selectra® 50 mg tablet (Sanovel), Lustral® 50 mg tablet (Pfizer), and Misol® 50 mg tablet (Nobel)) containing the active ingredient SRT were obtained from the Turkish pharmaceutical market.

Instruments and Chromatographic Conditions

For the analysis of SRT in pharmaceutical formulations, a UHPLC system manufactured by Thermo Scientific® Dionex Ultimate 3000 was employed. The system consisted of a degasser (SR-3000 Solvent Rack), a pump (LPG-3400SD), an autosampler (WPS-3000SL), a column oven (TCC-3000SD), and a diode array detector (DAD 3000). Chromelon software (Thermo Scientific®) was utilized for data acquisition and processing. For chromatographic separation, the Thermo Scientific® column (3 µm 120 Å, LC, 150 × 4.6 mm, C18) was used. The mobile phase consisted of methanol and deionized water containing 0.05% TFA (70 : 30, v : v). The column temperature was maintained at 40°C during analysis. A 20 µL sample was injected into the system, and the flow rate of the mobile phase was maintained at 1 mL/min. The DAD detector was set at a wavelength of 254 nm.

Preparation Stock, Standard, and Quality Control Solutions

Fifty milligrams of SRT active ingredient was weighed on a sensitive balance and dissolved in a 50 mL volumetric flask with some methanol. After dissolution, the solution was made up to 50 mL with methanol. By diluting this stock solution with methanol, standard working solutions were prepared to determine the calibration curve. Working solutions with concentrations of 0.5, 1, 5, 10, 25, 50, 75, and 100 µg/mL and quality control solutions with concentrations of 4, 40, and 80 µg/mL were prepared.

Preparation of Tablet Solutions

Ten tablets each of Lustral®, Selectra®, and Misol® 50 mg tablet pharmaceutical preparations containing SRT were taken. Each tablet was weighed individually. Thus, the weight of an average tablet was calculated. Ten tablets of each preparation were crushed in a mortar, and the amount of powder corresponding to an average tablet was transferred to a 50 mL volumetric flask. Some methanol was added to 3 volumetric flasks and vortexed. The solution was left to allow the excipients to precipitate. Methanol containing SRT was filtered, transferred to a different 50 mL volumetric flask, and made up to 50 mL with methanol. Thus, stock solutions of 3 pharmaceutical preparations with concentrations of 1 mg/mL were prepared. Two samples, each at 30 and 60 µg/mL concentrations, were prepared from these stock solutions and analyzed by the method.

Optimization of Mobile Phase, Column Temperature, and Detector Wavelengths

Many different experiments were carried out by making changes in the method parameters to determine the chromatographic method conditions. In mobile phase selection, different combinations and compositions of methanol, acetonitrile, water, and an aqueous solution containing 1% FA or 0.05% TFA were tried (Figure 2).

After mobile phase selection, experiments were carried out on different column temperatures (ambient temperature, 25°C, 30°C, and 40°C) to obtain the best peak resolution and retention time. In order to perform the analyses with high sensitivity, they were performed at different wavelengths (254 nm and 290 nm) to determine the wavelength with the highest absorbance (Figure 3).

Method Validation

The method was validated by testing its specificity, linearity, recovery values, LOD, LOQ, and within-day and between-day precision and accuracy. The validation was conducted according to the guidelines set by the ICH for validating analytical procedures.[24]

RESULTS

Specificity (Selectivity)

The blank sample containing only the mobile phase was analyzed. Thus, it was checked whether there was any interference from the mobile phase during the retention time of SRT. Subsequently, standard working solutions containing SRT were injected into the UHPLC system, and chromatograms were obtained. In the

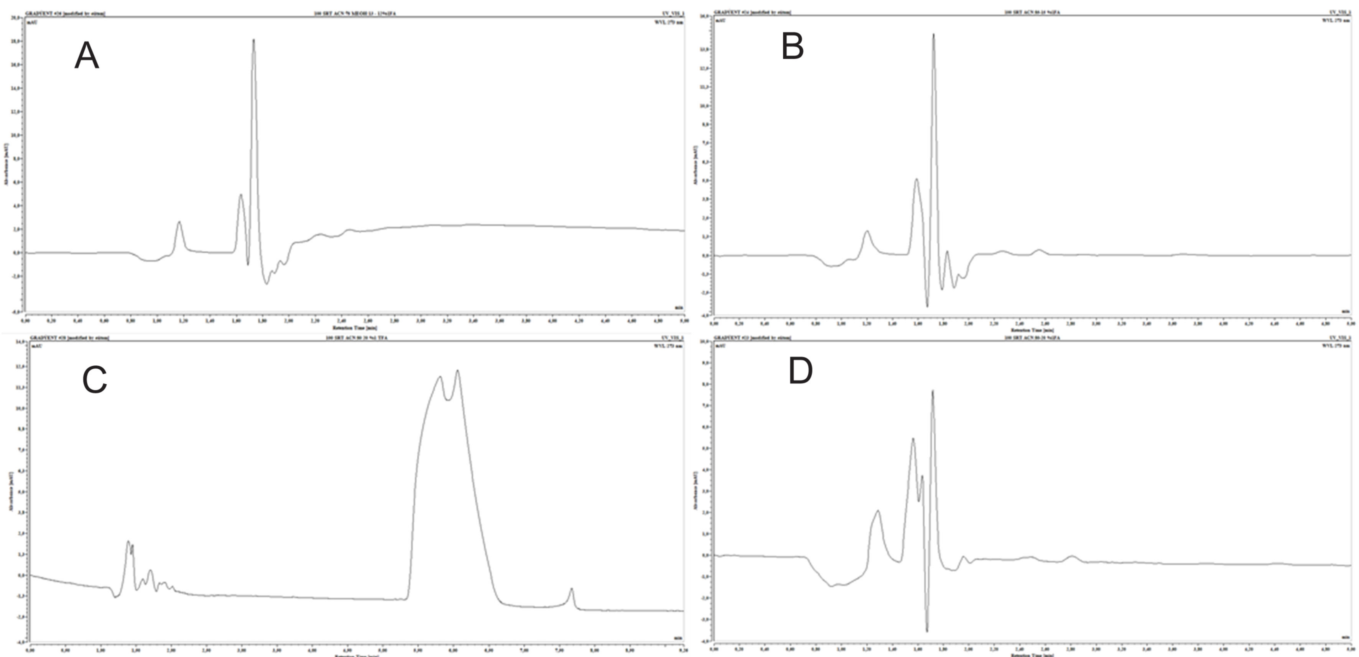

Figure 2. Chromatograms of 100 µg/mL sertraline in different mobile phase experiments. A. Methanol–acetonitrile–1% FA (75 : 12 : 13, v : v : v). B. Acetonitrile–1% FA (85 : 15, v : v). C. Acetonitrile–1% TFA (80 : 20, v : v). D. Acetonitrile–1% FA (80 : 20, v : v). FA, formic acid; TFA, trifluoroacetic acid.

chromatogram, the retention time of SRT was determined to be 2.1 minutes (Figure 4).

Linearity and Working Range

The area of the peak obtained from the chromatograms of the standard working solutions for each concentration was plotted against the concentration. The regression analysis of the calibration curve was performed to obtain the equation of the standard curve and the correlation coefficient. The correlation coefficient of the calibration curve formed by 0.3, 0.5, 1, 10, 25, 50, 75, and 100 µg/mL concentrations was found to be 0.9997, and the calibration curve equation was found to be $y = - 0.1096x - 0.0156$. The working range was selected as the concentration range where acceptable accuracy, precision, and linearity were achieved. Thus, it was determined that our method showed linearity between 0.3 and 100 µg/mL concentrations (Figure 4).

Accuracy and Precision

Quality control solutions prepared at low, medium, and high concentrations (20, 40, and 80 µg/mL) within the working range were analyzed 6 times with an interval of 3 days. Average concentrations and standard deviation values were determined. Accuracy was expressed as relative error (RE% = (found − added)/added × 100), and precision was expressed as relative standard deviation (RSD% = SD/mean × 100). Relative error percentage and RSD% of the intraday and interday concentration values were found to be less than 8%. Thus, it was determined that the UHPLC method we developed had high accuracy and precision (Table 1).

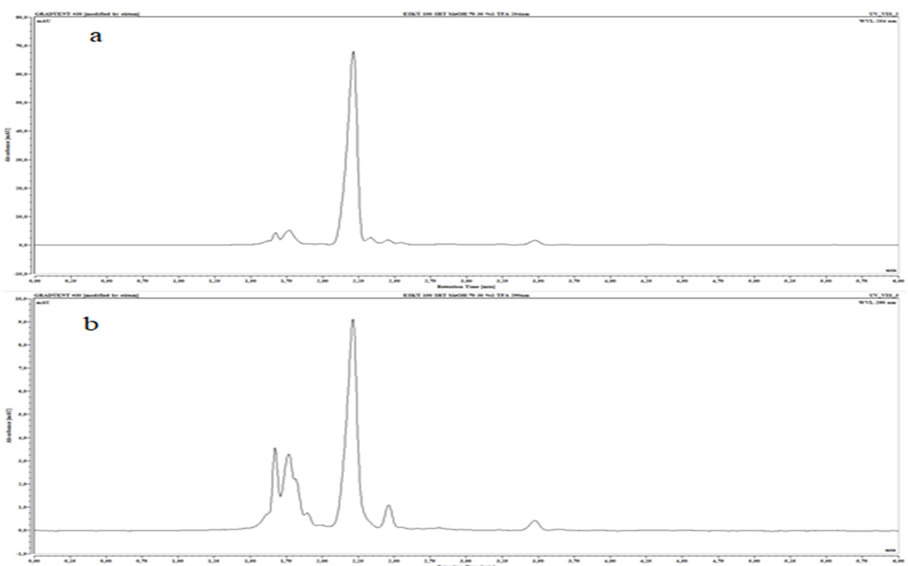

Figure 3. Chromatograms of 100 µg/mL sertraline in different wavelengths. a. 254 nm. b. 290 nm.

