Research Article

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Antioxidant potential of some natural and semi-synthetic flavonoid derivatives and the extracts from *Maclura pomifera* (Rafin.) Schneider (osage orange) and its essential oil composition

Maclura pomifera'dan (Rafin.) Schneider elde edilen ekstre ve bazı flavonoit türevlerinin antioksidan aktivitesi ve uçucu yağ bileşimi

DOI 10.1515/tjb-2016-0125
Received April 29, 2015; accepted March 29, 2016; previously published online September 23, 2016

Abstract

Objective: The antioxidant potential of various extracts were obtained from the leaf and fruit of *Maclura pomifera* (Rafin.) Schneider (Moraceae) along with its major isoflavonoids; osajin and pomiferin, their semi-synthetic derivatives; *iso*-osajin and *iso*-pomiferin and macluraxanthone, which were assayed.
Methods: The extracts and compounds were subjected to six experimental models including 2,2′-diphenyl-1-picrylhydrazyl (DPPH), *N,N*-dimethyl-*p*-phenylendiamine ($DMPD^+$), and nitric oxide (NO) radical scavenging activity, metal-chelating capacity, ferric- (FRAP) and phosphomolibdenum-reducing antioxidant power (PRAP) assays by using ELISA methods.
Results: The fruit aqueous extract exerted higher scavenging activity against DMPD and NO radicals, while the fruit ethyl acetate extract was the most active against DPPH radical (68.61 ± 2.53%). Among the tested compounds, the highest DPPH (91.74 ± 0.26%) and DMPD (30.63 ± 1.31%) radical scavenging effect was observed with macluraxanthone, while pomiferin and *iso*-pomiferin exhibited better activity than osajin and *iso*-osajin in all assays except the metal-chelation capacity assay. Phytol was the major compound in both the leaf oils, while the fruit essential oil contained β-caryophyllene as the main component (69.3%).
Discussion and Conclusion: The current study covers particularly antioxidant capacity of *iso*-osajin, *iso*-pomiferin, and macluraxanthone by the aforementioned methods and, among them; pomiferin seems to be a natural possible antioxidant agent.

Keywords: *Maclura pomifera*; Antioxidant; Osajin; Pomiferin; Macluraxanthone; Essential oil.

Özet

Amaç: Mevcut çalışmada, *Maclura pomifera'nın* (Rafin.) Schneider (Moraceae) yaprak ve meyvalarından elde edilen çeşitli ekstreler ile başlıca flavonoitleri olan osajin ve pomiferin, bunların yarı-sentetik türevleri olan *izo*-osajin ve izo-pomiferin ile makluraksanton'un antioksidan potansiyeli incelenmiştir.
Metot: Ekstreler ve bileşiklere, 2,2′-difenil-1-pikrilhidrazil (DPPH), *N,N*-dimetil-*p*-fenilendiamin ($DMPD^+$) ve nitrik oksit (NO) radikal süpürücü aktivite, metal-şelasyon

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kapasitesi, demir- (FRAP) ve fosfomolibdenyum-indirgeme antioksidan gücü (PRAP) dahil altı metot ELISA mikrotiter yöntemi ile uygulanmıştır.
Bulgular: Meyva etil asetat ekstresi DPPH radikaline karşı en aktif ekstre iken (%68.61 ± 2.53), meyvanın sulu ekstresi DMPD ve NO radikallerine karşı daha yüksek süpürücü etki göstermiştir. Test edilen bileşikler içinde, metal-şelasyon haricindeki tüm testlerde, pomiferin ve izo-pomiferin, osajin ve izo-osajin'den daha iyi aktivite gösterirken, en yüksek DPPH (%91.74 ± 0.26) ve DMPD (%30.63 ± 1.31 %) radikal süpürücü etki makluraksanton'da görülmüştür. Erkek ve dişi ağaçların yaprakları ile meyvanın uçucu yağı GC-MS ile analiz edilmiştir. Meyva uçucu yağı β-karyofillen'i (%69.3) ana bileşen olarak taşırken, her iki yaprakta da fitol ana bileşendir.
Tartışma ve Sonuç: Bu çalışma, bahsigeçen yöntemler kullanılarak özellikle izo-osajin, izo-pomiferin ve makluraksanton'un antioksidan kapasitesini kapsamaktadır ve pomiferin doğal muhtemel bir antioksidan ajan olarak görünmektedir.

