Volume 13, Issue 4, Page 23-28, 2024 https://doi.org/10.46810/tdfd.1455825 Research Article

BİNGÖL UNIVERSITY

Türk Doğa ve Fen Dergisi
Turkish Journal of Nature and Science

www.dergipark.gov.tr/tdfd

Phytochemical Analysis and Determination of Antioxidative, Antimicrobial And *In Vitro* Cytotoxic Properties of Black Rosehip (*Rosa Pimpinellifolia* L.) Fruits Growing in The Northeast of Türkiye

Büşra KICIK[1], Hamit Emre KIZIL[2*], Sinan BAYRAM[2]

[1] Bayburt University, Engineering Faculty, Food Engineering Department, Bayburt, Türkiye
[2] Bayburt University, Vocational School of Health Services, Department of Medical Services and Techniques, Bayburt, Türkiye
Büşra KICIK ORCID No: 0000-0002-2053-2229
Hamit Emre KIZIL ORCID No: 0000-0001-6193-3734
Sinan BAYRAM ORCID No: 0000-0002-2156-1566

**Corresponding author: ekizil@bayburt.edu.tr*

(Received: 20.03.2024, Accepted: 12.09.2024, Online Publication: 30.12.2024)

Keywords
Rosa pimpinellifolia (L.), MCF-7, Antibacterial, Antioxidant, Cytotoxicity

Abstract: The aim of this study was to determine the phenolic content of *Rosa pimpinellifolia* (L.) fruit extract and antibacterial, antioxidant activity and show the cytotoxic effects of different concentrations of *Rosa pimpinellifolia* L. fruit extract obtained by ultrasonic assisted method on MCF-7 cell line by WST-8 assay. Antibacterial effects analyzed by disk diffusion method and determined that there was no antibacterial effect in 500 µg/mL *Rosa pimpinellifolia* L. fruit extract dose. In addition, the total phenolic content was determined by the Folin-Ciocalteu method and found that total phenolic content is 37.5±0.2 mg GA/g. Antioxidant activity value was determined as 32.06±1.1 mg TE/g by ABTS method and 23.47±1.6 mg TE/g by DPPH method. The highest cytotoxic effect of *Rosa pimpinellifolia* L. fruit extract was determined in dose of 40 µg/mL on MCF-7 cell.

Türkiye'nin Kuzeydoğusunda Yetişen Siyah Kuşburnu (*Rosa Pimpinellifolia* L.) Meyvelerinin Fitokimyasal Analizi ve Antioksidatif, Antimikrobiyal ve *In Vitro* Sitotoksik Özelliklerinin Belirlenmesi

Anahtar Kelimeler
Rosa pimpinellifolia (L.), MCF-7, Antibakteriyal, Antioksidan, Sitotoksisite

Öz: Bu çalışmanın amacı, *Rosa pimpinellifolia* (L.) meyve ekstraktının fenolik içeriğini ve antibakteriyel, antioksidan aktivitesini belirlemek ve ultrasonik destekli yöntemle elde edilen farklı konsantrasyonlarda *Rosa pimpinellifolia* (L.) meyve ekstraktının MCF-7 hücre dizisi üzerindeki sitotoksik etkilerini WST-8 analizi ile göstermektir Antibakteriyel etkiler disk difüzyon yöntemiyle analiz edilmiş ve 500 µg/mL *Rosa pimpinellifolia* (L.) meyve ekstraktı dozunda antibakteriyel etkinin olmadığı belirlenmiştir. Ayrıca toplam fenolik içerik Folin-Ciocalteu yöntemiyle belirlenmiş ve toplam fenolik içeriğin 37.5±0,2 mg GA/g olduğu bulunmuştur. Antioksidan aktivite değeri ABTS yöntemiyle 32.06±1,1 mg TE/g, DPPH yöntemiyle ise 23.47±1.6 mg TE/g olarak belirlenmiştir. *Rosa pimpinellifolia* (L.) meyve ekstraktının MCF-7 hücresi üzerindeki en yüksek sitotoksik etkisi 40 µg/mL olarak belirlenmiştir.