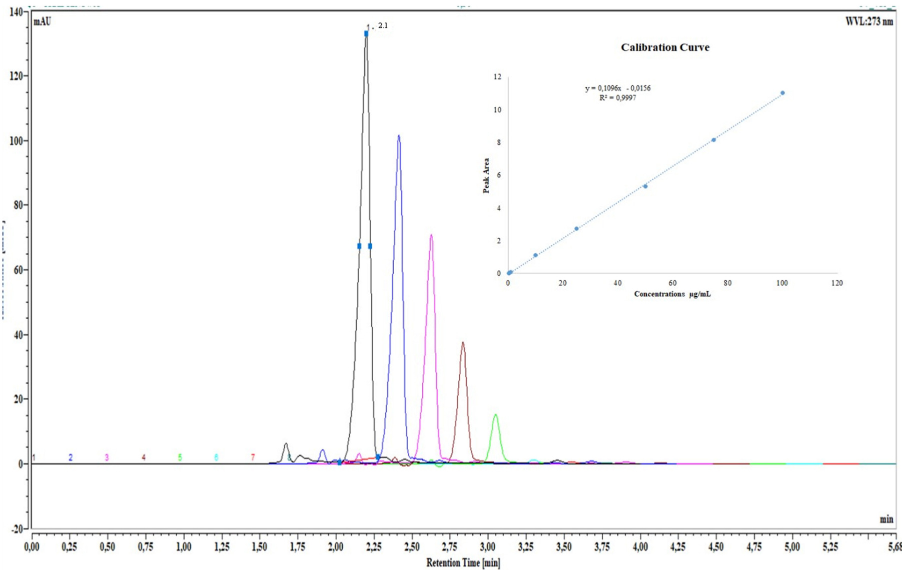

Figure 4. Chromatograms of standard working solutions containing sertraline (0.3-100 µg/mL).

Limit of Detection and Limit of Quantitation

Standard SRT solutions were prepared and analyzed at concentrations lower than the smallest concentration of the working range. By examining the chromatograms, the concentration corresponding to a signal/noise ratio of 3 was determined as 0.10 µg/mL, while the concentration corresponding to a signal/noise ratio of 10 was determined as 0.300 µg/mL (Figure 5).

Analytical Recovery

To conduct analytical recovery studies on a pharmaceutical preparation, the standard addition method was used. First, tablet solutions were prepared as per the instructions given in the "Preparation of Tablet Solutions" section. Chromatograms were obtained for the tablet solutions at a concentration of 5 µg/mL, and the peak areas were determined. Next, standard working solutions at 3 different concentrations (10, 40, and 85 µg/mL) were added to these tablet solutions separately. After obtaining the chromatograms and determining the peak areas, the analytical recovery values were calculated. To obtain these values, the concentrations of the added standard solutions (10, 40, and 85 µg/mL) were subtracted from the total solution concentration (tablet solution + standard solution) and then related to the concentration of the tablet solution (5 µg/mL). Table 2 shows that the average analytical recovery value was 100.2%.

Stability

A stability study of the stock and working solutions of SRT was carried out. After keeping the solutions containing SRT at 30, 60, and 90 µg/mL concentrations at the ambient temperature of 4 and −20°C for 24 and 48 hours, their chromatograms were taken using the HPLC method. By comparing the peak areas with standard solutions with the same concentrations, the results are given as % recovery in Table 3.

Application of the Ultrahigh-Performance Liquid Chromatography with Diode Array Detection Method to Pharmaceutical Preparations

To prove that the developed and validated UHPLC method was applicable to real samples, quantification was performed in 3 different pharmaceutical preparations containing SRT. For this, 3 tablet solutions at 1 mg/mL concentration were diluted, and 2 samples at 30 and 60 µg/mL concentrations for each tablet were analyzed by UHPLC methods and their chromatograms were taken (Figure 6).

DISCUSSION

Chromatography is a technique used in many different fields to separate and quantify chemical components in mixtures. High-performance liquid chromatography, a type of chromatography, stands out with its high accuracy, precision, repeatability, selectivity, sensitivity, recovery, ability to analyze very small sample volumes, rapid determination of results, and low cost compared to alternative techniques. These distinctive features make the HPLC method indispensable in the pharmaceutical industry, especially for the quantitative evaluation of pharmaceutical formulations and the examination of active drug ingredients in different matrices.

In the HPLC technique, the interaction of the components with the stationary and mobile phases, depending on their polarity, determines the way and time they arrive at the detector. Therefore, chromatographic conditions need to be optimized to both separate the components in a short time and obtain peaks with high resolution and short retention times. In this study, during the optimization phase of the UHPLC method developed for the determination of SRT, parameters such as mobile phase, stationary phase, flow rate, temperature, wavelength, and injection

Table 1. Accuracy and Precision Results of the Proposed Method

	Intra-day			Interday		
Added (µg/mL)	Found ± SD (µg/mL)	Accuracy (RE %)	Precision (RSD %)	Found ± SD (µg/mL)	Accuracy (RE%)	Precision (RSD %)
20	20.58 ± 1.12	0.37	1.34	19.88 ± 1.47	−0.74	2.43
40	39.73 ± 0.14	−0.16	2.04	41.37 ± 2.73	2.03	3.15
80	79.63 ± 3.12	−0.39	1.76	80.73 ± 1.36	0.36	1.26

RE%, relative error; RSD%, relative standard deviation; SD, standard deviation of 6 replicate determinations.

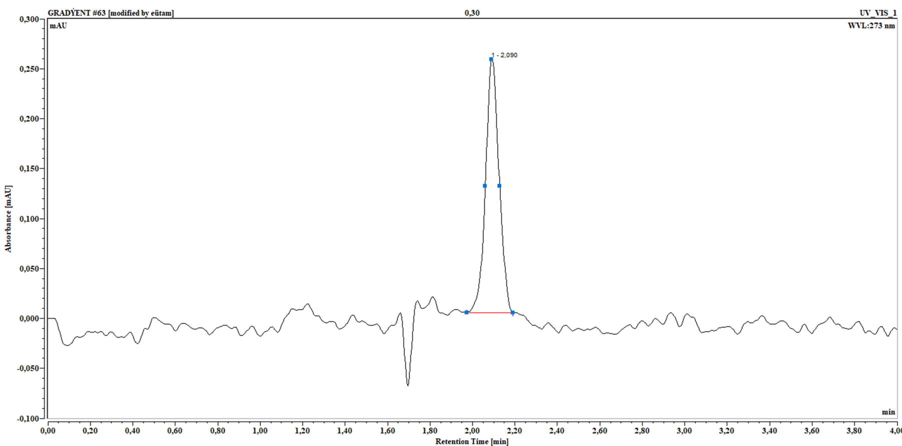

Figure 5. Chromatogram of the sample (0.300 μg/mL) of the limit of quantitation value of the ultrahigh-performance liquid chromatography with diode array detection method.

volume were examined with different modifications by looking at the literature. A reversed-phase C18 column (3 μm, 150 × 4.6 mm) was operated at temperatures of 25, 30, 35, and 40°C as the stationary phase. Flow rates of 0.8-1.2 mL/min and injection volumes of 5-40 μL were tried. Different wavelengths between 240 and 290 nm were tried with the DAD. In mobile phase selection, acetonitrile and methanol solvents were introduced into the system in binary and triple combinations with water, the aqueous solution containing 0.05% TFA, and the aqueous solution containing 1% formic acid. In all these experiments, standard solutions were analyzed one by one, and the results were evaluated. Thus, a new UHPLC method was developed for the quantification of SRT as an alternative to the methods reported in the literature. The developed method was validated by subjecting it to validity tests. The stability of SRT at room temperature, 4°C, and −20°C, at 24- and 48-hour standing conditions showed that more than 95% of SRT remained intact under all conditions compared to standard solutions.

In the study, the retention time of SRT was 2.1, and the analysis time was 4 minutes. Peak areas determined from chromatograms of standard SRT were plotted against concentration. A calibration curve showing linearity over a wide range was derived without the need for derivatization steps applied in spectrophotometric methods reported in the literature. In the

Table 2. Analytical Recovery Values from Tablets

Tablet	Tablet Solutions (μg/mL)	Added Standard Solutions (μg/mL)	Found ± SD (μg/mL)	Analytical Recovery %	RSD%
Lustral® 5 μg/mL	5	10	9.92 ± 0.62	99.9	1.37
		40	40.13 ± 0.16	100.3	2.37
		85	85.92 ± 0.21	100.6	3.61
Selectra® 5 μg/mL	5	10	10.04 ± 0.97	100.1	1.43
		40	41.50 ± 0.53	101.9	2.72
		85	84.10 ± 3.42	98.7	3.47
Misol® 5 μg/mL	5	10	9.81 ± 1.02	99.2	1.04
		40	39.15 ± 2.78	98.9	1.56
		85	86.72 ± 3.47	102.1	2.81

RSD%, relative standard deviation; SD, standard deviation.

Table 3. Stability Studies

Concentrations (μg/mL)	Ambient Temperature 24 hours	Ambient Temperature 48 hours	4°C 24 hours	4°C 48 hours	−20°C 24 hours	−20°C 48 hours
30	98.1 ± 0.14	97.8 ± 3.35	99.8 ± 3.62	98.7 ± 1.59	98.7 ± 0.49	101.3 ± 1.47
60	99.3 ± 0.72	101.3 ± 2.23	99.3 ± 1.57	96.6 ± 2.43	98.3 ± 2.91	99.5 ± 0.93
90	100.2 ± 2.06	99.7 ± 2.68	99.5 ± 2.49	99.2 ± 1.90	99.4 ± 3.20	103.4 ± 0.76

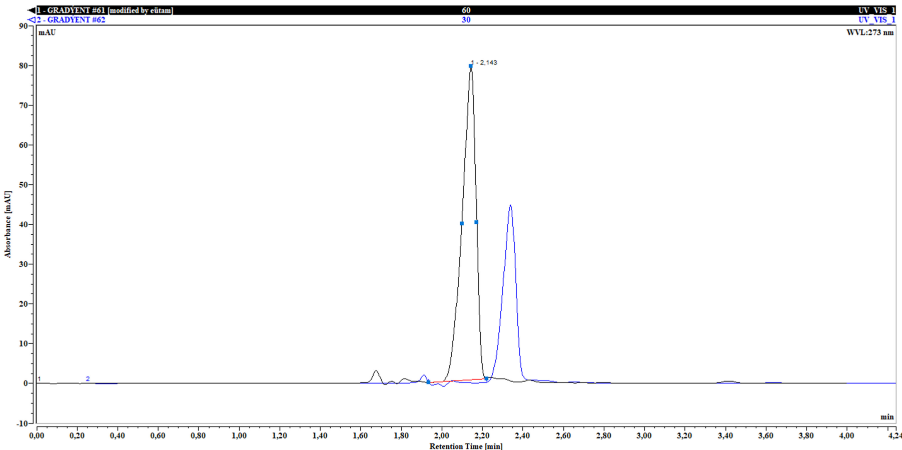

Figure 6. Chromatogram of samples with 30 and 60 μg/mL concentrations prepared from the stock solution of the Lustral® commercial preparation.

regression analysis, the correlation coefficient of the calibration curve was found to be 0.9997, and the calibration curve equation was $y = 0.1096x - 0.0156$. It was determined that the method showed linearity between 0.3 and 100 µg/mL. Using the signal/noise (S/N) ratio, the lowest concentration at which SRT could be detected (LoD) was 0.1 µg/mL (S/N = 3). The concentration at which SRT could be measured with appropriate accuracy and sensitivity (LoQ) was also determined to be 0.3 µg/mL (S/N = 10). In the intraday and interday analyses of quality control solutions prepared at 20, 40, and 80 µg/mL concentrations, RE% and RSD% were found to be lower than 8%. These values show that the UHPLC method has high accuracy and precision. The results of the analytical recovery from the pharmaceutical preparation were within the acceptance limits (85%-120% and RSD% < 8%).

