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Original Article

Antioxidant and prooxidant properties of selected herbs and *Citrus bergamia* Risso et Poiteau (bergamot) used for the management of hyperlipidemia

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ABSTRACT

Background and Aims: In recent years, there has been an increased interest in the search for herbs to aid the management of hyperlipidemia. There is currently very little data on the simultaneous evaluation of the antioxidant and prooxidant properties of antihyperlipidemic herbs. This study was designed to evaluate the antioxidant and prooxidant properties of four antihyperlipidemic herbal drugs and also of bergamot.
Methods: Antioxidant property was determined by ferric ions (Fe^{3+}) reducing capacity (IRC), DPPH radical scavenging activity (DPPH IC_{50}) and trolox equivalent antioxidant capacity (TEAC); deoxyribose degradation test was used for prooxidant property.
Results: The highest total phenolic content (TPC) was in the myrtle leaf (ML) (135.35±3.46 mg GAE/g, $p<0.05$) whereas the highest total flavonoid content (TFC) was in green tea (GT) (48.76±0.69 mg QE/g, $p<0.05$) both of which were maintained from a pharmacy. Among the bergamot samples, the highest TPC and TFC values were in filtered fruit juice (BFFJ) as 197.35±6.29 mg GAE/100 mL; and 94.14±1.39 mg QE/100 mL; $p<0.05$, respectively. GT showed the highest antioxidant capacity in IRC and TEAC assays (2.29±0.12 mM TE/g; and 2.32±0.07 mmol TE/mg, $p<0.05$). The lowest DPPH IC_{50} was identified in ML from a pharmacy (6.95±0.08 µg/mL; $p<0.01$). BFFJ had the highest IRC (2.94±0.031 mM TE/10µL), TEAC (5.14±0.084 mmol TE/10 µL) and the lowest DPPH IC_{50} value (10.561±0.17 µL). GT from a pharmacy and 1mg/mL concentration BFLFJ (filtered and lyophilized) were associated with the lowest hydroxyl radical scavenger activity (0.171±0.013 µM MDA equivalent, $p<0.05$ and 0.144±0.015 µM MDA equivalent, $p<0.05$).
Conclusion: BFLFJ and GT got the highest attention due to high TPC, TFC, antioxidant and low prooxidant properties. Our results highlight the necessity of clarifying the value of bergamot and GT in this field with further studies.

Keywords: Antioxidant, Bergamot, *Citrus bergamia*, Medicinal plants, Oxidative stress, Prooxidant

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INTRODUCTION

Cardiovascular (CV) diseases (CVD) are the leading cause of morbidity and mortality in the world (Mach et al., 2019). Hyperlipidemia (HL) is one of the major risk factors for CVD. Statins are effective in reducing CV events and are safe for almost all HL patients (Arca & Pigna, 2011). However, treatment plans do not always work properly, since there may be problems in patient adherence, statin intolerance, and doctors' attitudes to low CV risk patients (Blaha, Nasir, & Blumenthal, 2012; Redberg & Katz, 2012). Thus both patients and scientific societies are searching for natural products (NP) for HL treatment (Arca & Pigna, 2011). There are scientific studies supporting the use of NP alone or associated with other drugs in clinical practice (Cicero, Parini, & Rosticci, 2015). However, there is still an insufficient number of studies demonstrating morbidity and mortality outcomes(Cicero et al., 2017). Due to lack of outcome data, anti-HL NP are still accepted as food supplements or functional foods (Mach et al., 2019). Red yeast rice, bergamot, berberine, dietary fiber, green tea, phytosterols, spirulina and artichoke are the most studied NP for HL (Banach et al., 2018). There are studies for NP to be used alone or in conjunction with anti-HL pharmaceuticals (Patti et al., 2017). NP act through different mechanisms such as decreasing intestinal cholesterol absorption, inhibiting hepatic cholesterol synthesis, and decreasing hepatic low-density lipoprotein cholesterol uptake. In addition to anti-HL effects, NP also improve endothelial function and have anti-inflammatory, antioxidant, and anti-atherosclerotic activities (Cicero et al., 2017).

