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Physiological and Biological Potential of Dark-Red Autumn Leaves of Smilax Excelsa L.

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ABSTRACT

Protecting and enhancing plant biodiversity, as well as obtaining biofunctional substances derived from plants that are environmentally pure and do not harm plants during the vegetative and yield phases, are among the most pressing challenges of our day. In this study, the biopotential value of dark-red autumn leaves of *Smilax exselca L.* (DRALS) was examined.Biologically potent substances in bioextract were studied by GC-MS method, identification of anthocyanins by UV-Vis spectroscopy method in a comparative manner, mineral content of green and DRALS were studied by EDXRF method. As a result of the study, it was confirmed that the bioextract contains 18 biologically potent substances and 25 mineral chemical elements that have antioxidant, antimicrobial, antibacterial, antifungal, anticarcinogenic, immunomodulatory, desulphosinigrin as potential anti-diabetic drug against alpha-glucosidase, antiasthmatic and anti-inflammatory effects. The results of this research indicate that DRALS could be a significant potential source of natural chemical compounds that may serve as therapeutic agents in the treatment and prevention of various diseases, as well as dyes in the cosmetics industry and secondary metabolites in the food industry.

1-Introduction

The fruits, branches, roots, and leaves of various species of the *Smilax excelsa* plant, which grows in many countries, have been studied for their biologically active compounds obtained through various extracts, both in terms of quantity and quality, while their physiological and bioactive properties have also been investigated [1,2] However, the dark red autumn leaves of the *Smilax plant*, rich in anthocyanins, have not been widely studied (Figure 1).

Figure 1. Dark-red autumn leaves of *Smilax excelsa L*.

Anthocyanins and their derivatives are polyphenols that possess antioxidant, anticancer properties, neutralize damage of ultraviolet rays, and protect vision and brain aging, which leads to their widespread inclusion in daily diets [3,4,5]. Anthocyanins are pigments abundant in fruits and vegetables, and commonly applied in foods due to attractive colour and health-promoting benefits. However, instability of anthocyanins leads to their easy degradation, reduced bioactivity, and colour fading in food processing, limiting their application and causing economic losses. Stability of anthocyanins depends on their own structures and environmental factors. For structural factors, modification including copigmentation, acylation and biosynthesis are a potential solution to increase anthocyanin stability due to forming stable structures. With regard to environmental factors, encapsulation such as microencapsulation, and complexation with liposome and nanoparticles has been shown effectively to enhance the stability. We proposed the potential challenges and perspectives for the diversification of anthocyanin-rich products for food application, particularly, introduction of hazards, technical limitations, interaction with other ingredients in food system and exploration of pyranoanthocyanins. The integrated strategies are warranted for improving anthocyanin stabilization for promoting their further application in food industry [6]. The use of plant-based medicines and the acquisition of healthy and beneficial organic compounds for the organism leads to a decrease in the number of plant species and is not considered ecologically efficient [7].

Turkish scientists have conducted a comparative analysis of the antioxidant activity of extracts obtained from the green leaves of *Smilax excelsa L*. using water, infusion, ethanol, and ethyl acetate in their research [8].Primary metabolites participate in nutrition and reproduction in organisms, fulfilling essential metabolic functions. Secondary metabolites such as drugs, perfumes, flavorings, insecticides, and dyes hold significant biological and economic value. The sustainable utility of higher plants as natural new sources of pharmaceutical compounds is of great importance for the development of new technologies [9-11].

The aim of the research is to expand the successful biotechnological production of plant-based food additives, dyes, and pharmaceuticals using autumn leaves, as well as to achieve the conservation and propagation of plant biodiversity.

The current article investigates and presents the biological active substances and the mineral chemical elements of a bioextract rich in anthocyanins obtained from the DRALS, which has completed its growing season. Taking the above into account, we believe that a comprehensive study of the dark red autumn leaves of this valuable plant grown in Azerbaijan will contribute to expanding the possibilities of its use for therapeutic purposes in scientific medicine.

2-MATERIAL and METHODS

2.1. Plant Material

As a research subject, the green leaves and the dark red autumn leaves of the *Smilax excelsa L.* plant, which grows at an altitude of 750-800 meters above sea level in the Sheki region of Azerbaijan, were collected for study in December at the end of the vegetation period.

2.2. Extraction.

Five grams of dried and crushed leaves were placed in four chemical beakers. In the first container, 150 mL of distilled water was added, in the second container, 70% alcohol, in the third container, 1% citric acid, and in the fourth container, a ternary mixture solution of 1% citric acid and 70% ethanol (EtCitric) was added in a 1:1 ratio. Extraction was carried out using the infusion method at a temperature of 60°C for 120 minutes [12]. After all the extracts cooled down, they were filtered through filter paper and the transparent parts were collected for research by centrifuging at 3000 rpm for 15 minutes. Every 10 minutes, 3 ml was taken from each bioextract and the absorbance was checked in the spectrophotometer at a wavelength of 515-520 nm. For concentration and the analytical accuracy of the experiment, each was poured back onto itself.