When compared to other spectroscopic methods, the LOQ value of 0.3 µg/mL demonstrates higher sensitivity for the UHPLC method.[4,5] Since the method shows linearity at concentrations of 0.3-100 µg/mL, it offers a wider working range than the reported chromatographic and spectroscopic methods.[6,7,25] Additionally, the following mobile phases were used in previous HPLC studies: methanol: phosphate buffer (pH 4.5) (20 : 80 v/v),[6] acetonitrile and phosphate buffer,[9] acetonitrile–170 mM phosphate buffer, pH 3.0 (adjusted with 85% phosphoric acid) 68 : 32 (v/v),[10] methanol–acetate buffer (pH 2.8, 80 : 20, v/v),[7] and 0.1 M phosphate buffer and acetonitrile.[11] On the other hand, in this research, the use of an aqueous solution containing methanol and 0.05% TFA as the mobile phase provides a distinct economic advantage and convenience compared to the preparation of acetonitrile and buffer solutions. Along with economic considerations, the method also demonstrated higher sensitivity and a shorter analysis time compared to previous HPLC studies, with a detection limit of 0.3 µg/mL and an analysis time of 4 minutes.[6,7,11,26]

As a result of the study, a UHPLC method with high sensitivity and a wide working range conducted in a short analysis time, was introduced into the literature as an alternative method for the quantification of SRT in standard solutions and pharmaceutical preparations. The developed method gave acceptable results in the validity tests of selectivity, linearity, sensitivity, accuracy, precision, analytical recovery, and stability parameters. The method has been successfully applied to pharmaceutical preparations. Thus, the UHPLC method proved to be applicable for the quantification of SRT in standard solutions and pharmaceutical preparations.

Ethics Committee Approval: Ethical approval and informed consent are not required in our study as no research was conducted on human or animal specimens.

Peer-review: Externally peer-reviewed.

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Research Article

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Evaluating the Antioxidant Capacity of Rheum Ribes via Cupric Reducing Antioxidant Capacity, Ferric Reducing Antioxidant Power and 2,2-Diphenyl-1-picrylhydrazyl Methods

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ABSTRACT

Objective: It was aimed at determining the antioxidant capacity of the above-ground parts of the *Rheum ribes* species, known as "Iskın," which grows in the high parts of Erzurum province, using different methods.

Methods: Methanol extracts have been prepared to determine the antioxidant capacity of the *Rheum ribes* plant. The samples were then analyzed with cupric reducing antioxidant capacity, ferric reducing antioxidant power, 2,2-diphenyl-1-picrylhydrazyl radicals. As a reference sample, gallic acid compounds were reacted with the same radicals in different concentrations. The reference sample was determined for equivalent antioxidant capacity methanol extracts.

Results: Gallic acid was used as a reference standard along with the in vitro capacity measurements. The working range was determined where the absorbance graph was linear, and the calibration curves were plotted. The "Iskın" sample was then measured using the same workflow, with 6 consecutive samples. The antioxidant capacity of rhubarb samples was calculated in terms of gallic acid equivalent µg/mL.

Conclusion: In the study, the antioxidant capacity of rhubarb plant was determined using different methods. The response of the rhubarb plant against triphenyltetrazolinechloride, neocuproin, diphenyl picryl hydrazine radicals was determined by spectrophotometric measurements, and the findings were compared with the reference gallic acid sample. According to the results, it was determined that this endemic plant exhibited a very high antioxidant capacity. For this reason, it is thought that crop planting for this wild plant may lead to a remarkable contribution to the industry of the local region and may be used in the cosmetic and drug industries.

Keywords: Antioxidant, CUPRAC, DPPH, FRAP, *Rheum ribes*, spectrophotometer

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INTRODUCTION

Inorganic molecules or atoms containing one or more non-associated electrons in their outer orbit are called free radicals.[1] Free radicals are compounds with high activity. Enzyme reactions, autooxidation reactions, and life activities can be caused by endogenous sources and various environmental factors such as air pollution, cigarette smoke, ionized rays, ultraviolet (UV) radiation, and xenobiotics.[2] Free radicals in life cause very simple chain reactions with biological molecules such as nucleic acids, carbohydrates, and lipids.[3] Free radicals occur in organisms as a result of metabolic reactions; in macrophages, they are involved in many metabolic responses, such as the destruction of bacteria, electron transfer, and biosignal production. The presence of these reactive and excessive amounts of radicals in life can lead to diseases such as premature aging, neurological disorders, cardiovascular diseases, and cancer.[4]

Antioxidants are molecules that minimize the effects of free radicals, destroy their effects, and prevent reactions that can cause premature aging and various diseases. Antioxidants bind unassociated electrons in free radicals to form a stable structure.[5,6]

Free radicals affect the organism when the oxidative balance is notined. The antioxidants, on the other hand, provide a balance between the oxidant and the antioxidant, ensuring that tissues and cells maintain their structural integrity and fulfill their functions.[7]

The protective effects of naturally produced antioxidants in the human body are limited, and oxidative stress occurs when the production of reactive oxygen derivatives (ROS) exceeds the antioxidant capacity in biological systems. Increased ROS formation in tissues and cells for a variety of reasons is called oxidative stress.[8] Free oxygen radicals are formed in life by 3 basic mechanisms: metabolic, reactive, and energetic. The most important mechanism in the formation of free oxygen radicals is the metabolic pathway. Free oxygen radicals cause harmful effects in cells because they are highly reactive. It is important for each cell and tissue to maintain the balance between antioxidants and intrinsic oxidation within physiological limits. Oxidative stress causes damage to deoxyribose nucleic acid (DNA), carbohydrates, enzymes, and proteins. Neurodegenerative and cardiovascular diseases, autoimmune disorders, diabetes, and cancer form the molecular basis for the development of the cell membrane due to the damage caused by random bonding and rupture in the DNA chain and damage to structural proteins and enzymes.[9]

Antioxidants can be synthesized in the body or can be obtained from the outside. Antioxidant systems in living organisms are divided into 2 groups: those externally supplied by diet (exogenous) and those formed in the body (endogenic). While the endogenous defense systems are made up of enzymes, the exogeneous antioxidant defenses are divided into natural and synthetic. Synthetic antioxidants are compounds such as hydroxysianol, propyl galate, hydroxytoluene, and trolox. Natural antioxidants are mostly found in bacon, seeds, green vegetables, and fruits.[4]

Studies on plants show that plants are a good source of antioxidants. The natural sources of antioxidants are generally phenolic compounds in plants. It is associated with the antioxidant activity of the hydroxyl group found in phenolic molecules.[10] Plant phenolic compounds can reduce reactive oxygen species that have toxic effects on a range of biological and pathological processes.[11]

Rheum ribes is a perennial alpine plant in the Polygonaceae family.[12] The *R. ribes* plant, which grows on rocks and cliffs, grows in stress-induced natural environments, and its excessive consumption in Eastern Anatolia has led to research on the plant. It has some healing properties, such as vomiting, preventing hemorrhoids, reducing stomach pain, reducing symptoms of inflammation, measles, and diabetes, and increasing appetite. The root has laxative and bleeding-stop effects. It regulates the digestive system.[13]

R. ribes contains chemicals called polyphenols that help prevent cancer. These substances prevent the development of many cancers, especially leukemia.[14] Polyphenols are one of the most common groups of plant metabolites and constitute the most important single group of flavonoids. Phenolic compounds destroy free radical chains, exhibit antioxidant activity, and form chelates with metal ions that catalyze lipid peroxidation.[15]

Considering the phenolic component profile of *R. ribes*, especially its flavonoids, stilbens, and antrakinons, it is believed that they provide a potential source of antioxidants.[16] Our study aims at determining the antioxidant capacity of the aboveground portions of the species *R. ribes*, of the family Polygonaceae, known in our country as the "Iskın," using different methods.

METHODS

The study collected approximately 2 kg of surface samples of the *R. ribes* species. An antioxidant activity study was carried out by extracting methanol extract from these samples. For methanol extraction, samples were dried and powdered. The dust samples were then left to be macerated for 3 days. Extracts were obtained by rotating steam when the samples were kept at a temperature of under 2 hours in the fractional distillation scheme.

Ferric Reducing Antioxidant Power Method

In order to produce the ferric reducing antioxidant power (FRAP) radical, 100 mL balloons were first taken into a bowl containing 40 mM HCl acid in a 10 mM TPTZ solution and completed with a volume of pure water to 100 mL. Another container produced a 20 mM $FeCl_3$ solution. In a third container, an acetate buffer containing 0.3 M acetates at pH 3.6 was prepared. From these 3 solutions in equal volumes, 30 mL of FRAP solution was obtained. Subsequently, 285 μL of this solution was transferred to pleytes, and 15 μL of samples were added to it. After a 30-minute incubation phase, the dissolutions were measured at a wavelength of 593 nm.

Cupric Reducing Antioxidant Capacity Method

0.4262 grams of $CuCl_2$ are used to prepare the reactive first. $2H_2O$ was diluted and dissolved in 250 mL of pure water (10 mM). Then 19.27 grams of NH4Ac were dissolved in 250 mL of water to prepare the acetate buffer. For the preparation of a 7.5 mM neocuproin solution, a 25 mL balloon jojed is obtained by preparing 0.039 grams of the neocuproid compound with ethanol at 96% purity. The solution was then mixed with 60 μL acetate buffer, 60 μl CuCI2, 60 μ L neocuproin solution, and 66 μL sample. It was incubated for 30 minutes, and then 450 nm wavelength measurements were taken. This method is based on the conversion of the Cu (II) Neocuproin complex to Cu (I) Neo-Cuproine through compounds that have an antioxidant effect in the environment and its absorption in the composite complex at 450 nm wavelengths.

