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Research Article

Comparison of different drying methods for phytochemical quality of stevia (*Stevia rebaudiana* Bert.)

Yaşar ÖZYİĞİT[1], Esra UÇAR[2], Nuraniye ERUYGUR[3], Mehmet ATAŞ[4], Merve İNANIR[5], Halil BAL[4], Danial KAHRIZI[6], Kenan TURGUT[7]*

[1]*Akdeniz University, Vocational College of Korkuteli, Antalya 07800, Turkey; ozyigit@akdeniz.edu.tr*
[2]*Cumhuriyet University, Vocational College of Sivas, Sivas 58140, Turkey; eucar@cumhuriyet.edu.tr*
[3]*Selcuk University, Department of Pharmacognosy, Konya, 42250, Turkey; nuraniye.eruygur@selcuk.edu.tr*
[4]*Cumhuriyet University, Department of Pharmaceutical Microbiology, Sivas 58140, Turkey; atasmehmet@gmail.com; halilbal@cumhuriyet.edu.tr*
[5]*Cumhuriyet University, Department of Pharmacology, Sivas 58140, Turkey; ecz.merveinanir@gmail.com*
[6]*Tarbiat Modares University, Faculty of Agriculture, Agricultural Biotechnology Department, Tehran, Iran; dkahrizi@yahoo.com*
[7]*Akdeniz University, Department of Field Crops, Antalya 07058, Turkey; kturgut@akdeniz.edu.tr (*corresponding author)*

Abstract

This study examined the effects of different drying methods on the chemical content, antioxidant, antibacterial, enzymatic and anticancer activity values of stevia (*Stevia rebaudiana* Bert.) extract. Stevia leaves were dried using four different methods (in the sun, shade, air conditioner, and oven), and extracts were collected and analyzed. Based on extract content, 2-tetradecyl acrylate was the major ingredient in air-conditioned and oven-dried applications (25.00% and 21.47%, respectively). The same compound was detected in both sun-drying and shade-drying methods, but the content was low. The antioxidant activity values of the samples were evaluated with 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging tests. While the best result for DPPH (IC_{50} value 57.94 ± 0.63 µg/mL) was found in the shade-dried stevia samples, the best result in terms of ABTS test (IC_{50} value 44.03 ± 1.22 µg/mL) ml) was detected in oven-dried. When the samples were examined in terms of antimicrobial activity, it was seen that extracts of all drying methods were effective against *Staphylococcus aureus*. However, it was determined that the extracts obtained from plants dried in air conditioning and oven had a stronger effect. The cell viability assay was utilized to assess the antiproliferative effects of extract on L929 and MDA-MB-231 cell lines. The extracts did not significantly affect the L929 cell viability, while MDA-MB-231 remarkably reduced cell viability (sun drying, IC_{50} = 0.9 mg/mL; oven drying, IC_{50} = 0.65 mg/mL; shade drying, IC_{50} = 0.74 mg/mL; air conditioner drying, IC_{50} = 0.47 mg/mL). In particular, the extract obtained by the air conditioner drying method showed the most prominent cytotoxic effect. The results showed that drying using different methods had an impact on the quality standards of the stevia leaves.

Keywords: anticancer; antimicrobial; antioxidant; enzyme activity; *Stevia rebaudiana*

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Introduction

Stevia (*Stevia rebaudiana* Bertoni), also known as sweet herb, is a plant that includes essential oil and glycosides from the Asteraceae family, is a perennial herb. Originating from Paraguay and the northeast, the stevia was first used by the indigenous people of Guaranì (Andolfi *et al*., 2006; Shaafi *et al*., 2021). Stevia is cultivated for commercial purposes in the natural sweetener industries used in Taiwan, USA, Thailand, Malaysia, Indonesia, Australia, Tanzania, Canada and Russia (Ramesh *et al*., 2006; İnanç and Çınar, 2009). In warm regions, stevia leaves are harvested up to 4 times a year at 90-day intervals (Singh and Rao, 2005).