1. INTRODUCTION

Cancer, characterized by the uncontrolled growth and spread of cells in the body, poses a significant global public health concern, with its prevalence on the rise [1-3]. According to data from the World Health Organization (WHO) on cancer statistics, breast cancer accounts for 12%, lung cancer 12%, colorectal cancer 11%, prostate cancer 8%, stomach cancer 6%, liver cancer 3%, with the remaining 48% encompassing other types of cancer. Among these, breast cancer is the most frequently diagnosed cancer in women, with projections indicating a doubling of the 8.1 million deaths recorded in 2012 by the year 2040. Particularly alarming is the diagnosis of breast

cancer in young women aged 20-59, who face an increased risk of mortality within this demographic [4, 5]. Various risk factors contribute to the etiology of breast cancer, including certain medical conditions, sedentary lifestyles, obesity, exposure to environmental toxins and trauma to breast tissue, and prolonged use of exogenous hormones [6]. Breast cancer presents a complex phenotype, encompassing both carcinoma in situ and invasive carcinoma, with diverse histological subtypes [7].

One promising avenue in cancer treatment lies in the use of medicinal aromatic plants. In this regard, rosehip fruit emerges as a potential alternative remedy. Belonging to the genus *Rosa* within the Rosaceae family, rosehip is characterized by its erect or shrubby stature, ranging in height from 1.5 to 3.5 meters, depending on the variety. The fruit typically exhibits an elliptical shape and is found in hues of yellow, red, and orange [8]. Rosehip boasts a spectrum of health-promoting properties, including anti-inflammatory, antioxidant, immunomodulatory, cardioprotective, anticancer, antidiabetic, neuroprotective, and antibacterial effects. Rosehip is renowned for its rich phytochemical composition, comprising phenolics, flavonoids, folic acid [9], vitamins such as α-tocopherol (Vitamin E) and γ-tocopherol [10], terpenes, carotenoids, galactolipids, minerals, and tannins [11]. Furthermore, it contains essential fatty acids such as oleic, linoleic [12], alongside catechin, chlorogenic acid, caffeic acid, and apigenin 7-O-glucoside [13].

Our study is conducted in the province of Bayburt, an area characterized by its natural landscape, nestled within the inner region of the Upper Coruh Basin, within the widened portion of the valley carved by the Coruh River. There is limited research on the biological activity of *Rosa pimpinellifolia* (L.) extracts naturally occurring in Bayburt province. This research aims to determine the phenolic content, antibacterial and antioxidant activity of *Rosa pimpinellifolia* (L.) fruit extract at varying concentrations, as well as its cytotoxicity on breast cancer cells.

2. MATERIAL AND METHOD

This study was conducted using the data of Büşra KICIK's master's thesis (YÖK ID: 701041).

2.1. The Methanolic Fruit Extract Preperation

Black fruit rosehip (*Rosa pimpinellifolia* L.) was gathered from the same plants and location in Bayburt Province, Central District Gümüşsu Village, at an altitude of 1.817 m. A portion of the collected *R. pimpinellifolia* (L.) plant was dried in a dark environment away from sunlight exposure for extraction processes. The completely dried 1 g fruit sample was pulverized, and added 10 mL of 80% methanol. The mixture was then subjected to an ultrasonic water bath at 40 ºC for 60 minutes. Then, it was centrifuged at 5000 rpm for 30 min, and transferred into a glass tube. The extraction process was conducted twice, and the resulting supernatants were pooled together to attain a total volume of 25 mL using methanol. The mixture was then passed through a 0.45 µm membrane filter and subsequently transferred to an amber bottle. The extract was stored at -20°C for further analysis, while the remainder was dried using a vacuum evaporator, lyophilized.

