

# Antioxidant Activity and Phenolic Composition of Ethanol Extracts of *Momordica charantia* and *Datura stramonium*

## Mehlika ALPER<sup>1</sup><sup>67</sup>, Cennet ÖZAY<sup>2</sup>

<sup>1</sup>Muğla Sıtkı Koçman University, Faculty of Science, Department of Molecular Biology and Genetics, Muğla 48000, Turkey, <sup>2</sup>Izmir Katip Celebi University, Faculty of Pharmacy, Department of Basic Pharmaceutical Sciences, Izmir 35620, Turkey <sup>1</sup>https://orcid.org/0000-0001-6193-346X, <sup>2</sup>https://orcid.org/0000-0002-1120-6122 Sciences, Izmir 35620, Turkey

## ABSTRACT

Medicinal plants in the world are natural antioxidant sources as they contain some secondary metabolites such as phenolic acids and flavonoids. Therefore, they are used to prevent or treat many diseases in many parts of the world. However, the use of antioxidants in cancer treatment is still controversial. Accordingly, the phenolic composition and antioxidant potential of the ethanol extracts of Momordica charantia L. (Cucurbitaceae) and Datura stramonium L. (Solanaceae), which are used traditionally in Turkey and have cytotoxic potential on human cancer cells, were investigated. The antioxidant assays (DPPH, metal chelating, phosphomolybdenum, and ferric reducing power) were applied to the ethanol extracts of the fruits of *M. charantia* and the leaves of *D.* stramonium. The total phenolic and flavonoid contents were determined. The phenolic compounds contained in ethanolic extracts were identified using HPLC method. Fifteen phenolic compounds were identified in the extracts. Caffeic acid was the major constituent in both extracts. The amount of caffeic acid was detected to be 6282.51 and 15183.36  $\mu$ g g<sup>-1</sup> extract in *M. charantia* extract and the D. stramonium extracts, respectively. D. stramonium leaf extract showed higher antioxidant activity than M. charantia fruit extract and this activity may be associated with high phenolic compound concentrations. Hence, further studies about screening of bioactive compounds from various part of these plants will be a great importance for obtaining of source of novel natural antioxidants.

#### **Research Article**

Article History	
Received	: 31.12.2020
Accepted	: 09.04.2021

#### Keywords

*Momordica charantia Datura stramonium* Antioxidant activity Total phenolic and flavonoid content HPLC

# *Momordica charantia* ve *Datura stramonium* Etanol Ekstraktlarının Antioksidan Aktivitesi ve Fenolik Kompozisyonu

#### ÖZET

Dünyadaki tıbbi bitkiler, fenolik asitler ve flavonoidler gibi bazı içerdikleri sekonder metabolitler için doğal antioksidan kaynaklarıdır. Bu nedenle dünyanın pek çok yerinde birçok hastalığı önlemek veya tedavi etmek için kullanılmaktadırlar. Bununla birlikte, antioksidanların kanser tedavisinde kullanımı hala tartışmalıdır. Bu doğrultuda, Türkiye'de geleneksel olarak kullanılan ve insan kanser hücreleri üzerinde sitotoksik potansiyele sahip Momordica charantia L. (Cucurbitaceae) ve Datura stramonium L. (Solanaceae) 'un etanol ekstraktlarının fenolik bileşimi ve antioksidan potansiyeli araştırılmıştır. Antioksidan deneyler (DPPH, metal selatlama, fosfomolibdenyum ve demir gücü), M. indirgeme charantia'nın meyvelerinin ve D. stramonium'un yapraklarının etanolik ekstraktlarına uygulandı. Toplam fenolik ve flavonoid içerikler belirlendi. Etanolik ekstraktlarda bulunan fenolik bileşikler HPLC yöntemi kullanılarak tespit edildi. Ekstraktlarda on beş fenolik bileşik tanımlandı. Kafeik asit, her iki ekstraktta da ana bileşendi. M. charantia ekstraktı ve D. stramonium ekstraktında kafeik asit miktarları sırasıyla 6282.51 ve

#### Araştırma Makalesi

Makale TarihçesiGeliş Tarihi: 31.12.2020Kabul Tarihi: 09.04.2021

#### Anahtar Kelimeler

*Momordica charantia Datura stramonium* Antioksidan aktivite Toplam fenolik ve flavonoid içerik HPLC 15183.36 μg g<sup>-1</sup> olarak tespit edildi. *D. stramonium* yaprak ekstraktı, *M. charantia* meyve ekstraktından daha yüksek antioksidan aktivite gösterdi ve bu aktivite yüksek fenolik bileşik konsantrasyonları ile bağlantılı olabilir. Bu nedenle, bu bitkilerin çeşitli kısımlarından biyoaktif bileşiklerin taranması ile ilgili daha ileri çalışmalar, yeni doğal antioksidan kaynaklarının elde edilmesi için büyük önem taşıyacaktır.

**To Cite:** Alper M, Özay C 2022. Antioxidant Activity and Phenolic Composition of Ethanol Extracts of *Momordica charantia* and *Datura stramonium*. KSU J. Agric Nat 25 (1): 1-9. DOI: 10.18016/ksutarimdoga.vi.851251.

