Research article

The Investigation of Phytochemical Contains, Antioxidant and Antimicrobial Activities of *Malus floribunda* Siebold ex Van Houtte From Eastern TURKEY

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ABSTRACT

Objective: *M. floribunda* is an ornamental and landscape plant in Turkey and it's small fruits are consumed as food. Antimicrobial, antioxidant activities and phytochemical contents of *M. floribunda* were investigated. **Material and Method:** *M. floribunda* was collected from different localities in the province of Elazig-Turkey. The nutritive value (flavonoid, sugar, fatty acid, vitamin, protein, element), antioxidant (lipid peroxidation, glutathione amounts and DPPH) and antimicrobial activities (on thirteen bacteria, three yeast and two dermatophyte) of *M. floribunda* were determined. **Results:** Vitamin (1.73-3.5 μg/g A, 1.32-1.44 μg/g D, 2.75-2.83 μg/g E, 2.64-3.30 μg/g K), flavonoid (33-253 μg/g routine, 93-123 μg/g morin, 0.22-0.32 μg/g quercetin, 292-356 μg/g naringenin), sugar (97-820 μg/g), protein (1.4-1.6 μg/g) and phytosterol (0.27-1.74 μg/g ergosterol, 26-36 μg/g stigmasterol, 95-121 μg/g β-sitosterol and 0.21-0.35 μg/g retinol) levels can vary. *In vitro* medium, it is determined that in FeCl group, LPO amounts increases in a large ration with respect to the control group and LPO levels in groups which includes plant extracts and $FeCl_2$, decreases in certain amounts. It was determined that the radical scavenging activity of the fruits increased due to the dose. Four different fatty acids were identified in the fruit. Fruits were found to different levels of sugar. Vitamin E in the examples were determined high rate. Protein found up to 1.686 mg/g. Ca content was the most detected in the all samples. Extracts inhibited the development of microorganisms at different rates. **Conclusion** Although studies on this type were limited, similarities and differences were seen in the results compared to other studies.

Key words: *M. Floribunda*, Flavonoids, Phytosterol, Apple, Antioxidant Effect, Antimicrobial Activities.

INTRODUCTION

Apples contains are phytochemicals which potent antioxidant and antimicrobial activity. The majority of these phytochemicals consist of phenolic compounds.[1] Flavonoids from the phenolic components of apple are quercetin and glycosides.[2] In addition to antimutagenic and potent antioxidant effects of *M. domestica* and its juice, it has been determined to be protective against to cancer, diabetes, obesity, cardiovascular diseases, asthma and other lung diseases.[3] Apple consumption has been found to reduce the risk of colorectal cancer. This effect was thought to be due to was rich in flavonoid and other polyphenol contents.[4]

M. floribunda is an ornamental and landscape plant in Turkey and small fruits are consumed as food. Especially, this fruit is consumed by diabetic patients as a sugar reducing agent in Elazığ, Turkey. In this study, medicinal (antimicrobial and antioxidant) effects and phytochemical contents of *M. floribunda* was investigated.

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MATERIAL AND METHOD

Fruit Samples

M. floribunda was collected from different regions of Elazığ, placed in sterile bags and kept refrigerated at -20 ºC until analysis.

Preparation of Fruits Extracts

Fruit samples were extracted at a ratio of 1:5 (g/mL) with methanol. After blotting, all groups were centrifuged. The supernatant obtained at the end of centrifugation was removed from the solvent medium using rotovapor. Methanol was added to prepare an extract.

Determination of resveratrol and flavanoid content

Analysis of the flavanoid and resveratrol were performed on a HPLC with according to the method determined by Rodriguez-Delgado *et al.*[5]

Extraction and Analysis of Phytosterols and ADEK Vitamins

Fruit was weighed and homogenized with a 3/2 mixture of hexane/isopropyl alcohol and after hydrolysis at 85ºC with 5% KOH, extractions of phytosterols were done with n-heptane. Fruit extracts were prepared according to the litterature to determine the amount of vitamins. The content of phytosterol and vitamins were analyzed by HPLC.[6,7]

Free Radical (DPPH) Activity

Free radical 25 mg/l DPPH methanol was prepared. 10, 25, 50 and 100 µg/µl extracts and 3.9 ml DPPH solution were added to the test tubes, respectively. The mixtures were allowed to incubate for 30 minutes at room temperature in a dark place, and the absorbance at 517 nm was read on a spectrophotometer against blanks at the end of the incubation.[8] The reduced absorbance, the amount of DPPH remaining, was determined as the free radical scavenging activity. The results were calculated according to the following formula: % = [(Control$_{ABS}$-Sample$_{ABS}$) / Control$_{ABS}$)] * 100.6 mm, 5 µ.) Column.

Lipid Peroxidation (LPO) Measurement *In vitro*

After taking 1 ml of the sample, 1 ml of TBA solution of 0.6%, 1 ml of TCA solution of 20%, 1 ml of 4% HCI and 1 ml of distilled water was added and vortexed. It was then incubated at 95ºC for 60 min. and the resulting pink color was extracted with 3 mL of n-butanol. Samples were centrifuged and the supernatant fraction obtained at the end of centrifugation was read spectrophotometrically at a wavelength of 532 nm against to blanka. 1,1,3,3-Tetraethoxypropane (TEP) was used as standard and the readings were calculated according to calibration curve. The results were given in nmol/µl.

