Original Research Article

Total Phenolics, Flavonoids Contents, Antioxidant Activity and DNA Protective Effect of Lenten Rose (*Helleborus orientalis)*

ABSTRACT

The aims of the present study were to evaluate the antioxidant activity and DNA Protective effect of *Helleborus orientalis* (HO) leaf extract against oxidative damage, and to determine the total phenolic and flavonoid contents of the plant species studied.

Methods: The total phenol content (TPC) of *H. orientalis* (*Ranunculaceae*) extract was determined using the Folin-Ciocalteu technique. The aluminum chloride colorimetric assay in the determination of The total flavonoid content (TFC) and was used, Analysis of Phenolic Acids was identified by High-Performance Liquid Chromatography (HPLC). Antioxidant activity was analyzed by the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Assay. Protective effect of *H. orientalis* leaf extract against to H_2O_2 was evaluated by using TAS, TOS methods and Comet assay.

Key findings: *H. orientalis* ethanol extracts contain high amounts of antioxidants. The HO leaf methanol extract (LME) decreased the DNA damage at all tested concentrations in a dose-dependent manner ($r=0.86$ $p<0.01$) against to H_2O_2.

Conclusions: The total phenol content in the extracts of different parts of the plant varied from 4.00 mg GAE/1 gr to 19.42 mg GAE/1 gr. The flowers had the highest phenol content (19.42 mg GAE/1 gr sample) and followed by the leaves (17.20 mg GAE/1 gr sample). The total flavonoid content in the extracts from different parts of the plant varied from 2.57 mg QE/1 gr to 11.88 mg QE/1 gr. The flowers had the highest flavonoid content (11.88 mg QE/1 gr sample) and followed by the leaves (10.21 mg QE/1 gr sample).

Antioxidant activity of fractions was explained as a percentage of DPPH radicals' scavenging and IC_{50} values (µg/ml). Leaf and flowers of HO are richer in antioxidant than its root and stem. As the concentration of leaf extracts used increased, the DNA

protective effect increased and it was statistically significant at overdoses of 2500 µg/mL. Total antioxidant status (TAS) levels were significantly ($p < 0.05$) decreased in the H_2O_2 group (3.4±0.21) but *H. orientalis* was significantly ($p<0.05$) increased TAS levels in this group. When the concentration of leaf extracts used increased, the protective effect has also increased and statistically significant at overdoses of 2500 µg / mL (6.3±0.67). Total oxidant status (TOS) levels were significantly ($p < 0.05$) increased in the H_2O_2 group (25.3±0.74) and *H. orientalis* was significantly ($p<0.05$) decreased TOS levels in groups.

Keywords: Antioxidant Activity, Flavonoids, *Helleborus orientalis,* Oxidative DNA Damage, Phenolics, Protective Effect.

INTRODUCTION

There is little information on the therapeutic properties of *Helleborus* (family Ranunculaceae) species known as ornamental plants. For this reason, studies on the phytochemical structure and phytochemical properties of *Helleborus species* have been of interest in the last few years. There are studies including inherent extracts from these species which indicate that *Helleborus* plants are a helpful origin of chemical compounds with excellent therapeutic potency years (Maior and Dobrotă, 2013).

Some *Helleborus* extracts have immuno-stimulatory molecules and are used in anticancer treatment (Bussing and Schweizer, 1998). In vitro application of *H. niger* water extract resulted in a minor induction of sister chromatid exchanges in blood mononuclear cells from a healthy person. Additionally, Lindholm et al. (2002) noticed a powerful antitumor potential of some *Helleborus species* (*H. cyclophyllus* Boiss. *H. multifidus* and *H. hercegovinus*) (Lindholm et al., 2002). In cancer cells, the Reactive oxygen species (ROS) is maintained at higher levels than normal cells and mainly exerts its proliferative actions. When ROS levels are further increased by pro-oxidants so as to exceed a borderline level, the pro-apoptotic effects of ROS may exceed its proliferative effects and cytotoxic effects display in cancer cells, whereas the ROS levels in normal cells remain below the borderline level which is nontoxic to normal cells. Antioxidants treat cancers as cancer inhibiting agents (Jin et al., 2014).

Polyphenolic antioxidants are flavonoids and phenolic acid. These compounds most important natural bioactive secondary metabolites in plants (Ghasemzadeh and

Ghasemzadeh, 2011, Pehlivan et al., 2018). In addition, phenolic antioxidant compounds reduce the risk of cancer by clearing free superoxide radicals. Phenols include both hydroxyl group and aromatic ring. It has been reported that phenols are involved in the defense system against microorganisms. Phenols directly cleans free radicals or act as electron donors against hydrogen peroxide in reactions catalyzed by ascorbate peroxidase. Thus they reduce the oxidative stress in the cell (Morina et al., 2008, Pehlivan et al, 2018). In recent years it has been determined that flavonol glycoside and phenolic glucoside derivatives isolated from some *Helleborus species* and exhibit significant anti-proliferative and potent antioxidant activity (Braca A et al., 2014; Prieto et al., 2006; Vitalini et al., 2011).