Anahtar Kelimeler: *Maclura pomifera*; Antioksidan; Osajin; Pomiferin; Makluraksanton; Uçucu yağ.

Introduction

Maclura pomifera (Rafin.) Schneider (Moraceae), known by local names such as "osage orange, hedge apple, box wood, and horse apple", is a tree species natively grown in the United States. However, it is widely cultivated throughout the world for ornamental purposes and, therefore, it is practically naturalized in Turkey. *Maclura pomifera* (MP) is a dioecious plant which means it has separate male and female sexes. The fruit of MP that contain sticky white latex sap is well-known for its rich isoflavonoid and xanthone content [1–4]. The extracts of the plant as well as its flavonoid derivatives have so far been reported to possess several desired biological activities including insecticidal [5] (Peterson et al. 2002), estrogenic [6], protective effect on ischemic reperfusion [7], anti-inflammatory and antinociceptive [8], anticholinesterase [9], anticancer [10] and antidiabetic [11] effects.

We previously worked on the anti-inflammatory, antinociceptive and cholinesterase inhibitory effect of the isolated compounds and extracts of MP growing in Turkey and the Czech Republic [8, 9, 12]. In continuation of our studies on MP, the present study was designed to evaluate the in vitro antioxidant potential of the ethyl acetate, ethanol, aqueous, and *n*-butanol extracts prepared from the fruit of MP along with the leaf ethanol extract of the female tree using six experimental models including 2,2-diphenyl-1-picrylhydrazyl (DPPH), *N*,*N*-dimethyl-*p*-phenylendiamine ($DMPD^{+}$), and nitric oxide (NO) radical scavenging activity, metal-chelating capacity, ferric- (FRAP) and phosphomolibdenum-reducing antioxidant power (PRAP) assays adapted to ELISA microtiter methods. Besides, the major isoflavonoids; osajin and pomiferin, their semi-synthetic derivatives; *iso*-osajin and *iso*-pomiferin, and macluraxanthone (Figure 1) isolated from MP were also assayed in the same manner. In addition, the essential oils obtained from the leaves of the female and male trees as well as the fruit of MP were analyzed by capillary gas chromatography-mass spectrometry (GC-MS).

Materials and methods

Plant material

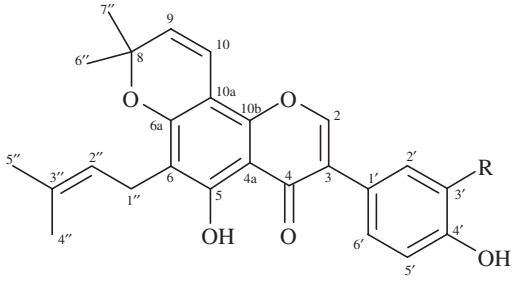
The leaf and fruit parts of MP used in this study were collected separately from the male and female trees growing in the Garden of Herbal Plants of the Department of Natural Drugs, Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic in 2007. Voucher specimen (MPF 001030) of the plant material is kept in the Herbarium of the Department of Natural Drugs (Brno, Czech Republic).

Preparation of the extracts

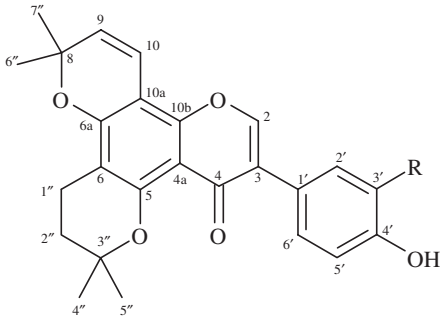
The leaf and fruit of MP were chopped by knife and dried at room temperature. Later on, each plant part was sequentially extracted with ethyl acetate (EtOAc), ethanol (EtOH), distilled water (H_2O), and *n*-butanol which were then evaporated in vacuo to afford their corresponding crude extracts.