2.3. Valuation of anthocyanins.

The absorption of anthocyanins in each extract was measured every 10 minutes using a UV-vis spectrophotometer (UV-2700 Shimadzu), and records were taken. The results were compared by plotting the relevant graphs using Origin Pro8 software. The measurements have been repeated three times. After the evaluation of the extracts, the bioextract with a high anthocyanin value was identified using Cyanidin-3-Glucoside (Sigma Aldrich) as a control in a UV-vis spectrophotometer (UV-2700 Shimadzu) at 515-520 nm. For the preparation of the C-3-G solution, a ternary mixture solution with a pH of 3.31 was used as the solvent, specifically EtCitric in a 1:1 ratio (S220-KIT SevenCompact pH/ion meter, Mettler Toledo) [13].

2.4. Gas-Chromatography-Mass Spectrometry Analysis (GC-MS).

Biologically active compounds in the extracts were identified by mass spectroscopy and gas chromatography. The Agilent Technologies 6890 N Network GC System, a chromatograph with a mass spectrometer and a 5975 inert Mass Selective Detector mass spectrometer, as well as a detector with injection-split, split/splitless, low mass threshold of 150, high mass threshold of 400, and inlet pressure of ≈60 kPa were utilized for this purpose. A 30 m quartz capillary column "HP-5MS 5% Methyl Siloxane" with an internal diameter of 0.25 mm and a stationary phase thickness of 0.25 μm was utilised for the tests. In temperature programming mode, analyses were carried out at 50 °C to 260 °C at a rate of 15 °C per minute. The column's temperature regime is as follows: a 40°C beginning temperature that remains constant for two minutes; a two-minute temperature rise from 15°C to 200°C; a ten-minute temperature rise from 15°C to 260°C; and however 3.38e-005 is HiVac. Diluted using a 1:1:2 (v:v:v) methanol, chloroform and water combination. The gas flows at a rate of 1.50 mL/min. The materials were identified using the standard mass spectroscopic NIST library. 35 minutes were spent on the analysis [14].

Based on the results of chromatography, the recorded substances have been identified individually according to literature references for their therapeutic properties, physiological indicators, and other effective characteristics.

2.5. Determination of minerals.

A non-destructive method for determining major and minor elements in a variety of sample types, the Energy Dispersive X-ray Fluorescence (EDXRF) technique is used for the elemental analysis of medicinal plant samples. To analyze macro and microelements, a sample of 3 g of ground green and dark red autumn leaf powder was placed in a polypropylene cuvette using the Rigaku NEX QC+ (Austin, TX, USA) EDXRF spectrometer, equipped with a maximum power of 4 W, a 50 kV X-ray tube, and an SDD semiconductor detector. Each sample was scanned under the same conditions with two repetitions and a duration of 40 seconds.

3. RESULT AND DISCUSSION.

Before investigating the organic and mineral composition of DRALS for the purpose of the research work, it was determined which solvents were effective for the concentration of anthocyanins in the leaf. In the selection of solvents, economically efficient, safe for human health, and commonly used solvents have been utilized. Considering that anthocyanins have a polyphenolic structure, and due to their more stable properties in hydrophobic, hydrophilic, and acidic environments, it was deemed more appropriate to select a ternary mixture of water-organic solvent-acid as the best solvent for them [9,15]. In Figure 2, the time dependence of anthocyanin absorption in various solvents is shown. Measurements were taken at a wavelength of 515-520 nm for each experiment. Every 10 minutes, 3ml from each bioextract was taken and the absorbance was checked using a spectrophotometer, and for the concentration and analytical accuracy of the experiment, each was poured back onto itself. According to the Beer-Lambert law A=ϵlc, the absorbance of a solution is directly proportional to its concentration [16], the concentration of anthocyanins in EtCitric extract was observed to increase up to 25±3 minutes, 35±2 minutes in 1% citric acid, 45±3 minutes in 70% ethanol, and 55±4 minutes in distilled water. In the following minutes, it stabilized. In Figure 3, the absorbance of anthocyanins in each bioextract is presented separately. Thus, $A_{(EtCitric)}$=0.2441, $A_{(1\%\ citric)}$=0.2278, $A_{(70\%\ ethanol)}$=0.2122, $A_{(water)}$=0.1991. The pH value of citric solvent being 3.37 has resulted in the concentration of anthocyanins remaining stable against environmental influences [17].The bioextract obtained in EtCitric was identified with a 40µM Cyanidin-3-Glucoside (in EtCitric) solution, and as shown in Figure 4, an approximate 99% match was observed in the 515-520 nm range.