H. orientalis is a perennial plant and is indigenous to Greece and Turkey (Kazuki et al., 2003). Same antioxidant compounds have nowadays been isolated from plants of this genus but information on the antioxidant activity of HO is limited. In order to use this plant in modern medicine, it must be determined whether or not it causes antioxidant effects in the cell. Comet assay is a technically easy, fast and highly sensitive test to detects the small changes in the DNA structure (Liman, 2013). According to our literature search, antioxidant effects of *H. orientalis* have not been examined yet by comet assay.

The aims of this study are to evaluate the antioxidant activity using different methods and to determine the protective effect of *H. orientalis* extract against oxidative DNA damage by employing a Comet assay.

MATERIALS AND METHODS

Reagents and Plant material

H. orientalis (*Ranunculaceae*) were collected from Piraziz, Giresun, Turkey (Latitude 40.924° - Longitude 38.128° - Height 290 m) in April 2017. The plant was identified by Professor Mustafa Kargıoğlu, from the Department of Biology, Faculty of Science and Literatures, Afyon Kocatepe University. An authenticated voucher specimen (AKU9324) was deposited in the Herbarium of Faculty of Science and Literatures Afyon Kocatepe

University. Its leaves and branches were dried at 27±2 °C in a dark room for two weeks. Dried materials were milled (80–100 mesh) before extraction.

All the chemicals (Sigma-Aldrich) used in this study were filtered through a 0.45 Millipore membrane filter.

Ultrasound-assisted extraction

Extraction was performed utilizing Wise bath brand ultrasonic bath with 50 kHz frequency. 1 g dried part of plant material powdered was extracted with 30 mL of a methanol solution (70% methanol in distilled H_2O) in an ultrasonic bath at 27 °C temperature for 30 min. It was watched out that the solvent and water level of flask in the ultrasonic bath remained the same. After fixing the ultrasonic bath temperature and time value, extraction proces was started. When the extraction was complete, the mixture was first filtered with Whatman filter paper and then filtered with a 0.45 micron membrane filter.

Determination of total phenolic content (TPC)

The Folin-Ciocalteu technique protocol was done according Kähkönen et al. (1999). Absorbance was measured at 765 nm by a UV-Vis spectrophotometer and compared to a Gallic acid (GA) calibration curve. The amount of the absorbance measured in the extract was calculated from the equation of the standard curve prepared with GA. TPC in the extract is expressed in terms of "mg GA/g sample".

Determination of total flavonoid content (TFC)

The TFC of the extract was determined by the aluminum chloride colorimetric assay (Chang et al., 2002). The absorbance of the samples was read at 510 nm. The same processes were used for quercetin used as standard and flavonoid contents of the samples were calculated as equivalent to quercetin (mg QE / g sample).

Analysis of Phenolic Acids by High-Performance Liquid Chromatography (HPLC)

Determination of phenolic acids in extract was carried out with HPLC system (Agilent 1260 series). Instrument control and data analysis were performed using Agilent

HPLC Chemstation 10.1 edition through Windows 2000. Zorbax Extend-C18 (5 µm, 4.6 mm -150 mm, Agilent) column was used. The flow rate of the mobile phase was adjusted at 0.5 mL/min. Mobile phase A was 0.02% Trifluoroacetic acid (TFA) solution in water, and phase B was 0.02% TFA solution in methanol. The gradient conditions were as follows: 0-5 min, 25% B; 5-10 min, 25-30% B; 10-16 min, 30-45% B; 16-18 min, 45% B; 18-25 min, 45-80% B; 25-30 min, 80% B; 30-40 min, 80-25% B (The column temperature: 25 °C, injection volume: 10 µL). As standard Vanillic acid (254), gallium and syringic acid (275, 305), and rosmarinic acid (320 nm) were used (Wen et al., 2005).