Isolation of osajin and pomiferin and semi-synthesis of *iso*-osajin and *iso*-pomiferin

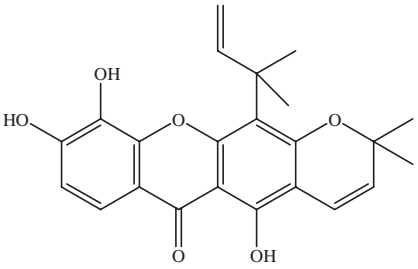
The fruits of MP were chopped by knife, frozen by liquid nitrogen, powdered and then extracted by methanol using Soxhlet extraction. After cooling down, a crude crystallic yellow product and parent solution were obtained. Later on, osajin and pomiferin were isolated following the



1 R= H (Osajin)
2 R= OH (Pomiferin)



3 R= H (*Iso*-osajin)
4 R= OH (*Iso*-pomiferin)



Macluraxanthone

Figure 1: Chemical structures of osajin and pomiferin (1,2), their *iso*-derivatives (3,4), and macluraxanthone.

method of Wolfrom and Mahan [13]. Cleanness of the compounds obtained was checked by HPLC.

Osajin and pomiferin used as the starting material were isomerized in the presence of formic acid and kept at room temperature for 2 days, then water was added and the whole mixture was extracted with chloroform. New derivatives were identified as *iso*-osajin and *iso*-pomiferin based on NMR data, as described in our former publication [9].

Antioxidant microtiter assays

Radical scavenging-based antioxidant activity assays

DPPH radical scavenging assay

The hydrogen atom or electron donation capacity of the corresponding samples was computed from the bleaching property of the purple-colored methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). The stable DPPH radical scavenging activity of the extracts was determined by the method of Blois [14]. The samples (2700 µL) dissolved in ethanol (75%) were mixed with 300 µL of DPPH solution (1.5×10^{-4} M). The remaining DPPH amount was measured at 520 nm using a Unico 4802 UV-visible double beam spectrophotometer (Dayton, NJ, USA). The results were compared to that of gallic acid employed as the reference.

DMPD radical scavenging assay

Principal of the assay is based on reduction of the purple-colored radical $DMPD^+$ (*N*,*N*-dimethyl-*p*-phenylendiamine) [15]. According to the method, a reagent comprising of 100 mM DMPD, 0.1 M acetate buffer (pH = 5.25), and 0.05 M ferric chloride solution, which led to the formation of the DMPD radical, was freshly prepared and the reagent was equilibrated to an absorbance of 0.900 ± 0.100 at 505 nm. Then, the reagent (1000 µL) was mixed with 50 µL of the sample and reference solutions dissolved in ethanol (75%) and absorbance was taken at 505 nm using a Unico 4802 UV-visible double beam spectrophotometer (USA). Quercetin was employed as the reference and the experiments were done in triplicate.

Nitric oxide (NO) radical scavenging activity

The scavenging activity of the extracts against NO was assessed by the method of Marcocci et al. [16]. Briefly, the sample and reference dilutions were mixed with 5 mM sodium nitroprusside and left to incubate for 2 h at 29°C. An aliquot of the solution was removed and diluted with Griess reagent (1% sulfanilamide in 5% H_3PO_4 and 0.1%

naphthylethylenediamine dihydrochloride). The absorbance of the occurred chromophore was measured at 550 nm using a Unico 4802 UV-visible double beam spectrophotometer (USA).

Metal-based antioxidant activity assays

Fe^{2+}-ferrozine test system for metal-chelation

The metal-chelating effect of the samples by Fe^{2+}-ferrozine test system was estimated in consistent with Chua et al.'s method [17]. Accordingly, 740 µL of ethanol and 200 µL of the samples dissolved in ethanol (75%) were incubated with 2 mM $FeCl_2$ solution. The reaction was initiated by the addition of 40 µL of 5 mM ferrozine solution into the mixture, shaken vigorously, and left standing at ambient temperature for 10 min. The absorbance of the reaction mixture was measured at 562 nm. The ratio of inhibition of ferrozine-Fe^{2+} complex formation was calculated as given in "Data processing for antioxidant activity assays" and ethylenediaminetetraacetic acid (EDTA) was employed as the reference in this assay.