2.1.1. Evaluation of total phenolic content

The total phenolic content was determined following the Folin-Ciocalteu method as outlined by Magalhães et al. (2010), with gallic acid employed as the reference standard. In summary, 50 µL of fruit extract was combined with 50 µL of Folin-Ciocalteu reagent (1:5, v/v) and 100 µL of sodium carbonate (Na_2CO_3) solution (0.35 M) in a microplate well. The mixture was allowed to react for 3 minutes, after which the absorbance was measured at 760 nm using a spectrophotometer (Multiskan GO Thermo). All measurements were conducted in triplicate. The outcomes were quantified and expressed as gallic acid equivalent (mg GAE/g).

2.1.2. Determination of *in vitro* antibacterial effect

For the assessment of antibacterial activity, 24-hour-old cultures were employed. Pathogenic strains, preserved as stock cultures at -20°C, were introduced into tryptic soy agar (TSA) medium using the streak plate inoculation technique. After inoculation, single colonies were selected from the strains and incubated at 37°C for 24 hours. These colonies were then transferred to sterile tryptic soy broth medium in 15 mL Falcons and incubated for 18 hours. The resulting strains were adjusted to 0.5 McFarland standard turbidity and used as the inoculum [14]. The in vitro antibacterial activities of *Rosa pimpinellifolia* (L.) extracts were evaluated using the disc diffusion method. The solvent was evaporated from the methanol extract, previously prepared using ultrasonic extraction, using a nitrogen volatilization device. After quantifying the obtained active substance, dimethyl sulfoxide (DMSO) was added to achieve a dose of 500 µg/mL. Subsequently, 10 µL of this extract was taken and impregnated into 6 mm diameter blank antimicrobial susceptibility discs (OXOID). The impregnated discs were then allowed to dry in a sterile cabinet for two hours. Following this drying period, pathogenic microorganisms were inoculated onto TSA media using sterile cotton-tipped swabs. Immediately after inoculation, the discs impregnated with water and methanol extracts, prepared using microwave and ultrasonic extraction methods, were carefully positioned in the petri dishes [15, 16].

2.1.3. Determination of antioxidant activity

For the 2,2-azino-bis 3-ethylbenzthiazoline-6-sulphonic acid (ABTS) test, the ABTS reagent was prepared at a concentration of 7 mM by dissolving it in water. The solution was subsequently mixed with 2.45 mM potassium persulfate, and the resulting mixture was kept in the dark at room temperature for 12-16 hours prior to utilization.To perform the test, 1 mL of methanolic fruit extract and 1 mL of ABTS solution were diluted with methanol (80%) to a total volume of 4 mL. After sealing

the tubes, they were left at 25 C for 6 minutes. Following this incubation period, the absorbance was measured at 734 nm. All measurements were conducted in triplicate, and the results were expressed as trolox equivalent (TE mg/g). For the 1,1-diphenyl-2-picrylhydrazyl (DPPH) test, DPPH solution was prepared by dissolving it in 185 µL methanol, to which 15 µL of fruit extract was added and vortexed for 10 seconds. This mixture was then kept at room temperature in the dark for 45 minutes. After the incubation period, the absorbance was measured at 515 nm using a microplate reader (Multiskan Go, Thermo). All measurements were performed in triplicate, and the results were expressed as trolox equivalent (TE mg/g) [17,18].