Fourier Transform Infrared Spectrophotometer (FTIR)

FTIR Analysis has been applied to determine functional groups present in the methanol extracts of *H. orientalis* About 1 mg of dried material was analyzed by FTIR (spectra frequency zones 3500-500 cm- 1) (Perkin Elmer Lambda 35)

LC-Mass Analyze

LC-Mass Analysis was applied to determine unknown substances in the structure of *H. orientalis*. The dried ground samples were weighed 1 g and extracted in an ultrasonic bath with 30 mL of 70% methanol. The analysis was performed by using the 6460 mass detector with the C18 column at 325 ° C of the ionization temperature using the Jetstream ionization technique. The mobile phase A was 20 % acetonitrile and mobile phase B was 80% distilled water containing 0.1% formic acid. The analysis was performed with Triple Quadrupole LC-MS-MS device. Separation process was performed with a 1200 model UPLC device. The injection volume was 10 µL.

Antioxidant activity by DPPH test assay

The DPPH assay protocol was carried out according to the method of Thaipong et al. (2006). The absorbance of the samples (Shimadzu UV Mini 1240) was read at 515 nm. Antioxidant activity is expressed as % inhibition of DPPH.

Protective effect of leaf extract against to H_2O_2 by Comet assay

The alkaline comet assay was done according to Singh et al. (1998) Negative and positive controls (1xPBS and H_2O_2) were also included, and exposure time is in parallel with

HO leaf extract dissolved in 1XPBS (500, 1000, 2500, 5000 and 10.000 µg/mL) for 0.5h at 37 °C. While supernatant was used for The oxidative stress index (OSI) determination, the pellet was used for Comet assay. Electrophoresis was then carred out for 20 min at 25 V (1 V cm^{-1}) at 4 ^{o}C. Following electrophoresis, slides washed with neutralization buffer (0.4 M Tris, pH = 7.5) three times. The slides were covered with coverslip after staining with 60 µl of 20 µg/ml ethidium bromide. Totally 300 comets per concentration were analyzed using a fluorescence microscope (BAB-TAM-F, Turkey). Scores of slides were classified according to Koçyiğit et al (2005). The mean ± standard deviations of the obtained data were calculated. . Significance levels in different treatment groups were analyzed using Duncan multiple range tests (SPSS 23.0 version).

Measurement of total oxidant status and oxidative stress index

When Comet assay DNA damage study was carried out, the last supernatants were taken into Eppendorf tubes and stored at -20 ˚C for used in TOS and TAS studies. The total oxidant (530 nm) and oxidant (660 nm) level was estimated utilizing a completely automated colorimetric technique created by Erel. TOS (530 nm) and TAS (660 nm) levels were evaluated as spectrophotometric (Elisa Thermo Scientific) using Rel Test Kit RL0024 and RL0017(Erel, 2004; Erel, 2005). Stress index was counted up according to the following formula; TOS: (ΔAbsSample)/ (ΔbsStandard) X Conc. of standard TAS: ((ΔAbs H_2O) - (ΔAbs Sample)) / ((ΔAbsH_2O) - (ΔAbs Standart)).

The oxidative stress index (OSI) of samples were determined with the ratio of TOS to TAS. Standard deviations of the data obtained from the test samples were calculated and analyzed using Duncan post-hoc one-way variance analysis (ANOVA).

RESULTS

Phenolic contents

TPC in plant extracts of *H. orientalis* were given in Table 1. TPC in plant extracts ranged from 19.42 to 4.39 mg GAE/1 g sample. The highest concentration of TPC was measured at FME (Flowers Methanol Extract). TPC in LME (Leaves Methanol Extract). was determined as 17.20 mg GAE/1 g sample. A graph of the absorbance values versus the Gallic acid concentration was drawn and a curve of Gallic acid calibration was

generated. The calibration curve for Gallic acid was given in figure 1. Linearity was defined as (y=98.316 x+39.945) and regression coefficient was defined as (R^2=0.9990).

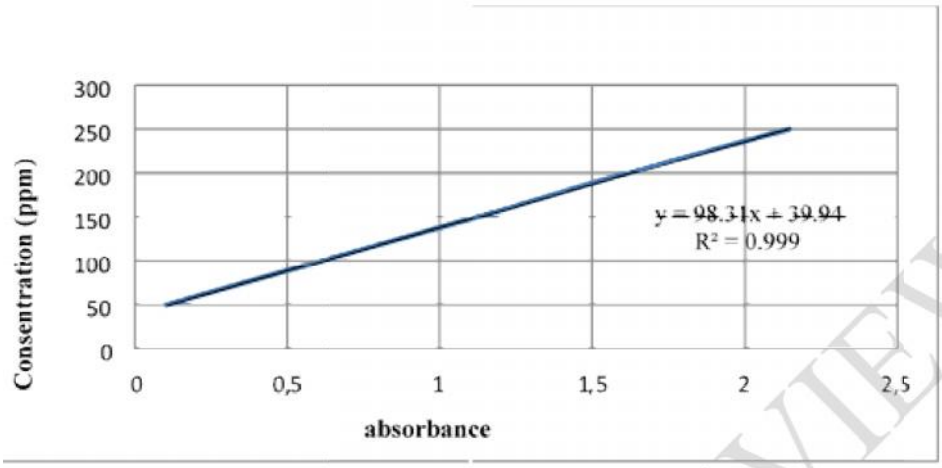

Fig1. TPC linearity graph

Table 1 The total phenolic contents of various plant extracts parts of *H. orientalis*