## INTRODUCTION

Free radicals are generated by mitochondria, peroxisomes, inflammatory processes, ischemia and physical exercise during normal metabolism (Lobo et al., 2010). It is known that the balance between the production and neutralization of Reactive Oxygen Species (ROS) with antioxidant systems is essential. When an imbalance occurs, cells can be exposed to oxidative stress due to increased ROS production (Krishnaiah et al., 2011; López-Alarcón and Denicola, 2013). This imbalance might harm molecules (e.g. nucleic acids, lipids, proteins,), causing metabolic disorders and diseases like diabetes, cancer, atherosclerosis, neurological disorder, rheumatoid arthritis, hypertension stroke due to oxidative stress (Carocho and Ferreira, 2013; Alam et al., 2013).

Plants produce secondary metabolites with different biological and pharmacological properties. Due to this, antioxidant molecules, which may be obtained from fruits and vegetables, are important to keep oxidative balance (Armendáriz-Barragán et al., 2016). In this way, the identification of compounds is an important study field together with their isolation (Rajendran et al., 2014).

Momordica charantia L. (Cucurbitaceae), which is a common plant in tropical and sub-tropical regions, including Brazil, India, China, Africa, Australia and Turkey (Krishnaiah et al., 2011; Güneş et al., 2019), are known as bitter melon or bitter gourd. M. charantia has been widely used as food and medicine great and contains a diversity of secondary metabolites such  $\mathbf{as}$ polyphenols, flavonoids. triterpenoids, saponins and alkaloids. Its diverse biological activities include anti-diabetic, anti-cancer, anti-inflammatory, antimicrobial, anti-helminthic, antioxidant and anti-ulcer activities (Villarreal-La Torre et al., 2020). Studies on M. charantia have revealed that its components with pharmaceutical importance are phenolic compounds and triterpenes (Cuong et al., 2018).

Datura L. genus is a member of Solanaceae, possesses poisonous properties, and is represented in Turkey by three species, *Datura stramonium* L., *D. metel* L. and *D.innoxia* Mill. *Datura stramonium* L. is a widespread medicinal herbaceous plant throughout the World, which is commonly known as thorn-apple or jimson weed (Öz Arık, 2017). Researches have determined that *D. stramonium* contains alkaloids, phenols, glycosides, tannins and saponins. Alkaloids, atropine, and scopolamine are the primary biologically active substances in D. stramonium. Some medicinal features of *D. stramonium* containing its different activities like antimicrobial, antiantioxidant, anticancer, antiviral, inflammatory, antiepileptic, antiasthmatic, insecticidal, and analgesic were investigated (Singh and Singh, 2013; Al-Snafi, 2017; Alper, 2019).

Although studies have been conducted on the alkaloids of D. stramonium (Berkov et al., 2006; El Bazaoui et al., 2011; Ally and Mohanlall, 2020), studies regarding its phenolic compounds are relatively few. It is known that the biosynthesis and accumulation of secondary metabolites depend upon different environmental factors as well as genetic, morphogenetic features. ontogenic, Content of different secondary metabolites does not stay steady since several biotic (fungi, bacteria, nematodes, etc.) and abiotic (location, temperature, soil, etc.) factors play a significant role in their synthesis (Verma and Shukla, 2015). Also, the uses of antioxidants in cancer therapy have still been debated (Fuchs-Tarlovsky, 2013; Dastmalchi et al., 2020). Among the ethanolic extracts of the aerial parts of *M. charantia* (Günes et al., 2019) and D. stramonium (Alper, 2019) collected from Edirne, it was determined that the fruit extract of *M. charantia* and, the leaf extract of *D. stramonium* have more cytotoxic potential on human cancer cells tested, unlike seed extracts. All these factors considered, we aimed to determine phenolic contents of ethanol extracts of M. charantia fruits and D. stramonium leaves from Edirne (Turkey) and also to assign their potential antioxidant properties. In addition, for two extracts obtained from similar geography originating from our country, with this study, the potential of *D. stramonium* extract, whose bioactive properties are less known than *M. charantia* extract, may able to evaluated comparatively.

## MATERIALS and METHODS

## Chemicals

All chemicals and reagents used in the antioxidant assays were bought from Sigma-Aldrich (Germany) and Merck (Germany).

M. charantia and D. stramonium were gathered from the Center of Edirne-Turkey in 2015. M. charantia and D. stramonium were collected from Hıdırağa village and, İstasyon neighbourhood and its surroundings, respectively. The botanical identity of the both species was authenticated by Prof. Dr. Fatma Güneş from Trakya University (Edirne, Turkey). Voucher specimens were preserved at her herbarium. The fruits of *M. charantia* and the leaves of D. stramonium were air-dried in shadow, brought separately into fine powder. These plant parts were individually extracted with ethanol. The filtered extracts were concentrated with a rotary evaporator (IKA RV10D, Staufen, Germany). After the lyophilization process, the crude extracts were placed in light-protected bottles and stored at -20°C until use.

## Antioxidant Activity Assays

DPPH Radical Scavenging Activity: For studied of the DPHH (2,2-diphenyl-1-picrylhydrazyl) activity of the extracts, each extract solution (1 mL) was mixed with methanolic DPPH radical solution (0.004%, 4 mL). Then, the mixture was left to incubate at room temperature for 30 minutes protected from light. The same processes were done in the standard Trolox. The absorbances at 517 nm were taken. The radical scavenging activity of the extracts was given as equivalent of Trolox (mg TEs g<sup>-1</sup> extract) (Ceylan et al., 2016).