2,2-Diphenyl-1-picrylhydrazyl Method

This method is used to measure the antioxidant capacity of a radical called diphenyl-1 picryl hydrazyl (DPPH). This substance is a stable and affordable nitrogen radical that absorbs at 515 nm wavelengths. During scanning, the DPPH solution loses its color and can be easily measured on a spectroscopic photometer. When the DPPH method is studied closely, a 390 μL and 25 mg/L DPPH solution is prepared in methanol and mixed with a sample solution of 10 μL volume according to the original DPPH procedure. The final solution, prepared until the absorbance value is stable, is measured in a 515 nm wavelength spectroscopic photometer. This measurement can last up to 30 minutes in some samples. The percentage of non-reacting DPPH is determined by the following formula:

DPPH% = (DPPH remaining/DPPH first added) × 100

There is a correlation between DPPH inhibition and the concentration of antioxidant substance.

RESULTS

Ferric Reducing Antioxidant Power Method

Gallic acid was used as a control sample in the study. The average absorption values for each aliquot are shown in Table 1. Calibration curves were derived from the 1-50 μg/mL working range, where the absorbance graph was linear (Figure 1) and statistical calculations were made (Table 2). Subsequently, samples of *R. ribes* were measured by the same method to be 6 square samples, and the antioxidant capacity of the gallic acid equivalent was calculated. Average equivalent levels of gallic acid are shown

Table 1. Absorbance Values Vs. Concentration for the Ferric Reducing Antioxidant Power Method

Concentration µg/mL	1	2.50	5.00	7.50	10.00	15.00	20.00	25.00	30	35	40	45.00	50.00
ABSORBANCE	0.052	0.121	0.158	0.244	0.329	0.442	0.621	0.741	0.892	1.027	1.145	1.325	1.481
	0.053	0.124	0.154	0.240	0.334	0.451	0.625	0.744	0.878	1.036	1.154	1.331	1.480
	0.048	0.125	0.161	0.243	0.338	0.448	0.632	0.742	0.881	1.031	1.146	1.340	1.460
	0.048	0.122	0.160	0.251	0.330	0.453	0.630	0.750	0.895	1.035	1.157	1.323	1.492
	0.051	0.116	0.152	0.253	0.339	0.452	0.636	0.752	0.886	1.041	1.159	1.317	1.484
	0.049	0.113	0.159	0.252	0.327	0.450	0.631	0.741	0.891	1.032	1.137	1.330	1.472
Average	0.050	0.122	0.158	0.245	0.335	0.449	0.629	0.747	0.885	1.033	1.150	1.328	1.476

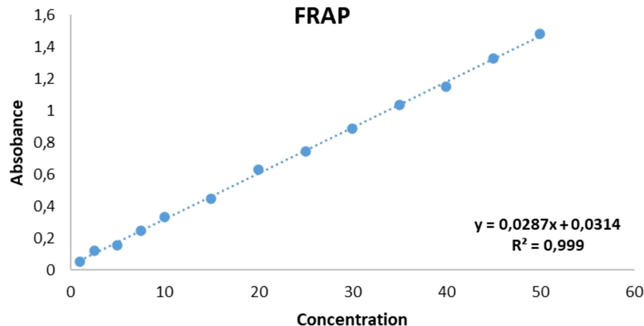

Figure 1. Concentration versus Absorbance Plot for Ferric Reducing Antioxidant Power Method.

Table 2. Statistical Values of the Calibration Curve for the Ferric Reducing Antioxidant Power Method

Method	WR (mg/mL)	LR[a]	Sa	Sb	R^2
FRAP	1-50	y = 0.0287 × 0.0314	4×10^{-4}	7×10^{-5}	0.999

LR, linear regression; R2, correlation coefficient; Sa, standard deviation of the shift in the regression curve; Sb, standard deviation of the slope of the regression curve; WR, working range.[a]6 calibration curves.

Table 3. Average Equivalent Amount of Gallic Acid for Ferric Reducing Antioxidant Power Method

	Gallic Acid equivalent Antioxidant Capacity (µg/mL)
Rheum Ribes	48.0 ± 0.8

in Table 1. Measurements showed that 30 µg/mL of extrene was approximately equivalent to 48 µg/mL of gallic acid (Table 3).

Cupric Reducing Antioxidant Capacity Method Method

Gallik acid was used as a control sample for the CUPRAC method. Six different gallic acid samples were taken from each concentration, and the absorption of these samples was measured. The average absorbance values for each concentration are shown in Table 4. Calibration curves were derived from the 1-50 µg/mL working range, where the absorbance graph was linear (Figure 2) and statistical calculations were made (Table 5). Then the Iskın sample was measured using the same method, making it 6 square samples. The gallic acid equivalent antioxidant capacity was calculated. The average equivalent levels of gallic acid are shown in Table 6.

2,2-Diphenyl-1-picrylhydrazyl Method

Gallic acid samples, which were different from the DPPH method, were left to wait for 30 minutes, and after this incubation phase, measurements were made of each sample at a wavelength of 515 nm. Gallic acid was used as a control sample. The percentage of inhibition values for each concentration are shown in Table 7. Calibration curves were found (Figure 3) with a linear absorbance chart for the 1-20 µg/mL working range and calculated statistically. (Table 8). The measurements were done using the same method, with 6 samples from the *R. ribes* samples. Gallic acid-equivalent antioxidant capacities have been measured. The average equivalent inhibition levels of gallic acid are shown in Table 9.

DISCUSSION

Antioxidants are chemicals that protect cells by either blocking the formation of free radicals or cleaning out existing free radical.[17] Its structures usually contain phenolic components. Antioxidant activity refers to the process that occurs when antioxidant molecules and free radicals interact. Antioxidant capacity is defined as the reaction of an antioxidant chemical mixture with a radical.[18]

Oxidative stress is caused by oxygen-using metabolic pathways and is the result of a disturbance of the prooxidant and antioxidant balance in the body.[19]

Since free radicals interact with biological macromolecules in conditions of oxidative stress, causing a variety of diseases, the total definition of antioxidants that destroy these free radicals in biological samples is important. Many analytical methods have been developed to measure the antioxidant capacity.[11]

In this study, the antioxidant activity of the methanol extraction of the *R. ribes* plant, which grows in high places in the Erzurum region, were determined by various methods of in vitro activity.

The antioxidant levels of Iskın extracts have been determined with FRAP, an in vitro anti-oxidant method commonly used in the literature. In order to produce the FRAP sample, 100 mL of the balloon was initially taken into a bowl containing 40 mM HCl acid in a 10 mM TPTZ solution and completed with a volume of 100 mL pure water. Another container produced a 20 mM $FeCl_3$ solution. In a third container, an acetate buffer containing 0.3 M acetates at pH 3.6 was prepared. From these 3 solutions in equal volumes, 30 mL of FRAP solution was obtained. Subsequently, 285 µL of this solution was transferred to pleytes, and 15 µL of samples were added to it. After a 30-minute incubation phase, the dissolutions were measured at a wavelength of 593 nm. Gallic acid was used as a control sample in the study. Calibration curves were derived for the absorbance of reference solutions prepared in the

Table 4. Average Absorbance Values Corresponding to Concentration for the Cupric Reducing Antioxidant Capacity Method

Concentration µg/mL	1	2.50	5.00	7.50	10.00	15.00	20.00	25.00	30	40	50
ABSORBANCE	0.1327	0.1864	0.2746	0.3907	0.5560	0.6620	0.8677	1.0373	1.3033	1.7832	1.9921
	0.1325	0.1860	0.2740	0.3901	0.5564	0.6622	0.8675	1.0371	1.3030	1.7830	1.9925
	0.1330	0.1865	0.2743	0.3905	0.5559	0.6624	0.8679	1.0374	1.3035	1.7828	1.9927
	0.1320	0.1862	0.2745	0.3906	0.5557	0.6618	0.8680	1.0370	1.3034	1.7836	1.9920
	0.1322	0.1869	0.2749	0.3910	0.5563	0.6615	0.8671	1.0376	1.3036	1.7835	1.9918
	0.1333	0.1866	0.2741	0.3912	0.5561	0.6623	0.8672	1.0377	1.3037	1.7831	1.9923
Average	0.1326	0.1864	0.2744	0.3907	0.5561	0.6620	0.8676	1.0374	1.3034	1.7832	1.9922

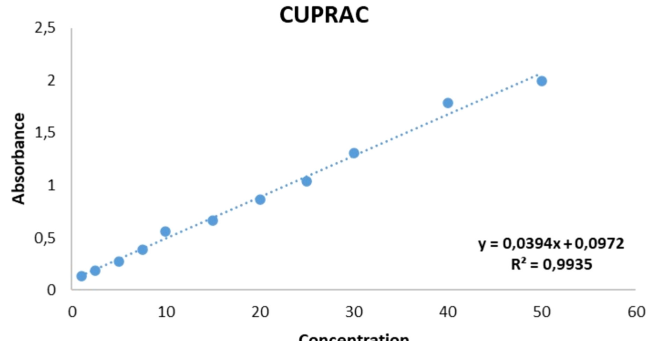

Figure 2. Concentration Versus Absorbance Pot for the Cupric Reducing Antioxidant Capacity Method.

Table 5. Statistical Values of the Calibration Curve for the Cupric Reducing Antioxidant Capacity Method

Method	WR (mg/mL)	LR[a]	Sa	Sb	R^2
CUPRAC	1-50	y = 0.0394 × 0.0972	4×10^{-4}	7×10^{-5}	0.9935

CUPRAC, cupric reducing antioxidant capacity; LR, linear regression; R2, correlation coefficient; Sa, standard deviation of the shift in the regression curve; Sb, standard deviation of the slope of the regression curve; WR, working range.[a]6 calibration curves.

Table 6. Average equivalent amounts of gallic acid for the Cupric Reducing Antioxidant Capacity Method

	Gallic Acid equivalent Antioxidant Capacity (μg/mL)
Rheum ribes	46.7 ± 0.45

range of 1-50 μg/mL. The correct equation of the calibration curve is y = 0.0287 × 0.0314. The Iskın extract was then measured at the same wavelength, and the gallic acid equivalent antioxidant activity was calculated at 48 μg/mL. It is one of the topics in the literature where emodine, aloe emodin, and flavonoids like parietine, antrachinons, and curcumin can show very high antioxidant activity.