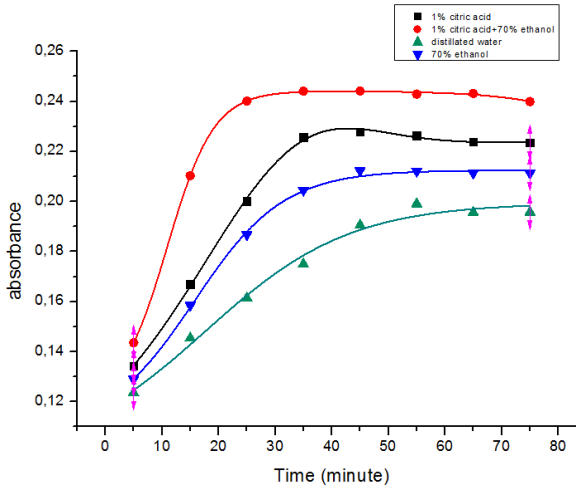


Figure 2. The time dependence of anthocyanin absorption in various extractants

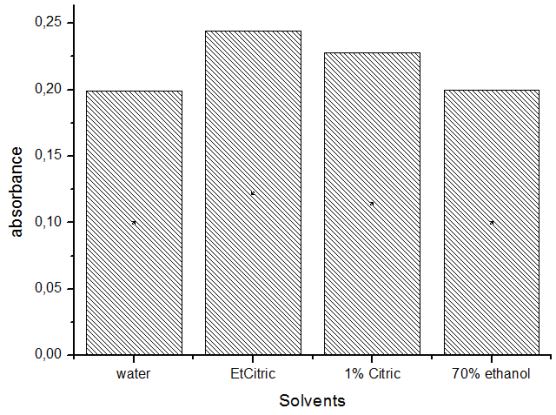


Figure 3. Absorption of anthocyanins in various extractants

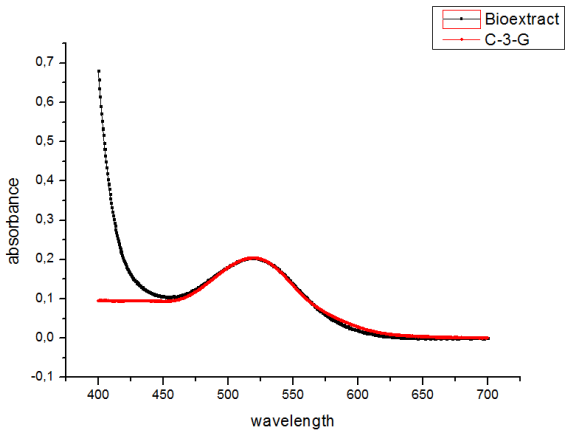
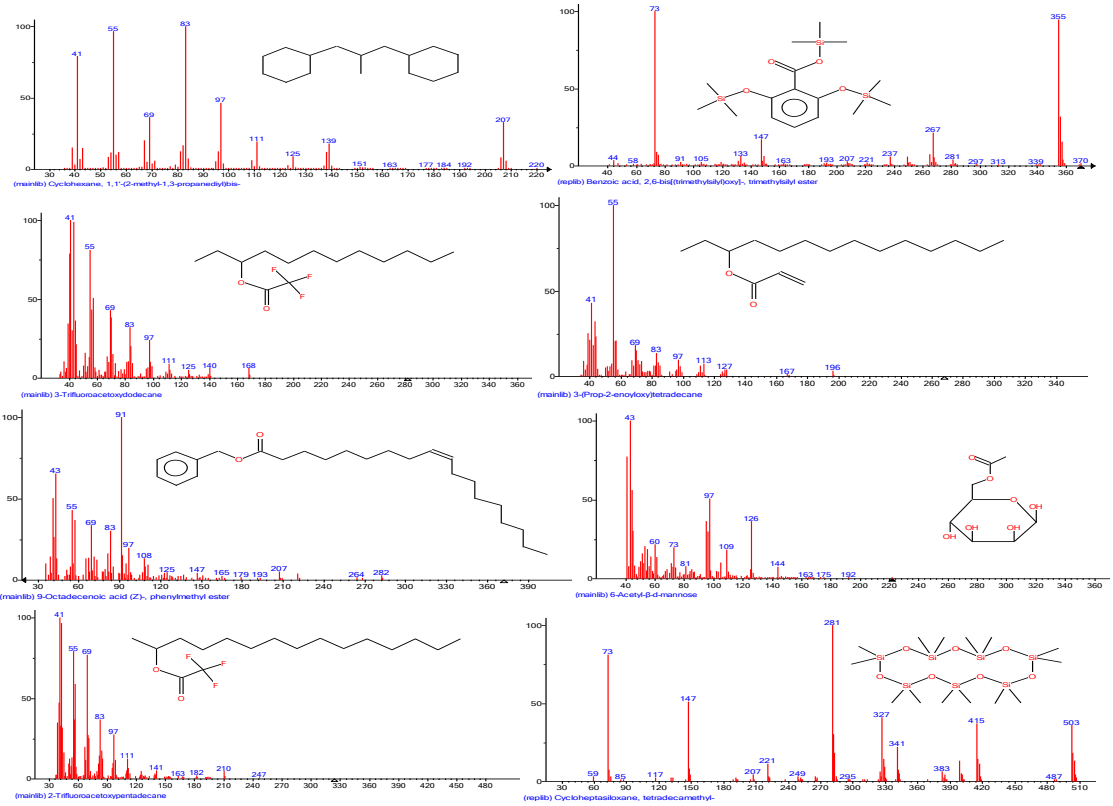