Determination of Element Contents

The samples were dried at room temperature for about 2 week, then grinded and stored at 105ºC for 24 hours. 1 g samples was weighed out and 10 mL of a mixture of HNO_3: H_2SO_4: H_2O_2 (10:1:1) was added. The samples were incubated at 100ºC until dissolved. Then the dH_2O was added to the cooled samples as 50 mL and filtered with filter paper. The element contents of the samples were determined by atomic absorption and atomic emission spectrophotometer.[9]

Measurement of Protein Amount

The total protein level of the fruits were determined spectrophotometrically at 750 nm using a commercial kit.[10]

Antimicrobial activity

Preparing Extracts

Plant groups were extracted with methanol at a ratio of 1/10 (g/ml). Fruits extracts were stored at +4ºC during experimental runs.

Preparation of microorganism cultures

Bacterial strains (*Listeria monocytegenes*, *Salmonella enterica typhimirium*, *Enterococcus faecium*, *Proteus mirabilis*, *Staphylococcus cohnii*, *Staphylococcus aureus* COWAN 1, *Bacillus megaterium* DSM32, *B. subtilis*, *Klebsiella pneumoniae* FMC 5, *Escherichia coli* ATCC 25922, *Proteus vulgaris* FMC1, *Enterobacter aeregenes* CCM 2531, *Pseudomonas aeruginosa* DMS50071) and yeast (*Candida albicans* FMC17, *C. glabrata* ATCC 66032, *C. tropicalis* ATCC1380, *Trichophyton* sp. and *Epidermophyton* sp.) in nutrient buyyon and malt extract was inoculated, respectively (48 hours at 25±1°C for yeast, 24 h at 35±1°C for bacteria).

Disc Diffusion Method

The impregnated discs were placed on the solidified agar by lightly pressing. Petri dishes prepared in this manner were incubated at 4°C for 1.5-2 h, then incubated for 24 h at 37±1°C with bacteria-inoculated plates and plates at yeast and dermophyte-grafted plates at 25±1°C for 3 days. The inhibition zones (mm) formed on the feeder at the end of the period were evaluated. Standard antibiotic discs were used for control group. The antimicrobial activity test was repeated three times.

Statistical analysis

For statistical analysis, SPSS 15.0 for Windows package program was used. Comparison between control and experimental groups was performed using One way ANOVA and LSD tests. The results were expressed as mean ± SEM. The differences between the groups were $p>0.05$, $p<0.05$, $p<0.01$, $p<0.001$ and $p<0.0001$.

RESULTS

Flavonoid and Resveratrol Contents of Fruit

Routine, morin, quercetin, naringenin were found in all samples. Routine was determined the most in A1, the lowest in the A2 ($p>0.05$) show Table 1. Also, morin was determined the most A1. Quercetin was found to be less than other flavonoids. Naringenin was found more than other flavonoids but A1.

Phytosterol Contents of Fruit

Ergosterol, stigmasterol, β-sitosterol and retinol were found in all groups. β-sitosterol was determined the most abundant phytosterol. Ergosterol was determined to be highest in A1 group and lowest in A2. Stigmasterol was found to be most abundant in A2, but retinol was found to be less than other phytosterols ($p>0.05$) (Table 2).

Vitamin Content of Fruits

Vitamin A was determined the highest in A1, but lowest in A3. The amount of vitamin D in all samples was determined to be the lowest ($p>0.05$) (Table 3).

Sugar Contents of Fruit

Fructose, glucose, sucrose and maltose were found to be common in all groups. Sucrose was determined as the most abundant sugar while maltose was determined the lowest ($p>0.05$) see in Table 4.

The Effect of Scavenging DPPH Radicals of *M. floribunda*

It was determined that the effect of free radical scavenging (DPPH) increased with parallel to increasing amount of concentration of fruit extract (Figure 1). When the concentration-dependent radical scavenging effect of the fruit sample was compared statistically. The difference between 50 µL - 100 µL and 100 µL - 200 µL was not significant ($p>0.05$). In addition, the activity at 5 µL and 25 µL concentrations was significantly lower than the other concentrations ($p<0.05$) see Figure 1.

In vitro Antioxidant Effects (LPO) of *M. floribunda*

Lipid oxidation level was significantly increased in all groups compared to the control group show Figure 2 ($p<0.05$). There was a significant decrease in lipid oxidation level in fruit groups ($p<0.05$). When samples collected from different localizations were compared among themselves. No difference was observed between A2 and A3 ($p>0.05$), whereas A1 was found to be less effective ($p <0.05$) compared to those two examples see Figure 2.