Ferric Reducing Antioxidant Power (FRAP) Assay: The method was done by the method of Zengin and Aktumsek (2014) after slight modifications. Sample solutions were mixed with freshly prepared working FRAP reagent (acetate buffer–0.3 M, TPTZ (2,4,6tripyridyl-s-triazine–10 mM, FeCI<sub>3</sub>–20 mM). After 30 minutes of incubation at room temperature was completed, the absorbance at 593 nm was recorded. The same procedures were achieved for Trolox, which is used as a standard. FRAP activity of the extracts was reported as equivalents of Trolox (mg TEs g<sup>-1</sup> extract)

Phosphomolybdenum Assay: Total antioxidant capacities of the extracts were evaluated by phosphomolybdenum method explained by Berk et al. (2011). In short, each sample solution was reacted with reagent solution (0.6 M H<sub>2</sub>SO<sub>4</sub>, 28 mM Na<sub>3</sub>PO<sub>4</sub> and 4 mM (NH<sub>4</sub>)<sub>2</sub>MoO<sub>4</sub>), and held at 95°C for 90 minutes. The absorbance at 695 nm was recorded after each mixture was brought to room temperature. The antioxidant property of each extract was given as Trolox equivalents (mg TEs g<sup>-1</sup> extract).

Metal Chelating Activity : For this assay, sample

solutions were mixed with  $\text{FeCl}_2$  solution (0.05 mL, 2 mM). The absorbance of the reaction starting after adding 5 mM ferrozine was read at 562 nm following incubation at room temperature for 10 minutes. The results for the extracts were stated as EDTA (mg EDTAEs g<sup>-1</sup> extract) equivalents (Zengin et al., 2016).

## Total Phenolic and Flavonoid Contents

Colourimetric assays were employed to achieve total phenolic and flavonoid contents in the extracts (Ozay and Mammadov, 2016). Folin–Ciocalteu reagent (1 mL) was mixed with the extract (1 mg/mL) and sodium carbonate (2%). After 2 h incubation, the formation of the coloured mixture is determined spectrophotometrically at 760 nm and the total phenolic content was given as gallic acid equivalents (mg GAEs g<sup>-1</sup>). After mixing the extract solution (1 mL) with an equal volume of aluminium trichloride solution (1mL), the absorbance at 415 nm was read. The outcomes were reported as quercetin equivalents (mg QEs g<sup>-1</sup> of extract).

## Phenolic Compound Analyses

Analysis of phenolic contents of the extracts was conducted by reversed-phase High Performance Liquid Chromatography (RP-HPLC, Shimadzu, Japan) according to the procedure of Caponio et al. (1999) with some modifications. HPLC system was furnished with pump (LC-20AT), Photo-diode array detector (SPDM20A), and auto sampler (SIL-20ACHT). The analysis was done with the C-18 column (Agilent Eclipse XDB, 250 mm × 4.6 mm, 5 µm) by a mobile phase at 30°C. The mobile phase consisted of a mixture of two solvents, solvent A (the formic acid solution 3%) and solvent B (methanol). 0.2 g of sample dissolved in the mobile phase were filtered (0.45  $\mu$ m) before injection (10  $\mu$ L) into the HPLC. The gradient conditions were as stated in a previous study (Alper et al., 2021). 15 compounds shown in Table 2 were standards. Phenolic compounds in the extracts were given as ug per gram of the extract.

## **Statistical Analysis**

Statistical analysis was done with the software SPSS version 22.0 program. Statistical significance between the extracts was analyzed with One-Way ANOVA and Tukey's multiple comparison test. Data were presented as a mean  $\pm$  SE. Significance was considered as  $*P \leq 0.05$ .

## **RESULTS and DISCUSSION**

## Determination of Antioxidant Capacity, Total Phenolic and Flavonoid Contents

The different antioxidant assays were performed with the ethanol extracts of the fruits of *M. charantia* and the leaves of *D. stramonium*. The results of these assays are presented in Table 1. All antioxidant assays applied in our study were significantly different between the two plants ( $P \le 0.05$ ). D. stramonium extract had higher antioxidant activity compared to M. charantia extract.

Table 1. Antioxidant activities of M. charantia and D. stramonium extracts (mean ± SE)	
Circles 1 M describer D describer later later a distribution of the later describer and	

Çizelge 1. M. charantia ve D. stramonium ekstraktlarının antioksidan aktiviteleri (ortalama ± SE)						
Plants	DPPH	FRAP assay	Phosphomolybdenum assay	Metal chelating activity		
	(mg TEs g <sup>-1</sup> )	(mg TEs g <sup>-1</sup> )	$(mg TEs g^{-1})$	(mg EDTAEs g <sup>-1</sup> )		
M. charantia	$50.07 \pm 0.18^{b}$	$57.32 \pm 0.23^{b}$	$41.02 \pm 0.13^{b}$	$08.47 \pm 0.02^{b}$		
D. stramonium	$63.19 \pm 1.02^{a}$	$71.02 \pm 1.10^{a}$	$65.11 \pm 0.35^{a}$	21.52±0.09ª		

TEs: Trolox equivalents, EDTAEs: EDTA equivalents. Different letters in each column demonstrate significant difference (\* $P \le 0.05$ )

Also, the total phenolic and flavonoid contents for each extract were detected. Total phenolic and flavonoid contents of the ethanolic extracts of M. *charantia* and D. *stramonium* were given in Figure 1. When these results were examined, the ethanolic extract obtained from D. *stramonium* was determined to have the highest total phenolic content (25.77 mg GAEs g<sup>-1</sup>). Total flavonoid contents of the extracts from these plants were not significantly different (P>0.05), but their phenolic contents were significantly different (P≤0.05) (Figure 1).