Phytonutrients are candidate sources of anti-HL actions. However, there is very little data on anti-HL actions of NP in this study. Green tea extract suppressed HL and non-alcoholic fatty liver disease in mice (Kang et al., 2011). Hawthorn extract were found to be capable of decreasing glucose production and triacylglycerol synthesis (Shih, Lin, Lin, & Wu, 2013). Ethanolic extracts of calyces and leaves of *Hibiscus sabdariffa* L. were found to possess significant antioxidant and anti-HL activities (Ochani & D'Mello, 2009). In a review on anti-HL actions of *Citrus bergamia* Risso et Poiteau some studies showed significant decrease in total cholesterol, triglycerides, low-density lipoprotein cholesterol and an increase in high-density lipoprotein cholesterol (Lamiquiz-Moneo et al., 2020).

Oxidant and antioxidant balance is crucially important in maintaining healthy biological systems. This balance seems to be in favor of mild prooxidant to achieve proper signaling through cells for a strong immune reaction (Ho, Karimi Galougahi, Liu, Bhindi, & Figtree, 2013). CVD such as HL leads to an increase in the reactive oxygen species and oxidative stress, thus the vascular endothelial integrity becomes disrupted (Jamwal & Sharma, 2018). Antioxidant properties of anti-HL NP may have important roles against this mechanism. Flavonoids and other phenolic compounds have strong antioxidant properties and the ability to protect cells from reactive radicals (Shayganni, Bahmani, Asgary, & Rafieian-Kopaei, 2016). Although antioxidant activities are well known, exogenous antioxidants also show prooxidant activity at high doses and particularly in the presence of metal ions such as iron and copper (Azam, Hadi, Khan, & Hadi, 2004). Antioxidant and prooxidant effects of NP have been studied in the literature. To the best of our knowledge, there are as yet no data on the simultaneous evaluation of antioxidant and prooxidant capacities of anti-HL NP. Therefore, in the present study, we aimed to screen the antioxidant and prooxidant properties of *Citrus bergamia* juice and bergamot albedo fragment along with selected plant extracts.

MATERIAL AND METHODS

Methodology

Plant material

Various pharmacy and herbal market commercial samples of hawthorn (*Crataegus* L. spp.) flower-leaf (CFL), hibiscus (*Hibiscus sabdariffa*) flower (HF), green tea (*Camellia sinensis* L.) (GT) and myrtle (*Myrtus communis* L.) leaf (ML) were analyzed in our study. Fruit juice and albedo fragments of two different commercial samples of bergamot fruit harvested in Antalya, Turkey, (named as *Citrus bergamia* rissofemminello and Native A41) were also tested. Codes used for the extracts prepared from plant material and for bergamot albedo and fruit juice samples are listed in Table 1.

Preparation of extracts

Methanol was used to prepare the extracts from dried CFL, HF, GT, ML and albedo fragment of bergamot fruit. First, the plant material was ground to powder to obtain fine particles. 10 g of powdered plant material was extracted with 100 mL methanol in an ultrasonic bath for 30 minutes at 30°C. The extract was

Table 1. Extracts prepared from herbal drug specimens and bergamot fruit.

Herbal drug specimens	**From pharmacy**	**From herbal market**
Hawthorn flower-leaf	CFL-1	CFL-2
Hibiscus sabdariffa flower	HF-1	HF-2
Green tea (loose)	GT-1	GT-2
Myrtle leaf	ML-1	ML-2
Bergamot fruit	***Citrus bergamia* rissofemminello**	**Native A41**
Bergamot albedo fragment	BA-1	BA-2
Bergamot Filtered &Lyophilized Fruit Juice	BFLFJ-1	BFLFJ-2
Bergamot Filtered Fruit Juice	BFFJ-1	BFFJ -2

CFL, hawthorn (*Crataegus* L. spp.) flower-leaf; HF, hibiscus (*Hibiscus sabdariffa* L.) flower; GT, green tea (*Camellia sinensis* L.); ML, myrtle (*Myrtus communis* L.) leaf; BA, bergamot albedo fragment; BFLFJ, Bergamot Filtered & Lyophilized Fruit Juice; BFFJ, Bergamot Filtered Fruit Juice.

filtered. This procedure was repeated three times. The filtrates were pooled and then concentrated using a rotary evaporator. The extracts were aliquoted and stored at -20°C until use.