The methanol extract of *H. orientalis*	mg GAE/1 gr of sample
Flower (FME)	19.42
Leaf (LME)	17.20
Body (BME)	6.86
Root (RME)	4.39

Flavonoid contents

The TFC in the extracts of different parts of the plant were given in Table 2 as quercetin equivalent (QE). The highest amount of flavonoid content was found in FME (11.880 mg QE/g) followed by LME (10.212 mg QE/g) BME (3.116 mg QE/g) and RME (2.567 mg QE/g). A graph of the absorbance values versus the quercetin concentration was drawn and a curve of quercetin calibration was generated. The calibration curve for quercetin was given in Figure 2. Linearity was defined as (y=2438x+0.218) and regression coefficient was defined as (R^2=0.9998).

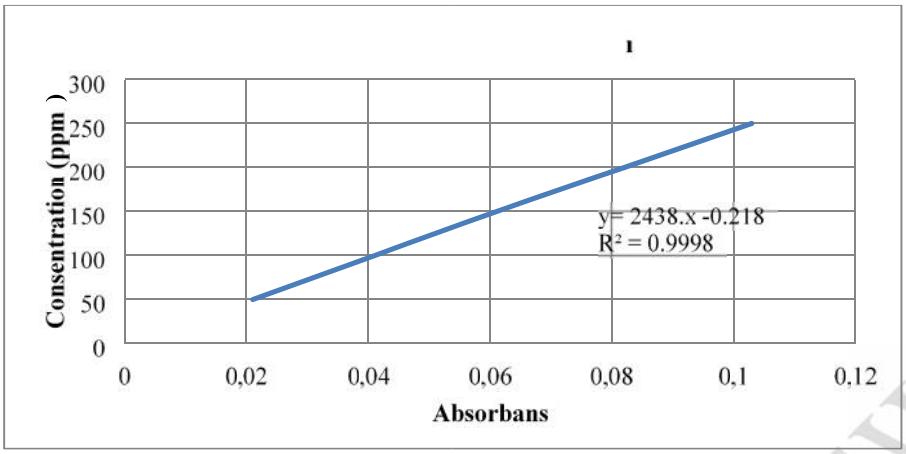

Fig. 2. TFC Linearity Graph

Table 2 The total flavonoid contents of different parts of *H. orientalis*

The methanol extract of *H. orientalis*	mg QE/ 1 g. of sample
Flower (FME)	11.880
Leaf (LME)	10.212
Body (BME)	3.116
Root (RME)	2.567

Phenolic Acid Contents of *H. orientalis*

The presence of Gallic acid, protoacetic acid, vanillic acid, caffeic acid, syringic acid, coumaric acid and rosmarinic acid in phenolic acids was investigated by HPLC method in the parts of *H. orientalis*. 26.52 ppm of Gallic acid in the flowers, 7.48 ppm of rosmarinic acid in the body, 20 ppm of caffeic acid in the leaves and 19 ppm of rosmarinic acid and 20 ppm of Gallic acid in the roots of *H. orientalis* plant were determined by HPLC

FT-IR Spectrum analysis

The FTIR spectrum of *H. orientalis* extracts is given in Fig 3 to 6. The data on the peak values and the probable functional groups were presented in Tables 3.

Table 3

FTIR spectral peak values and functional groups obtained for the *H. orientalis* extract

Simple	Frequency (cm^{-1})	Functional group	Possible compound
Stem	3304.0	N-H bending	Amine and Amide Alkaloids
	2930	C-H group	Phenolic Aromatic Compounds
	1723.4	C=O carbonyl group	Saponins
	1592	C=C group	Phenolic Aromatic Compounds
	1035.9/1252.6	C-O-C stretching	Glycosides
Root	3307.1	N-H bending	Amine And Amide Alkaloids
	2855/2921.9 and 3012.8	C-H group	Phenolic Aromatic Compounds
	1707.3	C=O carbonyl group	Saponins
	1035.9/1156.3/1196.4 and 1274	C-O-C stretching	Glycosides
Flower	3248.3	N-H bending	Amine And Amide Alkaloids
	2847/2919.2	C-H group	Phenolic Aromatic Compounds
	1394.3	CH_3	Alkaloids
	1308/1035.9	C-O-C stretching	Glycosides
Leaf	3288.4	N-H bending	Amine and Amide Alkaloids
	2857.7/2921.9	C-H group	Phenolic Aromatic Compounds
	1512	C=C group	Phenolic Aromatic Compounds
	1038.5/1260.6	C-O-C stretching	Glycosides