Figure 1. Total phenolic and flavonoid contents of *M. charantia* and *D. stramonium* extracts. Each bar indicates the mean of contents ( $\pm$  SE) in each species. (GAEs: Gallic acid equivalents, QEs: Quercetin equivalents). \* $P \le 0.05$ .

Şekil 1. M. charantia ve D. stramonium ekstraktlarının toplam fenolik ve flavonoid içerikleri. Her çubuk, her türdeki içeriğin ortalamasını (± SE) gösterir. (GAEs: Gallik asit eşdeğerleri, QEs: Kuersetin eşdeğerleri). \*P≤ 0.05.

Phenolics are the most widely distributed secondary metabolites in plants. Plant polyphenols have been reported to take increased attention owing to their effective antioxidant features and their pronounced effects in preventing various oxidative stress-related diseases (Dai and Mumper, 2010). Cai et al. (2006) revealed that conventional Chinese medicinal plants related to anticancer had a great variety of natural phenolic compounds with different constructive properties as well as drastically different antioxidant activity.

It was found that *M. charantia* and *D. stramonium* possess antioxidant activity based on the results of various antioxidant assays *in vitro*. The antioxidant activity of *M. charantia* was confirmed in previous studies (Kubola and Siriamornpun, 2008; Choi et al.,

2012; Ozusaglam and Karakoca, 2013; Chen et al., 2019). Antioxidant compounds in *M. charantia* pulp showed potential natural antioxidant activity to inhibit the lipid peroxidation (Padmashree et al., 2010). It was reported that a wild *M. charantia* alcohol variety and aqueous extract had effects in the elimination of DPPH free radical at 300  $\mu$ g mL<sup>-1</sup>, and metal chelating activity at 100, 250, 500  $\mu$ g mL<sup>-1</sup> compared with Vitamin E (Wu and Ng, 2008). In another study, total phenolic contents of the ripe fruit of *M. charantia* extracted in 80% ethanol were detected as 14.9 mg GAEs g<sup>-1</sup> extract (Horax et al., 2010).

*D. stramonium* has both poisonous and medicinal properties and has been proven to have important pharmacological potency with a great benefit and usage in traditional folk medicine in nearly all parts

of the World. (Soni et al., 2012). The methanol extracts of D. stramonium leaves were reported to show the DPPH scavenging activity with the IC50 value of 6.7  $\mu$ g mL<sup>-1</sup> (Sreenivasa et al., 2012). Benabderrahim et al. (2019) investigated the antioxidant activity and total phenolic and flavonoids contents of Datura innoxia, collected from Isparta, Turkey. The total phenolics and flavonoids results and DPPH, FRAP, phosphomolybdenum assay results of *D. innoxia* methanolic aerial parts were found to be higher than D. stramonium, which we examined in this study. However metal chelating activity of D. stramonium was found to be higher than Datura innoxia. In a previous study, where the methanolic leaf extracts of D. innoxia and D. metel were examined for their total phenolics and flavonoids content, the highest total phenolic and total flavonoid content were found in D. innoxia leaf extract as 70.26  $\pm$  1.12 mg GAE g<sup>-1</sup> and 34.24  $\pm$  1.28 mg RE g<sup>-1</sup> respectively (Bhardwaj et al., 2016).

The antioxidant activity of the plants in this study may be explained by the phenolic compounds they contain. Caffeic acid was determined to be a major constituent in both extracts and therefore, it may be mainly responsible for the antioxidant activity observed (Masek et al., 2016). There are various studies in the literature that reveal a significant relationship between total phenolic contents and antioxidant activities (Ozay and Mammadov, 2016; El-Hawary et al., 2019).

## **Determination of Phenolic Composition**

The phenolic compound amount determined by HPLC analysis in these extracts was summarized in Table 2 and the chromatograms were illustrated in Figure 2 and 3. All of the 15 phenolic compounds used as the standards were detected in varying amounts according to the extracts. Caffeic acid was clearly the most abundant phenolic acid in both extracts although its amount was very different (6282.51 µg g<sup>-1</sup> extract in *M. charantia* fruit extract and 15183.36 µg  $g^{-1}$  extract in *D. stramonium* leaf extract). The main phenolic compounds of M. charantia fruit extract, after caffeic acid were 2,5 dihidroxybenzoic acid, epicatechin, gallic acid and 3,4-dihydroxybenzoic acid, respectively. However, 2,5 dihidroxybenzoic acid, epicatechin, vanillic acid, gallic acid and chlorogenic acid were detected as the main phenolic compounds of D. stramonium leaf extract, depending on their amounts, respectively following caffeic acid.