Preparation of bergamot fruit juice

Bergamot fruit juice (BFJ) was obtained by mechanical pressure. The freshly squeezed juices were filtered. Half of the BFFJ was lyophilized then stored at -20°C until use. The other half of the BFFJ was directly stored at -20°C in small aliquots (25 mL).

Determination of total phenolic content (TPC)

TPC was determined by colorimetric Folin–Ciocalteau method (McDonald, Prenzler, Antolovich, & Robards, 2001). Briefly, 500 µL of crude extract (0.1 mg/mL) was mixed thoroughly with 5 ml of %10 Folin–Ciocalteau reagent (1:10 with distilled water) and incubated for 30 minutes; then 4 mL of 1M Na_2CO_3 was added. The mixture was allowed to stand for 60 min in the dark, and absorbance was measured at 760 nm. Gallic acid which was prepared in different concentrations (25, 50, 100, 150, 250 and 250 mg/mL) was used as standard. The TPC was calculated from the gallic acid calibration curve, and the results were expressed as mg of gallic acid equivalent (GAE)±Standard Deviation (SD) per g extract and mg of GAE±SD per 100 mL BFJ.

Determination of total flavonoid content (TFC)

TFC was determined by the colorimetric aluminum chloride method(Chang, Yang, Wen, & Chern, 2002). In brief, 1.5 mL methanol, 0.1 mL of 10% $AlCl_3$ and 0.1 mL of 1M CH_3CO_2Ksolutions were added in the same order to 500 µL of samples (1 mg/mL). Distilled water was added into the tube and the solution was made up to 5 mL. The tubes were incubated for 30 minutes at room temperature and absorbance was measured at 415 nm. TFC of extracts were calculated from the quercetin standard curve (12.5, 25, 50, 75 and 100 µg/mL) and the results were expressed as mg of Quercetin Equivalent (QE)±SD per g extract and mg of QE±SD per 100 mL BFJ.

Determination of antioxidant properties

The antioxidant properties were determined by three methods.

Ferric ions (Fe^{3+}) reducing capacity (IRC)

IRC was determined by a colorimetric method (Oyaizu, 1986). In order to perform IRC assay, 0.4 mL of the sample solution (150 µg/mL and 300 µg/mL) was mixed with 0.4 mL of 1% $[K_3Fe(CN)_6]$. The mixture was incubated at 50˚C for 20 minutes. 0.4 mL TCA (10%) was added to the mixture and centrifuged at 3000xg for 10 minutes. 0.5 mL of the supernatant was mixed with 0.5 mL $FeCl_3$ 1% and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing capacity. The results were expressed as mM Trolox Equivalent (TE) per g extract±SD and mM TE±SD per 10 ul BFJ.

Trolox equivalent antioxidant capacity (TEAC)

TEAC was measured by decolorization of the ABTS radical cation ($ABTS^{+}$)(Re et al., 1999). 7.4 mM ABTS stock solution and 2.45 mM $K_2S_2O_8$ solutions were mixed within the ratio of 2:1 to produce 7 mM $ABTS^{+}$ radical cation. This radical solution was left in the dark at room temperature for 16 hours to reach stable absorbance at 734 nm. $ABTS^{+}$ solution was diluted with 5 mM PBS (pH.7.4) until it had an absorbance of 0.700±0.02 at 734 nm. Then, 1 mL of radical solution was added to the well and the absorbance was measured at 734 nm using 5 mM PBS (pH:7.4) as blank. The absorbance value was recorded as $A_{ABTS^{+}}$.1 mL of radical solution was mixed with 10 µL test solution (0.1 mg/mL) and the absorbance was recorded at 6 minutes after the initial mixing. Percentage of inhibition was calculated as follows:

Inhibition% = $[(A_{ABTS^{\cdot+}} - A_{6.min}) \times / A_{ABTS^{\cdot+}}] \times 100$ where $A_{ABTS^{\cdot+}}$ is the absorbance of the $A_{ABTS^{\cdot+}}$ at 734 nm (0.70±0.02) and $A_{6.min}$ is the absorbance after the addition of the sample to the $A_{ABTS^{\cdot+}}$.

Trolox was used as an antioxidant standard (0, 0.25, 0.5, 1, 2 and 2.5 mM). The absorbances of the samples were compared to that of the trolox standard curve. The results were expressed as mmol TE/mg extract±SD and mmol TE/10 µL BFJ±SD.

DPPH radical scavenging activity (DPPH IC50)

Free radical scavenging activity was performed according to DPPH method (K.-J. Wang, Zhang, & Yang, 2006). The samples were reacted with the stable DPPH radical in a methanol solution. Samples or standard antioxidant (ascorbic acid) solution were prepared in methanol. In a 96-well plate, 100 µL of DPPH (200 µM) was mixed with 100 µL of different concentrations of samples or standard. Plates were covered and incubated at room temperature for 30 minutes in the dark. The absorbance of the residual DPPH solution was determined at 517 nm. The inhibition percentage of the samples was calculated as follows:

Inhibition% = $[(A_B - A_A) / A_B \times 100$ where A_B is the absorbance of DPPH radical and A_A is the absorbance of the sample or standard. The results were calculated as average Inhibition $Concentration_{50}$ (IC_{50})±SD and expressed as µg/mL±SD for the extracts and µL±SD for the BFJ.

Determination of prooxidant properties

Deoxyribose degradation test

Determination of prooxidant properties was carried out by deoxyribose degradation test(Mathew & Abraham, 2006). Briefly, 100 µL 2-deoxy- D-ribose (3.36 mM), 100 µL H_2O_2 (1 mM), 100µL $FeCl_3$ (1 mM), 100 µL EDTA (1 mM), 100 µL ascorbic acid (0.1 mM) were mixed with 100 µL sample solution (0.1 mg/mL or 1 mg/mL) in a test tube. The final volume was adjusted to 1 mL with potassium phosphate buffer solution (20 mM, pH 7.4). Tubes were incubated for 1 hour at 37°C. The extent of deoxyribose degradation was measured by the TBA method (Ohkawa, Ohishi, & Yagi, 1979). 1 mL TBA (1 % w/v) and 1 mL TCA (2.8% w/v) were added to the reaction mixture and heated in a water bath at 100 ˚C for 30 minutes. The absorbances of the reaction mixtures were measured spectrophotometrically at 532 nm. Chromogenic TBA reactive product (TBARP) concentrations were calculated from the corresponding absorbance values using MDA standard curve prepared at different concentrations of MDA (0, 0.1, 0.2, 0.4, 0.6, 0.8, and 1 µM). The results were expressed as µM MDA equivalent TBARP.

Statistical analysis

The experiments were performed in triplicate. The results were expressed as mean±SD. The statistical analysis of the results was carried out using GraphPad Prism 5 software (San Diego, CA) and statistical comparisons were made using analysis of variance (ANOVA). A value of $p < 0.05$ was considered as statistically significant.

RESULTS

TPC and TFC

Total phenol and flavonoid contents of the methanol extracts of the herbal drug specimens and bergamot samples are shown in Table 2 and 3, respectively. The highest TPC was detected in ML-1 (135.35±3.46 mg GAE/g extract, $p<0.05$) whereas the lowest was in HF-2 (42.30±1.05 mg GAE/g extract, $p< 0.05$). The highest TFC was measured in GT-1 (48.76±0.69 mg QE/g extract, $p<0.05$) whereas the lowest was in HF-2 (17.09±0.62 mg QE/g extract).