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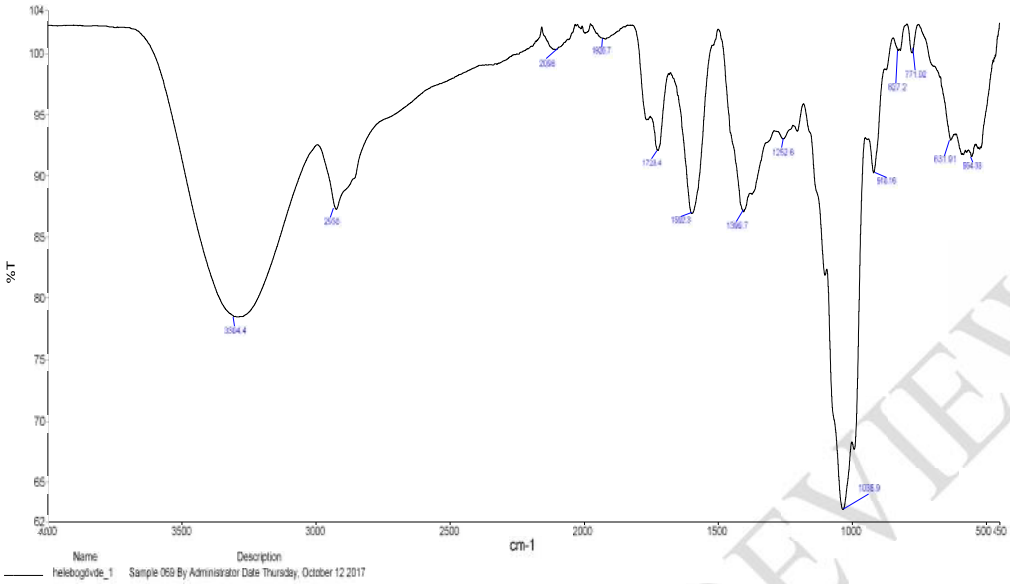

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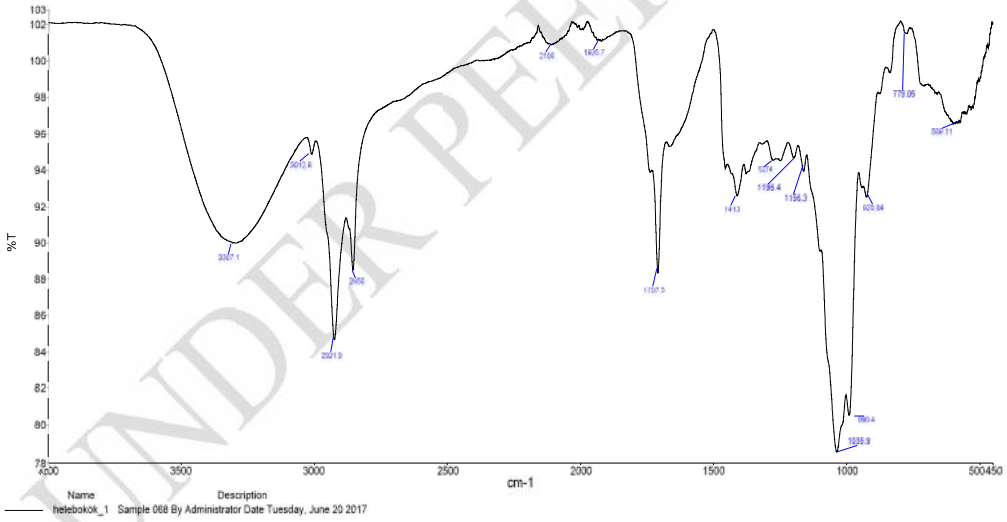
209 **Fig. 3.** FT-IR analysis of *H. orientalis* stem



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211 **Fig. 4.** FT-IR analysis of *H. orientalis* root