2.1.4. Cell culture and viability analysis

Black rosehip extract was dissolved in DMSO at 20 mg/mL and concentrations of 400, 300, 200, 100, and 40 µg/mL were prepared by serial dilution. The breast cancer cell line MCF-7 was cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) in 25 mL flasks to support cell proliferation. Cell transfer was carried out using trypsin-Ethylenediaminetetraacetic acid (EDTA) solution after rinsing with phosphate buffered saline (PBS) buffer to ensure viable cells at the flask base. Subsequently, flasks were incubated for 24-48 hours in a 5% CO_2 incubator at 37°C. Cell viability was assessed before each passage using trypan blue dye. After centrifugation, the cell suspension was quantified on a Thoma slide stained with trypan blue dye. Tetrazolium salts, which are organic compounds with a heterocyclic structure, undergo reduction by gaining electrons and transform into a formazan structure, resulting in a color change [19] due to active mitochondria breaking the tetrazolium rings [20]. This color change is absent in dead cells, leading to no formation of color. Therefore, the quantity of formazan dye produced is directly proportional to the number of living cells. Water-soluble tetrazolium-8 (WST-8) is reduced by dehydrogenases in cells to yield an orange-colored product soluble in cell culture medium. MCF-7 cells were seeded into 96-well plates and incubated at 37°C for 24 hours to allow for proper adherence to the well surfaces. Following the incubation period, the medium was aspirated from the wells. The cells were then exposed to varying concentrations of black rosehip extract (400, 300, 200, 100, and 40 µg/mL) in triplicate. A DMSO-treated group served as the negative control, while a group treated with medium containing 10% DMSO functioned as the positive control. Upon completion of the optimized incubation periods, cell viability was assessed using the Cell Viability Detection Kit-8 (CVDK-8, EcoTech Biotechnology) following the manufacturer's instructions. In brief, the culture medium from the treated cells was replaced with fresh medium containing 10% CVDK-8 solution, and changes in cell viability were quantified by measuring the optical density at 590 nm. [21].

2.1.5. Statistical analysis

The cytotoxicity investigation was performed with three repetitions, and the data were presented as mean ± standard deviation. Statistical analysis was conducted utilizing Student's t-test through GraphPad Prism software. A significance level of $p \leq 0.05$ was considered statistically significant.

3. RESULTS

In this study, the methanol extract prepared using *Rosa pimpinellifolia* (L.) fruit sample exhibited a total phenolic content of 37.5 ± 0.2 mg/g GA/g. However, when tested against 10 different pathogenic bacteria at a concentration of 500 µg/mL (Table 1), the extract showed no antibacterial effects, as evidenced by the absence of inhibition zones around the antimicrobial test discs.

Table 1. Antimicrobial effect of *R. pimpinellifolia* (L.) fruit methanolic extract depending on 500 µg/mL dose application

	No	Microorganisms	IZD	GEN	20%DMSO
GRAM (+)	P1	*Bacillus cereus* BC 6830	-	20	-
	P2	*Enterococcus faecalis* NCTC 12697	-	17	-
	P3	*Staphylococcus aureus* NCTC 10788	-	19	-
	P4	*Staphylococcus aureus* BC 7231	-	18	-
	P5	*Staphylococcus aureus* ATCC25923	-	17	-
GRAM (-)	P6	*Escherichia coli* NCTC 9001	-	15	-
	P7	*Escherichia coli* BC 1402	-	14	-
	P8	*Pseudomonas aeruginosa* NCTC 12924	-	13	-
	P9	*Salmonella* Typhimurium RSSK 95091	-	16	-
	P10	*Yersinia enterocolitica* ATCC 27729	-	19	-

IZD : Inhibition zone diameter (mm); GEN: Gentamicin = positive control; 20%DMSO = negative control

Furthermore, the antioxidant capacity of the *Rosa pimpinellifolia* (L.) fruit extract was assessed using the ABTS and DPPH test methods. The results showed an antioxidant capacity of 32.06 ± 1.1 mg TE/g by the ABTS test and 23.47 ± 1.6 mg TR/g by the DPPH test (Table 2).