Another method used is the CUPRAC method, which is widely used in literature. In order to produce the CUPRAC sample, 100 mL of the balloon was initially taken into a bowl containing 40 mM HCl acid in a 10 mM TPTZ solution and completed with a volume of 100 mL pure water. Another container produced a 20 mM $FeCl_3$ solution. In a third container, an acetate buffer containing 0.3 M acetates at pH 3.6 was prepared. From these 3 solutions, 30 mL of CUPRAC solution was obtained in equal volumes. Subsequently, 285 μL of this solution was transferred to pleytes, and 15 μL of samples were added to it. After a 30-minute incubation phase, the dissolutions were measured at a wavelength of 593 nm. Gallic acid was used as a control sample in the study. Calibration curves were derived for the absorbance of reference solutions prepared in the range of 1-50 μg/mL. The correct equation of the calibration curve was calculated as y = 0.0394 × 0,0972. The extraction of Iskın was then measured at 450 nm wavelengths, and the gallic acid equivalent antioxidant activity was detected at 46.7 μg/mL.

Another method used is DPPH, one of the antioxidant capacity determination methods commonly used in literature. This method is used to measure the capacity of a radical antioxidant called diphenyl-1 picryl hydrazyl. When the DPPH method was studied, according to the original DPPH procedure, a 390 μL and 25 mg/L DPPH solution was prepared in methanol and mixed with a sample solution of 10 μL. The absorbance value was measured at 515 nm wavelengths in the final solution spectroscopic photometer, prepared until stable, and the gallic acid equivalent antioxidant activity was detected at 16.7 μg/mL.

Iskın contains many anthraquinons and flavonoids. These are the anthraquinones and flavonoids emodin, aloe emodin, and chersetin, along with parietine. There are many opinions in the literature that these substances may have very high antioxidant activity due to their presence.[20,21] The study revealed similar findings to previous studies in the literature on the antioxidant properties of methanol extracts from *R. Ribes*.[22] It exhibited significant antioxidant activity, consistent with findings reported in the literature.

As expected, a high antioxidant capacity has been detected for Iskın samples grown in Erzurum in the study. According to this study, a sample of 30 μg/mL of the methanol extraction of the Iskın plant showed antioxidant activity equivalent to approximately 48 μg/mL of gallic acid by FRAP, which is about 1.5 times the reference effect on the neuproin radical. The CUPRAC method showed antioxidant activity equivalent to approximately 47 μg/mL of gallic acid, which is about 1.5 times the reference, for example. The DPPH method showed antioxidant activity equivalent to approximately 47 μg/mL of gallic acid, about half of the reference sample. This study suggests that the samples of Iskın collected in Erzurum have a very high content of flavonoids and anthraquinones. It is thought to be an important starting point for the future culture, standardization, and industrial use of the Iskın plant.

In the study, an endemic species, and ethnobotanical studies conducted in Erzurum, the antioxidant capacity of the Iskın plant used in the treatment of many diseases such as cancer and diabetes has been determined using different methods. The reaction of the Iskın plant to the radicals of triphenyl tetrazoline chloride, neokuproin, and Difenyl Pikril Hydrazine was detected by spectroscopic measurements, and the results were compared with the reference gallic acid sample. The results show that this endemic plant has very high antioxidant activity. The study is in line with similar studies in literature. According to these studies, it is believed that the Iskın plant found in our area has a very intense content of anthraquinones and flavonoids. Therefore, it is believed that the cultivation of this wildly grown plant, the standardization of its contents, and the introduction to the economically relatively underdeveloped East Anatolia and Southeast Anatolia economies can play a role in the development of both our region's industry and agriculture, as well as the extension to the industry of the compounds contained in its Iskın contents

Table 7. Average absorbance values corresponding to concentration for the DPPH method

Concentration μg/mL	1	2.50	5.00	7.50	10.00	12.50	15.00	17.5	20
ABSORBANCE	5.187	7.854	23.639	37.462	47.611	61.014	72.296	85.640	97.444
	5.213	7.850	323.608	37.412	47.650	61.040	72.282	85.624	97.440
	5.145	7.842	223.600	37.468	47.582	61.020	72.296	85.642	97.424
	5.223	7.874	223.650	37.476	47.610	61.032	72.264	85.618	97.480
	5.172	7.877	223.661	37.482	47.620	60.982	72.314	85.660	97.490
	5.201	7.891	223.642	37.470	47.620	61.024	72.318	85.640	97.512
Average	5,190	7,865	23,633	37,462	47,616	61,019	72,295	85,637	97,465

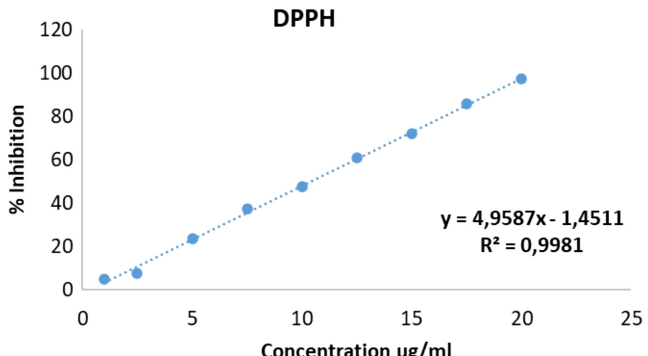

Figure 3. Absorbance Graph Corresponding to Concentration for the 2,2-Diphenyl-1-picrylhydrazyl Method.

Table 8. Statistical Values of the Calibration Curve for the 2,2-Diphenyl-1-picrylhydrazyl Method

Method	WR (mg/mL)	LR[a]	Sa	Sb	R^2
DPPH	1-20	y = 4.9587 × -1.4511	4×10^{-4}	7×10^{-5}	0.9981

DPPH, 2,2-Diphenyl-1-picrylhydrazyl; LR, linear regression; R2, correlation coefficient; Sa, standard deviation of the shift in the regression curve; Sb, standard deviation of the slope of the regression curve; WR, working range. [a]6 calibration curves.

Table 9. Average Equivalent Amounts of Gallic Acid for the 2,2-Diphenyl-1-picrylhydrazyl Method

	Gallic Acid equivalent Antioxidant Capacity (μg/mL)
Rheum Ribes	16.7 ± 0.38

used in the pharmaceutical and cosmetic industries. This work establishes a foundation for identifying, separating, and investigating the effectiveness of chemicals believed to be accountable for the antioxidant properties of the plant.

Ethics Committee Approval: Ethical approval and informed consent are not required in our study as no research was conducted on human or animal specimens.

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Investigation of the Antibacterial Activity of Different *Pelargonium Graveolens* Essential Oils Sold in the Market

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ABSTRACT

Objective: *Pelargonium graveolens* is a medicinal plant belonging to the Geraniaceae family. It is rich in essential oil. In this study, the antimicrobial activity of 5 different commercial *Pelargonium graveolens* essential oils was tested and compared.

Methods: The disc diffusion method was used for antimicrobial activity determination.

Results: Four essential oils were found to be effective against the gram-positive bacteria *Staphylococcus aureus*. One of the essential oils did not show any effect against any bacteria.

Conclusion: It has been observed that the essential oil of *Pelargonium graveolens* is especially effective against *Staphylococcus aureus*, and the herbal products available in the market do not show the same effect.

Keywords: Antimicrobial activity, essential oil, *Pelargonium graveolens*, *Staphylococcus aureus*

INTRODUCTION

Pelargonium graveolens (Geraniaceae) is a medicinal species with many biological activities, containing various compounds such as terpenes, flavonoids, steroids, alkaloids, tannins, phenols, saponins, glycosides, reducing sugars, and anthraquinones.[1, 2] It has been determined that massage and inhalation applied to the head and neck area in the morning and evening with *P. graveolens* essential oil is an effective application for reducing the perceived stress level.[3] *P. graveolens* has been used in traditional treatment for diseases such as hemorrhoids, dysentery, and cancer. It has some activities such as antioxidant, antibacterial, antifungal, and acaricidal. It contains a high amount of essential oil.[4] The main compounds were defined as citronellol, geraniol, and citronelly formate in essential oil.[5, 6] Moreover, kaempferol 3-O-rhamnoside-glucoside, kaempferol 3,7-di-O-glucoside, kaempferol 3-O-glucoside, quercetin 3-O-glucoside, quercetin 3-O-pentose, quercetin 3-O-rhamnoside-glucoside, quercetin 3-O-pentoside-glucoside, and myrisetin 3-O-glucoside-rhamnoside flavonoids were determined in methanolic and aqueous extracts, respectively.[6] Increasing antibiotic resistance in hospital and community-acquired infectious agents is an important problem. Resistance observed in microorganisms such as *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Enterobacter* spp., *Acinetobacter* spp., *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*. and *Mycobacterium tuberculosis* causes difficulties in treatment.[7] Antimicrobial resistance is an important problem threatening human and animal health all over the world. It is thought that new molecules waiting to be discovered in natural products will reduce the problem of antimicrobial resistance.[8] In this study, the antimicrobial activity of *P. graveolens* essential oils purchased from 5 different companies was investigated and compared.

METHODS

Five different brands of *Pelargonium graveolens* essential oil (100% purity) sold in the market were provided. Samples are numbered from 1 to 5. Antibacterial efficacy of oils against *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 9027, *Klebsiella pneumoniae* ATCC 700603, and *Proteus mirabilis* ATCC 25933 standard bacterial strains according to the Clinical and Laboratory Standards Institute (CLSI) determined by the Kirby–Bauer diffusion method.[9] Ciprofloxacin 5 μg (Oxoid, USA) was used as the standard antibiotic. Bacteria stored at −80 °C before the study were cultivated in tryptic soy broth medium and incubated at 37°C for 24 hours. Then, it was passaged into a solid medium suitable for growth (eosin–methylene blue agar, 5% sheep blood

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Table 1. Essential Oils Zone Diameters

	Bacteria				
Pelargonium graveolens Essential Oils	*Escherichia coli* ATCC 25922	*Klebsiella pneumoniae* ATCC 700603	*Pseudomonas aeruginosa* ATCC 9027	*Staphylococcus aureus* ATCC 29213	*Proteus mirabilis* ATCC 25933
1	-	-	-	13 mm	-
2	-	-	-	-	-
3	-	-	-	13 mm	-
4	-	-	-	15 mm	-
5	-	-	-	18 mm	-
Ciprofloxacin 5 µg	34 mm	26 mm	35 mm	24 mm	40 mm

agar) and incubated at 37°C for 24-48 hours. Essential oils were absorbed into empty and sterile antibiotic discs (10 µL for each disc). 0.5 MC Farland turbidity from all bacteria for disc diffusion test suspensions was prepared and cultivated on Mueller–Hinton agar. Essential oil-impregnated discs and ciprofloxacin discs were placed on the surface of the medium. After an overnight incubation at 37°C, the diameters of the zones formed around the discs were measured. The tests were repeated 3 times, and the averages of the zone diameters were calculated.