Table 2. Phenolic compounds of *M. charantia* and *D. stramonium* extracts ( $\mu g g^{-1}$  extract) (mean ± SE)

No	Phenolic Compounds	RT (min)	$UV_{max}$	M.charantia	D. stramonium
	(Fenolik Bileşikler)			(µg g <sup>-1</sup> extract )	(µg g <sup>-1</sup> extract )
1	Gallic acid	6.8	280	$549.34 \pm 3.83$	$680.89 \pm 4.64$
2	3,4-dihydroxybenzoic acid	10.7	280	$540.18\pm3.22$	$21.54\pm0.48$
3	4-hydroxybenzoic acid	15.7	280	$25.11\pm0.54$	$311.73\pm3.54$
4	2,5-dihydroxybenzoic acid	17.2	320	$1477.58 \pm 12.75$	$4721.82 \pm 80.55$
5	Chlorogenic acid	18.2	320	$81.28\pm0.76$	$410.70\pm2.88$
6	Vanillic acid	19.2	320	$101.90 \pm 1.41$	$1411.50 \pm 11.13$
7	Epicatechin	21.3	260	$599.45 \pm 4.01$	$1928.60 \pm 15.17$
8	Caffeic acid	22.7	280	$6282.51 \pm 135.5$	$15183.36 \pm 271.0$
9	<i>p</i> -coumaric acid	26.1	320	$1.18\pm0.02$	$31.83 \pm 0.23$
10	Ferulic acid	30.1	320	$3.15\pm0.09$	$45.42\pm0.38$
11	Rutin	45.6	360	$24.07\pm0.47$	$2.47\pm0.11$
12	Ellagic acid	47.7	240	$208.64 \pm 3.25$	$190.75 \pm 2.85$
13	Naringin	49.7	280	$18.00\pm0.45$	$5.18\pm0.11$
14	Cinnamic acid	67.8	280	$232.17 \pm 3.42$	$227.94 \pm 3.36$
15	Quercetin	71.1	360	$9.94 \pm 0.19$	$7.66 \pm 0.15$

RT: retention time (alıkonma zamanı)

When the main compounds aforementioned were compared, the amount of chlorogenic acid, 4hydroxybenzoic acid, 2,5 dihidroxybenzoic acid, vanillic acid, caffeic acid and epicatechin were remarkably higher in *D. stramonium* leaf extract than *M. charantia* fruit extract, whereas 3,4dihydroxybenzoic acid was importantly determined higher in *M. charantia* fruit extract than that of *D. stramonium* leaf extract. Also, both extract were found to be rich in ellagic acid and cinnamic acid. Quercetin was a little in both extracts. In addition, *p*  coumaric acid and rutin were detected in the lowest level of concentration in *M. charantia* fruit extract and *D. stramonium* leaf extract, respectively.

The phytochemical compounds in plants were demonstrated to have different biological activities like antioxidant, anticancer, antibacterial, antiinflammatory, cardioprotective properties, which have potential beneficial effects for human health. The research about phenolic compounds has increased in recent, due to their potential ability (Tungmunnithum et al., 2018).



Figure 2. The HPLC chromatogram of *M. charantia* extract *Şekil 2. M. charantia ekstraktının HPLC kromotogramı* 



Figure 3. The HPLC chromatogram of *D. stramonium* extract *Şekil 3. D. stramonium ekstraktının HPLC kromotogramı* 

There are various reports revealed the phenolic composition of *M. charantia*. Horax et al. (2010) extracted the phenolics, using ethanol and water solvent systems, from pericarp (fleshy portion) and seeds of *M. charantia* collected at three maturation stages as immature, mature, and ripe and reported that catechin, gallic acid, gentisic acid, chlorogenic acid and epicatechin were the main phenolic components in the extracts. Choi et al. (2012) evaluated the phytochemical contents from the 80% methanol extracts of roasted and unroasted M. charantia fruits, leaves, stems, and roots from Korea Farms. They expressed that epigallocatechin was the most abundant phytochemical compound in both extracts of M. charantia fruits and leaves. On the contrary our findings, they showed that the amount of caffeic acid in unroasted fruits (34.39±0.11 mg kg<sup>-1</sup> d.w) and in roasted fruits (46.16 $\pm$ 0.31 mg kg<sup>-1</sup> d.w) was significantly lower than that of our extract. Also, they were detected neither rutin nor quercetin in their fruit extract. In addition, gallic acid and epicatechin in our extract were relatively more than those found in their fruit extracts and the opposite was true for *p*-coumaric acid and ferulic acid. The amounts of vanillic acid and chlorogenic acid in their fruit extracts were similar to our extract. In another

chlorogenic acid, gallic acid, catechin, study, epicatechin, protocatechuic acid and gentisic acid were found as the main phenolic compounds in the fruit extract of *M. charantia* (Ghaima et al., 2013). In bitter melon (M. charantia) fruit extracts with increasing maturation, the contents of gallic acid, chlorogenic acid and catechin were stated to be increase, whereas the contents of ferulic acid, pcoumaric acid and caffeic acid were denoted to be reduce (Lee et al., 2017). In a different study, epicatechin was indicated to be a highly accumulated phenolic compound in flowers and fruits of bitter melon, while the highest content in leaves was reported to be rutin (Cuong et al., 2018). These differences in the results may be attributed to the location and harvesting time of the plants, chosen solvent, extraction procedure, analysis method and genetic variability.

Several previous reports demonstrated the phenolic composition of different *Datura* species. Fatima et al. (2015) notified, based on HPLC-DAD quantitative analysis, that catechin and apigenin were prominently detected in the methanol (5.41 and 2.11  $\mu$ g mg<sup>-1</sup> dry weight (DW), respectively) and methanol-chloroform (1.28 and 1.78 mg g<sup>-1</sup> DW, respectively) extract among the extracts of *Datura innoxia* leaves.