Table 2. Antioxidant activity (ABTS, DPPH) and total phenolic compounds assays results of *R. pimpinellifolia* (L.) methanolic fruit extract

R. pimpinellifolia L. Methanolic Fruit Extract	ABTS (mg TE/g)	DPPH (mg TE/g)	TPC (mg GA/g)
	32,0±1.1	23,4±1.6	37,5 ±0.2

Regarding cytotoxicity, different doses (400 µg/ml, 300 µg/ml, 200 µg/ml, 100 µg/ml, 40 µg/ml) of the methanol extract of *Rosa pimpinellifolia* (L.) fruit were evaluated for their effects on the breast cancer cell line (MCF-7) over a 72-hour period. All doses of the fruit extracts exhibited antiproliferative properties in MCF-7 cells. Notably, doses of 100 µg/ml and 40 µg/ml demonstrated higher antiproliferative effects compared to doses of 400

µg/ml, 300 µg/ml, and 200 µg/ml. Specifically, the highest cytotoxic effect was observed at a concentration of 40 µg/ml, while the lowest was found at 400 µg/ml. The cytotoxic effect was statistically significant at concentrations of 200 µg/ml, 100 µg/ml, and 40 µg/ml (Fig. 1) ($p < 0.05$).

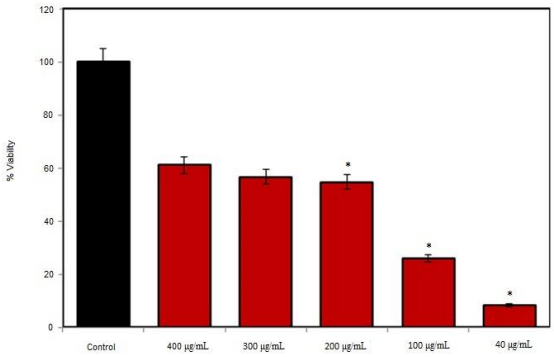

Figure 1. % Viability results of *R. pimpinellifolia* (L.) fruit extract on MCF-7 cells (*<0.05)

4. DISCUSSION AND CONCLUSION

The discrepancy between the findings of the study conducted by Öz et al. [22] and the current study regarding the antimicrobial activity of essential oils from *Rosa canina* (L.) and *R. pimpinellifolia* (L.) plants, particularly in relation to different plant parts, underscores the variability in antimicrobial properties within and between different plant species. While they observed limited and low-level antimicrobial activity in essential oils extracted from flower, leaf, and stem samples of *Rosa canina* (L.) and *R. pimpinellifolia* (L.) plants, no antimicrobial activity was detected in essential oils obtained from fruits. On the other hand, Gidik et al. [23] evaluated the antimicrobial activity of rosehip samples against various microorganisms and found antimicrobial effects at a concentration of 40 mg/mL. This contrasts with the current study's findings, where the extracts of *R. pimpinellifolia* (L.) fruit samples prepared using ultrasonic and microwave-assisted extraction methods showed no antibacterial effect against the selected target pathogens at a concentration of 500 µg/mL. The discrepancy between these studies could be attributed to several factors, including differences in the extraction methods, concentrations tested, variations in microbial strains used, and inherent differences in the chemical composition of the plant materials. Additionally, the specific antimicrobial assays employed in each study may have different sensitivities and specificities, leading to variations in the observed results. Overall, these findings highlight the importance of considering various factors when assessing the antimicrobial activity of plant extracts and emphasize the need for further research to elucidate the mechanisms underlying these activities and their potential applications. The comparison of the findings from our study with those of other research efforts sheds light on the variability in phenolic content and antioxidant activity across different *Rosa* species and extraction methods. The total phenolic content of *R. pimpinellifolia* (L.) fruit extract was determined to be 37.5 ± 0.2 mg/g, which is notably higher than the values reported by Shameh et al. [24] for various *Rosa* species, including *R. canina*. Additionally, the antioxidant activity of *R. pimpinellifolia* (L.) extract, as measured by ABTS and DPPH assays, was found to be 32.06 mg TE/g and 23.47 mg TE/g, respectively, demonstrating a substantial antioxidant effect and reported lower total phenolic content and antioxidant activity values for *Rosa* species, including *R. canina*, suggesting potential species-specific variations or differences in extraction methods and conditions. Similarly, Tahirović, Bašić [25] found varying phenolic content and antioxidant activity in *R. canina* extracts prepared using different solvents, with 80% methanol yielding lower phenolic content compared to 50% methanol. This indicates that the choice of solvent and its concentration can influence the extraction efficiency of phenolic compounds. Furthermore, Demir et al. [26] observed similar DPPH values for different *Rosa* fruit samples compared to our study's findings, indicating consistency in antioxidant activity within the genus *Rosa*. Overall, the discrepancies in phenolic content and antioxidant activity observed across different studies could stem from various factors, including genetic variability among *Rosa* species, environmental conditions, extraction methods, and analytical techniques used for assessment. Further research exploring these factors is necessary to better understand the composition and bioactivity of *Rosa* species extracts. The comparison of our study's findings with those of other research endeavors highlights the variability in total phenolic content and antioxidant activity among different *Rosa* species, as well as the influence of various extraction parameters. In our study, the antioxidant activity of *R. pimpinellifolia* (L.) fruit extract, determined by the ABTS method, was found to be 32.06 mg TE/g, which is substantially higher than the values reported by Gidik et al. [23] for different *Rosa* species collected from Bayburt, Türkiye. Similarly, they reported the total phenolic content reported for these *Rosa* species was higher compared to the values obtained in our study, indicating potential differences in phenolic composition among *Rosa* species from different regions. Moreover, the total phenolic content determined in the methanol extract of *R. pimpinellifolia* fruit in our study was lower than that reported by Güven [27] and Fattahi et al. [11] for the same species, suggesting variations in phenolic composition even within the same species. The discrepancy in antioxidant activity and total phenolic content among different studies can be attributed to several factors, including genetic variability among *Rosa* species, environmental conditions, harvesting time, extraction solvent type and ratio, as well as extraction methods and procedures. Additionally, variations in analytical techniques and assay conditions may contribute to differences in reported values. Furthermore, Öz et al. [22] highlighted the variability in antioxidant activities of essential oils obtained from different plant parts of *R. pimpinellifolia* (L.) and *R. canina* (L.), with higher antioxidant activities observed in essential oils from *R. pimpinellifolia* (L.). They also noted differences in total phenolic content between different harvest years,