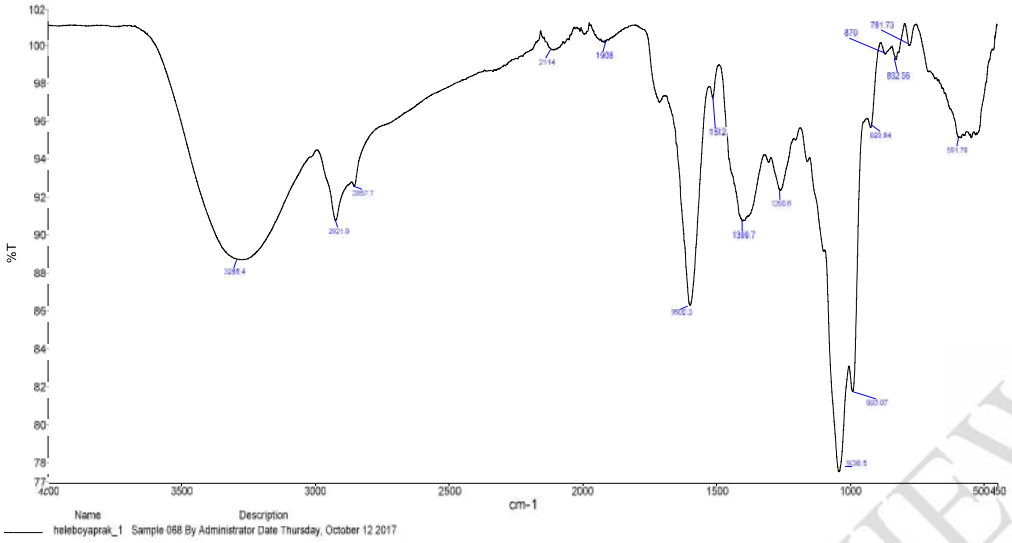

Fig. 5. FT-IR analysis of *H. orientalis* leaf

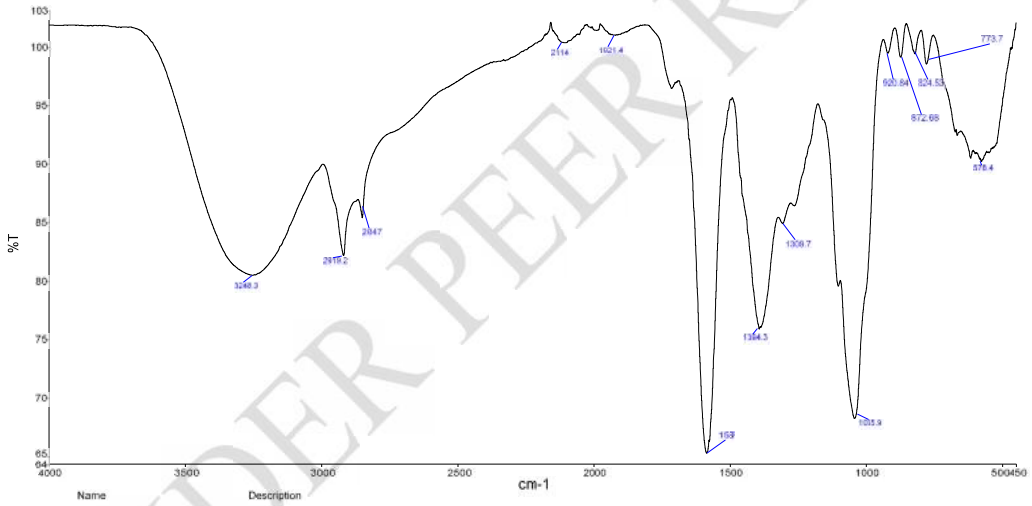

Fig. 6. FT-IR analysis of *H. orientalis* flower

LC-MS results

In LC-MS analysis, glycosides equivalent to the molecular weights of cardioactive glycosides were determined. The hellebrin, hellebrigenin in the leaves of the plant and hellebrigenin in the body, hellebrigenin in the root and the presence of helleborine were determined. In the leaves of the plant hydroxycinnamic acids and other

polar compounds containing caffeic acid, flavonol glycosides containing quercetin have been identified. In the roots of the plant, quercetin containing flavonol glycosides was found. The presence of quercetin-containing flavonol glycosides was determined in the body of the plant. Flowers of the plant have hydroxycinnamic acids and other polar compounds containing caffeic acid and flavonol glycosides quercetin were found.

Antioxidant activity by DPPH Assay

From the plot plotted against the % inhibition value calculated against different concentrations of antioxidants, linear regression resulted in Antioxidant concentrations which cause 50% inhibition was calculated by linear regression. The results are expressed as EC50 (µl) in table 4.

Table 4 EC50 (µl) values of different parts of *H. orientalis*

The methanol extract of *H. orientalis*	EC50 (µl/ml)
Flower (FME)	18.00
Leaf (LME)	13.00
Body(BME)	28.00
Root (RME)	40.00

TAS and TOS levels

There were significant increases of TOS levels whereas decreases TAS in the H_2O_2 group compared to the control group (Table 5). Additionally, treatment leaf extract decreased to their increased oxidant levels in the H_2O_2 group compared to the control group ($p<0.05$). These results supported that the administration of leaf extract decreased Oxidative Stress Index (OSI) compared to the H_2O_2 group ($P < 0.05$).