They also showed that catechin and apigenin were present in the ethanolic extract of *D. innoxia* fruit, and its amounts were 2.65 and 2.46 mg g<sup>-1</sup> dry weight respectively. Rahmoune et al. (DW), (2017)announced that D. stramonium included five (luteolin, quercetin, trans-caffeic acid, trans-ferulic acid and dihydroferulic acid) phenylpropanoid compounds, whereas D. innoxia contained eight (luteolin, quercetin, trans-caffeic acid, trans-ferulic acid, cis-caffeic acid, cis-4- hydroxy-cinnamic acid, trans-4-hydroxy-cinnamic acid and trans-sinapic acid) compounds, and the phenylpropanoids concentration in leaves of both species were emphasized to be notably higher than in the roots. Based on LC-ESI-MS/MS method, the major flavonoids identified in methanol extract of aerial parts of D. innoxia from Turkey were reported to be (+)-catechin, (-)epicatechin and hyperoside (Benabderrahim et al., 2019). According to their study, the phenolic compounds detected in very high concentrations in D. innoxia were (+)-catechin and (-)-epicatechin, also the amount of (-)-epicatechin (24147.64  $\pm$  2512.35 µg g<sup>-1</sup> of dry plant) was importantly higher than the extract of *D. stramonium* evaluated in the present study. Partap et al. (2019) determined and quantified of chlorogenic acid and caffeic acid from the root and seed parts of *Datura* spp for the first time by HPTLC analysis. The total concentration of phytotoxins (phenolics) in aqueous extract of *D.stramonium* was displayed as  $28.96 \text{ mg } L^{-1}$  and these compounds detected by HPLC analysis, among 11 different allelopathic compounds, were shown to be quercetin  $(0.66 \text{ mg } L^{-1})$ , chlorogenic acid  $(9.44 \text{ mg } L^{-1})$ , sinapinic acid (2.03 mg  $L^{-1}$ ), caffeic acid (6.67 mg  $L^{-1}$ ) and benzoic acid (10.16 mg  $L^{-1}$ ) (Raza et al., 2019). As far as we know, the study is the first one to report the contents of phenolic compounds of ethanol extracts of leaves of *D. stramonium* from Edirne Turkey. When all the results are evaluated together, it is clear that the antioxidant capacity and the contents of the phenolic compound may differ depending on plant species, the parts of the plants used, the region where the plants are obtained, and the solvent choosen for extraction.

# CONCLUSION

The results of the present work showed that the ethanol extracts of M. charantia fruit and D. stramonium leaf are a potential natural antioxidant source, and these plants may consider as prominent species in pharmaceutical research, because they contain different phenolic compounds associated with pharmaceutical and medicinal properties. Further studies are needed for the isolation and identification of active phytoconstituents in extracts that might be used for pharmaceutical use.

#### ACKNOWLEDGMENT

The authors would like to thank Burdur Mehmet Akif Ersoy University Scientific and the Technology Application and Research Center for HPLC analysis, and Scientific Research Projects (15/006) of Muğla Sıtkı Koçman University for financial support for HPLC analysis. They would also like to thank Prof. Dr. Hatice Güneş from Muğla Sıtkı Koçman University for her helpful advice throughout the work, and Prof. Dr. Fatma Güneş from Trakya University for providing the plant species. Some of the results given in the present study about ethanol extract of the leaves of *D. stramonium* were presented as a poster at the 3rd International Eurasian Biodiversity Symposium (SEAB-2017) as a summary.

## Author's Contributions

The contribution of the authors is equal.

## Statement of Conflict of Interest

Authors have declared no conflict of interest.

## REFERENCES

- Alam MdN, Bristi NJ, Rafiquzzaman Md 2013. Review on In Vivo and In Vitro Methods Evaluation of Antioxidant Activity. Saudi Pharm J 21(2): 143-152.
- Ally F, Mohanlall, V 2020. An Overview of Tropane Alkaloids from *Datura stramonium* L.. J Pharmacogn Phytochem 9(3): 5-13.
- Alper M, Özay C, Güneş H, Mammadov R 2021. Assessment of Antioxidant and Cytotoxic Activities and Identification of Phenolic and Compounds of Centaurea solstitialis Urospermum picroides from Turkey. Braz Arch Biol Technol 64: e21190530.
- Alper M 2019. Investigation of Potential Anti-cancer and Anti-inflammatory Effects of *Datura stramonium* Ethanolic Extracts Against Selected Human Cancer Cell Lines. Fresen Environ Bull 28(12): 8993-9003.
- Al-Snafi AE 2017. Medical Importance of *Datura* fastuosa (syn: Datura metel) and Datura stramonium - A Review. IOSR J Pharm 7(2): 43-58.
- Armendáriz-Barragán B, Zafar N, Badri W, Galindo-Rodríguez SA, Kabbaj D, Fessi H, Elaissari H 2016. Plant Extracts: From Encapsulation to Application. Expert Opin Drug Del 13(8): 1165-1175.
- Benabderrahim MA, Sarikurkcu C, Elfalleh W, Özer MS 2019. *Datura innoxia* and *Dipsacus laciniatus*: Biological Activity and Phenolic Composition. Biocatal Agric Biotechnol 19: 101163.
- Berk S, Tepe B, Arslan S, Sarikurkcu C 2011. Screening of The Antioxidant, Antimicrobial and DNA Damage Protection Potentials of The

Aqueous Extract of *Asplenium ceterach* DC. Afr J Biotechnol 10(44): 8902-8908.

