ChemistrySelect

Research Article
doi.org/10.1002/slct.202300130

Chemistry Europe
European Chemical Societies Publishing

www.chemistryselect.org

Determination of Antioxidant Activities of *Viscum album* L.: First Report on Interaction of Phenolics with Survivin Protein using *in silico Analysis*

Safiye Elif Korcan,[a] Nevin Çankaya,*[a] Serap Yalcın Azarkan,[b] İbrahim Bulduk,[c] Emre Can Karaaslan,[d] Mustafa Kargıoğlu,[d] Muhsin Konuk,[e] and Gencer Güvercin[f]

Mistletoe, *Viscum album* L., (*VA*) known as "Ökse Otu" in Turkey, is a hemiparasitic shrub that grows especially on the trunks and crowns of broadleaf trees. This study is aimed to determination of the *VA*'s antioxidant compounds and evaluate their *in vitro* activities growing on twelve different host trees and determination of *in silico* approach of compounds against survivin protein. At the same time, the active ingredients determined in HPLC analysis, were performed with molecular docking and molecular dynamics analysis on survivin protein, an apoptosis inhibitor protein in cancer. Antioxidant activities of methanolic extracts of organs of *VA* were determined. Phytochemical properties of different organs of *VA* samples taken from different hosts varied as well. Total phenolic content was determined at the highest rate in fruits, followed by leaves and branches, respectively. Total flavonoid content was determined in the highest amounts in the leaves, followed by branches and fruits, respectively. Antioxidant activity values were evaluated with DPPH (using DPPH as a radical), ABTS (using ABTS as radical cation) and FRP tests. The antioxidant activity of the branches of the plant was determined at the highest rate, followed by leaves and fruits, respectively. During *in silico* analysis, Quercetin, Rosmarinic acid and Catechin were found to bind to survivin protein effectively. These substances can have the potential to become active pharmaceutical ingredients by working with different proteins in the future.

Introduction

Medicinal plants and their extracts have been used by all cultures and civilizations for the treatment of various ailments since ages. The World Health Organization (WHO) reported that eighty percent of the total world population uses medicinal plants as medicine.[1] It has been reported that plant-derived bioactive compounds have many In Vitro Biological Potentials such as antioxidant, anti-inflammatory, antibacterial, antifungal, antitumor,[2–4] pain modulators[5] and prevention and treatment of psychiatric disorders.[6–8] Because of these remarkable properties, herbal bioactive compounds are of great interest in nutrition and pharmacology.[9] Flavonoids, a group of natural bioactive compounds, have recently attracted attention with their potential in the treatment of diabetes, hyperglycemia[10] and especially cance.[11] In recent years, potential mechanisms of bioactive compounds in the treatment of diseases can be evaluated by examining computer-aided modeling.[12,13] As it is well known natural compounds including antioxidants have advantages over synthetic ones, even if they have slight or negligible side effects. Another advantage of is the plant bioactive compounds are being obtained easily and economically. It is reported that antioxidants like flavonoids are beneficial in the chemo prevention of various diseases, especially cancer associated with oxidative stress caused by free radicals in the body.[14,15] Fernández et al (2021) reported that Xanthohumol showed good antitumor activity, while apigenin and luteolin showed low antitumor activity in their study on human colorectal cancer (CRC) cell lines.[9]

Viscum album L. (*VA*) (Loranthaceae), known as mistletoe, is an ever green semi-parasitic plant, growing on various trees. Belghoul et al (2020) reported that the medium lethal dose (LD50) value of *V. tuberculatum* was higher than 2000 mg/kg bw.[16] Similarly, Manzoor Ullah et al (2022) reported that up to 2 g/kg of *VA* extract can be considered safe because of its LD50 value of more than 2 g/kg.[17] The results of toxicity studies indicate that *VA* plant extract can be classified under category 5 of the Globally Harmonized System of Classification and Labeling of Chemicals (UN 2011).[18]

As a result of pharmacological studies, different types of components were identified in *VA* such as viscotoxins, polysaccharides, lectins phenylpropanes, lignans,phenolic and flavonoids.[18–21] Studies have also shown that *VA* extracts are much more antioxidant than other herbal extracts. It also has antibacterial, antiepileptic, immunostimulatory and antiviral effects.[22–27]

[a] *S. E. Korcan, Assoc. Prof. Dr. N. Çankaya*
Vocational School of Health Services, Uşak University, 64200, Uşak, Turkey
E-mail: nevin.cankaya@usak.edu.tr
[b] *S. Y. Azarkan*
Department of Medical Pharmacology, Faculty of Medicine, Kırsehir Ahi Evran University, 40100, Kırşehir, Turkey
[c] *İ. Bulduk*
Faculty of Engineering, Department of Chemical Engineering, Afyon Kocatepe University, 64200, Uşak, Turkey
[d] *E. C. Karaaslan, M. Kargıoğlu*
Department of Molecular Biology and Genetics, Faculty of Science and Arts, Afyon Kocatepe University, 03200, Afyonkarahisar, Turkey
[e] *M. Konuk*
Molecular Biology and Genetics Department, Faculty of Engineering and Natural Sciences, Üsküdar University, 34662, Istanbul, Turkey
[f] *G. Güvercin*
Bioengineering Department, Yeditepe University, 34755, Istanbul, Turkey.

Table 1. *VA.* samples collected areas.

Plant Material	Collected Area at Afyonkarahisar Province	Altitude	Date	Herbarium Number
Pyrus communis L.	Sultadağı. Yakasenek.village	980 m	28.9.2018	9947
Crateagus orientalis Pall. Ex M.Bieb. subsp.*orientalis*	Kocatepe National Park	1450 m	30.9.2018	9948
Pyrus amygdaliformis Vill. var. *amygdaliformis*	Dazkırı village Cedit plateau	1075 m	5.10.2018	9942
Celtis planchoniana K.I. Chr	North of Çay District	980 m	2.11.2018	9958
Pinus nigra J.F.Arnold. subsp. *pallasiana* (Lamb.) Holmboe var. *Pallasiana*	Dazkırı District. İdris village	1075 m	5.10.2018	9952
Robinia pseudoacacia L.	North of Çay District	980 m	2.11.2018	9955
Prunus dulcis (Mill.) D.A. Webb	Sultadağı. Yakasenekvillage.	980 m	28.9.2018	9946
Salix alba L. subsp. *Alba*	Düzağaç Town	1130 m	21.10.2018	9953
Pyrus elaeagnifolia Pall. subsp. *elaeagnifolia*	Kocatepe National Park	1450 m	30.9.2018	9949
Morus nigra L.	North of Çay District	980 m	20.11.2018	9956
Mespilus germanica L.	Büyükkalecik Town	1450 m	30.9.2018	9950
Prunus armeniaca L.	North of Çay District	980 m	2.11.2018	9960

However, the most important use of *VA* extracts is cancer treatment. It has been reported that it can be used for therapeutic purposes in many cancer types such as breast, pancreatic, bladder, laryngeal cancer, lymphoblastic leukemia and medulloblastoma cells.[22,28–32] The main molecules thought to show antitumor properties in *VA* are lectins and viscotoxins. In addition, it has been reported in recent studies that secondary metabolites, especially phenolic compounds, triterpenoids, and non-polar compounds can be used in cancer treatment.[33,34] It has also been reported that whole *VA* extract is more potent in inhibiting tumor cells than isolated compounds, and this may be due to the synergistic effect of some molecules.[35] Its anticancer effect in a variety of ways, including immune enhancement, tumor prevention, malignant tissue inhibition, alleviation of chemotherapeutic side effects, and DNA protection.[22,28–32]

Many of the polyphenols induce apoptosis, which has proven to play a key role in the biological system in cancer therapy. The induction of apoptosis can be intrinsic or extrinsic. The extrinsic pathway is activated by internal cell signaling, primarily in the mitochondria. The intrinsic pathway results in the activation of caspase-8. As a result, the anticancer activity of phenols arises from different mechanisms such as protecting the cell from oxidative stress, inducing apoptosis in the cell, inhibiting immunosuppression, stopping the cell cycle, inhibiting cell migration and proliferation.[36] However, the therapeutic use of *VA* for cancer treatment and its anticancer mechanism are still poorly explored.[37]

Survivin (BIRC5) is the smallest member of the Apoptosis Inhibitor protein (IAP) family that plays a role in cell cycle regulation. Survivin directly interact with caspase-3 and 7, and also regulate cytokinin to inhibit apoptosis.[38] Survivin is over expressed in almost all human malignancies, but the expression regulatory mechanisms of this protein are not yet fully understood.[39]

The aims of this study are: (i) screening of antioxidant compounds from leaves, fruit, and stems of *VA* growing on different host trees in Afyonkarahisar province, (ii) evaluation of the antioxidant compounds from methanolic extracts by DPPH, ABTS, and FRP. iii) to analyze *in silico* the relationship of active substances with proteins that inhibit apoptosis.

Material and Methods

VA samples of 12 different hosts (*Pyrus communis, Pyrus amygdaliformis, Pinus nigra, Robinia pseudoacacia, Prunus dulcis, Salix alba, Pyrus elaeagnifolia, Morus nigra, Mespilus germanica, Prunus armeniaca, Crataegus orientalis, Celtis planchoniana*) in Afyonkarahisar province at the Central Anatolian region (Turkey) were collected in September, October and November 2018. Species identification of the plants hosting *VA* was made using the work named Flora of Turkey and East Aegean Island.[40] Samples diagnosed have been kept at Afyon Kocatepe University Molecular Biology and Genetics Department with Herbarium their record numbers (Table 1and Figure 1).