In nowadays, stevia is used as a natural sweetener in many countries (Yıldız and Karhan, 2021; Judickaitė *et al*., 2022; Yang *et al*., 2023). This feature is due to the substances found in its leaves. Because, stevia leaf extracts contain important substances such as flavonoids, alkaloids, free sugars, amino acids, essential oils and trace elements, as well as stevioside, rebaudioside A, B, C, D, E and dulcoside which are sweeter than sucrose. Stevioside has not been reported to have a harmful effect on humans to date (Komissarenko *et al*., 1994; Carneiro *et al*., 1997; Zayova *et al*., 2013). Due to its zero-calorie feature, it is convenient to use for diabetics and dieters (Fronza and Folegatti, 2003).

The plants show biological activity properties due to have flavonoids, alkaloids, glycosides. Recently, due to its antioxidant properties, medicinal and aromatic plants that contain phenolic components, are attracting attention (Kim *et al*., 2011). These compounds play a very important role in both human health and the preservation of nutrients, as they delay the oxidative stress that occurs in the cells (Yashin *et al*., 2017; Patra *et al*., 2016; Charles, 2013; Sama *et al*., 2014).

However, these compounds contained in plants are affected by some agronomic processes. Especially the wrong processes applied after the harvest negatively affect the product content and thus the quality. One of the most important of these processes is the drying process. In medicinal and aromatic plant, if drugs are not to be extracted directly, they must be dried so that they can be stored without spoiling. Thanks to the drying process, microbial growth and biochemical changes are prevented (Garcia, 2014). During drying, some evaporation is seen in some components together with the water in the drug. The amount of evaporation of the existing components with water may vary according to the drying method. This situation, may affect the quality and quantity of essential oil (Moyler, 1994; Khalid *et al*., 2008).

The most common methods used to dry medicinal and aromatic plants are sun and shade drying. In addition, drying in the oven, solar and microwave are used as modern methods (Abbas *et al*., 2021, Khallaf and El-Sebaii, 2022). But, the effects of these methods on quality factors may vary from plant to plant. Medicinal and aromatic plants, which are rich in secondary metabolites, require the determination of suitable drying methods—a matter of great importance. This study aimed to investigate whether different drying methods have an effect on the biological activities of the stevia leaves. In light of the data obtained, the most suitable drying method for the stevia plant was tried to be determined. There are not many studies on the drying of this plant. Therefore, this study is important.

Materials and Methods

Plant material and preparation of the extracts

The plant samples were obtained from the trial field of Akdeniz University and 150 g stevia leaves were dried in sun, shade, air conditioner and oven by applying four different drying methods. The average temperature, average humidity and daily sunshine duration of the application areas are given in Table 1. The samples were weighed continuously and the application was terminated when the weight reductions were fixed. Dry matter ratios were determined as 27±0.5% in all samples after drying. Then, this sample was extracted and

their chemical composition structures of them were evaluated in the laboratories of CUTAM (Cumhuriyet University Advanced Technology Research Center), Cumhuriyet University, Sivas in 2020.

Table 1. The average temperature, average humidity and daily sunshine duration of the application areas

	Average temperature °C	Average humidity (%)	Sunshine duration (hour/day)
Sun drying	34.20	52.12	9.71
Shade drying	26.05	66.21	0
Air conditioner drying	22.00	49.30	0
Oven drying	45.00	25.00	0

The plant samples were weighed 10 g and were powdered by laboratory type miller. These samples were extracted with 50 mL of ethanol 80% by maceration process. Then, the crude extract was filtered and ethanol 80% is evaporated under reduced pressure.