Table 5 TAS, TOS levels and OSI index in groups.

	TAS (mmol Trolox Equiv./ L.)	TOS (mM H_2O_2 Equiv. / L.)	OSI
Control	7.3 ± 0.02^a	11.2 ± 0.85^a	3.21 ± 1.2^a
H_2O_2	3.4 ± 0.21^b	25.3 ± 0.74^b	8.14 ± 3.12^b

500 µg/L + H_2O_2	3.2±0.14[b]	24.12±0.72[b]	8.10±3.11[b]
1000 µg/L + H_2O_2	3.1±0.11b[c]	23.17±0.69[b]	8.10±3.14[b]
2500 µg/L + H_2O_2	6.3±0.67[cd]	19.07±0.67[c]	4.25±2.14[c]
5.00µg/L + H_2O_2	6.1±0.64[de]	19.21±0.64[dc]	4.29±2.11[dc]
10.000µg/+ H_2O_2	6.2±0.61[e]	19.11±0.81[dc]	4.32±3.01[dc]

*Means with the same letter do not differ statistically at the level of 0.05. SD: Standard Deviation

Protective effect of leaf extract against to H_2O_2

The content of phenolic acid in the fruit fell in the first and second phase of the flowering phase while the phase increased in the fourth (Sahri et al., 2011). For this reason, leaf extracts were used in the Comet experiment. Protective effect of HO leaf extract against to H_2O_2 is given to Table 6. Exposure of HO leaf extract decreased the DNA damage at all concentrations in a dose-dependent manner ($r=0.86$ $p<0.01$) against to H_2O_2. While the highest DNA damage was observed the positive control (305±5.51), the lowest one observed in the negative control (6±1). The decreasing DNA damage showed statistically significant results ($p<0.05$) above the 2500 µg/mL of HO leaf extract.

Table 6 Protective effect of *H. orientalis* leaf extract against to H_2O_2

Treatment	**DNA Damage (Arbitrary Unit ±SD)***
Control	6±1[a]
H_2O_2 200 µM	305.33±5.51[b]
500 µg/mL + H_2O_2	304.33±3.21[b]
1000 µg/mL + H_2O_2	302±5.57[bc]
2500 µg/mL + H_2O_2	296.33±2.52[cd]
5.000 µg/mL + H_2O_2	293.33±3.06[de]
10.000 µg/mL + H_2O_2	288.67±3.51[e]

* Means with the same letter do not differ statistically at the level of 0.05. SD: Standard Deviation

DISCUSSION

Over the past few years, different *Helleborus species* have been the subject of phytochemical investigations because of their potential to produce important secondary metabolites. However, studies in the literature have been limited to a few studies with *H. orientalis* species. To the best of our knowledge, no data have been published on total phenolic and flavonoid content *H. orientalis* and relationship protective effect of leaf extract against to H_2O_2 by Comet assay. Therefore the aim of our study was to examine the possible antioxidant potential of *H. orientalis* Lam species with distribution in Piraziz, Giresun, Turkey.

Kumar and Laltha show that (2014) ethanol extract of *H. niger* exhibits excellent antioxidant activity in streptozotocin and nicotinamide-induced diabetic rats induced type 2 diabetes model. In our study, ethanol was used as the solvent in *H. orientalis* extraction Roman et al (2010) results revealed that the concentrated *H. purpurascens* extracts have a high antioxidant activity. Similar to the findings we have in our study, Paun et al (2014) evaluated that *H. purpurascens* and *H. officinale* polyphenolic extracts and they show that the plant flowers had the highest phenol content followed by the plant leaves. Cakara et al. (2011) reported that the antioxidative properties of *H. orientalis* leaf extracts were caused by high levels of phenolic and flavonoids.

In this study, the concentration of TPC in *H. orientalis* ethanol extracts ranged from 19.42 to 4.39 mg GAE/1 gr of the sample. The plant flowers had the highest phenol content (19.42 mg GAE/1 gr) followed by the plant leaves (17.20 mg GAE/1 gr) (Table 2). The highest amount of flavonoid content was found in FME (11.880 mg QE/g) followed by LME (10.212 mg QE/g) (Table 4). The maximum total flavonoid content of *H. orientalis* methanol extracts was found in flowers and leaves (10.212 mg QE / 1 g) in spectrophotometric measurement (11.880 mg QE / 1 g).