Figure 4. Identification of Cyanidin-3-glucoside and bioextract(EtCitric)

GC-MS or gas chromatography-mass spectrometry is a useful technique for the precise identification of bioactive substances. Using GC-MS analysis, a total of 29 components were detected in the EtCitric extract of dark red autumn leaves of *Smilax excelsa L.* of the study, and 18 substances with high peak areas were selected and identified. 18 biologically potent substances consist of 62.07% in bioextract, such as 3 antioxidant, 6 antibacterial, 5 antimicrob, 5 anticanserogen, 4 antifungal, 4 antidiabetic, 3 antiinflammator, 3 cardiovascular analeptic agent, 1 antiviral, 1 antihypertensive, 1 hypocholesterolemic, 1 agent against for lungs diseases and Antiasthmatic, 1 agent for the treatment of estrogen dependent diseases. Based on Figure 5, Table 1 lists the active ingredients along with their Retention Time (RT), molecular weight, molecular formula, and therapeutic effect.



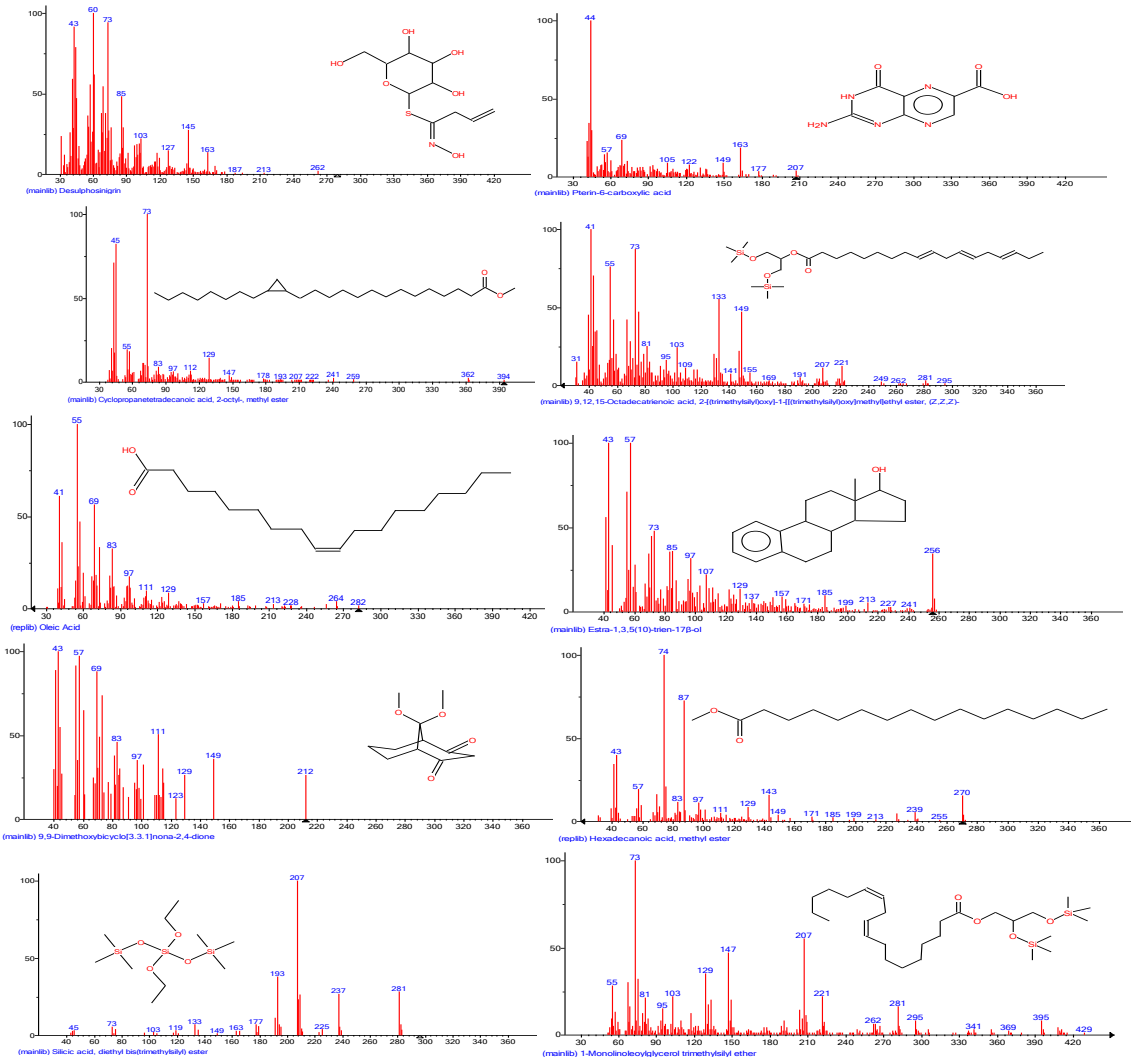


Figure 5. GC-MS spectra of Etanol-citric-extracted dark-red autumn leaves from *Smilax excelsa L.*, where the x-axis represents run time and the y-axis relative abundance.