Gas Chromatography-Mass Spectrometry (GC/MS) characterization of the extracts

The Gas Chromatography/Mass Spectrometer was exploited to identify the components of the extracts and determine the relative percentages (Sacchetti *et al*., 2005). GC-MS analyses were worked with a mass spectrometer detector. The technique, in which helium gas at 1.5 ml constant flow rate was used as the carrier gas, was programmed in split mode with an injection volume of 1 µl and a rate of 5 per minute among 80-300. Post-run was set at 300 °C for 2 min. The total run time was 60 minutes (Eruygur and Dural, 2019). The chemical composition of the obtained extract was searched with three different libraries (W9N11.L, NIST05a.L, and wiley7n.I).

Antioxidant activities

The DPPH radical scavenging activity of the extract was evaluated in accordance with the Blois method with minor modification (Blois, 1958). For determination of DPPH Radical Scavenging Activity, 150 µL of the extract with different concentration was mixed with 50 µL of 1.0×10-3 M freshly prepared DPPH methanol solution in 96 well plate. Methanol was used as control of the experiment. After 30 min of incubation at 25 °C, the reduction of the DPPH free radical was measured reading the absorbance at 517 nm with microplate reader (Epoch, USA). BHT used as positive controls. The ABTS radical scavenging activity was evaluated by the method of Re *et al.* (1999) with minor modifications. Briefly, the stock solution of ABTS was made by reacting 7 mM ABTS solution with 2.4 mM of potassium persulfate solution in equal volume for 16 h. working solution was then prepared by diluting the stock ABTS solution with methanol to give an absorbance of 0.7 ± 0.02 units at 734 nm using a microplate reader (Epoch, USA). In each experiment, the ABTS solution was prepared freshly. 50 µL of extract was mixed with 150 µL ABTS working solution and stand for 10 min at dark place. All the analyses were conducted in triplicate and the results expressed as mean ± standard deviation. Total phenolic content was determined with the spectrophotometric method (Clarke *et al*., 2013), and expressed as gallic acid equivalents. Briefly, 20 µL of extract in DMSO was mixed with 100 µL freshly 1/10 diluted F-C reagent with distilled water. After five min, the solution was mixed with 80 µL of 7.5% Na_2CO_3 solution, and incubated for 30 min at 25 °C. The measurement of absorbance was done at 650 nm in a microplate reader (Epoch, USA). All the analyses were performed in triplicate and the results expressed as mean ± standard deviation. Total flavonoid content was determined with the aluminum chloride colorimetric method of Molan and Mahdy (2014). 25 µL of 1 mg/mL test sample solution, 100 µL of dd. H_2O and 7 µL of 5% $NaNO_2$ were mixed together in 96-well plates. After 15 min of incubation at room temperature, 7 µL of 10% $AlCl_3$ was added. After 5 min, 50 µL of 1 M NaOH and 60 µL of distilled water were added to each well. Then the absorbance was measured at 490 nm in a microplate reader (Epoch, USA). The total ingredient of

flavonoids was defined as a milligram catechin equivalent corresponding to one gram of the dry weight of the extract.