Ultrasound-Assisted Extraction

500 mg of dried, ground plant material were individually weighed with precision. 50 mL of 70% methanol solution in ultra-pure water was added. It was extracted in an ultrasonic bath for 15 minutes. After the extraction was completed, it was filtered through white band filter paper.

Determination of Total Phenolic and Flavonoid Content

Total phenolic contents (TPC) of methanolic extracts were performed using the Folin-Ciocalteu method.[41] After adding 500 μL of sample extract, 250 μl of Folin-Ciocalteu reagent (2 N, Sigma Aldrich), 7250 μL of ultrapure water to a 10 ml test tube, it was kept in the dark for 5 minutes. 2000 μL of 7.5% Na_2CO_3 solution was added and kept in the dark for 30 minutes. After 30 minutes of incubation, the absorbance value against ultrapure water was measured at 765 nm with a spectrophotometer (Shimadzu UV-1800 spectrophotometer, Japan) instrument. TPC was expressed as mg gallic acid equivalent (GAE) per 1.00 g dried plant material.

The total flavonoid content (TFC) of the samples was determined using the aluminum chloride colorimetric

Figure 1. VA samples of 12 different hosts. (a) Pyrus amygdaliformis Vill. var. Amygdaliformis,. (b) Prunus armeniaca L., (c) Pyrus communis L., (d) Salix alba L. subsp. Alba,.(e) Mespilus germanica L., (f) Crateagus orientalis Pall. Ex M.Bieb. subsp.orientalis, (g) Pyrus elaeagnifolia Pall. subsp. Elaeagnifolia, (h)Robinia pseudoacacia L.,. (ı) Prunus dulcis (Mill.) D.A. Webb,. (j) Morus nigra L.,. (k) Celtis planchoniana K.I. Chr, (l) Pinus nigra J.F.Arnold. subsp. pallasiana (Lamb.)

method.[42] For this, 50 μL of sample extract, 950 μL of methanol, 6400 μL of ultra-pure water, 300 μL of $NaNO_2$ solution (5% in ultra-pure water), and 300 μl of $AlCl_3$ solution (10% in ultra-pure water) were added to a 10 mL test tube and kept in a dark environment for 5 minutes. Then, 2000 μl of NaOH solution (4% in ultra-pure water) was added and it was kept in the dark for 15 minutes again. The absorbance of the mixture against ultrapure water at a wavelength of 510 nm was measured using a spectrophotometer (Shimadzu UV-1800 spectrophotometer, Japan). Total flavonoid content was expressed as mg quercetin equivalent (QE) per 1 g dried plant material.

Determination of Total Anthocyanin Content

Determination of the total antioxidant content of the extract was carried out by following the method of Giusti et al., (1999).[43] The absorbances (A) were recorded at 510 ($A_{510(pH1.0)}$ and $A_{510(pH4.5)}$) and 700 nm ($A_{700(pH1.0)}$ and $A_{700(pH4.5)}$) at two different pH values, pH 1.0 and pH 4.5. The total amount of anthocyanin (TAC) was calculated according to the following formula using the cyanide-3-glycoside equation.

$$TAC = [(A_{510(pH1.0)} - A_{700(pH1.0))} - (A_{510(pH4.5)]}\text{-}A_{700(pH4.5)})] \quad (1)$$

Results mg cyanide 3-glycoside/g (μg cg-3-glkt/g) dry weight.

Analysis of Flavonoid and Phenolic Substances by High Performance Liquid Chromatography (HPLC) Method

Agilent 1260 HPLC Device equipped with UV detector, quad gradient pump, automatic sampler and Chemstation software was used to determine the organic acid contents in the samples. An ACE-C18 (4.6 mm × 150 mm, 5 μm) column was used for the analyses. Ultrapure water containing 0.1% acetic acid (A) and acetonitrile (B) were used as mobile phases. The mobile phase flow rate was 1.0 mL min^{-1}, the column temperature was 25 °C, and the injection volume was 10 μL. Detection wavelengths were chosen considering the wavelengths at which the phenolic compounds to be analyzed have the maximum absorption. According to this, caffeic acid and chlorogenic acid were detected at 330 nm, p-coumaric acid at 305 nm, syringic acid, protocatechic acid and gallic acid at 280 nm, vanillic acid at 225 nm. The following gradient conditions were used: 0.00 min–3.25 min, 8–10% B; 3.25 min–8.00 min, 10–12% B; 8.00 min–15.00 min, 12–25% B; 15.00 min–15.80 min, 25–30% B; 15.80 min–25.00 min, 30–90% B; 25.00 min–25.40 min, 90–100% B; 25.40 min–30.00 min, 100% B.[44]

Antioxidant activity

DPPH (2,2′-diphenyl-1-picrylhydrazyl radical) assay

Antioxidant activity was determined using the DPPH (2,2-diphenyl-1-picrylhydrazil) radical method.[45] For this, 0.0024 g of DPPH was precisely weighed and a stock solution of DPPH reagent (6×10^{-5} M) was prepared by dissolving in 100 mL of methanol. A working solution of DPPH with a concentration (40 mg L^{-1}) was prepared from the stock reagent solution by diluting it with methanol. 300 μL of sample extract and 5700 μL of DPPH working solution were mixed in a 10 mL test tube. The mixture was incubated for 60 minutes at room temperature in a dark environment. The absorbance of the reaction mixture against ultrapure water was measured at 517 nm using a spectrophotometer (Shimadzu UV-1800 spectrophotometer, Japan). On the other hand, a control solution without sample extract was prepared and its absorbance against ultrapure water was measured at 517 nm in a spectrophotometer device. The antioxidant activity was calculated as:

$$A(\%) = (AC(O)_{517} - AA(t)_{517})/AC(O)_{517} \times 100 \quad (2)$$

where $AC(O)_{517}$is the absorbance of the control at t = 0 min and $AA(t)_{517}$is the absorbance of the antioxidant at t = 1 h.

ABTS (2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid) Radical Test

For the determination of ABTS radical quenching activity of plant extracts the method of Thaipong et al. (2006) was used. In these experiments, ascorbic acid was used as a positive control.[46] Sample antioxidant activity was expressed as % Inhibition of ABTS positive radical cation. Percent inhibition at

absorbance 734 nm was calculated according to the following formula.

$$\% \text{ Inhibition} = [A_0 - (A_t - B)]/A_0 \times 100] \quad (3)$$

Where A_0, the absorbance of the initial control, A*t*, 6 minutes absorbance of the sample, B, the absorbance of the blank solution.

Ferric Reducing Pover (FRP)

In this procedure, the method of Oyaizu (1986) was employed.[47] For this, 2.5 mL of phosphate buffer (0,2 M, pH = 6.6) with 2.5 mL of 1% $K_3Fe(CN)_6$ (100–200–300–400 μL) to different amounts of samples are mixed and incubated for 20 minutes at 50 °C, then 2.5 mL of 10% TCA was added and centrifuged at 2500 rpm for 10 minutes. After centrifugation, 2.5 mL of supernatants were taken and the total volume adjusted to 5 mL with 0.5 mL of 0.1% $FeCl_3$ solution, the absorbance values were determined spectrophotometrically at 700 nm.

Statistical analysis

To determine if there is a difference total phenolic and total flavonoid contents of *VA* growing in different hosts was done t test. The relationship between Total Phenolic Activity, Total Flavonoids, DPPH, ABTS and FRP was determined by Kendall's Tau-b Correlation test in SPSS 24 program.

Molecular docking and Molecular dynamic analyses

The 3D structures of the Survivin protein (ID:1xox) were obtained from the RCSB PDB protein database (https://www.rcsb.org/). The 3D structure of Quercetin, Rosmarinic acid and Catechin was found in the PubChem database. (https://pubchem.ncbi.nlm.nih.gov/). Molecular docking calculations were performed using Autodock.[48]

Interactions between ligands and proteins were visualized using Molegro Molecular Viewer program (http://molexus.io/molegro-molecular-viewer/).We determined the performance of MM/PB(GB)SA to determine the correct binding positions for ligands, including those from the Schrödinger package and the Amber package (http://cadd.zju.edu.cn/farppi).[49]

A dynamic simulation of the ligand and protein complex was performed using the WebGro application. A MD simulation was performed for 50 ns to study the stability of the ligand and protein complex.[50–54]

Results and Discussion

Total Phenolic and Total Flavonoid Content Quantification Analysis

The methanolic gallic acid solution was used as standard at different concentrations for the phenolic (150, 300, 450, 600, 750 mgL^{-1}). The absorbance value versus concentration was plotted and the calibration curve was generated. The linearity of the calibration curve (y = 0.0004X-0.001) and the regression coefficient (R^2 = 0.999) was determined. The total phenolic content of the extracts was expressed as gallic acid equivalent (GAE). The results of total phenolic contents are presented in Table 2.

For the flavonoid content of *VA* extract, quercetin's methanolic solution was used as standard at different concentrations (200, 400, 600, 800, 1000 mg L^{-1}). The absorbance value versus concentration was plotted and the calibration curve was generated. The linearity of the calibration curve (y = 0.0001X–0.002) and the regression coefficient (R^2 = 0.999) were determined. The total flavonoid content of the extract is expressed as a quercetin equivalent (QE). The values were figured out by using the formula given in the M&M section. Total flavonoid contents in different parts of *VA* are given in Table 2 as mg QE in 1 g sample.