RESULTS

Except for the number 2 essential oil, all essential oils were found to be effective against *Staphylococcus aureus*. No effect was observed against any other bacteria. Zone diameters are shown in Table 1.

DISCUSSION

In this study, the antimicrobial effect of 5 different commercial *Pelargonium graveolens* essential oils against *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus,* and *Proteus mirabilis* bacteria was tested by the disc diffusion method. Ciprofloxacin was used as a control. Essential oils 1, 3, 4, and 5 were effective only against *Staphylococcus aureus* (13 mm, 13 mm, 15 mm, and 18 mm zone diameter, respectively). The number 2 essential oil was not found to be effective against any bacteria. They showed higher activity against gram-positive bacteria than gram-negative bacteria.

In a previous study, the antimicrobial effect of *P. graveolens* essential oil was investigated by the disc diffusion method against *Listeria monocytogenes*, *Salmonella enteritidis*, *P. aeruginosa*, *E. coli*, *S. aureus,* and *Bacillus subtilis*. It was found to be effective against all bacteria except *L. monocytogenes*. It showed the highest effect against *S. aureus*.[10] Similar to these results in our study, samples 1, 3, 4, and 5 were found to be effective against *S. aureus*. Unlike in our study, no effects were observed against *P. aeruginosa* and *E. coli*. In another previous study, extracts of *P. graveolens* obtained by decoction and infusion methods showed antimicrobial activity against *S. aureus* (16 mm and 15 mm zone diameter, respectively).[11] In this study, most of the essential oils showed activity against *S. aureus*. Methicillin-resistant *S. aureus* has become an important problem all over the world since it was first identified in 1961. In recent years, isolates of Methicillin-resistant *S. aureus* have caused both hospital-acquired and community-acquired infections.[12] *S. aureus* is a pathogen frequently isolated from bloodstream infections, skin and soft tissue infections, and postoperative wound infections acquired in the hospital.[13] If the effect of *P. graveolens* essential oil against this bacterium is investigated with more detailed studies, it may be beneficial.

In this study, it was observed that different branded essential oils obtained from the same plant showed different effects. Therefore, it is important that medicinal plants are standardized in terms of safety, efficacy, and quality. The plant must be correctly identified.[14]

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Rational Drug Use and Community Pharmacy

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ABSTRACT

Rational drug use is the set of rules that should be followed to enable people to take drugs in accordance with their clinical needs, at doses that meet their personal requirements, within sufficient time, and at the lowest cost to themselves and society. Rational drug use is briefly defined as "the planning, implementation, and monitoring process that enables effective, safe, and economical implementation of drug therapy." Pharmaceutical care is personalized care for the patient using drugs and aims to improve treatment outcomes, including quality of life. In Europe, the concept of pharmaceutical care is based on "personalized professional care for the patient in the pharmacy." This concept is defined as the individualized coaching of patients by pharmacists regarding their drugs. The concept includes close monitoring of the patient's prescription and use and education about the medication. It is also the pharmacist's responsibility to monitor treatment progress, provide advice, and evaluate all outcomes of care. The pharmacist's pharmaceutical care responsibilities include: presenting medication to the patient, informing the patient about the medication, patient counseling, monitoring treatment, monitoring adverse drug reactions, developing medication protocols/guidelines, working in an integrated manner with other health-care professionals, and recording medication histories. Pharmacists are in a position to improve preventable drug-related problems. One way to do this is for them to be educated in the rational use of drugs during their undergraduate and on-the-job training.

Keywords: Community pharmacy, pharmacy services, rational drug use

INTRODUCTION

Over the past 50 years, significant advancements in medical science have transformed many once-incurable diseases into treatable conditions. The continuous development of novel and more effective drugs with improved safety profiles has naturally led to a substantial increase in drug consumption.[1] For instance, in Turkey, drug consumption has experienced a notable surge. In 2002, approximately 700 million boxes of drugs were consumed, whereas this figure soared to 1.7 billion boxes in 2011, with an average per capita drug consumption of 23 boxes.[2] Consequently, the importance of making rational and informed choices when it comes to drugs has become increasingly significant, prompting the rise of the concept of rational use of drugs.[1] On a global scale, it is estimated that more than half of all drugs are inappropriately prescribed, dispensed, or sold. In response to this concern, the World Health Organization (WHO) has conducted extensive research on irrational drug use, recognizing it as a crucial health issue in developed countries. The WHO has also issued a statement outlining 12 fundamental principles to address this matter.[3]

12 key policies recommended by WHO to promote RDU:

1. Establish a multidisciplinary national institution to coordinate drug use policies,
2. Clinical guidelines,
3. Basic drug lists based on treatment choices,
4. Presence of drug and treatment committees in districts and hospitals,
5. Problem-based pharmacotherapy education in undergraduate curricula,
6. Maintenance of continuing medical education as a licensing requirement,
7. Follow-up of audits, inspections, and feedback,
8. Use of independent information on drugs,
9. Educating the public about drugs,
10. Avoidance of unethical financial incentives,
11. Establish appropriate and binding regulations,
12. Adequate government spending to ensure the availability of drugs and personnel.

Rational drug use (RDU) is the set of rules that should be followed to enable people to easily take drugs in accordance with their clinical needs, at doses that meet their personal requirements,

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within sufficient time, and at the lowest cost to themselves and society. Rational drug use is briefly defined as "the planning, implementation, and monitoring process that enables effective, safe, and economical implementation of drug therapy."[4] On the other hand, Taşdemir defines RDU as "a systematic approach that includes making the correct diagnosis for the patient, carefully defining the problem, determining the treatment goals, selecting the treatment with proven efficacy (reliable) from different options, writing an appropriate prescription, starting the treatment by giving clear information and instructions to the patient, and monitoring and evaluating the results of the treatment."[5] In this definition, rational use of drugs is assessed in a process that starts with the diagnosis of the patient and ends with the analysis of the results of the treatment. The RDU concept can be summarized by the 7 principle:[6]

- Appropriate indication
- Appropriate drug
- Appropriate route, dose, duration
- Appropriate patient
- Appropriate patient counseling
- Appropriate evaluation
- Reasonable price

In modern health-care services, the predominant approach to treat illnesses involves the administration of drugs. However, this widespread usage of drugs has given rise to the issue of irrational use of pharmaceuticals. The core of this problem stems from 2 main factors: the incorrect selection of drugs by health-care professionals and/or the inappropriate use of medications by patients.[4] A study conducted by Patel et al[7] in the USA revealed that drug-related issues constituted 28% of emergency department admissions. Strikingly, 70% of these cases were deemed preventable. The preventable conditions were predominantly attributed to various factors, including inappropriate drug use (46%), incorrect prescribing and insufficient patient follow-up (44%), lack of patient education (8%), and errors made by pharmacists during prescription filling (2%).[7]

Diagnosis, being the initial and crucial step in the RDU, treatment, which involves the accurate application of drugs, and finally patient follow-up, collectively ensure the successful implementation of RDU principles.[8]

Rational Prescribing Process and Rational Treatment Steps

Rational drug use represents a significant health issue on a global scale, with particular prominence in developing countries.[9] One of the most important actors in RDU is the physician, who determines the diagnosis and treatment and prescribes the medication.[10] Patients taking medication without a physician's advice or prematurely discontinuing medication before the recommended treatment duration, constitutes one of the primary issues encountered in RDU.[11] Rational drug use in Turkey revealed some concerning statistics. Specifically, 46.6% of patients were found to take medication without a physician's recommendation,[12] 77.3% of patients discontinued their medication earlier than recommended by the physician,[11] and 24.5% of patients recommended a medication they were satisfied with to someone else.[13] Data from the literature consistently indicate that rational drug prescribing practices are not being adhered to, and there is a lack of adequate communication between physicians and patients regarding RDU.[14]

The WHO lists a lack of knowledge on the part of physicians, a lack of updating of their skills and knowledge, busy working hours, unlimited availability of drugs, profit for physicians from the sale of drugs, and inaccurate and incomplete explanation of drugs to patients as some of the reasons that hinder the rational prescribing phase.[15]

Iskit summarized the rational prescribing process as follows:[16]

- Identifying the patient's problem and setting treatment goals.
- The most critical question to answer is: "Is drug treatment necessary?" Not all diseases or conditions require drug treatment. If medication will improve the patient's quality of life, it should be given after diagnosis.
- The patient's other medications and habits should be questioned. All medications used should be known. Smoking and alcohol consumption can alter the effects of medications.
- Make a list of effective drug groups and select an effective drug according to the criteria: Four main characteristics should be considered when deciding which class of drug to use in treatment. In accordance with the principles of rational pharmacotherapy, drug options that can achieve the physician's treatment goals should be compared in terms of efficacy (adequate dose, duration), safety (side effects, drug interactions), suitability (contraindications, ease of storage use), and treatment cost, and the best option should be determined.
- Personal choice is required. A drug molecule should be chosen from the drug group decided as a result of the previous analysis, using the same criteria (efficacy, safety, suitability, cost).
- The minimum number of drugs should be sufficient. A small number of drugs with known properties should be used in treatment.
- Treatment should be started at a low dose and the dose should be increased as slowly as possible. The dose may need to be adjusted according to the patient's response to the drug (individualization of treatment).
- Medication regimens should be simplified to improve patient compliance. Liquid formulations of drugs or, if possible, single daily administration should be preferred. Adequate and comprehensible information (preferably in writing) about the drug should be provided to the patient and their family. An easy-to-understand prescription should be written and the patient's family should be empowered to monitor the use of the medication.
- Treatment should be reviewed regularly and unnecessary drugs should be removed from the regimen. Adding new medications should be preferred if it will improve the patient's quality of life; it should be remembered that new medications may cause new problems.
- Effective communication between the patient and/or their relatives and the physician is necessary.