In vitro enzyme inhibition assay

The acetylcholinesterase/butyrylcholinesterase inhibition analysis was carried out in accordance with the Ellman method as described by our previous study (Ergul *et al*., 2019). Briefly, the mixture consisting of 20 µL of test sample/reference standard of various concentrations, 140 µL of 0.1 mM phosphate buffer (pH 6.8), 10 µL of 3 mM 5,5'-dithio-bisnitrobenzoic acid (DTNB), and 20 µL of enzyme (0.22 U/mL for acetylcholinesterase/0.1 U/mL for butyrylcholinesterase) prepared in phosphate buffer was incubated for 5 min at 25 °C. Following preincubation, 10 µL of the substrate (0.71 mM acetylthiocholine iodide/0.2 mM butyrylthiocholine chloride in phosphate buffer) was added to start the reaction, followed by further incubation for 10 min. The developed yellow colour was measured at 412 nm (Epoch, USA). Galantamine was used as the positive control. The α-glucosidase inhibitor activity analysis was performed with the technique presented by Kumar *et al*. (2012). 25 microliters of sample solution diluted with buffer was mixed with 25 µL of α-glucosidase (0.5 U/mL) and incubated for approximately 10 min at 25 °C. Then 25 µL of 0.5 mM 4-nitrophenyl-β-D-glucuronide (pNPG) was added to each well as substrate and incubated for a 30 min at 37 °C. After the incubation period, 100 µL of 0.2 M sodium carbonate was added to terminate the reaction and the absorbance was read at 405 nm. The α-amylase enzyme inhibitory activity was determined by previous reported method (Kumar *et al*., 2013). The reaction mixture containing 50 µL of sample solution diluted with buffer and 25 µL of α-amylase from porcine pancreases (0.5 mg/mL) was incubated for approximately 10 min at 25 °C. Then 50 µL of freshly prepared 0.5% starch solution (w/v) was added to each well as substrate and incubated for 10 min at 25 °C. After the incubation period, 100 µL of 1% 3,5-dinitrosalicylic acid (DNS) was added as the colour reagent, followed by heating in a water bath for 10 min. The absorbance was read at 540 nm. Acarbose was defined as a positive control for both methods.

Antimicrobial activities (micro-well dilution assay)

In order to determine the Minimum inhibition concentration (MIC) of the extracts, the broth microdilution method was conducted according to the method described previously (Eloff 1998). For this purpose, *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 29213), *Pseudomonas aeruginosa* (ATCC 27853), *Bacillus cereus* (ATCC 11778), *Candida albicans* (ATCC 10231) and *Candida tropicalis* (DSM 11953) strains were used. The extracts were dissolved in 50% DMSO (50 mg/mL). 10 µL extract was added to the first line of the microtiter plate, which was diluted with 90 µL broth. Serial two-fold dilutions were made in 96-well plate. The concentration of the extracts in the wells ranged from 2.5 to 0.004 mg/mL. Mueller Hinton Broth (Accumix® AM1072) was used for bacteria and Saboraud Dekstroz Broth (Himedia ME033) for *Candida*. Bacteria and yeast suspension (50 µL) were added to the prepared samples. The final inoculum size was 5×10^5 CFU/mL at bacteria and $0.5\text{-}2.5\times10^3$ CFU/mL at *Candida* every well (CLSI, 2012). The 11th well was used for sterility control and the last well-containing broth and microorganism without extracts was used as growth control. The covered microplates were incubated for 24 h at 37 °C. The lowest concentration of extract that was capable of inhibiting the visible growth of the microorganism was accepted as the MIC value.

Cell viability assay

MDA-MB-231 cell line (human breast carcinoma, ATCC, HTB-26) and L929 cell line (mouse fibroblast cells, ATCC) were cultured in DMEM including 10% (v/v) heat-inactivated fetal bovine serum (FBS) and 1% penicillin/streptomycin, in a 25 cm^2 cell culture flask and incubated at 37 °C in a 5% CO_2 humidified atmosphere until they reached approximately an 80-90% confluence.

The cell viability of the ethanol extracts of different drying conditions of stevia was assessed using the XTT cell viability assay (Roche Diagnostic, Germany) against the MDA-MB-231 and L929 cell lines. The extract was dissolved in Dimethyl sulfoxide (DMSO) and diluted with DMEM prior to exposure. Cells were seeded in 96-well plates at the density of 1.5×104 cells per well and were allowed to attach for nearly 6-8 h. After attachment, the cells were exposed to several concentrations (0.0625, 0.125, 0.25, 0.5, 1 mg/mL) of ethanolic extract of stevia for 24 h. Besides, non-treated cells and cells exposed to DMSO (0.5%) were used as negative control and solvent control respectively. At the end of the exposure time to the extract, a 50 µL XTT labelling mixture was added to each well for the determination of living cells and then the plates were incubated at 37 °C for another 4 h. After mixing, the absorbance of each well was measured using a microplate reader (Thermo, Germany) at 450 nm against the control. All experiments were conducted in three independent experiments, and the cell viability was expressed in % related to control (100% of viability).