Table 1:Biologically potent substances in contents of dark-red autumn leaves of *Smilax excelsa L.*

Peaks	RT	Compound name	Formula of compound	m/z	therapeutic action
1	5.263	Cyclohexane, 1,1'-(2-methyl-1,3-propanediyl)bis-	$C_{16}H_{30}$	222	Adjuvant for insulin [18]
2	9.104	Benzoic acid, 2,6-bis[(trimethylsilyl)oxy]-, trimethylsilyl ester	$C_{16}H_{30}O_4Si_3$	370	Antifungal, antibacterial [19]
3	9.981	3-Trifluoroacetoxydodecane	$C_{14}H_{25}F_3O_2$	282	antibacterial, antifungal, antioxidant, anticancer, and anti-inflammatory [20]
4	10.181	3-(Prop-2-enoyloxy)tetradecane	$C_{17}H_{32}O_2$	268	antioxidant, anticarcinogenic, anti-inflammatory, antidiabetic, hepato- and gastro-protective, antiviral, neuroprotective,

					cardioprotective, and anti-hypertensive [21]
5	10.392	9-Octadecenoic acid (Z)-, phenylmethyl ester	$C_{25}H_{40}O_2$	372	hypocholesterolemic, anticancer, lungs diseases, emulsifying agent [22]
6	10.469	6-Acetyl-β-d-mannose	$C_8H_{14}O_7$	222	Antimicrob [23]
7	11.704	2-Trifluoroacetoxypentadecane	$C_{17}H_{31}F_3O_2$	324	Antimicrob, antibacterial [24]
8	12.604	Cycloheptasiloxane, tetradecamethyl-	$C_{14}H_{42}O_7Si_7$	518	antibacterial, antifouling, immunomodulator, antitumour [25], antifungal [26]
9	13.227	Desulphosinigrin	$C_{10}H_{17}NO_6S$	279	Anti-diabetic drug against alpha-glucosidase[27,28], Antiasthmatic [29]
10	13.404	Pterin-6-carboxylic acid	$C_7H_5N_5O_3$	207	for the treatment of erectile dysfunction[30]
11	14.257	Cyclopropanetetradecanoic acid, 2-octyl-, methyl ester	$C_{26}H_{50}O_2$	394	Antimicrob [31]
12	14.798	9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy]methyl]ethyl ester, (Z,Z,Z)-	$C_{27}H_{52}O_4Si_2$	496	Antioxidant, antidiabetic, anti-inflammator [32]
13	15.133	Oleic Acid	$C_{18}H_{34}O_2$	282	for the treatment of cardiovascular or autoimmune diseases, metabolic disturbances, skin injury and cancer[33], antitumor [34]
14	16.962	Estra-1,3,5(10)-trien-17β-ol	$C_{18}H_{24}O$	256	for the treatment of estrogen-dependent diseases [35]
15	17.709	9,9-Dimethoxybicyclo[3.3.1]nona-2,4-dione	$C_{11}H_{16}O_4$	212	Antifungal [36], cardiovascular analeptic agent [37]
16	19.682	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	Antimicrob, antibacterial [38]
17	32.479	Silicic acid, diethyl bis(trimethylsilyl) ester	$C_{10}H_{28}O_4Si_3$	296	Antibacterial [39]
18	36.255	1-Monolinoleoylglycerol trimethylsilyl ether	$C_{27}H_{54}O_4Si_2$	498	Antimicrob [40]

The change in the quantity of mineral elements in autumn leaves and the synthesis of anthocyanins often occur before the breakdown of chlorophyll, and the intensity of the color of red aging leaves increases with cool temperatures, high light, and moderate drought [43].In addition to the formation and accumulation of anthocyanins, the changes in the mineral composition of the dark red autumn leaves of *Smilax excelsa L*. were analyzed using Energy Dispersive X-ray Fluorescence (EDXRF) and are presented comparatively in Table 2.In addition to the formation and accumulation of anthocyanins, the changes in the mineral composition of the dark red autumn leaves of *Smilax excelsa L*. were analyzed using Energy Dispersive X-ray Fluorescence (EDXRF) and are presented comparatively in Table 2.