A statistical difference was found between TFC and TPC values in *Pinus nigra, Pyrus elaeagrifolia Robinia pseudoacacia, Prunus dulcis, Salix alba, Morus alba, Mespilus germanica* (Table 3).

The amount of TPC in *VA* methanol extracts ranged from 9.81 to 22.76 mg GAE/g. It was determined that the amount of

Table 2. Total phenolic and total flavonoid contents of *VA* growing in different hosts.

Host Plant (*hp*)	Total Phenolic Content (TPC) mg GAE/g (± SD)			Total Flavonoid Content (TFC) mg QE/g (± SD)		
	Branches	Leaves	Fruits	Branches	Leaves	Fruits
Pyrus communis	11.17(0.45)	12.45(0.26)	22.76(0.38)	10.02(0.68)	13.32(0.67)	3.42(0.33)
Crataegus orientalis	10.02(0.33)	13.38(0.51)	17.61(0.47)	8.82(0.71)	28.02(0.51)	3.42(0.46)
Pyrus amygdaliformis	11.66(0.21)	13.4(0.72)	13.52(0.25)	9.72(0.33)	15.12(0.40)	2.52(0.27)
Celtis planchoniana	9.81(0.62)	13.84(0.36)	20.32(0.84)	8.52(0.48)	15.72(0.61)	2.22(0.53)
Pinus nigra	12.26(0.47)	15.92(0.81)	11.2(0.68)	24.42(0.56)	53.82(0.73)	38.52(0.44)
Robinia pseudoacacia	10.87(0.60)	10.4(0.73)	16.66(0.53)	74.22(0.62)	23.52(0.33)	10.92(0.38)
Prunus dulcis	10.3(0.11)	12.34(0.44)	20.75(0.61)	34.92(0.44)	28.02(0.39)	22.62(0.36)
Salix alba	12.83(0.51)	14.62(0.53)	19.27(0.35)	23.22(0.57)	32.52(0.59)	21.72(0.66)
Pyrus elaeagrifolia	12.76(0.32)	14.41(0.63)	19.27(0.59)	28.32(0.27)	60.72(0.48)	24.42(0.65)
Morus alba	10.59(0..9)	10.18(0.58)	–	23.82(0.39)	20.52(0.51)	–
Mespilus germanica	12.42(0.53)	16.59(0.49)	22.19(0.69)	23.52(0.26)	97.92(0.51)	38.22(0.54)
Prunus armeniaca	11.33(0.09)	12.34(0.28)	17.03(0.38)	74.52(0.44)	20.82(0.37)	25.62(0.46)

Table 3. Difference total phenolic and total flavonoid contents of *VA* growing in different hosts (t test).

Host Plant (*hp*)	Total Phenolic Content (TPC) mg GAE/g (±SD)		Total Flavonoid Content (TFC) mg QE/g (±SD)			
	$\bar{X}$	SS	$\bar{X}$	SS	t	P
Pyrus communis	15.46	6.35	8.92	5.04	1.01	.416
Crataegus orientalis	13.62	3.80+	13.42	12.92	.030	.979
Pyrus amygdaliformis	12.86	1.04	9.12	6.32	.987	.427
Celtis planchoniana	14.65	5.30	8.82	6.75	.941	.446
Pinus nigra	13.12	2.47	38.92	14.70	3.45	*.027*[a]
Robinia pseudoacacia	12.64	3.48	36.22	33.50	−1.14	*.013*[a]
Prunus dulcis	14.46	5.53	28.52	6.15	−2.12	*.016*[a]
Salix alba	15.52	3.32	25.82	5.85	−2.29	*.014*[a]
Pyrus elaeagrifolia	15.48	3.38	37.82	19.92	−1.80	*.021*[a]
Morus alba	10.38	.289	22.17	2.33	−8.15	*.017*[a]
Mespilus germanica	17.06	4.90	53.22	39.40	−.1.59	*.021*[a]
Prunus armeniaca	13.56	3.04	40.32	29.71	−1.46	.281

[a] $P < .05$.

phenolic in the fruit extracts was higher than the branch and leaf extracts.Similarly, Skrypnik et al (2022) reported that TPC in mistletoe fruits was 3.0–3.2 times higher than in leaves and stems.[55]

The highest TPC was found in samples collected from *Pyrus communis* (22.76 mg GAE/g sample) at *VA* fruit methanol extracts. This was followed by *Mespilus germanica* (22.19 mg GAE/gfruit sample). The lowest TPC was detected in branch extracts samples collected from *Celtis planchoniana* (9.81 mg GAE/g sample). No fruit was found in the samples taken from *Morus alba*, therefore we were unable to study it.

Flavonoids contain hydroxyl attached to their ring structure. They are known to have a scavenging effect due to free radicals.[56] In this study the amount of TFC in *VA* methanol extracts ranged from 97.92 to 2.22 mg QE/g It was determined that the amount of flavonoid in the leaves and br-anched extracts were higher than the fruits extracts.

The lowest TFC was found in the flowers of *Celtis planchoniana* (2.22 mg QE/g). This was followed by *Pyrus amygdaliformis* flower extract with 2.52 mg QE/g and *Pyrus communis* and *Crataegus orientalis* flower extracts with 3.42 mg QE/g. The highest TFC was found in *VA* leaves samples collected from *Mespilus germanica* is 97.92 mg QE/g. It was followed by *Robinia pseudoacacia* (74.22 mg QE/g. *VA* branches extract) and *Pyrus elaeagrifolia* (60.72 mg QE/g. *VA* leaves extract) respectively (Table 2).

Flavonoids, including anthocyanins in plants, have effects such as improving signal biotic and abiotic stresses for micro-organisms, protecting against pathogens, affecting oxides transport and plant fertility, and making flowers visible to insects. Anthocyanins, which are flavonoid derivatives, are glucosides of anthocyanidins produced by the phenylpropanoid pathway.[65] Since anthocyanin molecules will change color depending on the pH of the environment they are in, they can act as pH indicators.[66] They show antioxidant properties by signaling free radical scavenging pathway, cyclooxygenase pathway, mitogen-activated protein kinase pathway and inflammatory cytokines.[65] Anthocyanins generally accumulate in flowers but are found in some genera in leaves and braces and baskets. Therefore, in our study *VA* branch leaf, fruit extracts were evaluated for anthocyanin (equation 1).. In our study, anthocyanin could not be determined in the samples.

In this study, the results of HPLC analysis show that the content of phenolic and flavonoids varies depending on the host tree and mistletoe organ. For example, gentisic acid was not detected in methanol extracts of *VA*'s leaves growing on *Pinus nigra*, *Salix alba* and fruit extract of *Prunus armeniaca*, *Celtis planchonianas*. The highest value of gentisic acid was found in the methanol extract of leaf *VA* growing on *Pyrus amygdaliformis* (1970.50 mg/100 g). This was followed by the *VA* leaf growing on *Mespilus germanica* (1882.05 mg/100 g). Quercetin and Rosmarinic acid (Except the fruit of the *VA* sample from *Pinus nigra*) were detected in all methanol extracts of *VA*. The highest amount of Quercetin was found in the leaf of *VA* growing on *Pyrus elaeagnifolia* (1522.62 mg/100 g) and followed by *Mespilus germanica* (1396.50 mg/100 g) and *Prunus dulcis* (1380.84 mg/100 g), respectively. The highest Rosmarinic acid value was determined in the leaf of *VA* growing on *Pyrus amygdaliformis* (489.06 mgL^{-1}). This sample was followed taken from *Salix alba* (319.68 mg/100 g). The highest value of p-coumaricacid was determined as 268.53 mg/100 g at *VA* branch on *Salix alba*. Vanicic acid and protocatechuic acid were not determined in any of the extracts of *VA*. The highest Sinapinic acid value was determined in the leaf of *VA* growing on *Pyruselaeagnifolia* (115.47 mg/100 g) (Table 4).HPLC chromatogram of phenolic standards is given in Figure 2.

Quercetin and Rosmarinic acid (Except the fruit of the *VA* sample from *Pinus nigra*) were detected in all methanol extracts of *VA* The highest amount of Quercetin was found in the leaf of *VA* growing on *Pyrus elaeagnifolia* (1522.62 mg/100 g) and followed by *Mespilus germanica* (1396.50 mg/100 g) and *Prunus dulcis* (1380.84 mg/100 g), respectively. It has been reported in recent studies that quercetin inhibits cancer through its apoptosis, suppresses signal transduction and reduces the proliferation, invasion and metastasis potential of tumor cells, protecting cellular DNA from cancer-causing mutations.[33,56–58] Although rosmarinic acid is known to be a potential therapeutic agent against cancer types, its mechanism has not been

Table 4. HPLC Analysis of *VA* Plant Material.