TPC of BFLFJ-1 was as 130.35±8.91 mg GAE/g extract ($p< 0.05$) and TFC of BFLFJ-2 was as 65.93±4.31 mg QE/g extract, ($p<0.05$). BFLFJ samples had the highest TPC and TFC capacity among non-liquid bergamot samples. BFFJ-1 values of TPC

Table 2. TPC, TFC, antioxidant and prooxidant properties of herbal drug specimens.

Herbal Drug Specimens	TPC (mg GAE/g extract)	TFC (mg QE/g extract)	IRC (mM TE/g extract)	TEAC (mmol TE/mg extract)	DPPH IC_{50} (µg/mL)	TBARS	
						0.1 mg/mL extract	1 mg/mL extract
CFL-1	116.22±3.25	41.140±1.39	0.77±0.02	1.66±0.09	27.32±0.18	0.754±0.013	0.261±0.024
CFL-2	83.61±1.32	36.381±2.27	0.65±0.03	1.55±0.12	49.80±0.29	0.811±0.022	0.303±0.011
HF-1	47.09±2.11	17.570±1.01	0.87±0.06	0.73±0.02	41.38±0.49	0.837±0.026	0.594±0.033
HF-2	42.30±1.05	17.090±0.62	0.68±0.03	0.59±0.01	48.13±0.81	0.823±0.022	0.551±0.027
GT-1	115.78±2.74	48.760±0.69 *	2.12±0.10	2.3±0.07*	9.72±0.67 **	0.203±0.007 *	0.171±0.013*
GT-2	114.48±3.49	43.760±1.42	2.29±0.12 *	1.76±0.04	25.19±0.23	0.284±0.009	0.211±0.008
ML-1	135.35±3.46 *	46.620±0.97	1.81±0.05	2.01±0.08	6.95±0.08*	0.803±0.015	0.285±0.016
ML-2	110.56±4.17	39.950±1.98	1.29±0.08	1.69±0.15	8.57±0.43	0.871±0.019	0.337±0.100

Values expressed are means±SD of three parallel measurements. * denotes significant differences ($p<0.05$); BA, bergamot albedo fragment; BFLFJ, Bergamot Filtered & Lyophilized Fruit Juice; BFFJ, Bergamot Filtered Fruit Juice; CFL, hawthorn (*Crataegus* L. spp.) flower-leaf; DPPH IC_{50}, DPPH radical scavenging activity; HF, hibiscus (*Hibiscus sabdariffa* L.) flower; GAE, Gallic Acid Equivalent; GT, green tea (*Camellia sinensis* L.); ML, myrtle (*Myrtus communis* L.) leaf; IRC, Ferric ions (Fe^{3+}) reducing capacity; QE, Quercetin Equivalent; TEAC, Trolox equivalent antioxidant capacity; TE, Trolox Equivalent; TFC, total flavonoid content; TPC, total phenolic content.

Table 3. TPC, TFC, antioxidant and prooxidant properties of bergamot albedo and fruit juice.

Bergamot Albedo and Fruit Juice	TPC (mg GAE/g extract)	TFC (mg QE/g extract)	IRC (mM TE/g extract)	TEAC (mmol TE / mg extract)	DPPH IC_{50} (µg/mL)	TBARS (µM MDA equivalent)	
						0.1 mg/mL extract	1 mg/mL extract
BA-1	39.26±3.70	20.19±0.37	2.11±0.04	1.3±0.047	25.19±0.37	0.709±0.033	0.567±0.029
BA-2	48.83±3.64	39.65±0.74	2.04±0.08	1.24±0.039	26.65±0.74	0.26±0.027	0.598±0.038
BFLFJ-1	130.35±8.91*	50.68±3.38	2.73±0.12*	1.86±0.086*	26.65±0.96	0.254±0.025*	0.151±0.011
BFLFJ-2	80.62±6.54	65.93±4.31*	1.98±0.18	1.19±0.067	14.18±0.59*	0.220±0.016	0.144±0.015*
	TPC (mg GAE/100 mL BFJ)	TFC (mg QE/100 mL BFJ)	IRC (mM TE/10 µL BFJ)	TEAC (mmol TE/10 µL BFJ)	IC_{50} (µL)	TBARS (µM MDA equivalent)	
						10 µL	100 µL
BFFJ-1	197.35±6.29 *	71.14±1.04	2.94±0.031*	5.14±0.084*	12.735±0.08	0.251±0.026	0.073±0.006
BFFJ-2	185.61±8.14	94.14±1.39*	2.69±0.047	4.29±0.091	10.561±0.1*	0.287±0.017	0.091±0.003