The bands at 1700-1600 cm-1 in the FTIR spectrum are caused by the stretching of the carbonyl groups C = O and C = C or also associated with phenolic molecules (Tahir et al., 2017). It has been reported that the vibration in the spectral region of 1540 2121175 cm-1 may be from O-H, C-O, C-H, and C = C deformation [213], or flavonol and phenol [Tahir et al., 2017; Masek et al., 2014; Nickless et al, 2014). Masek et al. reported that the vibrations in the spectral region of 940-1175 cm^{-1} were due to C-OH groups, C-O in phenol, carbohydrate-linked C-C and C-O (Nickless et al, 2014). In our study, C-H, C = O, C-O-C, C methanol OH, C = C bond stress in root, stem, flower, and leaf methanol extracts. This shows that phenolic

290 compounds are present in the methanol extracts of *H. orientalis*. In this study, the results of
291 HPLC analysis data, LC- Mass, FTIR spectral findings and spectrophotometric phenolic/
292 flavonoid substance support each other.

293 DPPH is considered to be an easy and fast method for the correct determination of
294 antioxidant activity (Katalinic et al, 2004). In this study, the sweeping activity of DPPH
295 radical is found in the highest flower extract. The sweeping activity of the lowest DPPH
296 radical was determined at the root. Plant extracts have higher antioxidant activity than many
297 antioxidant substances. (Table 6). This is probably related to depends on redox properties of
298 phenolic compounds (Adedapo et al., 2009; Katalinic et al, 2004; zheng and Wang, 2001).

299 The presence of phenolic acids (ferulic acid, caffeic acid and chlorogenic acid) and
300 flavonoids (kaempferol and quercetin derivatives) in the methanolic extract of Helleborus
301 atrorubens leaves was shown. (Cakara et al., 2011; Shahri et al., 2011). Many studies
302 demonstrated that caffeic acid has anti-mutagenic and anti-carcinogenic properties. Chen et al
303 (1995) show that caffeic acid is decrease lipoperoxyl radicals by inhibiting the reaction of
304 lipid peroxidation chain.

305 In our study, 26.52 ppm of gallic acid in the flowers, 7.48 ppm of rosmarinic acid in
306 the body, 20 ppm of caffeic acid in the leaves and 19 ppm of rosmarinic acid and 20 ppm of
307 gallic acid in the roots of *H. orientalis* plant were determined by HPLC. Also, we couldn't
308 determine some peaks in HPLC because of didn't use the standards. Although all of the
309 obtained data show that *H. orientalis* has got rich antioxidant content, no literature on the
310 Comet assay of *H. orientalis* plant extracts was found in the literature review. Although, *H.*
311 *odorus* and *H. hercegovinus* extracts have strong antioxidant activity, *H. multifidus* has
312 stronger antiproliferative activity (Cakara et al.1995, Chen et al, 1995). The leaves extracts
313 have better antioxidant activity and secondary metabolites are present in higher amounts than
314 the root extracts. On the other hand, *H. hercegovinus* root extracts exhibit high antitumoral
315 activity. Moreover, Kumar and Lalitha findings directly, verified at the first time that the
316 ethanol extract of *H. niger* root exhibited perfect activities type 2 diabetic rats (Kumar and
317 Lalitha., 2014).

318 *H. orientalis* flower development was divided into six stages and the amount of
319 phenolic content varies according to the flowering period (Zheng and Wang, 2001). For this
320 reason, Leaf extract was used for determination of protective effect ın this study.

Protective effect against oxidative stress of *H. orientalis* was determined using TAS, TOS method and Comet assay. Exposure of *H. orientalis* leaf extract decreased the DNA damage at all concentrations in a dose-dependent manner ($r=0.86$ $p<0.01$) against to H_2O_2 ın the Comet essay. The decreasing DNA damage showed statistically significant results ($p<0.05$) above the 2500 µg/mL of HO leaf extract. OSI value was found to decrease significantly ($p<0.05$) at 2500 4.2g / L of HO leaf extract compared to positive control (4.25 ± 2.14).

CONCLUSIONS

This is the first report on the activity of protective effect of leaf extract of *H. orientalis* against H_2O_2. The study shows that *H. orientalis* leaf and flowers extracts have a high antioxidant activity, thus it can be considered a good source for further medicinal applications. The following investigations should be based on the purification and chemical identification of *H. orientalis* extract.

The results of TAS, TOS and Comet Assay showed that *H. orientalis* had a protective effect against oxidative stress. *H. orientalis* leaf extract also reduced DNA damage. While there are many chemicals and other factors that cause DNA damage, the existence of plants that correct DNA damage is promising for the future.

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