Ferric-reducing antioxidant power (FRAP) assay

The FRAP of the extracts and reference was tested using the assay of Oyaizu [18] based on the chemical reaction of Fe(III) ≥ Fe(II). Different concentrations of the extracts dissolved in ethanol (75%) were added into 2500 µL of phosphate buffer (pH 6.6) and 2500 µL of potassium ferricyanide [$K_3Fe(CN)_6$] (1%, w/v). Later, the mixture was incubated at 50°C for 20 min and then 2500 µL of trichloroacetic acid (10%) was added. After the mixture was shaken vigorously, this solution was mixed with 2500 µL of distilled water and $FeCl_3$ (100 µL, 0.1%, w/v). After 30 min incubation, absorbance was read at 700 nm using a Unico 4802 UV-visible double beam spectrophotometer (Dayton, NJ, USA). Analyses were achieved in triplicate. Chlorogenic acid was the reference in this assay.

Phosphomolibdenum-reducing antioxidant power (PRAP) assay

In order to perform PRAP assays on the extracts, the samples were mixed with 10% phosphomolybdic acid solution in ethanol (w/v) [19]. Then, each solution was subsequently subjected to incubation at 80°C for 30 min and the absorbance was read at 600 nm using a Unico 4802 UV-visible double beam spectrophotometer (USA) and compared to that of quercetin as the reference.

Data processing for antioxidant activity assays

Inhibition of DPPH, DMPD, and metal-chelation capacity was calculated as given below and the results were expressed as percent inhibition (I%):

$I\% = [(A_{blank} - A_{sample})/A_{blank}] \times 100$, where A_{blank} is the absorbance of the control reaction (containing all reagents except the test sample), and A_{sample} is the absorbance of the extracts. Analyses were run in triplicate and the results were expressed as average values with SEM. For FRAP and PRAP assays, the analyses were also achieved in triplicate and increased absorbance of the reaction meant increased reducing power in both assays.

Statistical analysis of data

Data obtained from in vitro enzyme inhibition and antioxidant experiments were expressed as the mean standard error (±SEM). Statistical differences between the reference and the sample groups were evaluated by ANOVA (one way). Dunnett's multiple comparison tests were used as post hoc tests. $p < 0.05$ was considered to be significant [*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, ****$p < 0.0001$].

Determination of total phenol and flavonoid contents in the extracts of MP

Amount of phenolics in the extracts was determined in accordance with Folin-Ciocalteau's method [20]. In brief, a number of dilutions of gallic acid dissolved in ethanol (75%) were obtained to prepare a calibration curve. The extracts and gallic acid dilutions were mixed with 750 µL of Folin-Ciocalteau's reagent and 600 µL of sodium carbonate in test tubes. The tubes were then vortexed and incubated at 40°C for 30 min afterward, absorption was measured at 760 nm at a Unico 4802 UV-visible double beam spectrophotometer (USA). Total flavonoid content of the extracts was calculated by aluminum chloride colorimetric method [21]. To sum up, a number of dilutions of quercetin dissolved in ethanol (75%) were obtained to prepare a calibration curve. Then, the extracts and quercetin dilutions were mixed with 95% ethanol, aluminum chloride reagent, 100 µL of sodium acetate as well as distilled water. Following incubation for 30 min at room temperature, absorbance of the reaction mixtures was measured at wavelength of 415 nm with a Unico 4802 UV-visible double beam spectrophotometer (USA). The total phenol and flavonoid contents of the extracts were expressed as gallic acid and quercetin equivalents (mg g^{-1} extract), respectively.

Isolation of the essential oils from MP

The fresh leaves from female and male trees and fruits of MP were hydrodistilled for 3 h using a Clevenger-type apparatus to produce a small amount of essential oil for each which was trapped in *n*-hexane.

GC-MS analysis

The GC-MS analysis was carried out with an Agilent 5975 GC-MSD system. Innowax FSC column (60 m × 0.25 mm, 0.25 μm film thickness) was used with helium as a carrier gas (0.8 mL min^{-1}). GC oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4°C min^{-1}, kept constant at 220°C for 10 min, and then programmed to 240°C at a rate of 1°C min^{-1}. Split ratio was adjusted to 40:1. The injector temperature was set at 250°C. Mass spectra were recorded at 70 eV. Mass range was from *m/z* 35 to 450.

GC analysis

The GC analysis was carried out using an Agilent 6890N GC system. FID detector temperature was 300°C. To obtain the same elution order with GC-MS, simultaneous auto-injection was done on a duplicate of the same column applying the same operational conditions. Relative percentage amounts of the separated compounds were calculated from FID chromatograms.