Table 2: Amounts of mineral elements in green leaf and Dark-red autumn leaf

Mineral elements	Amount, %	
	green leaf	dark-red autumn leaf
Potassium	5.984	3,434
Sodium	3.011	2.732
Magnesium	7.623	3.713
Calcium	2.855	3.443
Titanium	0.026	0.043
Vanadium	0.003	0.003
Chromium	0.006	0.007
Manganese	0.018	0.029
Iron	0.043	0.049
Nickel	0.015	0.016
Copper	0.022	0.020
Zinc	0.008	0.010
Gallium	0.001	0.001
Zirconium	0.008	0.009
Strontium	0.019	0.012
Yttrium	0.002	0.002
Scandium	0.002	0.002
Aluminum	1.999	0.153
Silicon	0.647	0.557
Phosphorus	0.281	0.280
Sulfur	0.068	0.065
Barium	0.019	0.023
Lead	0.001	0.002
Niobium	0.006	0.008
Rubidium	0.003	0.002
Total	**22.668**	**14.615**

As seen in Table 2, the amount of mineral elements in the leaves that have undergone the growing season is approximately 35.52% lower. In autumn leaves, the amount of Ca among macroelements has increased compared to green leaves, while the amount of P has remained stable. Among the microelements, only the amounts of Fe, Mn, Zn, and Ni increased, while the amounts of I, Sc, V, and Ga remained stable. A sharp decrease was observed in other remaining macro and micro elements. Despite the overall decrease in the quantity of microelements, there has been an increase in the amounts of antioxidant microelements such as Zn, Mn, and Cr [42-44]. Due to the high content of anthocyanins in the bioextract obtained from DRALS in EtCitric solution, the autumn leaves of *Smilax excelsa L.* can be considered to have higher antioxidant properties compared to green leaves [45,46].

4. CONCLUSION

The EtCiric bioextract obtained from autumn leaves was more productive and of higher quality in terms of anthocyanin extraction and richness in biologically potent substances compared to water, 70% alcohol, and 1% citric bioextracts. Compared to the green leaves of *Smilax excelsa L.,* despite a 35.52% decrease in mineral elements in autumn leaves, an increase in microelements such as Zn, Mn, and Cr, which have

high antioxidant properties, has been observed. Eighteen biologically potent substances make up 62.07% of the bioextract, including 3 antioxidants, 6 antibacterial agents, 5 antimicrobials, 5 anti-carcinogenic substances, 4 antifungals, 4 antidiabetics, 3 anti-inflammatories, 3 cardiovascular analeptic agents, 1 antiviral agent, 1 antihypertensive, 1 hypocholesterolemic agent, 1 agent for lung diseases and asthma, and 1 agent for the treatment of estrogen-dependent diseases. Referring to the results of the research, we can say that the dark red autumn leaves of the *Smilax excelsa L*. plant are rich in a wide range of biologically active substances and minerals, and they have potential uses as an effective preventive agent in the treatment of various diseases.

5.REFERENCES

[1] Al Yassine, D., El Massri, N., Demircan, G., Bulut, G., Akin, D., Tacer-Caba, Z. 2023. *Total Antioxidant Potential, Total Phenolic Profile and Cytotoxic Activity Against Brain Cancer: Melocan and Galdirik*. Food Technology and Biotechnology. 61(4):p. 475–84.

[2] Gürbüz, İ., Özçelik, B., Günbatan, T., Akkol, E., Sahinoz, M., Akaydın, G. 2021. *Antibacterial, antifungal and enzyme inhibitory effects of selected plants from Turkey*. Pakıstan journal of pharmaceutıcal scıences [Internet]. 34(3).

[3] Atayeva, V., Aslanov, R. 2022. *EPR-based study to monitor Free Radicals in Treated Silk Fibroin with Anthocyanins*. JOTCSA. 9(4):p. 1055–1062.

[4] Pei, Z., Huang, Y., Ni J., Liu, Y., Yang, Q. 2024. *For a Colorful Life: Recent Advances in Anthocyanin Biosynthesis during Leaf Senescence*. Biology. 13(5):p. 329.

[5] Atayeva, V., Salmanova, A., Shukurlu, Y., Valibeyov, Kh. *Feedıng mulberry sılkworms wıth autumn leaf extracts*. The scientific heritage. No 135 (2024) DOI: 10.5281/zenodo.11044783

[6] Cai, D., Li, X., Chen, J., Jiang, X., Ma, X., Sun, J., et al. 2022. *A comprehensive review on innovative and advanced stabilization approaches of anthocyanin by modifying structure and controlling environmental factors.* Food Chemistry. 366:p. 130611.

[7] Rawat, U.S., Agarwal, N.K. 2015. *Biodiversity: Concept, threats and conservation*. ECJ. 16(3):p. 19–28.

[8] Ozsoy, N., Can, A., Yanardag R, Akev N. Antioxidant activity of *Smilax excelsa* L. leaf extracts. 2008. Food Chemistry. 110(3):p. 571–83.

[9] Pagare, S., Bhatia, M., Tripathi, N., Pagare, S., Bansal, Y.K. 2015. *Secondary Metabolites of Plants and their Role: Overview*. 9(3):p. 293-304.

[10] Mohammad, N., Behrooz, A.B., Zahra, N., Farshid, Z. 2023. *Antimicrobial activity between* Coriandrum sativum *seed and* Cuminum cyminum *essential oils against foodborne pathogens: A multi-ligand molecular docking simulation.* LWT. 185:p. 115217.