Host Plant Material HPLC Analysis (mg/100 g Dry Weight)	Gallic Acid	Gentisic Acid	Vanylic Acid	Protocolate Acid	Syringic Acid	Caffeic Acid	Coumaric Acid	Sinapinic Acid	Catechin	Quercetin	Rosmarinic Acid
Pyrus communis L											
Branch	22.68	702.95	–	–	–	14.01	93.45	13.44	2949.00	378.00	53.88
Leaf	1.72	–	–	–	8.46	7.08	–	21.19	–	92.95	21.30
Fruit	–	59.30	–	–	–	–	–		420.00	98.13	12.24
Crataegus orientalis											
Branch	–	770.40	–	–	6.21	34.50	85.74	16.98	–	520.17	81.48
Leaf	–	1290.93	–	–	17.49	34.86	193.56	41.46	–	901.08	131.40
Fruit	–	335.02	–	–	–	4.29	153.42	5.52	–	306.81	15.48
Pyrus amygdaliformis											
Branch	–	1347.49	–	–	–	56.16	197.22	68.91	–	327.54	318.12
Leaf	–	1970.50	–	–	25.74	54.84	196.41	35.46	–	784.74	486.06
Fruit	–	149.29	–	–	–	3.42	158.97	–	–	350.13	53.28
Celtis planchoniana											
Branch	26.85	618.25	–	–	–	9.09	169.11	42.69	–	306.12	101.64
Leaf	45.30	1637.56	–	–	–	28.56	114.45	66.57	–	135.54	168.48
Fruit	–	–	–	–	–	–	–	–	–	126.90	14.52
Pinus nigra											
Branch	–	–	–	–	–	43.50.	156.93	47.64	–	12.51	13.02
Leaf	–	559.63	–	–	106.44	14.01	133.83	39.54	–	53.49	9.18
Fruit	25.47	–	–	–	14.52	–	–	–	171.54	75.30	–
Robiniapseudoacacia											
Branch	20.64	626.73	–	–	–	–	98.67	32.70	–	262.59	62.10
Leaf	24.36	634.09	–	–	35.94	–	–	42.06	–	413.49	150.24
Fruit	–	565.36	–	–	–	–	–	–	–	182.58	26.10
Prunus dulcis											
Branch	17.43	623.16	–	–	–	–	70.92	30.30	–	849.15	180.72
Leaf	52.92	1249.54	–	–	21.81	–	134.19	108.51	–	1380.84	170.10
Fruit	49.20	35.73	–	–	–	–	54.84	–	–	283.59	31.92
Salix alba											
Branch	–	1062.85	–	–	36.45	–	268.53	68.13	–	468.75	183.42
Leaf	–	1231.43	–	–	51.99	–	123.81	68.97	–	466.95	319.68
Fruit	–	–	–	–	–	–	161.01	–	–	285.99	11.16
Pyruselaeagnifolia											
Branch	–	711.99	–	–	–	40.48	128.07	30.51	–	399.75	168.00
Leaf	–	1429.80	–	–	33.93	–	–	115.47	–	1522.62	376.68
Fruit	–	266.36	–	–	–	–	–	–	–	42.87	25.56
Morusnigra											
Branch	42.15	560.28	–	–	–	20.58	71.22	71.25	–	97.44	49.92
Leaf	153.51	493.56	–	–	13.95	–	–	69.78	–	211.62	106.68
Fruit	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]
Mespilusgermanica											
Branch	–	1319.37	–	–	–	38.04	121.98	42.69	–	500.40	136.50
Leaf	–	1882.05	–	–	–	15.15	114.39	61.44	–	1396.50	253.86
Fruit	–	235.35	–	–	–	–	–	–	–	21.84	10.62
Prunusarmeniaca											
Branch	–	860.22	–	–	55.86	–	89.73	26.46	–	768.72	169.44
Leaf	11.40	–	–	–	–	–	146.64	68.34	–	852.87	137.40
Fruit	–	–	–	–	–	–	–	–	–	417.63	89.04

[a] ND (not determinate)

fully elucidated. However, there are studies on it inhibiting cell proliferation and selectively inducing cancer cell apoptosis.[59]

This sample was followed taken from *Salix alba* (319.68 mg/100 g). Catechin was detected only in branches (2949.00) and fruit (420.00) of *VA* collected from *Pyrus communis* L, and in fruits (171.54) of *VA* collected from *Pinus nigra*. Catechins show antioxidant properties by chelating metal ions, scavenging reactive oxygen derivatives and increasing the levels of antioxidant enzymes.[60–62] In another study, it was shown that catechin induces apoptosis by inducing DNA fragmentation in prostate cancer cells.[64] It was also reported to induce apoptosis in HT29 colon cancer cells by increasing caspase3, caspase7, caspase8, and caspase9.[66]

Antioxidant Activity Tests

The literature survey indicated that *VA* has many biological activities like anticancer, antiviral, antioxidant, apoptosis-induction and immunomodulatory properties.[22,67–72] It has been used in cancer treatment for about 80 years. Therefore, the research

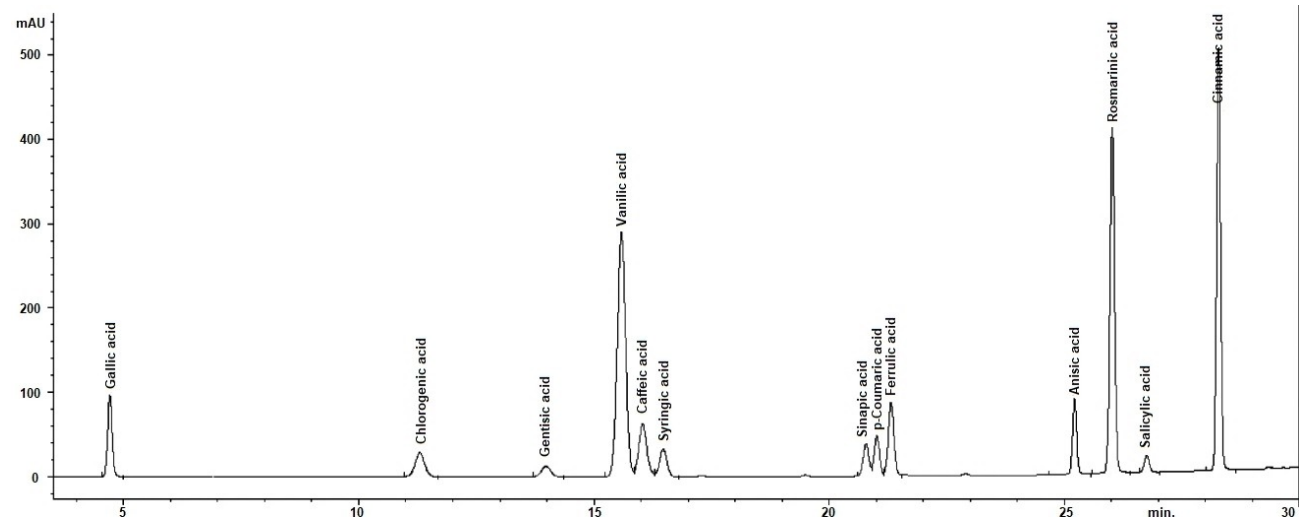

Figure 2. HPLC chromatogram of phenolic standards.

studies were intensified on the antioxidant activity of *VA* in the last decades due to its phenolics, especially phenolic, flavonoid and carotenoid contents.[73,74]

Determination of Antioxidant Activity (DPPH Test)

The DPPH Inhibition% values of *VA* branch, leaf and fruit samples are given in Table 5 (equation 2). The highest % inhibition values were found in *VA* branch samples and % inhibition values ranged from 91.74–84.68. Ascorbic acid solution (500 $\mu g\,mL^{-1}$) was used as the control solution in the antioxidant test. The capacity of ascorbic acid solution to inhibit DPPH radical was determined as 95%. It was determined that the lowest DPPH % Inhibition was in the flower extracts. In order from lowest to highest *Prunus armeniaca* (67.30) < *Robinia pseudoacacia* (68.59) < *Prunus dulcis* (75.9) < *Celtis planchoniana* (77.62).The highest value was determined from pine *VA* branch samples, respectively, 91.14% pear, 91.05% hawthorn, 90.79% followed by branch samples over the rice. As in the branch samples, leaf (89.59%) and fruit (87.52%) samples also had the highest % inhibition value on *VA* pine samples. The lowest values of *VA* grown on apricot were found in the branch (84.68%), leaf (79.00%) and fruit (67.30%) samples. DPPH% inhibition values of fruit and leaf samples of *VA* were also found to be quite high. Uçar et al reported that *VA* has free radical scavenging activity and protective effect against hydroperoxide formation.[27] They found that the antioxidant capacity of the extract may vary depending on the harvest time of the *VA* and the nature of the host tree.

While there was a negative and significant correlation ($r = -1.00$ $p < .01$) between *VA* Phenolic Content value and DPPH value of *Prunus dulcis.* It is seen that there is a positive and significant correlation ($r = 1.00$ $p < .01$) between the Flavonoid Content value and the DPPH value. There is a negative and significant correlation ($r = -1.00$ $p < .01$) between *VA* Phenolic

Table 5. DPPH and ABTS (%) activity of *VA* growing in different hosts.