Identification of essential oil components

Compositional identification of the essential oils was carried out by comparison of their relative retention times with those of authentic samples or by comparison of their relative retention index (RRI) to series of *n*-alkanes. Computer matching against commercial (Wiley GC/MS Library, Adams Library, MassFinder 3 Library) [22, 23] and in-house "Baser Library of Essential Oil Constituents" built up by genuine compounds and components of known oils, as well as MS literature data [24, 25] was used for the identification.

Results

Antioxidant results of the compounds and extracts of MP

In the current study, the EtOAc, EtOH, H_2O, and *n*-BuOH extracts from the fruit and the EtOH extract prepared from the leaves of the female tree of MP were subjected to six antioxidant assays using ELISA microtiter assays at 250 μg mL^{-1}, while osajin, pomiferin, their *iso*-derivatives, and macluraxanthone were also assessed in the same manner at the concentration of 100 μg mL^{-1}. Among the compounds tested, macluraxanthone was the most active in scavenging DPPH (91.74 ± 0.26%) and DMPD (30.63 ± 1.31%) radicals, while the highest NO scavenging effect was observed with *iso*-pomiferin (64.31 ± 0.70%) (Figure 2). Pomiferin and its *iso*-derivative have shown better antioxidant effects than osajin and *iso*-osajin in all cases except metal-chelating

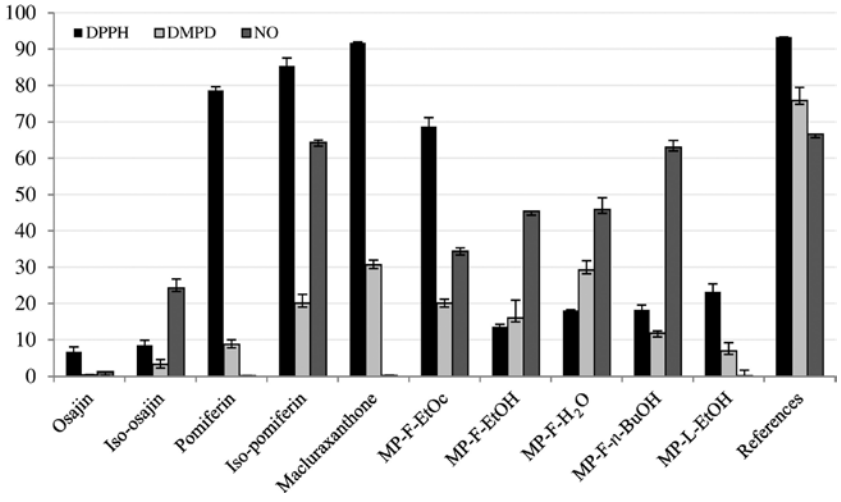


Figure 2: Radical scavenging activity of the flavonoid derivatives and the extracts of MP. F, fruit; L, leaf; references: quercetin for DPPH and ascorbic acid for DMPD and NO radical scavenging activity.

Table 1: FRAP and PRAP results of the flavonoid derivatives and the extracts from MP.

	Ferric-reducing antioxidant power[a] (FRAP[b]) (Absorbance at 700 nm ± SEM[c])	Phosphomolibdenum-reducing antioxidant power[a] (PRAP[b]) (Absorbance at 600 nm ± SEM)
Osajin	0.354 ± 0.007[h]	0.076 ± 0.002[h]
Pomiferin	0.926 ± 0.018[g]	0.144 ± 0.002[h]
Iso-osajin	0.205 ± 0.017[h]	0.130 ± 0.006[h]
Iso-pomiferin	1.263 ± 0.051[g]	0.652 ± 0.018[e]
Macluraxanthone	0.942 ± 0.038[g]	0.082 ± 0.002[h]
MP-F-EtOAc	1.237 ± 0.048[g]	0.266 ± 0.009[h]
MP-F-EtOH	0.401 ± 0.011[h]	0.129 ± 0.001[h]
MP-F-H_2O	0.484 ± 0.009[h]	0.192 ± 0.004[h]
MP-F-*n*-BuOH	0.580 ± 0.008[h]	0.203 ± 0.005[h]
MP-L-EtOH	0.516 ± 0.016[h]	0.221 ± 0.005[h]
Quercetin[d]	2.027 ± 0.032	0.594 ± 0.018

[a]Higher absorbance indicates greater antioxidant activity. [b]Final concentration in microplate well is 125 μg mL^{-1} for FRAP and 625 μg mL^{-1} for PRAP assays. [c]Standard error mean (n = 3). [d]Reference tested at 125 μg mL^{-1} for FRAP and 625 μg mL^{-1} for PRAP assays. [e]$p < 0.05$; [f]$p < 0.01$; [g]$p < 0.001$; [h]$p < 0.0001$.

capacity, while the extracts exerted varying levels of antioxidant activity according to the method used (Table 1, Figures 2 and 3).