[11] Jalil, S.S., Alizadeh, B.B., Hojjati, M., Vasiee, A., Noshad, M. 2023. *Evaluation of the constituent compounds, antioxidant, anticancer, and antimicrobial potential of Prangos ferulacea plant extract and its effect on Listeria monocytogenes virulence gene expression*. Front Microbiol [Internet]. 14.

[12] Salamon, I., Mariychuk, R., Grulova, D. 2015. *Optımal extractıon of pure anthocyanıns from fruıts of sambucus nıgra.* ISHS Acta Horticulturae. (1061):p 73–78.

[13] Alizadeh, B.B., Noshad, M., Falah, F., Zargari, F., Nikfarjam, Z., Vasiee, A. 2025. *First report on the synergy of Nepeta menthoides and Nepeta cephalotes essential oils for antimicrobial and preservation applications: A multi-ligand molecular docking simulation.* Applied Food Research. 5(1):p. 100707.

[14] Craig, A.P., Fields, C.C., Simpson, J.V. 2014. *Development of a Gas Chromatography-Mass Spectrometry method for the quantification of glucaric acid derivatives in beverage substrates*. International Journal of Analytical Chemistry. 2014(1):p. 402938.

[15] Mattioli, R., Francioso, A., Mosca, L., Silva, P. 2020. *Anthocyanins: A comprehensive review of their chemical properties and health effects on cardiovascular and neurodegenerative diseases*. Molecules. 25(17):p. 3809.

[16] Chemistry LibreTexts [Internet]. 2013. The Beer-Lambert Law.

[17] Zannou, O., Oussou, K.F., Chabi, I.B., Awad, N.M.H., Aïssi, M.V., Goksen, G., et al. 2023. *Nanoencapsulation of cyanidin 3-o-glucoside: purpose, technique, bioavailability, and stability*. Nanomaterials. 13(3):p. 617.

[18] Femi-Olabisi, F.J., Ishola, A.A., Faokunla, O., Agboola, A.O., Babalola, B.A. 2021. *Evaluation of the inhibitory potentials of selected compounds from Costus spicatus (Jacq.) rhizome towards enzymes associated with insulin resistance in polycystic ovarian syndrome: an in silico study.* Journal of Genetic Engineering and Biotechnology. 19(1):176.

[19] Hugar, A.L., Londonkar, R.L. 2017. *GC-MS profiling of bioactive components from aqueous extract of Pterocarpus marsupium*. International Journal of ChemTech Research. 10(9):p. 557-567.

[20] Kaizal, A,.F., Hussein, H.J. 2019. *Detectıon of phytochemıcal compounds of dutch ırıs (ırıs hollandıca) by usınggas chromatographyand mass spectrometry (GC-MS) technıque.* Plant Archives.19(2):p. 3904-3910

[21] Alkhafaji, H.H.K., Altameme, H.J.M., Alsharifi, S.M.H. 2022. *Detectıon of bıoactıve chemıcal compounds ın the methanolıc extract of azolla fılıculoıdes lamark fern by GC-MS technıque.* Iraqi Journal Agricultural Sciences. 53(4):p. 922–930.

[22] Hussein, A.O., Mohammed, G.J., Hadi, M.Y., Hameed, I.H. 2016. *Phytochemical screening of methanolic dried galls extract of Quercus infectoria using gas chromatography-mass spectrometry (GC-MS) and Fourier transform-infrared (FT-IR).* JPP. 8(3):p. 49–59.

[23] Ezekwe, S., Chikezie, P. *GC–MS analysis of aqueous extract of unripe fruit of Carica papaya.* Journal of Nutrition & Food Sciences. 2017. 7(3).

[24] Radin, M.S.R.M., Adel, A.S.A.G., Apandi, N.M., Amir, H.M.K., Jais, N.M. 2018. *Biomass quality of Scenedesmus sp. cultivated in wet market wastewater*. Malaysian Journal of Microbiology: p. 120–123.

[25].Rasyid, A., Putra, M.Y., Yasman. 2023. *Antibacterial and antioxidant activity of sea cucumber extracts collected from Lampung waters, Indonesia*. Kuwait Journal of Science. 50(4):p. 615–621.

[26] Hai, T.N., Diep, N. C. 2022. *Antimicrobial compounds of one streptomyces celluloflavus strain isolated from can Gio mangrove soil, Vietnam.* GSC Biol and Pharm Sci. 19(3):p. 120–126.

[27] Ashar, M..SA.A., Putra, W.E., Rifa'i, M., Sustiprijatno, S., Salma, W.O., Susanto, H., et al. 2023. C*alculating the stability of molecular interface between the ligand-complex and solvent molecule: A study of Averrhoa bilimbi bioactive compounds as anti-diabetic agent.* AIP Conference Proceedings. 2634(1):020023.

[28] Sari, A.N., Putra, W.E., Rifa'i, M., Sustiprijatno, Susanto, H., Salma, W.O., et al. 2023. *Profiling the coulomb energy of chimanine D and desulphosinigrin as potential anti-diabetic drug against alpha-glucosidase*. AIP Conference Proceedings. 2634(1):020012.