Values expressed are means±SD of three parallel measurements. * denotes significant differences ($p<0.05$); BA, bergamot albedo fragment; BFLFJ, Bergamot Filtered & Lyophilized Fruit Juice; BFFJ, Bergamot Filtered Fruit Juice; DPPH IC_{50}, DPPH radical scavenging activity; GAE, Gallic Acid Equivalent; IRC, Ferric ions (Fe^{3+}) reducing capacity; QE, Quercetin Equivalent; TEAC, Trolox equivalent antioxidant capacity; TE, Trolox Equivalent; TFC, total flavonoid content; TPC, total phenolic content.

were found to be higher than BFFJ-2 (197.35±6.29 mg GAE/100 mL BFJ; 185.61±8.14 mg GAE/100 mL BFJ, p<0.05 respectively). BFFJ-2 had higher TFC than BFFJ-1 (94.14±1.39 mg QE/100 mL BFJ, 71.14±1.04 mg QE/100 mL BFJ, p<0.05 respectively). Since the other bergamot samples were in non-liquid form, TPC and TFC of BFFJ-1 and BFFJ-2 were not compared and no further analysis was done.

Antioxidant properties

GT-2 had the highest IRC among the methanol extracts of herbal drug specimens (2.29±0.12 mM TE/g extract *p*<0.05). The highest TEAC values were measured in GT-1 (2.32±0.07 mmol TE/mg extract, p<0.05). The lowest DPPH IC_{50} was identified in ML-1 (6.95±0.08 µg/mL; p<0.01). The second lowest IC_{50} was detected in GT-1 (9.72±0.67 µg/mL; p<0.05). Antioxidant properties of herbal drug specimens are shown in Table-2.

The highest IRC among BA and BFLFJ samples was identified in BFLFJ-1 (2.73±0.12 mM TE/g extract, *p*<0.05). The highest TEAC among BA and BFLFJ samples was measured in BFLFJ-1 (1.86±0.086 mmol TE/mg extract, p<0.05). The lowest DPPH IC_{50} values were detected in BFLFJ-2 (14.18±0.59 µg/mL, p<0.05) among BA and BFLFJ. The IRC of BFFJ-1 was 2.94±0.031 mM TE/10µL (*p*<0.05); TEAC value of BFFJ-1 was 5.14±0.084 mmol TE/10µL, (p<0.05), and the DPPH IC_{50} value of BFFJ-2 was 10.561±0.17 µL, (p<0.05) among the BFFJ samples. The antioxidant capacity of BFFJ was not compared with other bergamot samples since they were in non-liquid form and therefore any further analysis was not performed. The antioxidant properties of bergamot albedo and fruit juice samples are presented in Table-3.

Prooxidant properties

Among all the non-liquid samples, GT-1 and BFLFJ-2 with 1mg/mL concentration were associated with the lowest TBARP (0.171±0.013 µM MDA equivalent, p<0.05; 0.144±0.015 µM MDA equivalent, p<0.05 respectively). The prooxidant capacity of BFFJ-1 was 0.073±0.006 µM MDA equivalent however, since the other bergamot samples were in non-liquid form no further analysis was done. Prooxidant activity of herbal drug specimens and bergamot samples are shown in Table 2 and Table 3, respectively.