Host Plant	DPPH[a] % Inhibition			ABTS[b] (%)		
	Branches	Leaves	Fruits	Branches	Leaves	Fruits
Pyrus communis	91.14	87.52	84.94	97.45	97.18	80.00
Crataegus orientalis	91.05	86.49	84.94	97.27	96.81	96.72
Pyrus amygdaliformis	89.00.	88.64	80.98	98.36	98.45	78.45
Celtis planchoniana	90.79	88.12	77.62	99.90	96.36	78.81
Pinus nigra	91.74	89.59	87.52	97.09	97.00	67.45
Robinia pseudoacacia	89.76	82.79	68.59	96.63	95.00	87.54
Prunus dulcis	87.69	81.93	75.9	97.27	97.36	94.54
Salix alba	89.07	87.01	82.79	98.54	95.45	95.54
Pyrus elaeagnifolia	88.21	86.75	85.89	98.90	98.18	94.63
Morus nigra	85.54	81.50	–	98.54	95.00	ND
Mespilus germanica	86.32	83.05	84.6	97.09	98.54	97.81
Prunus armeniaca	84.68	79.00	67.30	96.54	96.81	90.54

[a] DPPH: 1,1-Diphenyl-2-picrylhydrazyl free radical scavenging,
[b] ABTS: (2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid) radical scavengingcapacity.

Content value and DPPH value in *Prunus armeniaca* and *Salix alba*. (Table 7)

ABTS (2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid) Radical Test

Interesting results were noted for the *VA* against ABTS radicals (Table 5) (equation 3). It was observed that of *VA* methanol extract showed a markedly high ability to scavenge ABTS radicals (99.90 % to 67.54 %). All of *VA* branche and leaf extracts have ABTS radicals scavenge effect of 95 %≤. It was determined that the ABTS radical scavenging effect of fruit extracts was less than that of branch and leaf extracts. It was determined that the least effect was in the flower extract collected from *Pinus nigra* (67.45 %) (Table 5).

It was determined that there was a positive and significant correlation (r1.00 $p < .01$) between the *VA* Phenolic Content value of *Morus alba* and the DPPH value, and a positive and significant correlation ($r = 1.00$ $p < .01$) between the Phenolic Content value and the ABTS value. A negative and significant correlation ($r = -1.00$ $p < .01$) was found between Phenolic Content value of *VA* collected from *Pyrus elaeagrifolia, Celtis planchoniana, Pyrus amygdaliformis, Crataegus orientalis, Pyrus communis* and DPPH and ABTS values. A positive significant correlation ($r = 1.00$ $p < .01$) was found between the Flavonoid Content value of the *VA*s collected from *Robinia pseudoacacia* and *Morus alba*, and the DPPH and ABTS values. There is a negative and significant correlation ($r = -1.00$ $p < .01$) between *VA* Flavonoid Content value and DPPH value of *Mespilus germanica*. There is a positive and significant correlation ($r = 1.00$ $p < .01$) between the *VA* Flavonoid Content value of *Mespilus germanica* and the ABTS value. It was determined that there was a negative and significant correlation ($r = -1.00$ $p < .01$) between the *VA* Flavonoid Content value of *Pyrus amygdaliformis* and the ABTS value (Table 7). A strong positive correlation was found between the accumulation of polyphenol compounds and the antioxidant activity of the extracts.[75] Based upon the conducted research, it has been found that all mistletoe extracts (aqueous or ethanol, leaf or stem) have the ability of scavenging cation-radicals ABTS+.[18,76,77] In this study the ABTC and DPHH values are higher than the values some studies reported in the literature It was concluded that the tree on which the plant lives, the region, the method of obtaining the plant extract and the solvent difference may have an effect on this situation.[78] In general, ABTS values were found to be higher than DPPH. It is known that DPPH is hypophilic reagent, whereas ABTS is more hydrophilic reagent because it is cation. In this case, it can be said that the antioxidants in the *VA* extract are more hydrophilic.

Ferric Reducing Power (FRP)

The presence of reductants such as antioxidant substances in the antioxidant samples causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form. Kazazic et al. (2006) investigated the ligation of Fe^{+} and Cu^{+} ions with flavonoids in the gas phase. They determined that metal ions bind sequentially with or without simultaneous loss of parts of neutral flavonoid molecules such as H, CO, H_2. They reported that the part of flavonoids that is mainly responsible for antioxidant activity is the C ring.[79] It was reported that the compounds with structures containing two or more of the following functional groups: OH, SH, COOH, PO_3H_2, C,O, NR_2, S and O in a favorable structure-function configuration can show metal chelation activity. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity.[77] Increased absorbance of the reaction mixture indicates an increase in FRP.[80,81]

In this study, it was determined that there was an increase in FRP due to the increase in concentration in all tested fruit, branch and leaf extracts of *VA* (Table 6).According to data obtained from the present study, the distribution of phenolic and flavonoids content on *VA* leaves, branches and fruit varied depending on the growing trees. DPPH% inhibition values of fruit and leaf samples of *VA* were found to be quite high.

Table 6. FRP results of *VA* organs based at 700 nm spectrophotometric measurements.

Host Plant	Organs of *VA*	Extracts (500 mg dried, ground plant material with 50 mL of 70 % methanol) 100 µl	200 µl
Pinus nigra	Leaf	0.407	0.719
	Fruit	0.195	0.278
	Branch	0.307	0.526
Pyrus communis	Leaf	0.316	0.586
	Fruit	0.225	0.320
	Branch	0.370	0.710
Crataegus orientalis	Leaf	0.372	0.760
	Fruit	0.287	0.496
	Branch	0.349	0.584
Celtis planchoniana	Leaf	0.427	0.690
	Fruit	0.283	0.493
	Branch	0.252	0.401
Pyrus amygdaliformis	Leaf	0.344	0.778
	Fruit	0.213	0.352
	Branch	0.342	0.604
Robinia pseudoacacia	Leaf	0.230	0.434
	Fruit	0.243	0.466
	Branch	0.297	0.541
Prunus dulcis	Leaf	0.311	0.582
	Fruit	0.265	0.436
	Branch	0.283	0.454
Salix alba	Leaf	0.368	0.760
	Fruit	0.254	0.346
	Branch	0.334	0.595
Pyrus elaeagnifolia	Leaf	0.453	0.747
	Fruit	0.265	0.500
	Branch	0.323	0.570
Morus nigra	Leaf	0.233	0.433
	Fruit	[a]	[a]
	Branch	0.241	0.473
Mespilus germanica	Leaf	0.401	0.778
	Fruit	0.272	0.414
	Branch	0.344	0.506
Prunus armeniaca	Leaf	0.313	0.520
	Fruit	0.327	0.399
	Branch	0.235	0.430

[a] ND: (not determinate).

Table 7. The relationship between Total Phenolic Activity, Total Flavonoids, DPPH, ABTS and Fe^{3+} values was determinate by Kendall's Tau-b Correlation test.

Host Plant		TPC	TFC	DPPH	ABTS	Fe^{3+}(200 µl)
Pyrus communis	TPC	1	−0.33	*−1.00*[a]	*−1.00**	0.33
	TFC	−0.33	1	0.33	0.33	*−1.00*[a]
Crataegus orientalis	TPC	1	−0.33	*−1.00*[a]	*−1.00*[a]	−0.33
	TFC	−0.33	1	0.33	0.33	−0.33
Pyrus amygdaliformis	TPC	1	−0.33	*−1.00*[a]	*−1.00*[a]	−0.33
	TFC	−0.33	1	0.33	*−1.00*[a]	−0.33
Celtis planchoniana	TPC	1	−0.33	*−1.00*[a]	*−1.00*[a]	*−1.00*[a]
	TFC	−0.33	1	0.33	0.33	0.33
Pinus nigra	TPC	1	0.33	0.33	0.33	−0.33
	TFC	0.33	1	−0.33	−0.33	−1.00[a]
Robinia pseudoacacia	TPC	1	−0.33	−0.33	−0.33	0.33
	TFC	−0.33	1	*1.00*[a]	1.00[a]	*−1.00*[a]
Prunus dulcis	TPC	1	*−1.00*[a]	*−1.00*[a]	−0.33	−0.33
	TFC	*−1.00**	1	*1.00*[a]	0.33	0.33
Salix alba	TPC	1	−0.33	*−1.00*[a]	−0.33	−0.33
	TFC	−0.33	1	0.33	−0.33	−0.33
Pyrus elaeagrifolia	TPC	1	−0.33	*−1.00*[a]	*−1.00*[a]	−0.33
	TFC	−0.33	1	0.33	0.33	−0.33
Morus alba	TPC	1	*1.00*[a]	*1.00*[a]	*1.00*	–
	TFC	*1.00*[a]	1	*1.00*[a]	*1.00*[a]	–
Mespilus germanica	TPC	1	0.33	−0.33	0.33	−0.33
	TFC	0.33	1	*−1.00*[a]	*1.00*[a]	*−1.00*[a]
Prunus armeniaca	TPC	1	−0.33	*−1.00*[a]	−0.33	−0.33
	TFC	−0.33	1	0.33	−0.33	*1.00*[a]

[a] Correlation is significant at the 0.01 level (2-tailed).

Similarly, *VA* methanol extract showed a remarkably high ABTS radical scavenging activity. There was an increase in FRP due to the increase in the amount of extract (from 100 µl to 200 µl) in all tested fruit, branch and leaf extracts of *VA*. Increasing the amount of extract caused the FRP to nearly double. This can be explained by the increase in the amount of phenolic and flovonaid obtained as a result of HPLC analysis, depending on the increase in the amount of extract to 200 µl.