underscoring the impact of temporal variations on chemical composition and biological activities. Overall, these findings emphasize the need for standardized methods and careful consideration of extraction parameters when evaluating the chemical composition and biological activities of *Rosa* species extracts. Additionally, further research exploring the effects of environmental factors and harvesting time on the bioactivity of *Rosa* species is warranted to better understand and harness their therapeutic potential. The literature on the anticancer and cytotoxic properties of black rose hips (*Rosa pimpinellifolia*) is relatively sparse. However, a notable study by Demir et al. in 2021 investigated the antioxidant potential and cytotoxic effects of *R. pimpinellifolia* extract on human colon (WiDr), liver (HepG2), and lung (A549) cancer cell lines, in comparison to normal fibroblast (BJ) cells. The researchers quantified the extract's total phenolic content as 16.4±0.4 mg gallic acid equivalent, total flavonoid content as 5.2±0.2 mg quercetin equivalent, and reducing power as 34.3±2.4 mg trolox equivalent per gram of sample. Their findings revealed that the extract exerted a selective cytotoxic effect on all three cancer cell lines in a dose-dependent manner, underscoring its potential therapeutic applications [28]. In our study, it was observed that cytotoxicity decreased as the concentration increased, confirming the need to use low concentrations of active substances in cytotoxicity studies. This is because the administration of high concentrations of the substance may have a nutritive effect on cancer cells or may have an unreasonably high cytotoxic effect. Therefore, when calculating the concentration, it is important to conduct preliminary trials and observe the effects at low concentrations.