Irrational Use of Drugs

Today, the pharmacist plays an active role in ensuring that patients are properly informed and monitored about their drugs.[8,17] Failure at any of these stages will change the benefit of the drug, making it ineffective or even harmful.[6] Drug information is needed at every stage where decisions are made about RDU. Drug information should be reliable, unbiased, complete, and up-to-date.[18,19]

In addition to prioritizing the safe and effective use of drugs, it is also important to provide them at the lowest possible cost.[20] Irrational drug use reduces the quality of medical care and wastes

resources.[5] The use of drugs outside the principles of the RDU leads to unnecessary use of drugs and places a burden on the national economy in terms of health expenditure.[21] The psychosocial impact on patients of irrational use of the drug and the burden on the national budget are some of the main reasons for the emergence of RDU.[4]

Common examples of irrational patterns of drug use include:[6]

- Prescribing more than 1 drug to a patient (polypharmacy)
- Use of injectables when oral formulations would be more appropriate.
- Use of antimicrobials in inadequate doses or duration or for infections of nonbacterial origin, leads to antimicrobial resistance
- Failure to adhere to prescriptions
- Inappropriate self-administration of drugs or noncompliance with prescribed treatments

Rational Drug Use in Community Pharmacy

In Turkey, pharmacy is defined as "*a health service that carries out activities related to the preparation and presentation of various pharmaceutical types of drugs from natural and synthetic drug raw materials used in the diagnosis and treatment of diseases and the prevention of diseases; analysis of the drug, monitoring in terms of the continuity of its pharmacological effect, safety, effectiveness, and cost; ensuring standardization and quality assurance related to the drug; and informing patients about problems related to the use of drugs and reporting the problems that arise.*"[22] In recent years, the pharmaceutical approach to pharmacy has changed to put the patient at the center. The profession is evolving from a supplier of drugs to a provider of information and services, and ultimately to a provider of pharmaceutical care.[23]

A pharmacist must have good business skills as well as in-depth knowledge of drugs and the ability to communicate well. Pharmaceutical care can only be provided if a good relationship with the patient is established and pharmacists are able to communicate well with the patient about pharmacotherapy and related issues. In 1997, the American Society of Health-System Pharmacists published guidelines on pharmacist-led patient education and counselling, which emphasize that building a caring relationship with the patient in the pharmaceutical care process involves not only the technical aspects of information provision and communication but also the emotional aspects and empathy.[24]

Irrational use of drugs is a widespread problem in many countries, particularly in developing countries[9], because in most developing countries, community pharmacies are the main source of drugs.[25] The step where the pharmacist's role is most evident in the RDU is the prescription fulfillment stage.[18] Although it is the physicians who make the diagnosis and write the prescription, the pharmacist is the last person the patient communicates with before starting treatment. The patient will often remain in contact with the pharmacist until the next medical check-up.[8] The potential of community pharmacies to reach patients is considerable. To realize this potential, pharmacists are expected to fill the prescription while encouraging the patient to use the drugs effectively and safely.[26] Pharmacists are uniquely placed to ensure the safe and effective use of drugs.[25] Potential medication errors can be identified and minimized through pharmacist intervention.[27]

In the study conducted by Toklu et al[25] in 2002 in Istanbul, in which pharmacy practice in 84 community pharmacies was evaluated by questionnaire and simulated scenario application, it was found that only 43% of prescriptions were adequately labeled on the drug cabinet and only 6.5% of patients were informed about drug interactions in simulated prescription scenarios. In addition, 32% of pharmacists were not present in the pharmacy during the study and only 40.5% of prescriptions were filled by pharmacists. In a study conducted in community pharmacies in Ankara, it was found that pharmacy staff gave the opposite information to that given by the pharmacist on a topic that the pharmacy staff answered to the patient themselves, without referring the patient to the pharmacist.[28] As a result of the studies, it is observed that pharmacy practice in community pharmacies is inadequate in terms of good pharmacy practice.[25,28]

The involvement of pharmacists and the health-care team in the treatment process makes a significant contribution to improving the quality of prescribing and reducing medication-related problems.[29] However, communication between physicians and pharmacists, the 2 main pillars of treatment, is very limited and there is no dialogue between the 2 unless there is a problem with the prescription.[30] In particular, WHO wants pharmacists to be more involved in the overall health system and to utilize their broad academic experience.[23]

Barriers to the Diffusion of Rational Drug Use in Community Pharmacies

- Wrong medication given to the patient by the pharmacist[7,8]
- Inadequate indication information in pharmacy education[30]
- The fact that pharmacy education is not geared towards rational use of drugs and the practical shortcomings in this regard[31]
- Insufficient benefit from pharmacy placements during training[30]
- The reluctance of pharmacists to keep up with professional innovations[24]
- Failure to adapt to the new pharmacy service model and practices[30]
- Professional knowledge is outdated, and in parallel no internal training is organized[19]
- Problems related to the adaptation of knowledge acquired in undergraduate education to practice[31]
- Community pharmacists unable to practice due to problems with reimbursement systems and bureaucracy[30]
- The pharmacist does not feel the need to obtain information about the patient's medical history and treatment[8,30]
- Due to pharmacy workload, lack of time to spend with patients during the medication education phase and lack of communication between patient and pharmacist[8]
- Incomplete/incorrect information written or not written on drug boxes[8,25]
- Fulfillment of prescriptions by pharmacy assistants and technicians[25]
- Another fundamental problem is that the pharmacist is not present in his pharmacy[25]
- Pharmacists' lack of knowledge about adverse reaction reporting[30]
- Financial concerns and a lack of job satisfaction among pharmacists are also among the issues raised by pharmacists.[30]

Pharmacy Education and Rational Drug Use

The length and content of pharmacy education vary from country to country. Although the basic pharmacy courses are similar, the concept of pharmaceutical care varies according to the practice of community pharmacy in the region or country.[32,33]

In recent years, the place of rational drug use, medication management, and pharmaceutical care in the curricula of pharmacy schools in many countries has increased.[17] However, courses on ethics, clinical pharmacy, social pharmacy, communication, and health promotion are still insufficiently included in pharmacy faculty training in many European countries. As a result, pharmacists are thought to be reluctant to change the way they practice pharmaceutical care. Potential pharmacist trainees are reported to lack the knowledge and skills relevant to their new roles. The main problem in this area is that most universities still focus on training pharmacists only in their classical functions (compounding, developing, manufacturing, analyzing, and dispensing drugs). In particular, social pharmacy helps students learn theoretical issues from a community-oriented perspective.[23]

Rational Drug Use and Pharmaceutical Care

Pharmaceutical care was defined by Hepler and Strand as "*the professional responsibility of the pharmacist to achieve therapeutic outcomes that improve the quality of life of patients.*"[34] Pharmaceutical care is based on the relationship between the patient and the health-care professionals who take responsibility for the patient's care.[35] The ultimate goal of all health-care professionals, including pharmacists, is to improve the quality of life of patients.[36] Pharmaceutical care involves the active participation of the patient and the health-care professional in decisions about medication therapy. Pharmaceutical care has 3 main functions: identifying potential and existing drug-related problems, solving existing drug-related problems, and preventing drug-related problems.[35]

Pharmaceutical care is personalized care for the patient using the drugs and aims to improve treatment outcomes, including quality of life. In Europe, the concept of pharmaceutical care is based on "personalized professional care for the patient in the pharmacy." This concept is defined as the individualized coaching of patients by pharmacists regarding their drugs. The concept includes close monitoring of the patient's receipt, use, and education about the medication. It is also the pharmacist's responsibility to monitor treatment progress, provide advice, and evaluate all outcomes of care.[24]

The pharmacist's pharmaceutical care responsibilities include: presenting medication to the patient, informing the patient about the medication, counseling the patient, monitoring treatment, monitoring adverse drug reactions, developing medication protocols/guidelines, working in an integrated manner with other health-care professionals, and recording medication histories.[35]

Pharmacists providing sufficient and accurate information about prescription or nonprescription drugs recommended by the physician, being aware of side effects, following up on the patient's treatment, and referring the patient to the physician when necessary are the factors that will directly influence the success of treatment in the rational use of drugs. Pharmacists have an important responsibility to provide accurate information in order to prevent pharmacy-related and patient-related errors.[20,30,37]

Pharmacists need training and support to improve their practical skills to provide comprehensive pharmaceutical care and manage the rational use of drugs.[35] Many countries have active programs to introduce pharmacy-based pharmaceutical care.[23]

It seems that pharmacists especially play the role of dispensing drugs. In the near future, this activity will be carried out by the internet, machines, and/or technicians with minimal training. The fact that pharmacists have an academic education gives them a responsibility to serve society better than they do now. Pharmacists are in a position to improve preventable drug-related problems. It is thought that one way to do this is to be trained in the rational use of drugs during their undergraduate and professional training.

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Case Report

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Impact of Coronavirus Disease Diagnosis on Maternal and Neonatal Health Outcomes in Pregnancy: Two Case Reports

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ABSTRACT

The new coronavirus, named severe acute respiratory syndrome coronavirus 2, is a major public health problem. When exposed to infection, alterations in the immune system, decreased respiratory capacity, and vascular and hemodynamic changes put pregnant women at high risk of complications. The fetus/newborn may be harmed. This study aimed to determine the maternal and neonatal outcomes of 2 pregnant women diagnosed with coronavirus disease 2019 (COVID-19). These 2 cases contribute to the growing evidence of the potential adverse maternal and neonatal outcomes of (COVID-19) infection during pregnancy.