It is seen that there is a negative and significant correlation ($r = -1.00$ $p < .01$) between *VA* Flavonoid Content value and FRP of *Robinia pseudoacacia, Pinus nigra, Mespilus germanica* and *Pyrus communis*. There is a negative and significant correlation ($r = -1.00$ $p < .01$) between *VA* Phenolic Content value of *Celtis planchoniana* and FRP ($r = -1.00$ $p < .01$), a positive and significant correlation between *VA* Flavonoid Content value of *Prunus armeniaca* and FRP ($r = 1.00.$ $p < .01$) was found (Table 7).

Molecular Docking and Dynamic Analyses

Since ancient times, plant active ingredients have been important in the development of drugs for the treatment of various diseases. Despite the existence of many chemotherapeutic drugs for cancer treatments today, experimental, and clinical studies continue due to the side effects of these drugs. The obtained data confirm that survivin is a preferable target in cancer treatment and an important prognostic marker. Flavonoids contain hydroxyl attached to their ring structure. They are known to have a scavenging effect due to free radicals. However, recent studies suggest that it can block the cell cycle and inhibit the proliferation of cancer cells by inducing apoptosis. Flavonoids contain hydroxyl attached to their ring structure. They are known to have a scavenging effect due to free radicals.[56] In this way, it prevents healthy cells from turning into malignant cancer cells. There are types of promising strategies based on targeting reduction of survivin expression level to inhibit the growth of tumor cells and drive them into apoptosis. Several common proteins (Hsp90, HBXIP, XIAP, cIAP1, Smac) interact with survivin and modulate survivin stability and/or function. Investigating the transcriptional and post-transcriptional control mechanisms of survivin in cancer cells will reveal clues for the development of new approaches for cancer-specific therapy.[82,83] Molecular docking results from this study showed strong interactions between survivin and quarcetin, rosmarinic acid and catechin (Table 8).

Table 8. Results of molecular docking of ligands with Survivin protein.

Ligands	Binding Energy (kcal/mol)
Gallic Acid	−4.5
Gentisic Acid	−6.2
Syringic Acid	−5.0
Caffeic Acid	−6.2
Choumatic Acid	−4.3
Sinapinic Acid	−5.2
Catechin	−8.6 (H bound number: Phe 93, Glu 94, Gln 92)
Quercetin	−8.3(H bound number: Glu 40, Lys 91, Phe 13)
Rosmarinic Acid	−9.4 (H bound number: Asp 16, Arg 18(x2), Gln 92)

Our results indicated that quercetin, catechin, and rosmarinic acid can interact with Survivin molecule the best minimum binding energies belong to −8.3 kcal/mol, −8.6 kcal/mol, and −9.4 kcal/mol, respectively.

The lowest binding energy refers to the most stable bond between the survivin quercetin and, catechin, and rosamic acid. All docked structures were visualized in Molegro Molecular Viewer (Figure 3–5).

Molecular docking scoring does not provide an acceptable estimate of ligand binding affinities. Therefore, MM/PB(GB)SA analyzes were used to estimate binding affinities. The graph of the ligands MM/PB(GB)SA is shown in the Figure 3–5.

A MD simulation was performed using the WebGro to check the stability of ligand-protein docked complexes of survivin and ligands. 3 docked complexes (Survivin-Quercetin, survivin-Rosaminic acid, Survivin-Catechin) were chosen to run with the molecular dynamic simulations. The conformational stability of the protein backbone and ligand-protein complexes were determined using the RMSD technique (Figure 6).

Conclusion

The data obtained from this study revealed that *VA* extracts are quite rich in terms of phenolics and flavonoids. According to statistical analyzes (by Kendall's Tau-b Correlation test), a correlation ($P < 5$) was found between the increase in DPPH% and ABTS% and the phenolic flavonids contents in the extract. The results obtained show the effect of *VA*

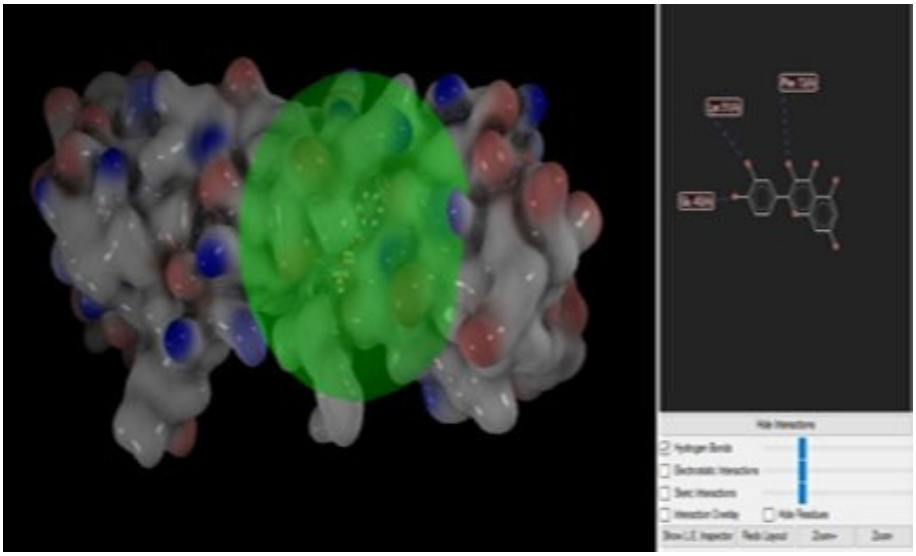

Figure 3. (A) Interaction between Survivin protein and Quercetin ligand and (B) H bond location (Glu 40, Lys 91, Phe 13).

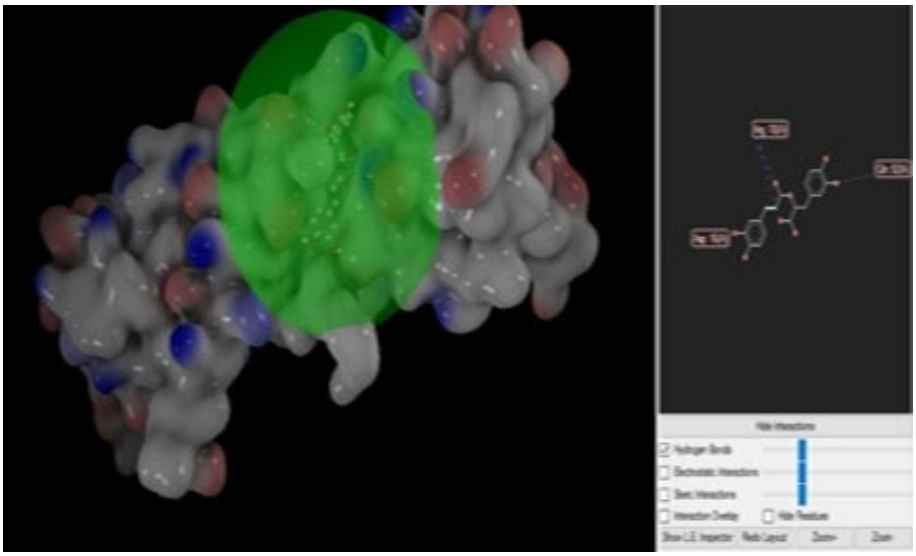

Figure 4. (A) Interaction between Survivin protein and Rosamarinic acid ligand and (B) H bond location (Asp 16, Arg 18(x2), Gln 92).

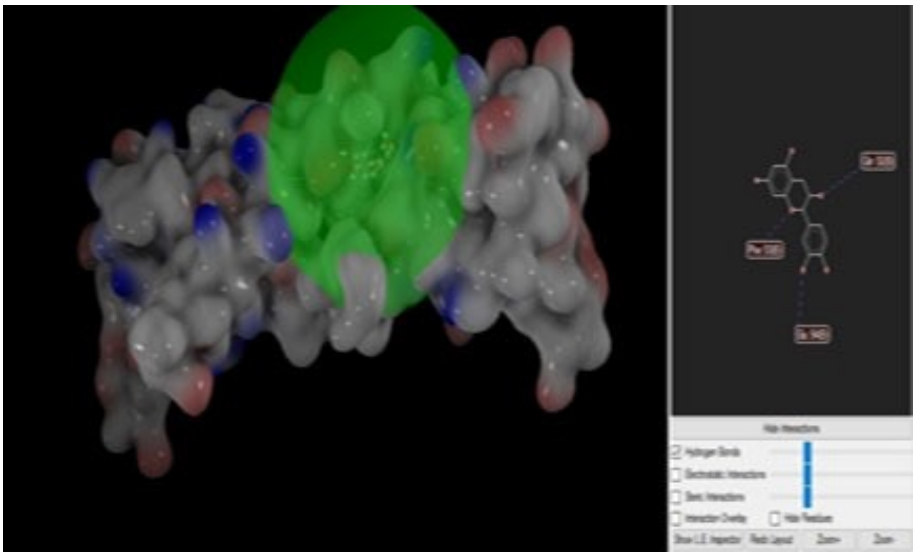

Figure 5. (A) Interaction between Survivin protein and Catechin ligand and (B) H bond location (Phe 93, Glu 94, Gln 92).