In summary, the results of this study suggest that *R. pimpinellifolia* (L.) fruits hold promise for applications in various industries, including food and pharmaceuticals, owing to their notable antioxidant capacity and total phenolic content. However, further research is necessary to delve into the specific phenolic compounds present in the plant and their roles in conferring antioxidant and antiproliferative effects. Understanding the individual phenolic profiles of *R. pimpinellifolia* (L.) fruits could provide insights into their potential health benefits and aid in the development of novel products with enhanced bioactivity. Additionally, elucidating the mechanisms underlying the antioxidant and antiproliferative properties of these fruits could pave the way for the development of targeted therapies for various ailments, including cancer. Moreover, exploring the variability in phenolic composition among different harvests and geographical locations may uncover factors influencing the bioactivity of *R. pimpinellifolia* (L.) fruits, thereby facilitating optimized cultivation and harvesting practices to maximize their therapeutic potential. In conclusion, while this study sheds light on the promising attributes of *R. pimpinellifolia* (L.) fruits, further research is warranted to unlock their full potential and capitalize on their valuable bioactive compounds for the benefit of human health and well-being. Moreover, it is crucial to perform additional molecular biology experiments on the extract that demonstrated cytotoxic effects in cell culture studies. Specifically, it is important to ascertain the mechanism of cell death, particularly by identifying whether it occurs through apoptotic pathways. In this regard, the expression levels of genes associated with apoptosis can be analyzed to provide a deeper understanding of the underlying processes. Furthermore, positive outcomes from *in vitro* studies must be proven *in vivo* animal experiments to confirm the therapeutic potential and biological relevance of the findings.

Acknowledgement

We would like to express our deepest gratitude to the entire laboratory team for their moral support during the preparation of this master thesis.

Conflicts of Interest

The authors declare that there is no conflict of interest.

REFERENCES

[1] Rozenblatt-Rosen O, Regev A, Oberdoerffer P, Nawy T, Hupalowska A, Rood JE, et al. The human tumor atlas network: charting tumor transitions across space and time at single-cell resolution. Cell. 2020;181(2):236-49.

[2] Feinberg AP, Levchenko A. Epigenetics as a mediator of plasticity in cancer. Science. 2023;379(6632):eaaw3835.

[3] Bayraktar B. Investigation of The Synthesis of Cyrinary Thyradyen Ritim and Cortizol Thycradium In Cancer. In: Sebahattin Çiftçi MU, Erdal Hamarta, Coşkun Arslan, editor. Bilim ve Teknoloji Araştırmaları 2019. Konya: Çizgi Kitabevi; 2019.

[4] Brenner DR, Brockton NT, Kotsopoulos J, Cotterchio M, Boucher BA, Courneya KS, et al. Breast cancer survival among young women: a review of the role of modifiable lifestyle factors. Cancer causes & control. 2016;27:459-72.

[5] Cuthrell KM, Tzenios N. Breast Cancer: Updated and Deep Insights. International Research Journal of Oncology. 2023;6(1):104-18.

[6] Tomasetti C, Li L, Vogelstein B. Stem cell divisions, somatic mutations, cancer etiology, and cancer prevention. Science. 2017;355(6331):1330-4.

[7] Cecil KM, Schnall MD, Siegelman ES, Lenkinski RE. The evaluation of human breast lesions with magnetic resonance imaging and proton magnetic resonance spectroscopy. Breast cancer research and treatment. 2001;68:45-54.

[8] Mulligan B. Manual of cultivated trees and shrubs hardy in North America. Nature Publishing Group UK London; 1941.

[9] Strålsjö L, Alklint C, Olsson ME, Sjöholm I. Total folate content and retention in rosehips (*Rosa* ssp.) after drying. Journal of agricultural and food chemistry. 2003;51(15):4291-5.

[10] Guimarães R, Barros L, Calhelha RC, Carvalho AM, Queiroz MJR, Ferreira IC. Bioactivity of different enriched phenolic extracts of wild fruits from Northeastern Portugal: A comparative study. Plant Foods for Human Nutrition. 2014;69:37-42.