Total phenol and flavonoid contents of the extracts and essential oil compositions of MP

Calibration equations were established as $y = 2.6698x + 0.1194$ ($r^2 = 0.9983$) for total phenol and $y = 3.1586x + 0.1018$ ($r^2 = 0.9971$) for total flavonoid contents in the present study. As displayed in Table 2, the fruit EtOAc extract of MP was determined to have the richest total phenolic and flavonoid contents. Considering the GC-MS analysis of the essential oils from MP, chemical composition of the female and male leaf essential oils was found to be very similar to each other, where phytol (*syn*. 3,7,11,15-tetramethylhexadec-2-en-1-ol), an acyclic diterpenic alcohol, was the major component in both of them (Table 3). On the other hand, β-caryophyllene (69.3%) was identified as the main compound in the fruit essential oil of the plant. Palmito-γ-lactone was established as the second major compound in all three essential oils analyzed.

Discussion

MP is a well-known species for its prosperous phenolic content, especially prenylated isoflavonoids, which prompted us to examine antioxidant potential of the

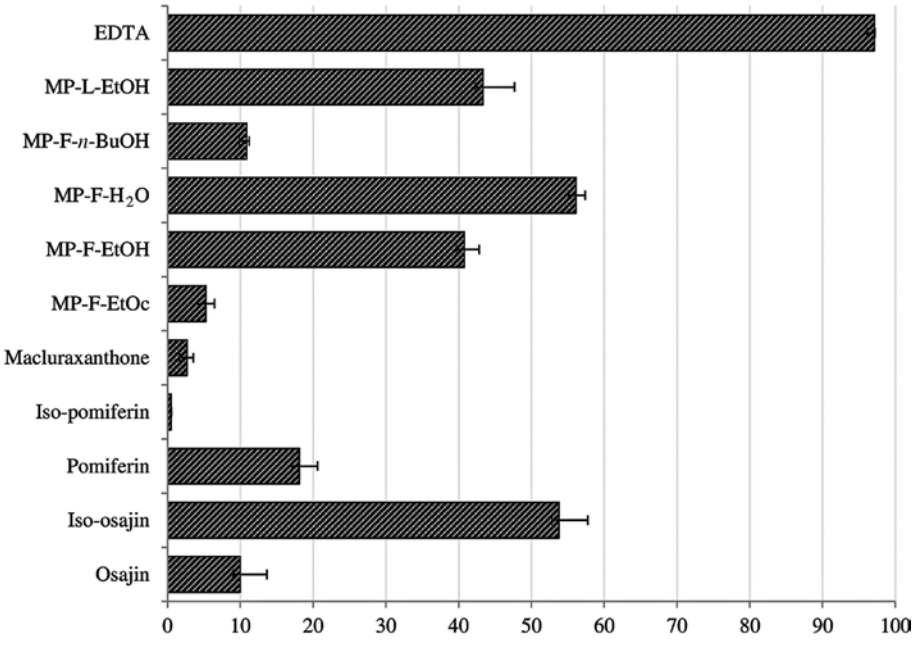


Figure 3: Metal-chelating capacity of the flavonoid derivatives and the extracts of MP. F, fruit; L, leaf; reference: EDTA.

Table 2: Total phenol and flavonoid amounts of the extracts from MP.