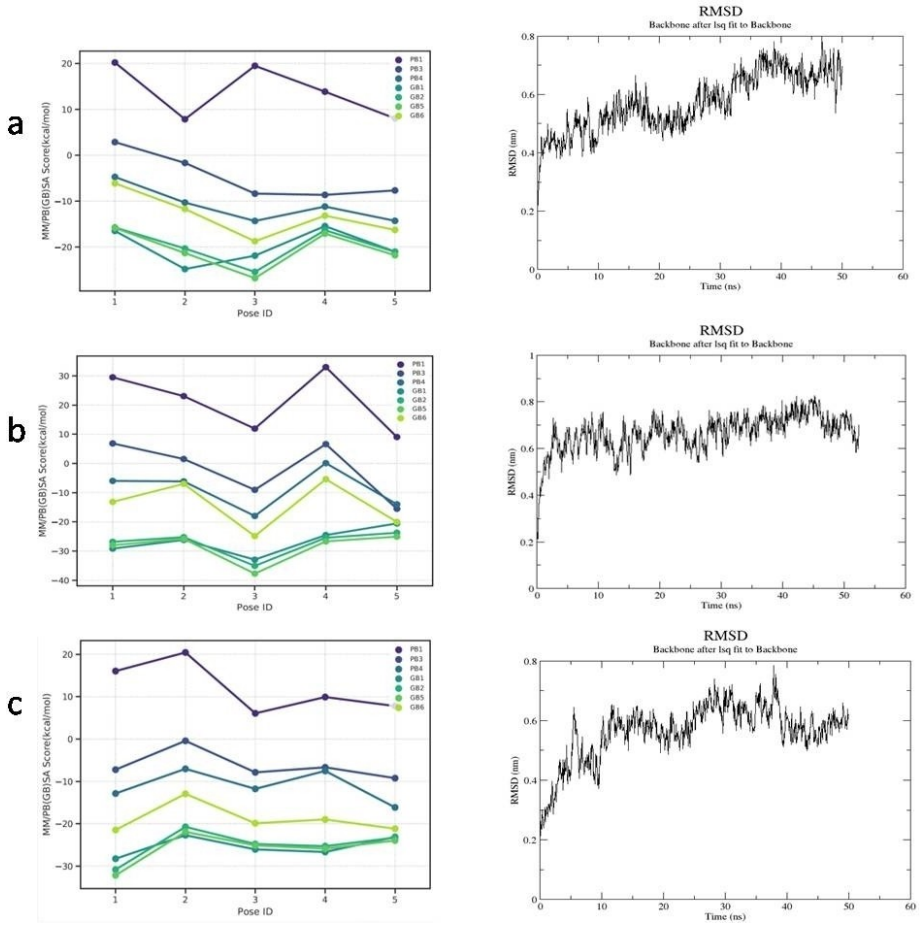

Figure 6. Molecular dynamic results and binding free energy graph (a) Quercetin (b) Rosmarinic Acid (c) Catechin.

extracts on cancer cells by showing antioxidant properties. In addition, as a result of docking analysis, quercetin, rosmarinic acid, and catechin in the extract were found to interact with survivin proteins, which are thought to be effective in

cancer formation and apoptosis. In conclusion, *VA* extracts act on cancer cells by multiple mechanism. It is concluded that among the compounds investigated, quercetin, rosmarinic acid, and catechins may act as the most suitable potential inhibitors of survivin. The possible clinical significance of our findings is that cancer cells can be promoted to apoptosis by abolishing the inhibition of the apoptotic pathway with quercetin, rosmarinic acid, and catechins. Therefore, they have been proposed as potential anticancer agents that require further investigation. However, its relationship with more antiapoptotic proteins should be investigated at *in silico, in vitro*, and *in vivo* levels.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability Statement

Research data are not shared.

Keywords: Antioxidant Activity · Flavonoid · Mistletoe · Phenolic · *Viscum album* L

[1] M. F. Khan, F. B. Kader, M. Arman, S. Ahmed, C. Lyzu, S. A. Sakib, S. M. Tanzil, I. U. Zim, A. S. Imran, T. Venneri, B. Romano, A. Haque, R. Capasso, *Biomed. Pharmacother.* **2020**, *131*, 110774.
[2] S. Mitra, R. Das, T. B. Emran, R. K. Labib, N. Tabassum, F. Islam, R. Sharma, I. Ahmad, F. Nainu, K. Chidambaram, F. A. Alhumaydhi, D. Chandran, R. Capasso, P. Wilairatana, *Front. Pharmacol.* **2022**, *13*, 943967.
[3] S. Ahmed, H. Khan, M. Aschner, H. Mirzae, A. E. Küpeli, R. Capasso, *Int. J. Mol. Sci.* **2020**, *21*, 5622.
[4] A. E. Küpeli, Y. Genç, B. Karpuz, E. Sobarzo-Sánchez, R. Capasso, *Cancers (Basel).* **2020**, *12*, 1959.
[5] M. N. U. Chy, M. Adnan, M. R. Chowdhury, E. Pagano, A. T. M. M. Kamal, K. K. Oh, D. H. Cho, R. Capasso, *J. Ethnopharmacol.* **2021**, *276*, 114182.
[6] J. Iqbal, B. A. Abbasi, R. Ahmad, M. Mahmoodi, A. Munir, S. A. Zahra, A. Shahbaz, M. Shaukat, S. Kanwal, S. Uddin, T. Mahmood, R. Capasso, *Biomedicine* **2020**, *8*, 117.
[7] E. K. Akkol, I. T. Çankaya, G. Ş. Karatoprak, E. Carpar, E. Sobarzo-Sánchez, R. Capasso, *Front. Pharmacol.* **2021**, *12*, 669638.
[8] O. Goni, M. F. Khan, M. M. Rahman, M. Z. Hasan, F. B. Kader, N. Sazzad, M. A. Sakib, B. Romano, M. A. Haque, R. Capasso, *J. Ethnopharmacol.* **2021**, *268*, 113664.
[9] J. Fernández, B. Silván, R. Entrialgo-Cadierno, C. J. Villar, R. Capasso, J. A. Uranga, F. Lombó, R. Abalo, *Biomed. Pharmacother.* **2021**, *143*, 112241.
[10] H. Mechchate, W. Ouedrhiri, I. Es-safi, A. Amaghnouje, F. Z. Jawhari, D. Bousta, *Biologics* **2021**, *1*, 154–163.
[11] D. Ağagündüz, T. Ö. Şahin, B. Yılmaz, K. D. Ekenci, Ş. D. Özer, R. Capasso, *J. Evidence-Based Comp. Altern. Med.* **2022**, *2022*, 1534083.
[12] G. Vieira, J. Cavalli, E. C. D. Gonçalves, S. P. F. P. Braga, R. S. Ferreira, A. R. S. Santos, M. Cola, N. R. B. Raposo, R. Capasso, R. C. Dutra, *Biomol. Eng.* **2020**, *10*, 792.
[13] I. Jahan, M. F. Khan, M. A. Sayeed, L. Arshad, M. A. Hossen, M. Jakaria, D. Ağagündüz, M. A. Haque, R. Capasso, *Curr. Issues Mol. Biol.* **2022**, *20(44)*, 2335–2349.
[14] M. Valko, D. Leibfritz, J. Moncol, M. T. D. Cronin, M. Mazur, J. Telser, *Int. J. Biochem. Cell Biol.* **2007**, *39*, 44–84.
[15] W. Pietrzak, R. Nowak, *Molecules* **2021**, *26*, 3741.
[16] M. Belghoul, A. Baghiani, S. Khennouf, L. Arrar, *S. Afr. J. Bot.* **2020**, *133*, 91–97.
[17] M. Ullah, S. Mehmood, R. A. Khan, M. Ali, F. Fozia, M. Waqas, R. Ullah, A. Alotaibi, M. A. Sultan, *J. Food Qual.* **2022**, ID 5691379, 1–9.
[18] A. Yirgu, Y. Mekonnen, A. Eyado, A. Staropoli, F. Vinale, *Pharm. Biol.* **2023**, *61*, 30–36.
[19] L. Burdejova, D. Moravcova, D. Strouhalova, K. Lunerova, *J. Pharm. Biomed. Anal.* **2021**, *195*,113850.
[20] S. A. A. Jafri, Z. M. Khalid, M. R. Khan, S. Ashraf, N. Ahmad, A. M. Karami, E. Rafique, M. Ouladsmane, N. M. S. Suliman, S. Aslam, *J. King Saud Univ. Sci.* **2023**, *35*, 102562.
[21] H. Yousaf, *bioRxiv* **2023** https://doi.org/10.1101/2023.01.03.522539.
[22] D. D. Orhan, M. Aslan, N. Sendogdu, F. Ergun, E. Yesilada, *J. Ethnopharmacol.* **2005**, *98*, 95–102.
[23] G. S. Son, W. S. Ryu, H. Y. Kim, S. U. Woo, H. K. Park, J. W. Bae, *J. Breast Cancer* **2010**, *13*, 1418.
[24] M. E. Shahaboddin, M. Pouramir, A. Moghadamnia, M. Lakzaei, S. M. Mirhashemi, *Afr. J. Pharm. Pharmacol.* **2011**, *5*, 432–436.
[25] S. Garg, U. K. Patil, T. P. Shrivastava, *Res. J. Pharm. Technol.* **2013**, *6*, 649–651.
[26] M. Marvibaigi, E. Supriyanto, N. Amini, F. A. A. Majid, S. K. Jaganathan, *BioMed Res. Int.* **2014**, ID:785479.
[27] E. Ö. Uçar, A. Karagöz, N. Arda, *Fitoterapia* **2006**, *77*, 556–560.
[28] A. P. Simões-Wüst, N. Hunziker-Basler, T. J. Zuzak, J. Eggenschwiler, L. Rist, A. Viviani, *Phytomedicine* **2007**, *14*, 49.
[29] G. Seifert, P. Jesse, A. Laengler, T. Reindl, M. Lüth, S. Lobitz, G. Henze, A. Prokop, *Cancer Lett.* **2008**, *264*, 218–228.
[30] W. Tröger, D. Galun, M. Reif, A. Schumann, N. Stankovi'c, M. Mili'cevi'c, *Eur. J. Cancer* **2013**, *49*, 3788–3797.
[31] M. Shakeel, A. Trinidade, S. Geider, K. W. Ah-See, *J. Laryngol. Otol.* **2014**, 128.
[32] Y. Kwon, S. Chun, M. Kim, H. Nan, C. Lee, S. Kim, *Am. J. Chin. Med.* **2021**, *49*, 487–504.
[33] A. Szurpnicka, A. Kowalczuk, A. Szterk, *Arch. Pharmacal Res.* **2020**, *43(6)*, 593–629.
[34] E. Kleszken, A. V. Timar, A. R. Memete, F. Miere, S. I. Vicas, *Pharmacophore* **2022**, *13(1)*, 10–26.
[35] F. E. Felenda, C. Turek, F. C. Stintzing, *J. Ethnopharmacol.* **2019**, *236*, 100–107.
[36] M. O. Hazafa, A. Iqbal, U. Javaid, M. B. K. Tareen, D. Amna, A. Ramzan, S. Piracha, M. Naeem, *Clin. Transl. Oncol.* **2022**, *24*, 432–445.
[37] A. Hmadcha, A. Martin-Montalvo, B. R. Gauthier, B. Soria, V. Capilla-Gonzalez, *Front Bioeng. Biotechnol.* **2020**, *8*, 43.
[38] M. Mobahat, A. Narendran, K. Riabowol, *Int. J. Mol. Sci.* **2014**, *15*, 2494–516.
[39] P. K. Jaiswal, A. Goel, R. D. Mittal, *Indian J. Med. Res.* **2015**, *141*, 389–397.
[40] P. H. Davis, *Flora of Turkey and the East Aegean Islands*, Edinburgh University Press, Edinburgh, **1988**, pp. 590.
[41] A. A. Elzaawel, S. Tawata, *Asian J. Crop Sci.* **2012**, *4*, 32–40.
[42] C. C. Chang, M. H. Yang, M. H. Wen, J. C. Cern, *J. Food Drug Anal.* **2002**, *10*, 178–182.
[43] M. M. Giust, L. E. Rodriguez-Saonaand, R. E. Wrolstad, *J. Agric. Food Chem.* **1999**, *47*, 4631–4637.
[44] D. Wen, C. H. Li, H. Di, Y. Liao, H. A. Liu, *J. Agric. Food Chem.* **2005**, *53*, 6624–6629.
[45] D. Villano, M. S. Fernandez-Pachon, M. L. Moya, A. M. Troncoso, M. C. Garcıa-Parrilla, *Talanta* **2007**, *71*, 230–235.
[46] K. Thaipong, U. Boonprakob, K. Crosby, L. Cisneros Zevallos, D. H. Byrne, *J. Food Compos. Anal.* **2006**, *19*, 669–675.
[47] M. Oyaizu, *Japanese J. Nutr.* **1986**, *44*, 307.
[48] O. Trott, A. J. Olson, *J. Comput. Chem.* **2010**, *31*, 455–461.
[49] X. Wang, H. Zhang, X. Chen, *Cancer Drug Resist.* **2019**, *2*, 141–60.
[50] H. Bekker, H. Berendsen, E. Dijkstra, S. Achterop, R. V. Drunen, D. V. Spoel, A. Sijbers, H. Keegstra, B. Reitsma, M. Renardus, *Phys. Comput.* **1993**, *92*, 252–256.
[51] C. Oostenbrink, A. Villa, A. E. Mark, W. F. Van Gunsteren, *J. Comput. Chem.* **2004**, *25*, 1656–1676.
[52] E. Lindahl, P. Bjelkmar, P. Larsson, M. A. Cuendet, B. Hess, *J. Chem. Theory Comput.* **2010**, *6*, 459–466.
[53] K. Lindorff-Larsen, S. Piana, K. Palmo, P. Maragakis, J. L. Klepeis, R. O. Dror, D. E. Shaw, *Bioinform.* **2010**, *78*, 1950–1958.
[54] M. J. Abraham, T. Murtola, R. Schulz, S. Páll, J. C. Smith, B. Hess, E. Lindahl, *SoftwareX* **2015**, *1–2*, 19–25.