[29] Kadhim, M., Fauzi, A., Hameed, I. 2017. *Determination of bioactive compounds of methanolic extract of vitis vinifera using GC-MS.* Internatıonal journal of toxıcologıcal and pharmacologıcal research; 9.

[30] Wopara, I., Mobisson, P.S., Pius, E., Uwakwe, A., Wegwu, M., Qureshi, N. 2020. *Inhibition of phosphodiesterase 5 enzyme by pterine-6 carboxylic acid from baphia nitida - related to erectile dysfunction: computational kinetic.* Research Journal of Medicinal Plant. 31:p. 49–55.

[31] Amaechi, N., Onyejekwe, A. 2023. *Identification of fatty acids and other compounds in coconut oil extracted by different methods using Gas Chromatography – Mass Spectrometry*. Asian Basic and Applied Research Journal. 7(1):p. 41–48.

[32] Ram, M., Ram, K.R.M., Anisha, G., Prabhu, K., Shil, S., Nagarajan, V. 2019. *Preliminary phytochemical and gas chromatography- mass spectrometry study of one medicinal plant Carissa carandas.* Drug Invention Today. 12:p. 1629–30.

[33] Sales-Campos, H., Reis de Souza, P., Crema, P.B., Santana, S.J., Ribeiro, C. C. 2013. A*n overview of the modulatory effects of oleic acid in health and disease.* Ingenta Connect [Internet]. 13(2):p. 201-210.

[34] Carrillo, P.C., Cavia C.M.M., Alonso, T.S. 2012. *Antitumor effect of oleic acid; mechanisms of action. A review.* [Internet]. 27(6):p. 1860-1865

[35] Lespérance, M., Roy, J., Djiemeny, N.A., Maltais, R., Poirier, D. 2021. *Synthesis of 16β-derivatives of 3-(2-bromoethyl)-estra-1,3,5(10)-trien-17β-ol as inhibitors of 17β-HSD1 and/or steroid sulfatase for the treatment of estrogen-dependent diseases.* Steroids. 172:p. 108856.

[36] Rolta,. R, Shukla, S., Kashyap, A., Kumar, V., Sourirajan, A., Dev, K. 2022. P*hytocompounds of Bistorta macrophylla (D. Don) Sojak. as bioavailability enhancers of fluconazole and amphotericin B to better manage Candida species infections* [Internet].

[37] Dassamiour, S., Bensaad, M.S., Hambaba, L., Melakhessou, M.A., Sami, R., Al-Mushhin, A.A.M., et al. 2022. *In silico investigation of some compounds from the n-butanol extract of centaurea tougourensis Boiss. & Reut.* Crystals. 12(3):p. 355.

[38] Shaaban, M.T., Ghaly, M.F., Fahmi, S.M. 2021. *Antibacterial activities of hexadecanoic acid methyl ester and green-synthesized silver nanoparticles against multidrug-resistant bacteria.* Journal Basic Microbiol. 61(6):p. 557–68.

[39] Juliet, Y.S., Kalimuthu, K,. Vajjiram, C., Ranjitha, V. 2018. *Evaluatıon and comparıson of phytochemıcal, gcms and ftır analysıs of wıld and mıcropropagated Cadaba frutıcosa (L.).* World Journal of Pharmaceutical Research. 7(14):p. 746-760.

[40] Narmatha, M., Mani, P., Maneemegalai, S. 2018. I*solation, purification and identification of an antimicrobial compound from the ethanol seed extract Of Syzygium Cumini*. 6(6):p. 529-535.

[41] Feild, T.S., Lee, D.W., Holbrook, N.M. 2001. *Why Leaves Turn Red in Autumn. The Role of Anthocyanins in Senescing Leaves of Red-Osier Dogwood.* Plant Physiology. 127(2):p. 566–574.

[42] Coassin, M., Ursini, F., Bindoli, A. 1992. *Antioxidant effect of manganese.* Archives of Biochemistry and Biophysics. 299(2):p. 330–333.

[43] Haq, Z., Jain, R.K., Khan, N., Dar, M.Y., Ali, S., Gupta, M., et al. 2016. *Recent advances in role of chromium and its antioxidant combinations in poultry nutrition: A review*. Vet World. 29(12):p. 1392–1399.
[44] Powell, S.R. 2000. *The Antioxidant Properties of Zinc*. The Journal of Nutrition. 130(5):p. 1447S-1454S.
[45] Mosallaie, F., Pirnia, M., Dehghan, Z., Falah, F., Sabbaghzadeh, R., Behbahani, B.A., et al. 2024. *Unveiling the chemical composition, antioxidant and antibacterial properties, and mechanistic insights of Convolvulus arvensis extract through molecular docking simulations.* Applied Food Research. 4(2):p. 100580.
[46] Alizadeh, B. B., Noshad,. M, Falah, F., Zargari, F., Nikfarjam, Z., Vasiee, A. 2024. *Synergistic activity of Satureja intermedia and Ducrosia anethifolia essential oils and their interaction against foodborne pathogens: A multi-ligand molecular docking simulation.* LWT. 205:p. 116487.