	Total phenol content[a] ± SEM[b]	Total flavonoid content[c] ± SEM
MP-F-EtOAc	196.06 ± 2.75	66.13 ± 4.30
MP-F-EtOH	24.41 ± 0.31	1.25 ± 0.26
MP-F-H_2O	47.66 ± 1.17	11.23 ± 2.42
MP-F-*n*-BuOH	64.76 ± 1.62	10.22 ± 0.45
MP-L-EtOH	39.34 ± 0.42	≤0.025

[a]Data expressed in mg equivalent of gallic acid to 1 g of extract. [b]Standard error mean (n = 3). [c]Data expressed in mg equivalent of quercetin to 1 g of extract.

extracts and three isolated compounds from MP along with their two *iso*-derivatives. Without any doubt, reactive oxygen species are able to trigger many diseases such as diabetes, Alzheimer's and other neurodegenerative diseases, atherosclerosis, etc. [26] and, hence, a great gratitude toward antioxidants has been presented for prevention and treatment of numerous diseases and upkeeping of human health. It is critical to note that no single experiment is satisfactory to describe antioxidant status of any plant extract and, therefore, it is rather reasonable to employ a wide variety of assays acting through different mechanisms. For instance, DPPH purple-colored solution bleaching may basically help to explain electron donation ability of any natural products, whilst compounds capable of transferring a hydrogen atom to DMPD cause a decolorization of the solution [27]. NO is well-known as an imperative chemical mediator usually produced by endothelial cells, macrophages, and neurons in association with the regulation of several pathological processes and also a potent pleiotropic inhibitor of physiological processes. As the metabolic track of NO gives rise to reactive nitrogen species, quenching of this radical is also important. In our study, *iso*-pomiferin displayed the highest radical scavenging effect than other isoflavones (osajin and *iso*-osajin) tested and a very close effect to that of macluraxanthone. It was generally more active than its parent compound (pomiferin).

To date, only a few studies have been performed on the antioxidant effect of MP and its constituents and, accordingly, pomiferin was previously reported be a more effective antioxidant than osajin in various antioxidant experimental models including DPPH and peroxynitrite radical scavenging, inhibition of lipid peroxidation, FRAP, β-carotene-linoleic acid model system, and autooxidation of purified sunflower triacylglycerols [28–30], which are all in complete agreement with our current findings with these two compounds.

Table 3: Composition of the essential oils of MP by GC-MS.

RRI[a]	Compound	Female tree-leaf (%)[b]	Male tree-leaf (%)[b]	Fruit (%)[b]
1244	2-Pentyl furan	–[c]	–	0.6
1266	(*E*)-β-Ocimene	–	–	0.2
1327	(*Z*)-3-Hexenyl acetate	–	–	0.2
1391	(*Z*)-3-Hexenol	0.7	0.1	–
1400	Nonanal	1.1	0.5	0.7
1466	α-Cubebene	–	–	tr
1497	α-Copaene	–	–	0.7
1500	Pentadecane	–	–	0.1
1612	β-Caryophyllene	0.5	0.9	69.3
1687	α-Humulene	–	–	1.1
1688	Selina-4,11-diene	–	–	0.8
1726	Germacrene D	–	–	0.1
1758	(*E*,*E*)-α-Farnesene	2.1	1.8	1.0
1773	δ-Cadinene	–	–	1.6
1785	7-*epi*-α-Selinene	–	–	0.1
1786	Kessane	–	–	0.2
1803	Liguloxide	–	–	2.3
1838	(*E*)-β-Damascenone	0.1	0.1	0.1
1900	Nonadecane	tr[d]	tr	–
1933	Tetradecanal	0.8	1.0	–
1992	Neophytadiene	0.3	0.2	–
2008	Caryophyllene oxide	–	–	0.8
2036	2-Pentadecanone	–	–	0.1
2041	Pentadecanal	tr	0.2	tr
2050	(*E*)-Nerolidol	0.3	0.3	0.2
2071	Humulene epoxide-II	–	–	tr
2094	*p*-Cresol	0.2	0.2	–
2096	Elemol	–	–	0.5
2100	Heneicosane	0.5	0.2	–
2131	Hexahydrofarnesyl acetone	0.3	0.3	–
2187	T-Cadinol	–	–	0.1
2242	2-Heptadecanone	–	–	0.4
2250	α-Eudesmol	–	–	tr
2257	β-Eudesmol	–	–	0.1
2300	Tricosane	0.4	0.1	tr
2400	Tetracosane	tr	tr	–
2500	Pentacosane	1.0	0.8	–
2503	Dodecanoic acid	tr	tr	tr
2551	Geranyl linalool	1.2	1.3	tr
2622	Phytol	61.5	51.4	tr
2670	Tetradecanoic acid	tr	0.1	0.6
2700	Heptacosane	3.2	2.9	–
2857	Palmito-γ-lactone	9.6	11.8	12.8
2900	Nonacosane	9.1	13.0	–
2931	Hexadecanoic acid	6.6	11.5	4.0
	Total	99.5	98.7	98.7

[a]RRI, relative retention indices calculated against n-alkanes. [b]% Calculated from FID data. [c]Not detected. [d]Trace (<0.1%).