[55] L. Skrypnik, P. Feduraev, A. Golovin, P. Maslennikov, N. Belov, M. Matveev, A. Pungin, *Plants* **2022**, *11*, 2686.
[56] R. Molani, S. Kheirouri, *Nutr. Cancer* **2022**, *74*, 405–422.
[57] D. X. Hou, T. Kumamoto, *Antioxid. Redox Signaling* **2010**, *13*, 691–719.
[58] B. Ramachandran, C. Jeyarajpandian, J. M. Jeyaseelan, D. Prabhu, S. Rajamanikandan, P. Boomi, R. Venkateswari, J. Jeyakanthan, *Struct. Chem.* **2022**, *33*, 1867–1893.
[59] M. S. Hossan, S. Rahman, A. Bashar, R. Jahan, A. Al-Nahain, M. Rahmatullah, *World J. Pharm. Pharm.* **2014**, *3*, 57–70.
[60] T. Farkhondeh, H. S. Yazdi, S. Samarghandian, *Curr. Drug Discovery Technol.* **2019**, *16*, 57–65.
[61] J. Bernatoniene, D. Kopustinskiene, *Molecules* **2018**, *23*, 965–975.
[62] G. Özduran, E. Becer, H. S. Vatansever, S. Yücecan, *Neurol. Res.* **2022**, *44*, 511–523.
[63] J. Choi, C. Yang, W. Lim, G. Song, H. Choi, *Mol. Cell. Toxicol.* **2022**, *18*, 193–203.
[64] F. Hajiaghaalipour, M. S. Kanthimathi, J. Sanusi, J. Rajarajeswaran, *Food Chem.* **2015**, *169*, 401–410.
[65] H. E. Khoo, A. Azlan, S. T. Tang, S. M. *Food Nutr. Res.* **2017**, *13(61)*, 1361779.
[66] U. K. Ibrahim, I. I. Muhammad, R. M. Salleh, *J. Appl. Sci.* **2011**, *11*, 2406–2410.
[67] A. Büssing, M. Schietzel, *Anticancer Res.* **1999**, *19*, 23–28.
[68] G. Maier, H. H. Fiebig, *Anti-Cancer Drugs* **2002**, *13*, 373–379.
[69] E. Karagöz, N. A. Önay, A. Kuru, *Phytother. Res.* **2003**, *17*, 560–562.
[70] A. A. Oluwaseun, G. Obah, *Afr. J. Biotechnol.* **2007**, *7*, 3138–3142.
[71] N. E. Gardin, *Phytother. Res.* **2009**, *23*, 407–411.
[72] L. Sabová, M. Pilátová, K. Szilagyi, R. Sabo, J. Mojžiš, *Phytother. Res.* **2010**, *24*, 365–368.
[73] M. I. Choudhary, S. Mahe, A. Begum, A. Abbaskhan, S. Ali, A. Khan, S. Rehman, A. Rahman, *Chem. Pharm. Bull.* **2010**, *58*, 980–982.
[74] C. Steinborn, A. M. Klemd, A. S. Sanchez-Campillo, S. Rieger, M. Scheffen, B. Sauer, M. Garcia-Käufer, K. Urech, M. Follo, A. Ücker, G. S. Kienle, R. Huber, C. Gründemann, *PLoS One* **2017**, *12*, e0187786.
[75] O. M. Vergun, O. V. Grygorieva, J. Brindza, O. V. Shymanska, D. B. Rakhmetov, V. H. Sedlaalovb, O. A. Korablova, V. V. Fishchenko, E. Ivanicov, *Plant Introd.* **2019**, *3*, 87–96.
[76] S. I. Vicaş, D. Rugină, C. Socaciu, *J. Med. Plants Res.* **2011**, *5*, 2237–2244.
[77] V. Simona, D. Rugină, C. Socaciu, *Bulletin UASVM, Agriculture* **2008**, *65*, 1843–5386.
[78] W. Pietrzak, R. Nowak, U. Gawlik-Dziki, M. Lemieszek, W. Rzeski, *Molecules* **2017**, *22*, 624.
[79] S. P. Kazazic, V. Butkovic, D. Srzic, D. Klasinc, *J. Agric. Food Chem.* **2006**, *54*, 8391.
[80] I. Gülçin, H. A. Alici, M. Cesur, *Chem. Pharm. Bull.* **2005**, *5*, 281.
[81] M. E. Büyükokuroğlu, I. Gülçin, M. Oktay, Ö. I. Küfrevioğlu, *Pharmacol. Res.* **2001**, *44*, 491.
[82] M. Zhang, J. Yang, F. Li, *J. Exp. Clin. Cancer Res.* **2006**, *25(3)*, 391–402.
[83] X. Chen, N. Duan, C. Zhang, W. Zhang, *J. Cancer* **2016**, *7(3)*, 314–323.

Submitted: January 10, 2023
Accepted: March 2, 2023