- Berkov S, Zayed R, Doncheva T 2006. Alkaloid Patterns in Some Varieties of *Datura stramonium*. Fitoterapia 77(3): 179-182.
- Bhardwaj K, Kumar S, Ojha S 2016. Antioxidant Activity and FT-IR Analysis of *Datura innoxia* and *Datura metel* Leaf and Seed Methanolic Extracts. Afr J Tradit Complement Altern Med 13(5): 7-16.
- Cai YZ, Sun M, Xing J, Luo Q, Corke H 2006. Structure-Radical Scavenging Activity Relationships of Phenolic Compounds from Traditional Chinese Medicinal Plants. Life Sci 78(25): 2872-2888.
- Caponio F, Alloggio V, Gomes T 1999. Phenolic Compounds of Virgin Olive Oil: Influence of Paste Preparation Techniques. Food Chem 64(2): 203-209.
- Carocho M, Ferreira ICFR 2013. A Review on Antioxidants, Prooxidants and Related Controversy: Natural and Synthetic Compounds, Screening and Analysis Methodologies and Future Perspectives. Food Chem Toxicol 51: 15-25.
- Ceylan R, Katanić J, Zengin G, Matić S, Aktumsek A, Boroja T, Stanić S, Mihailović V, Guler GO, Boga M, Yılmaz MA 2016. Chemical and Biological Fingerprints of Two Fabaceae Species (*Cytisopsis dorycniifolia* and *Ebenus hirsuta*): Are They Novel Sources of Natural Agents for Pharmaceutical and Food Formulations? Ind Crop Prod 84: 254-262.
- Chen F, Huang G, Yang Z, Hou Y 2019 Antioxidant Activity of *Momordica charantia* Polysaccharide and Its Derivatives. Int J Biol Macromol 138: 673-680.
- Choi JS, Kim HY, Seo WT, Lee JH, Cho KM 2012. Roasting Enhances Antioxidant Effect of Bitter Melon (*Momordica charantia* L.) Increasing Inflavan-3-ol and Phenolic Acid Contents. Food Sci Biotechnol 21(1): 19-26.
- Cuong DM, Kwon SJ, Jeon J, Park YJ, Park JS, Park SU 2018. Identification and Characterization of Phenylpropanoid Biosynthetic Genes and Their Accumulation in Bitter Melon (*Momordica charantia*). Molecules 23(2): 469.
- Dai J, Mumper RJ 2010. Plant Phenolics: Extraction, Analysis and Their Antioxidant and Anticancer Properties. Molecules 15(10): 7313-7352.
- Dastmalchi N, Baradaran B, Latifi-Navid S, Safaralizadeh R, Khojasteh SMB, Amini M, Roshani E, Lotfinejad P 2020. Antioxidants with Two Faces toward Cancer. Life Sci 258: 118186.
- El-Hawary SS, El-Hefnawy HM, Osman SM, El-Raey MA, Mokhtar Ali FA 2019. Phenolic Profiling of Different Jasminum Species Cultivated in Egypt and Their Antioxidant Activity. Nat Prod Res 14: 1-6.
- El Bazaoui A, Bellimam, MA, Soulaymani A 2011. Nine New Tropane Alkaloids from *Datura*

*stramonium* L. Identified by GC/M. Fitoterapia 82(2): 193-197.

- Fatima H, Khan K, Zia M, Ur-Rehman T, Mirza B, Haqi IU 2015. Extraction Optimization of Medicinally Important Metabolites from *Datura innoxia* Mill.: An In Vitro Biological and Phytochemical Investigation. BMC Complement Altern Med 15: 376.
- Fuchs-Tarlovsky V 2013. Role of Antioxidants in Cancer Therapy. Nutrition 29(1): 15-21.
- Ghaima KK, Nader MI, Taqi RA, Ghraibit SA 2013. Extraction and Identification of Phenol Compounds from Bitter Melon *Momordica charantia* Fruits and Their Role as Antioxidants. J Biotech Res Cent 7(1): 41-47.
- Güneş H, Alper M, Çelikoğlu N 2019. Anticancer Effect of The Fruit and Seed Extracts of *Momordica charantia* L. (Cucurbitaceae) on Human Cancer Cell Lines. Trop J Pharm Res 18(10): 2057-2065.
- Horax R, Hettiarachchy N, Chen P 2010. Extraction, Quantification, and Antioxidant Activities of Phenolics from Pericarp and Seeds of Bitter Melons (*Momordica charantia*) Harvested at Three Maturity Stages (Immature, Mature, and Ripe). J Agric Food Chem 58(7): 4428-4433.
- Krishnaiah D, Sarbatly R, Nithyanandam R 2011. A Review of The Antioxidant Potential of Medicinal Plant Species. Food Bioprod Process 89: 217-233.
- Kubola J, Siriamornpun S 2008. Phenolic Contents and Antioxidant Activities of Bitter Gourd (*Momordica charantia* L.) Leaf, Stem and Fruit Fraction Extracts In Vitro. Food Chem 110: 881-890.
- Lee SH, Jeong YS, Song J, Hwang KA, Noh GM, Hwang IG 2017. Phenolic Acid, Carotenoid Composition, and Antioxidant Activity of Bitter Melon (*Momordica charantia* L.) at Different Maturation Stages. Int J Food Prop 20: sup3, S3078-S3087.
- Lobo V, Patil A, Phatak A, Chandra N 2010. Free Radicals, Antioxidants and Functional Foods: Impact on Human Health. Phcog Rev 4: 118-126.
- López-Alarcón C, Denicola A 2013. Evaluating The Antioxidant Capacity of Natural Products: A Review on Chemical and Cellular-Based Assays. Anal Chim Acta 763: 1-10.
- Masek A, Chrzescijanska E, Latos M 2016. Determination of Antioxidant Activity of Caffeic Acid and *p*-Coumaric Acid by Using Electrochemical and Spectrophotometric Assays. Int J Electrochem Sci 11: 10644-10658.
- Ozay C, Mammadov R 2016. Assessment of Some Biological Activities of *Alyssum* L. Known as Madwort. Acta Pol Pharm 73(5): 1213-1220.
- Ozusaglam MA, Karakoca K 2013. Antimicrobial and Antioxidant Activities of *Momordica charantia* from Turkey. Afr J Biotechnol 12: 1548-1558.