DISCUSSION

TPC and TFC

Phenolic compounds are the most abundant antioxidants in the human diet. They have a considerable structural diversity. Flavonoids and other phenolic compounds are well-known plant secondary metabolites for their antioxidant, antibacterial, anti-cancer, cardioprotective, anti-HL, immune system promoting and anti-inflammatory effects (Tungmunnithum, Thongboonyou, Pholboon, & Yangsabai, 2018). In our study, the highest statistically significant TPC was detected in ML purchased from a pharmacy. Studies have shown that ML must be considered as good sources of phenolic compounds(Amensour, Sendra, Abrini, Pérez-Alvarez, & Fernández-López, 2010; Benchikh, Amira, & Benabdallah, 2018). Benchikh et al. found TPC as 149.25±3.11 mg GAE/g of dry extract resembling our result as 135.35±3.46 mg GAE/g of extract in ML (Benchikh et al., 2018).

In our study, the highest statistically significant TFC was measured in GT purchased from a pharmacy. According to Graham et al. GT had high amounts of flavonoids (Graham, 1992). Wang et al. reported that flavonoid was believed to be responsible for antioxidant, anticarcinogenic and anti-atherosclerotic activities (H. Wang & Helliwell, 2001).

Bergamot is a common Italian citrus fruit, cultivated almost exclusively to produce essential oils; the juice is considered a waste product (Pernice et al., 2009). However, other parts of bergamot have also drawn attention recently due to their polyphenolic, mainly flavonoid, content (Mannucci et al., 2017). Flavonoids from citrus fruits have many health benefits including anticancer, antiviral, and anti-inflammatory properties , as well as effects on capillary fragility, inhibition activity on human platelet aggregation, and prevention of diet-induced HL (Picerno et al., 2011).

In our study, among all the non-liquid samples, the highest TPC and TFC were identified in BFLFJ samples. BFFJ also had high TPC and TFC; however, these samples were not comparable due to liquid/non-liquid forms. Previous studies on bergamot juice were mostly conducted on its liquid form, to the best of our knowledge. Ercisli et al. showed similar results to our study in which the TPC of Turkish bergamot cultivated in Mersin was measured as "30.37±2.15 mg GAE/100 mL"(Sezai Ercisli et al., 2015). However, Yıldız Turgut et al. demonstrated a much lower TPC of Native A41 BJ than our study which was "30.37±2.15 mg GAE/100 mL" (Yıldız Turgut, D; Seçmen,Tuba; Tanır, 2018). TPC and TFC obtained by Pozzo et al. from lyophilized juices of "femminello" cultivars of *C. bergamia*, were also lower than the results of our study (Da Pozzo et al., 2018).

Antioxidant properties

In our study, GT-2 had the highest statistically significant IRC among the tested drug specimens. IRC is a good indicator for potential antioxidant activity and this is mainly based on reductones. One of the mechanisms of the antioxidant action of reductones is based on the breaking of free radical chain by donating a hydrogen to neutralize free radical(Jayaprakasha, Singh, & Sakariah, 2001). Simamora et al. found similar high IRC for methanol extract of GT(Simamora, Steven, Santoso, Rumiati, & Timotius, 2018). This study also suggested that GT extracts may act as electron donors which react with free radicals, converting them to more stable products, thus enabling a terminate radical chain reaction.

In the current study, the highest statistically significant TEAC was measured in GT-1 among herbal drug specimens. Zhao et al. showed TEAC as 1.89±0.31 mmol TE/g extract in aqueous extract of GT (Zhao et al., 2019). However, De la Luz Cádiz-Gurrea et al. demonstrated a high TEAC with methanol extract of GT, 9.66±1.27 mmol TE/g extract in accordance with our results (de la Luz Cádiz-Gurrea, Fernández-Arroyo, & Segura-Carretero, 2014).