[11] Fattahi S, Jamei R, Hosseini SS. Antioxidant and antiradical activities of *Rosa canina* and *Rosa pimpinellifolia* fruits from West Azerbaijan. 2012.
[12] Nowak R. Fatty acids composition in fruits of wild rose species. Acta Societatis Botanicorum Poloniae. 2005;74(3).
[13] Özçelik H, Gül A, Özgökçe F, Ünal M, Özkan G, Fakir H, et al. Türkiye *Rosa* L. (Gül) taksonlarının genetik çeşitliliğinin tespiti, ekonomiye kazandırılma olanaklarının araştırılması ve Süleyman Demirel Üniversitesi bünyesinde Rosaryum (Gülistan) tesisi. 2009.
[14] Ecem Bayram N, Gerçek YC, Bayram S, Toğar B. Effects of processing methods and extraction solvents on the chemical content and bioactive properties of propolis. Journal of Food Measurement and Characterization. 2020;14:905-16.
[15] Dhanasekaran S, Rajesh A, Mathimani T, Samuel SM, Shanmuganathan R, Brindhadevi K. Efficacy of crude extracts of *Clitoria ternatea* for antibacterial activity against gram negative bacterium (*Proteus mirabilis*). Biocatalysis and Agricultural Biotechnology. 2019;21:101328.
[16] qbal M, Bakht J, Shafi M. Phytochemical screening and antibacterial activity of different solvent extracted samples of *Arisaema jacquemontii*. Pakistan journal of pharmaceutical sciences. 2018;31(1).
[17] Herald TJ, Gadgil P, Tilley M. High-throughput micro plate assays for screening flavonoid content and DPPH-scavenging activity in sorghum bran and flour. J. Sci. Food Agric. 2012;92(11), 2326–2331.
[18] Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med. 1999;26(9–10), 1231–1237.
[19] Riss TL, Moravec RA. Use of multiple assay endpoints to investigate the effects of incubation time, dose of toxin, and plating density in cell-based cytotoxicity assays. Assay and drug development technologies. 2004;2(1):51-62.
[20] Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of immunological methods. 1983;65(1-2):55-63.
[21] Karataş, EA, Bayındırlı K. Enhanced Antıcancer Potency Of Gemcıtabıne In Combınatıon Wıth Propofol In Prostate Cancer. Hacettepe Journal of Biology and Chemistry. 2022; 50(1), 1-12.
[22] Öz M, Deniz I, Okan OT, Baltaci C, Karatas SM. Determination of the chemical composition, antioxidant and antimicrobial activities of different parts of *Rosa canina* L. and *Rosa pimpinellifolia* L. essential oils. Journal of Essential Oil-Bearing Plants. 2021;24(3):519-37.
[23] Gidik B, Akar Z, Can Z, Sefali A, Erturk O. Determination of antioxidant, antimicrobial activities, phenolic compounds of wild *Rosa* L. species Bayburt Turkey. Fresenius Environmental Bulletin. 2019;28(12A):9973-82.
[24] Shameh S, Alirezalu A, Hosseini B, Maleki R. Fruit phytochemical composition and color parameters of 21 accessions of five *Rosa* species grown in North West Iran. Journal of the Science of Food and Agriculture. 2019;99(13):5740-51.
[25] Tahirović A, Bašić N. Determination of phenolic content and antioxidant activity of *Rosa canina* L. fruits in different extraction systems. Radovi Šumarskog Fakulteta Univerziteta u Sarajevu. 2017;47(1):47-59.
[26] Demir N, Yildiz O, Alpaslan M, Hayaloglu A. Evaluation of volatiles, phenolic compounds and antioxidant activities of rose hip (*Rosa* L.) fruits in Turkey. Lwt-food science and technology. 2014;57(1):126-33.
[27] Güven L. *Rosa pimpinellifolia* yalancı meyve meyve ve köklerinde fitokimyasal ve biyolojik aktivite çalışmaları. health Sciences Institute. Erzurum: Ataturk University; 2016. p. 288.
[28] Demir EA, Demir S, Türkmen N, Turan, İ. The effect of *Rosa pimpinellifolia* extract on the proliferation of human tumor cells. KSU Journal of Agriculture and Nature. 2021; 24(6): 1170-1176.