Results and Discussion

Chemical composition

The chemical contents of extracts obtained from plants dried according to the different drying methods, were determined by GC-MS (Table 2). The chemical contents of the ethanol 80% extracts of plants showed significant differences in both the number and variety of components. The plants show biological activity properties due to having these chemical compounds. Components and amounts of plants vary according to the genus and species of the plant, as well as different growing environments of the same plant are also effective on these components. These differences cause changes in biological activity.

Table 2. The effects of different drying methods on the chemical composition of *Stevia rebaudiana*

Chemical components	RT	Sun dried	Shade dried	Air conditioner dried	Oven dried
(S-Nitroso) thiomethane	3.659		1.95		
Cineole	12.946	2.30			
Camphor	16.722	2.80	-	-	-
endo-Borneol	17.357	1.87	1.09	-	1.30
1,4:3,6-Dianhydro-.alpha.-d-glucopyranose	18.679	-	1.66	1.43	-
Phenol	26.015	1.26	-	-	-
5-Hexyl-2-furaldehyde	28.046	-	1.71	-	-
1-Dodecanol	28.263	-	6.50	-	-
Cyclododecane	28.264	11.45	-	-	-
Tetradecanol	28.269	-	-	6.83	6.76
4-(2,6,6-Trimethylcyclohexa-1,3-dienyl)but-3-en-2-one	28.601	1.36	-	-	-
2,4-Di-tert-butylphenol	29.374	4.82	4.60	2.45	4.55
(-)-Caryophyllene oxide	31.227	-	-	1.54	1.32
2-Propenoic acid, n-tetradecyl ester	33.459	5.98	9.06	25.00	21.47
(-)-Loliolide	35.118	1.53	1.72	1.03	-
Ethyl palmitate	38.849	-	-	1.07	2.17
Manoyl oxide	39.084	-	-	3.56	1.79
Cyclododecane	39.198	-	-	-	1.29
Stenol	40.262	-	-	1.17	-
Phytol	40.737	-	-	1.98	1.00
trans-.beta.-Ionone	43.684	-	-	-	7.46
Isosteviol methyl este	45.956	-	-	-	1.75

8.alpha.,13-Dihydroxy-4,15,16-trin or-labdane	46.007	9.37	-	-	9.00
Methyl steviol	46.036	-	-	10.14	-
Campherenone	46.328	9.16	-	-	-
Ledene	47.461	1.86	-	-	-
1-Chloro-1-ethyl-3-methyl-1-germacyclopent-3-ene	47.890	-	7.24	-	-
Total		53.76	35.53	56.2	59.86

According to the data obtained, 2-Propenoic acid, n-tetradecyl ester as a major component in air conditioning and oven drying applications (respectively, 25.00% and 21.47%) determined. Although this compound was available in other applications, its percentage was low. Drying under the direct sun can lead to losses in the components of the plants (Nurhaslina *et al*., 2022). Ropelewska *et al*. (2023) reported that when plant leaves are exposed to direct solar radiation, their colours become lighter and their aromas decrease. At the same time, the drying time can make a difference in the amount and variety of the components of the plants. Since drying in shade takes longer than other applications, it may have caused losses in the amount of this compound.