The lowest statistically significant DPPH IC_{50} value was identified in ML; GT was the second lowest among the herbal drug specimens. Free radical scavenging ability is an important antioxidant capacity. Oxidative stress, caused by reactive oxygen or free radicals, has been shown to be associated with the pro-

gression of many diseases including cancer, heart disease, and depression, among others (Kovacic & Jacintho, 2001) Benchikh et al. had found significantly low levels of DPPH IC_{50} values of aqueous and methanol extracts of ML, thus supporting our data (Benchikh et al., 2018). Another supporting data for powerful DPPH radical scavenging capacity of GT was demonstrated by Simamora et al. in different extracts of GT (Simamora et al., 2018).

In this study, BFLFJ-1 had the highest statistically significant IRC, TEAC and BFLFJ-2 had the lowest DPPH IC_{50} value among the non-liquid bergamot samples. Furthermore, among all the non-liquid samples, the highest antioxidant capacity was identified in BFLFJ samples. BFFJ also displayed remarkable antioxidant capacities, however these samples were not comparable since BFFJ was in liquid form. Most of the previous studies were conducted on BFFJ liquid form; Turgut et al. demonstrated a higher DPPH IC_{50} value of Native A41 BJ (Turgut, Demet Yıldız; Seçmen, Tuba; Tanır, 2018). The antioxidant potential of BJ was examined in the DPPH and ferric reducing power assays, the BJ showed a noticeable antioxidant effect in hypercholesterolemic diet-induced renal damage *in-vitro* models (Trovato et al., 2009). DPPH values of different cultivars of *C. bergamia* were found to be significantly associated with the TPC of BJ, thus supporting our data (Vincenzo, Sicari. Pellicanò, 2016). Pernice et al. also suggested that adding BJ to apricot and apple juices significantly increased the antioxidant capacity (Pernice et al., 2009).

Prooxidant properties

Although medicinal plant effects in prevention and treatment of disorders have been widely attributed to their antioxidant activities, there is increasing evidence pointing to their prooxidant hazardous effects, too. Polyphenols in medicinal plants can act as either antioxidants or prooxidants, depending on conditions such as the presence of oxygen or transition metalsand the concentration of the extract (Nasri & Rafieian-Kopaei, 2014). Therefore, prooxidant capacity was evaluated in our study via TBARS. GT-1 had the lowest statistically significant TBARS. There is an increasing amount of evidence suggesting epigallocatechin-3-gallate (EGCG), the main polyphenolic constituent in GT, has prooxidative properties (Elbling et al., 2005). High concentration of EGCG is suggested to make spontaneous H_2O_2 induced oxidative cell damage in *in vitro* models. Joubert et al. demonstrated *in vitro* prooxidant activity of potent antioxidant dietary supplement extracts via TBARS (Joubert, Winterton, Britz, & Gelderblom, 2005). In addition to having potent free radical scavenging activities and TEAC, GT-1 also have low prooxidant property. Our findings suggest that GT purchased from a pharmacy can be used as a safe source of antioxidant.

There is very little data on the prooxidant property of citrus plants. Simić et al. demonstrated the relationship between antioxidative and prooxidative activities and oxidation potentials among grapes, citrus, apple(Simić, Manojlović, Šegan, & Todorović, 2007). This study demonstrated the need for simultaneous evaluation of antioxidant and prooxidant capacities. Our data on prooxidant capacity showed that among the herbal drug specimens and the bergamot samples, GT purchased from a pharmacy and BFLFJ with 1mg/mL concentration were associated with the lowest prooxidant capacity.

CONCLUSION

Flavonoids and other phenolic compounds have recently been getting the attention of HL researchers. Beyond anti-HL actions, these phytochemicals also exhibit antioxidant activities which may further enhance anti-HL effects. However, recent evidence has shown that exogenous antioxidants also show prooxidant activity. Thus, selecting the least hazardous anti-HL NP with a balanced antioxidant and prooxidant capacity becomes important. Our study suggests that BFLFJ and GT may be used as safe antioxidant sources since they have high TPC, TFC, antioxidant and low prooxidant capacities. This study highlights the necessity of clarifying the value of bergamot and GT in this field with further studies.

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