In fact, only two studies demonstrated antioxidant potential of macluraxanthone. Consistent with our study, Teixeira et al. [3] stated that macluraxanthone was the responsible compound in MP according

to the results obtained from 2,20-azinobis-(3-ethylbenzothiazoline)-6 sulfonic acid (ABTS) and DPPH radical scavenging assays. The second study concluded that antioxidant activity of macluraxanthone isolated from the stem bark of *Rheedia acuminata* was greater than quercetin (reference) in DPPH, ABTS radical scavenging and trolox equivalent antioxidant capacity (TEAC) assays [31].

On the other hand, Fe^{3+} ions may create radicals from peroxides, whereas Fe^{2+}, which easily form complexes with ferrozine, is the most potent pro-oxidant among all the metal ions. Thus, practicality of metal-chelating activity of plant extracts/natural compounds is to uncover how their phytochemical content can catch ferrozine for ferrous ions [32]. In consequence, it was specified that phenolic compounds possibly present in extracts could donate to higher metal-chelating capacity [33]. Reliably, one can remark that higher metal-chelating capacity of the aqueous extract of MP can be assured to to be pertinent to its sufficient amount of phenolic compounds.

The antioxidant activity of phenolic compounds is considered to be principally due to their redox properties, which enable them to act as reducing agents. The antioxidant capacity of the compounds and extracts from MP was also measured through FRAP and PRAP methods. PRAP is based on the reduction of Mo (VI) to Mo (V) by the test sample and the subsequent formation of green phosphate/Mo (V) compounds. Many studies have pointed to the fact that the antioxidant potential of plant extracts in accordance with the mechanism of action is sturdily related with their total phenolic amount [34]. This statement is again in accordance with our current results as the fruit EtOAc extract of MP, which possessed the highest total phenol and flavonoid quantity, displayed the best FRAP and PRAP.

Despite the few studies previously mentioned on antioxidant activity of osajin, pomiferin, and macluraxanthone along with the extracts of MP, there has been no study on the semi-synthetic derivatives of osajin and pomiferin. In most of the experimental models applied herein for determination of antioxidant activity, *iso*-osajin and *iso*-pomiferin exhibited a higher effect than their parent compounds. The higher effect might be suggested to be related to ring formation of prenyl side chain at 1″-5″ in *iso*-derivatives (Figure 1).

Considering the essential oil composition of MP, only a few studies on the fruit of MP have been reported to date. In one of them, Peterson et al. [5] determined β-caryophyllene as the major component in the fruit of MP of US origin which agreed on our data, whereas the fruit essential oil of MP from Croatia contained eugenol (9.9%) and *p*-cresol (9.6%) as the main components [35].

In conclusion, the current study presents the results on the antioxidant effect of the extracts from the fruit and leaves of *M. pomifera* as well as two major isoflavonoids isolated; osajin and pomiferin, their *iso*-derivatives, and macluraxanthone. Among the tested compounds, pomiferin and its *iso*-derivatives, in particular, exerted an appreciable level of antioxidant effect. To the best of our knowledge, this is the first report on the NO radical scavenging activity, metal-chelating capacity, and PRAP of MP extracts as well as osajin, pomiferin, and macluraxanthone. We also disclose herein the first comparative data on antioxidant capacity of their semisynthetic *iso*-derivatives as well as the essential oil composition of the leaves of MP. As ROS are closely associated with pathogenesis of many serious illnesses, in the current study, the observed antioxidant activity of the extracts and compounds of MP might be convenient for the progress of novel and strong natural antioxidants.

Acknowledgments: F.S. Senol expresses her genuine appreciation to the Scientific and Technological Research Council of Turkey (TUBITAK) for the scholarship provided during her Ph.D. studies.

Conflict of interest statement: The authors have no conflict of interest.

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