Antioxidant activity

The DPPH and ABTS radical scavenging activity results are shown in Table 3, and the stevia samples dried in the shade had the best (IC_{50} value 57.94 ± 0.63 µg/mL) DPPH radical scavenging effect, followed by the air-dried stevia sample (IC_{50} value 62.08 ± 0.57 µg/mL), it was more active than the reference substance BHT (IC_{50} value 79.48 ± 0.60 µg/mL). As for the ABTS assay, the oven-dried stevia samples (IC_{50} value 44.03 ± 1.22 µg/mL) showed the best radical scavenging activity, it was followed by sun-dried (IC_{50} value 48.52 ± 1.24 µg/mL) and air dried (IC_{50} value 49.28 ± 1.20 µg/mL) stevia samples. The shade-dried stevia samples demonstrated the least ABTS radical scavenging activity (IC_{50} value 86.16 ± 1.21 µg/mL). This may be due to changes in the amount or degradation of the phytochemical compounds responsible for the radical scavenging activity in the extract, depending on the drying method. Researchers reported that the stevia contains high levels of phenolic and flavonoid substances and is a natural source of antioxidants. In addition, they evaluated that the antioxidant activity values can change according to the application of nitrogen fertilizer and the growing period of the plant (Ataş *et al*., 2018; Eruygur *et al*., 2019).

Total phenol (TPC) and flavonoid content (TFC)

When stevia samples obtained with different drying methods were compared in terms of phenolic and flavonoid compositions (Table 3), it was determined that the sample dried in the shade had the highest phenolic and flavonoid content (455.96 ± 4.59 mg GAE/g and 87.40 ± 7.26 mg QE/g), it was followed by air dried stevia samples (398.69 ± 4.58 mg GAE/g and 85.56 ± 4.77 mg QE/g). The stevia samples dried in the sun have the least phenolic and flavonoid content (129.82 ± 4.69 mg GAE/g and 64.83 ± 7.56 mg QE/g). From here it can say that the drying method has a great effect on the change in the amount of phenolic compounds in the stevia plant (Lemus *et al*., 2016).

Table 3. Total phenol and flavonoid contents, the antioxidant activity of stevia extract

Stevia sample	Total phenol content (mg GAE/g)	Total flavonoid content (mg QE/g)	Radical scavenging activities	
			DPPH (IC_{50} µg/mL)	ABTS (IC_{50} µg/mL)
Sun-dried	129.82 ± 4.69	64.83 ± 7.56	70.32 ± 0.23**	48.52 ± 1.24
Dried in shade	455.96 ± 4.59	87.40 ± 7.26	57.94 ± 0.63***	86.16 ± 1.21**
Air conditioner dried	398.69 ± 4.58	85.56 ± 4.77	62.08 ± 0.57***	49.28 ± 1.20
Oven-dried	373.27 ± 9.17	78.61 ± 3.06	79.73 ± 0.61**	44.03 ± 1.22
Reference standard			79.48 ± 0.60[b]	26.78 ± 3.435[c]

[a]IC_{50} values represent the means ± standard error meaning of three parallel measurements, [b]BHT, [c]BHA

Enzyme inhibition activity

Tyrosinase is a copper-contained common enzyme in skin and hair found in plants, microorganisms and animals. It plays a role in the synthesis of melanin, and it is responsible for melanisation in animals. Excessive formation of melanin pigment causes serious problems in the aesthetic appearance of the skin. Tyrosinase inhibitors are widely used in the cosmetic industry due to the reduction of melanin synthesis and skin whitening, and in the food industry because of preventing unwanted browning in foods (Zolghadri *et al*., 2019). In our study, when the effect of different drying techniques on tyrosinase enzymes in stevia samples was examined, stevia samples dried in the shade (76.33 ± 4.40%) and dried in the oven (67.67 ± 3.15%) showed the highest tyrosinase inhibition activity, as in antioxidant activity (Table 4). In addition, all stevia samples showed higher tyrosinase inhibition activity than the reference substance kojic acid (26.19 ± 0.62%). In a former study, the aqueous extract of stevia flowers was showed inhibitory effects on tyrosinase (IC_{50}=134.74 μg/mL) and α-glucosidase (IC_{50}=114.81 μg/mL) activities (So *et al*., 2019).