- Öz Arık U 2017. The Antifungal Effects of *Datura* stramonium L., *D. metel* L., *D. innoxia* Mill. in Flora of Turkey. Mugla J Sci Technol 3(2): 96-103.
- Padmashree A, Sharma GK, Semwal AD, Bawa AS 2010. Studies on Antioxygenic Activity of Bitter Gourd (*Momordica charantia*) and Its Fractions Using Various In Vitro Models. J Sci Food Agric 91: 776-782.
- Partap M, Gupta RC, Pradhan SK 2019. Comparative Analysis of Morphology and Phytochemical Constituents in Different Populations and Morphotypes of *Datura innoxia* Mill. and *Datura metel* L. from Punjab Plains. Asian J Pharm Clin Res 12(1): 193-199.
- Rahmoune B, Zerrouk IZ, Morsli A, Slaoui MK, Khelifi L, Do Amarante L 2017. Phenylpropanoids and Fatty Acids Levels in Roots and Leaves of *Datura stramonium* and *Datura innoxia*. Int J Pharm Pharm Sci 9(7): 150-154.
- Rajendran P, Nandakumar N, Rengarajan T, Palaniswami R, Gnanadhas EN, Lakshminarasaiah U, Gopas J, Nishigaki I 2014. Antioxidants and Human Diseases. Clin Chim Acta 436: 332-347.
- Raza A, Kamran M, Safdar ME, Ali HH, Abbas T, Asif M, Ali L, Rehman A 2019. Management Tactics for The Handling of *Parthenium hysterophorus* L. in Non-Native Environment Through Phytotoxic Compounds of Local Species. Int J Agric Biol 21(1): 215-222.
- Singh LR, Singh OM 2013. *Datura stramonium*: An Overview of Its Phytochemistry and Pharmacognosy. Res J Pharmacogn Phytochem 5(3): 143-148.
- Soni P, Siddiqui AA, Dwivedi J, Soni V 2012.
  Pharmacological Properties of *Datura stramonium*L. as A Potential Medicinal Tree: An Overview.
  Asian Pac J Trop Biomed 2(12): 1002-1008.

- SPSS 2013. IBM SPSS Statistics 22.0 for Windows. Armonk, NY
- Sreenivasa S, Vinay K, Mohan NR 2012. Phytochemical Analysis, Antibacterial and Antioxidant Activity of Leaf Extract of *Datura stramonium*. Int J Sci Res 1(2): 83-86.
- Tungmunnithum D, Thongboonyou A, Pholboon A, Yangsabai A 2018. Flavonoids and Other Phenolic Compounds from Medicinal Plants for Pharmaceutical and Medical Aspects: An Overview. Medicines 5(93): 1-16.
- Verma N, Shukla S 2015. Impact of Various Factors Responsible for Fluctuation in Plant Secondary Metabolites. J Appl Res Med Aromat Plants 2(4): 105-113.
- Villarreal-La Torre VE, Sagástegui Guarniz W, Silva-Correa C, Cruzado-Razco L, Siche R 2020. Antimicrobial Activity and Chemical Composition of *Momordica Charantia*: A Review. Pharmacog J 12(1): 213-22.
- Wu SJ, Ng LT 2008. Antioxidant and Free Radical Scavenging Activities of Wild Bitter Melon (*Momordica charantia* Linn. var. *abbreviata* Ser.) in Taiwan. LWT-Food Sci Technol 41: 323-330.
- Zengin G, Aktumsek A 2014. Investigation of Antioxidant Potentials of Solvent Extracts from Different Anatomical Parts of Asphodeline anatolica E. Tuzlaci: An Endemic Plant to Turkey. Afr J Tradit Complement Altern Med 11(2): 481-488.
- Zengin G, Nithiyanantham S, Locatelli, M, Ceylan R, Uysal S, Aktumsek A, Selvi PK, Maskovic P 2016. Screening of *In Vitro* Antioxidant and enzyme Inhibitory Activities of Different Extracts from Two Uninvestigated Wild Plants: *Centranthus longiflorus* subsp. *longiflorus* and *Cerinthe minor* subsp. *auriculata*. Eur J Integr Med 8(3): 286-292.