Keywords: Coronaviras disease 2019, pregnant woman, neonates, preterm birth, preeclampsia

INTRODUCTION

The coronavirus disease 2019 (COVID-19), which is a deadly disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), spread rapidly and led to a worldwide public health crisis in 2019.[1] The coronavirus disease 2019 infection risks the health of pregnant women and their fetuses in the perinatal period.[2] For this reason, pregnant women are considered a sensitive population regarding the strategies developed for and the importance given to preventing infection during infectious diseases.[3]

Pregnant women infected with COVID-19 have an increased risk of pregnancy complications, such as preeclampsia, eclampsia, hemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome, and referral to intensive care, compared to those not infected with COVID-19.[4] Furthermore, when infected with COVID-19 in the perinatal period, problems such as fetal distress, premature birth, respiratory distress, and death occur.[5] COVID-19 during pregnancy has also been associated with an increased likelihood of preterm birth.[6]

The risk of vertical transmission of COVID-19 infection during pregnancy is crucial for newborn health.[7] There is no common consensus on the vertical transmission of COVID-19 from mother to fetus during pregnancy. Still, it has been reported that it is transmitted to the newborn by the aerosol route.[8] Studies have not found any evidence of vertical transmission during the intrauterine period due to amniotic fluid and cord blood examination.[5,9,10] No evidence of the presence of SARS-CoV-2 was found in the postpartum breast milk analysis.[10] A study conducted in Turkey reported a SARS-CoV-2 infection in the placenta.[11]

It is vital to prevent infection during pregnancy, control infected pregnant women according to current guidelines, and closely monitor newborns at risk of COVID-19.[10-12] In addition, individual health guidelines, effective medical care, and anti-infection practices designed for the protection of health can prevent adverse maternal and fetal outcomes at the global level.[13]

This study describes the maternal and neonatal outcomes of 2 pregnant women infected with COVID-19 pneumonia.

CASE PRESENTATIONS

Case 71

A 36-year-old woman (gravida 1, para 1), 34 weeks pregnant, was admitted to the hospital for routine control. The ultrasound screening revealed that the baby was in breech presentation and diagnosed with polyhydramnios as a result of the measurements. It was decided that hospitalization was

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necessary due to the contractions observed as a result of the nonstress test performed to evaluate the fetus's health. The SARS-CoV-2 test was positive according to the diagnosis based on reverse transcription–polymerase chain reaction (RT-PCR) from the nasopharyngeal swab routinely performed during hospitalization. The patient was observed in an isolated room and did not demonstrate any symptoms of COVID-19. The patient's respiratory rate was 22 breaths per minute, oxygen saturation was 98%, blood pressure was 100/70 mmHg, pulse was 80 beats/minute, and temperature was 36.6°C. In the 27th week of her pregnancy, her husband was diagnosed with COVID-19.

Prenatal Treatment
Enoxaparin sodium 0.4 2 × 1 was given intramuscularly (IM) in 2 doses every 24 hours, while 50 mg of ritonavir and 200 mg of lopinavir 2 × 2 25 mg were given orally. Laboratory blood analysis showed that C-reactive protein was 0.11 mg/L. All laboratory results were within the normal range. Three days after hospitalization (gestation week 34+4), the patient's contractions started. Due to breech presentation, delivery was performed by cesarean section.

Postpartum Treatment
The patient was given favipiravir 2 × 3 200 mg, metronidazole 2 × 500 mg IV, diclofenac sodium 2 × 1 (PRN) IM, and Enoxaparin sodium 1 × 1 0.4 IU SC. The patient was discharged from the hospital at her own request, rejecting the tests and treatments to be conducted 24 hours after the cesarean section. The risks of early postpartum discharge were explained. The vital signs of the mother were measured to be within the normal range.

Newborn
A baby boy with a birth weight of 2190 g, a birth length of 49 cm, and a head circumference of 33 cm was born. The Appearance, Pulse, Grimace, Activity and Respiration (APGAR) scores at 1 and 5 minutes were found to be 1 and 1, respectively. There was no respiratory effort and no signs of crying in the newborn. Muscle tone was hypertonic, and flexion was observed in the hip and knee joints. The newborn died shortly after birth.

CASE 2

A 30-year-old (gravida 1, para 1) 31+6 weeks pregnant woman was hospitalized due to high blood pressure. Ten days ago, she was diagnosed with COVID-19 at a hospital in the outer center. The pregnant woman did not have any symptoms of COVID-19. The patient's respiratory rate was 24/minute, oxygen saturation was 95%, blood pressure was 140/100 mmHg, pulse was 84 beats/minute, and fever was 36.5 °C. The patient stated that she had blurred vision. The RT-PCR test was performed during hospitalization, and the test result was negative. Six days after the pregnant woman was diagnosed with COVID-19, she applied to another state hospital due to a headache, and her blood pressure was recorded as 170/100. She was admitted to our hospital due to persistent high blood pressure. While she was 26+4 weeks pregnant, her husband was diagnosed with COVID-19.

As a result of the urinalysis performed in our hospital, protein ++ was found in the urine. Preeclampsia was diagnosed because the pregnant woman had signs and symptoms such as high blood pressure, blurred vision, and headache.

Prenatal Treatment
Acetylsalicylic acid 100 mg (1 × 1) was given orally, Betamethasone sodium phosphate and betamethasone acetate (1 × 24) were administered in 2 doses with a 24-hour interval, and an oral treatment of methyldopa 3 × 1 was started.

Loading dose of magnesium sulfate ($MgSO_4$): It was administered by continuous intravenous infusion of 4 ampoules (6 g) of $MgSO_4$ in 100 mL of 0.9% isotonic sodium chloride in 15-20 minutes.

Magnesium sulfate maintenance therapy: 13 ampoules of 15% MgSO4 were placed in 1000 mL of lactated Ringer's and administered by an infusion pump so that 1.8 g (1-3 g) of MgSO4 in 100 mL of liquid would be given per hour.

On the second day of hospitalization (32+1 weeks), 4 ampoules (1 ampoule of 840 mg) of sodium bicarbonate placed in 500 mL of 0.9% isotonic sodium chloride were given as 20 cc/h IV due to Na:120 mEq/L revealed in the blood analysis. Since Na: 118 mEq/L was found in the blood analysis performed for control purposes, sodium chloride treatment was repeated. In the second control analysis, it was seen that Na was 120 mEq/L. It was concluded that an electrolyte imbalance was initiated due to the decrease in Na. Hematuria was detected, and the total protein in urine was 5.78 g, while hemoglobin was 14.4 g/dL, thrombocytopenia 10 000/mm3, uric acid 7.4 g/dL, and blood pressure was 140/100 mgHg. There was also increased electrolyte imbalance and edema in the legs and vulva. A diagnosis of severe preeclampsia was made, and a cesarean section was planned. After cesarean delivery, the patient was followed up in the intensive care unit, and intubation was not required. Our case was followed up in the intensive care unit for 4 days and discharged from the hospital after being followed up in normal patient service for 2 days.

Postpartum Treatment
She received methyldopa (2 × 1) 250 mg, nifedipine (2 × 1) 60 mg = orally, and cefuroxime axetil (2 × 1) 500 mg orally while enoxaparin sodium (1 × 1) was given through subcutaneous injection. Maintenance treatment was continued for 24 hours after delivery. Our case was followed up in the intensive care unit for 4 days, followed up in the normal patient service for 2 days, and then discharged.

Newborn
A baby girl with a birth weight of 1250 grams, a birth length of 39 cm, and a head circumference of 28 cm was born. The APGAR scores at 1 and 5 minutes were found to be 5 and 8, respectively. The baby was transferred to the neonatal intensive care unit due to premature birth and respiratory distress. The RT-PCR test was not performed on the newborn since the RT-PCR test performed during the mother's hospitalization was negative. No signs of respiratory distress were observed in the newborn on the second postnatal day. The baby continued to be followed up in the neonatal intensive care unit due to its premature birth. She was fed with breast milk (1 mL) for a week from the second day after delivery. The baby, weighing 2065 grams on the 34th day after birth, was discharged from the hospital. She is fed with 60 mL of milk on the 44th day after birth, and there is no problem with her health status. Postpartum 45. Rop examination and eye test screening results were found to be normal.

DISCUSSION

This study presents 2 cases of pregnant women who underwent emergency cesarean sections at 34+4 weeks and 32+1 weeks gestation. Both pregnant women and their spouses were diagnosed with COVID-19.

Pregnant women infected with COVID-19 during pregnancy are at high risk for complications of premature birth or stillbirth.[14] In a multinational cohort study, when pregnant women with and without a diagnosis of COVID-19 were compared, COVID-19 in pregnancy was associated with increases in severe maternal morbidity, mortality, and neonatal complications.[4] It was found that, of the pregnant women infected with COVID-19, 35.0% experienced preterm birth, while 30.5% experienced low birth weight.[3] A multicenter study conducted in Turkey reported that the prevalence of preterm birth was 26.4% and low birth weight was 12.8%.[15] A study in England reported that the number of stillbirths increased during the pandemic compared to the prepandemic period.[16] In our study, the postpartum death of case 1's baby was consistent with the study's findings. Our study includes the results of 2 women and their babies infected with COVID-19. Although both women had tested positive for COVID-19, the reason for their preterm births could not be determined. Case 1's baby died postpartum, while case 2's baby was discharged from the hospital in good health. Since no tests were conducted on the babies, the cause of the baby's death in case 1 could not be determined.

Preeclampsia is a disease characterized by proteinuria and high blood pressure after 20 weeks of gestation and is the cause of maternal and perinatal morbidity and mortality.[17] Risk factors for preeclampsia include primigravida, prepregnancy weight, age, history of preeclampsia, and lifestyle changes.[18] Severe acute respiratory syndrome coronavirus 2 may contribute to the development of preeclampsia by causing hypoxic injury.[19] In a study conducted with 23 pregnant women in England, preeclampsia occurred in 10.5% of the patients in the third trimester, with one developing liver dysfunction, HELLP, and Disseminated Intravascular Coagulation (DIC).[20] In a meta-analysis study, a significant difference was not found in preeclampsia risk between infected and uninfected women.[21] During pregnancy, COVID-19 is associated with preeclampsia, especially in nulliparous women.[22] Our findings are similar regarding the second case in our study, who was also nulliparous and was diagnosed with preeclampsia.

A case-control study reported that SARS-CoV-2 infection had little effect on neonatal outcomes.[23] In another study, receipt of breast milk at discharge was lower in neonates whose mothers were SARS-CoV-2 positive within the 10 days before birth.[24] Yet, another study found no evidence of vertical transmission in newborns of mothers positive for or with suspected SARS-CoV-2 infection.[25] Regarding the second case in our study, the baby was fed with breast milk on the second day after birth, and the process continued with no interruption. No symptoms of COVID-19 were observed in the newborn.

The role of COVID-19 in causing preterm birth remains inconclusive. The current study presents 2 case reports. The research results cannot be generalized to the population but provide important findings for future studies.

CONCLUSION

Our study presented the evaluation of 2 cases regarding maternal and neonatal outcomes and the associated risk factors. There is a need to provide the vulnerable population of pregnant women with evidence-based guidelines and information to manage their health and protect themselves against risks.

Informed Consent: Written informed consent was obtained from the two pregnant women who participated in this study.

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