Table 4. Enzyme inhibitory activities of different stevia extracts (at 2 mg/ml concentration)

Stevia sample	Tyrosinase	α-amylase	α-glucosidase
	Percentage (%) inhibition		
Sun-dried	55.89 ± 7.70	52.36 ± 5.43	N.A.
Dried in shade	76.33 ± 4.40	26.72 ± 1.17	N.A
Air conditioner dried	54.48 ± 6.37	10.30 ± 2.54	27.13 ± 2.13
Oven-dried	67.67 ± 3.15	24.14 ± 5.50	N.A
Reference standard	26.19 ± 0.62[b]	58.40 ± 0.63[c]	57.56 ± 0.52[c]

Note: [a]Each value of percent inhibition is presented as means ± SEM (n = 3). Abbreviations: SEM: standard error of mean; [b]Kojic acid; [c]Acarbose

Diabetes Mellitus (DM), is a type of disease that develops due to high blood sugar resulting from the inability of the pancreas to produce enough insulin for the body or the inability to use the insulin it produces effectively by the body. Glucose, which causes high blood sugar, occurs due to the breakdown of polysaccharides in meals by amylase and glucosidase enzymes, and with the inhibition of these two enzymes, glucose production can be reduced, and after-meal blood sugar rise can be prevented. For this reason, amylase and glucosidase inhibitors have become an important drug group in the treatment of diabetes.

In our study, when these two enzyme inhibition activities were examined on stevia samples obtained after different drying techniques, it was found that the sun-dried sample (52.36 ± 5.43%) had the highest amylase enzyme inhibitory effect, while the air-dried stevia sample (27.13 ± 2.13%) showed weak glucosidase inhibition, while the activity could not be detected in the other samples (Table 4). The reference drug acarbose showed 58.40 ± 0.63% and 57.56 ± 0.52% inhibition against amylase and glucosidase, respectively.

Antimicrobial activity

The antimicrobial activity of Stevia extracts (they were dried sun drying, shade drying, air conditioner drying and oven drying by applying four different drying methods) against *E. coli*, *S. aureus*, *P. aeruginosa*, *B. cereus*, *C. albicans* and *C. tropicalis* were detected using the broth microdilution technique at the concentration range 0.312 to >2.5 mg/mL (Table 5).

Table 5. Antimicrobial activity results of Stevia extracts (sun dried, shade dried, air conditioner dried and oven dried by applying four different drying methods)

Extract name	*E. coli* ATCC 25922	*S. aureus* ATCC 29213	*P. aeruginosa* ATCC 27853	*B. cereus* ATCC 11778	*C. albicans* ATCC 10231	*C. tropicalis* DSM 11953
Sun-dried	2.5	0.625	>2.5	2.5	>2.5	>2.5
Dried in shade	2.5	0.625	2.5	2.5	>2.5	>2.5
Air conditioner-dried	2.5	0.312	>2.5	>2.5	>2.5	>2.5
Oven-dried	2.5	0.312	>2.5	>2.5	>2.5	>2.5

Cell viability assay

As a result of the XTT cell proliferation experiment, the ethanol extracts at all concentrations remarkably inhibited MDA-MB-231 cell viability ($P<0.05$) in a dose-dependent manner (Sun Drying, IC_{50} = 0.9 mg/mL; Oven Drying, IC_{50} = 0.65 mg/mL; Shade Drying, IC_{50} = 0.74 mg/mL; Air Conditioner Drying, IC_{50} = 0.47 mg/mL), as seen in Figure 1. Additionally, the extracts displayed no significant cytotoxicity on the L929 cell line at the concentrations range (1-0.0625 mg/mL).

As a result of the cell viability experiment, we determined that the ethanol extracts of different drying conditions of stevia exhibited an effective cytotoxic activity at all concentrations. The extract obtained by the Air Conditioner drying method showed two times more cytotoxic effect compared to the extract obtained by the Sun drying method. It is thought that the antioxidant activity caused by the quantity of the polyphenolic components of the extract also causes the anti-cancer effect (Wölwer-Rieck, 2012). It remains the first study to indicate the cytotoxic effect of stevia leaf extracts on MDA-MB-231.

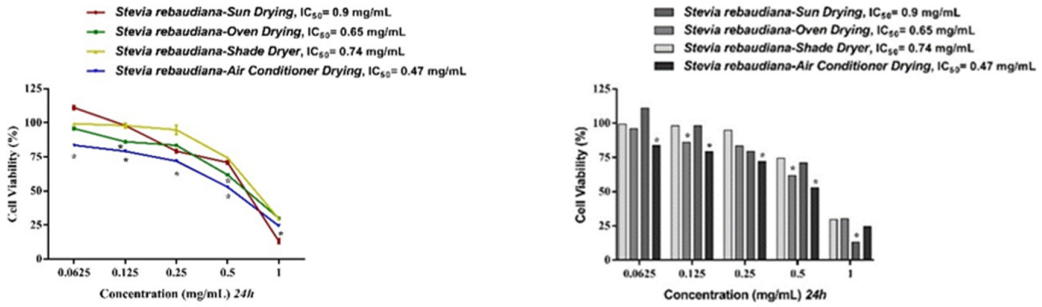

Figure 1. Effects of the ethanol extracts of different drying conditions of stevia on the viability of MDA-MB-231 cell line, after treatment with various concentrations (range: 0.065-1 mg/mL) for 24 h. The extract shows no toxicity in normal cells

The antimicrobial activity of plant extracts was accepted significant if the MIC value is 0.1 mg/ml or lower, moderate if 0.1 < MIC ≤ 0.625 mg/ml and weak if MIC > 0.625 mg/ml (Kuete, 2010; Awouafack *et al*., 2013;). According to these criteria, moderate antimicrobial activity was detected only on *S. aureus* (0.312 and 0.625 mg/mL). It was determined that different stevia extracts had weak antimicrobial activity on other bacteria and yeasts. According to Eruygur *et al*. (2019) reported that the stevia has antimicrobial activity thanks to its high content of phenolic and flavonoid substances.

Conclusions

The results of the study indicated that the ethanol extracts of different drying conditions of stevia have significant anticancer, antioxidant, and enzyme activity values. The 2-Propenoic acid, n-tetradecyl ester as a

major component in air conditioning and oven drying applications (respectively, 25.00% and 21.47%) determined. It has been determined that different drying methods cause differences in compound values. The best result for DPPH (IC_{50} value 57.94 ± 0.63 µg/mL) was found in the shade-dried stevia samples, while the best result in terms of ABTS test (IC_{50} value 44.03 ± 1.22 µg/mL) ml) was detected in the oven-dried stevia samples. When the samples were examined in terms of antimicrobial activity, it was seen that all drying methods were effective on *S. aureus*. In particular, the extract obtained by the air conditioner drying method showed the most prominent cytotoxic effect. Consequently, the ethanol extracts of different drying conditions of stevia may be considered a potential therapeutic agent for cancer. At the same time, it was determined that different drying methods made a difference in the chemical composition, antioxidant and antimicrobial activity values of stevia extracts, too.

Authors' Contributions

Data curation: Y.Ö., E.U., N.E., M.A., H.B., M.İ., D.K., K.T.; Formal analysis: Y.Ö., D.K.; Funding acquisition: K.T., E.U.; Investigation: Y.Ö., E.U., N.E., M.A., H.B., M.İ.; Methodology: Y.Ö., E.U., N.E., M.A., M.İ.; Project administration: K.T., Y.Ö., E.U.; Resources: Y.Ö., K.T.; Software: Y.Ö., D.K.; Supervision: K.T.; Validation: D.K.; Visualization: H.B., D.K.; Writing - original draft: Y.Ö., E.U., N.E., M.A., M.İ.; Writing - review and editing: Y.Ö., D.K., K.T.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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