Table 1: Flavonoid and resveratrol content of *M. floribunda* (µg/g)

Flavonoid	A1	A2	A3
Routine	253.05±2.8	33.55±1.63	103.95±1.59
Morin	123.7±5.22	103.05±5.06	93.48±2.28
Quercetin	0.22±0.26	0.30±0.1	0.32±0.3
Naringenin	352.9±4.02	292.11±2.61	356.16±3.4
Kamferol	-	-	-
Myricetin	-	-	-
Resveratrol	-	-	-

Mean±SD (n=3). A1: lokal. 1, A2: lokal. 2, A3: lokal. 3.

Table 2: Phytosterol content of *M. floribunda* (µg/g)

Phytosterol	A1	A2	A3
Ergosterol	1.74±0.06	0.27±0.02	0.89±0.04
Stigmasterol	28.96±0.49	36.61±1.16	26.71±1.93
β-sitosterol	121.7±2.67	102.8±1.71	95.6±0.71
Retinol	0.35±0.04	0.27±0.03	0.21±0.03

P>0.05, Mean±SD (n=3).

Table 3: Vitamin levels of *M. floribunda* collected from different localities (µg/g)

Vitamin	A1	A2	A3
A	3.50±0.28	2.29±0.26	1.73±0.11
D	1.44±0.31	1.40±0.05	1.32±0.08
E	2.82±0.10	2.83±0.11	2.75±0.19
K	2.79±0.18	3.30±0.25	2.64±0.09

P>0.05, Mean±SD (n=3). A1: lokal. 1, A2: lokal. 2, A3: lokal. 3

Table 4: Sugar Contents of *M. floribunda*

Sugar	A1	A2	A3
Fructose	453.5±7.99	450.68±6.93	460.75±19.74
Glucose	340.58±5.68	236.10±19.43	356.71±17.8
Sucrose	820.74±19.9	826.13±22.5	824.54±3.49
Maltose	97.75±15.17	112.63±7.51	98.65±2.21

P>0.05, Mean±SD (n=3). A1: lokal. 1. A2: lokal. 2. A3: lokal. 3.

Table 5: Effect of *M. floribunda* on methyl esters *in vitro* (µmol/ ml)

Methyl esters	A1	A2	A3
16:00	37.5±0.98	58.4±3.17	60.7±1.36
18:00	20.19±0.77	13.92±1.73	27.13±1.49
18:01	15.39±0.94	24.73±2.81	27.13±1.48
18:03	50.11±1.68	36.95±1.67	54.44±2.31
24:01	150±2.93	96.19±2.44	122.31±1.92

The results were given as mean ± SD (n = 3). A1: local. 1. A2: local. 2. A3: local.

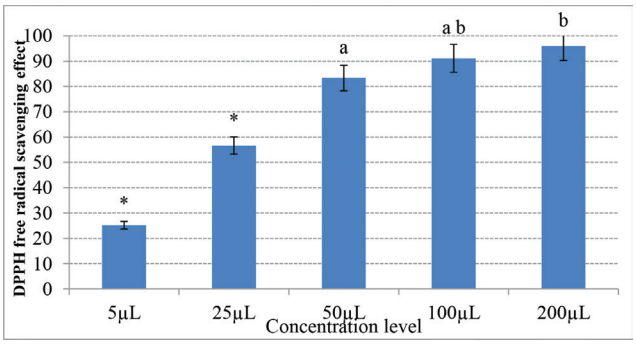

Figure 1: DPPH free radical scavenging effect (%) depending on the increasing concentration level of *M. floribunda*.

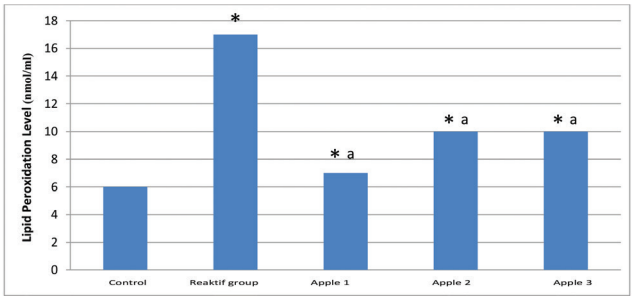

Figure 2: *In vitro* lipid peroxidation inhibition level *M. floribunda*. (The results were given as mean ± SD (n = 3). A1: loc.1. A2: loc. 2. A3: loc.3.* p <0.05 compared to other groups. [a] p <0.05 compared to reactive group.

Table 6: Protein amount of *M. floribunda*

Samples	Amount of protein
A1	1.412±0.35
A2	1.686±0.26
A3	1.611±0.62

Table 7: Element amounts of *M. floribunda*

Minerals	A1	A2	A3
Cu	8.44±0.14	8.49±0.16	9.70±0.11
Zn	5.28±0.17	4.78±0.23	4.50±0.27
Mn	4.80±0.19	5.00±0.14	4.45±0.22
Fe	28.56±1.14	25.51±1.19	24.45±1.28
Ca	101.4±2.1	90.60±1.93	85.43±1.86
Mg	80.0±1.67	72.67±1.78	75.56±1.70

Mean±SD (n=3).

Effects of *M. floribunda* on Fatty Acid Methyl Esters *in vitro*

Palmitic acid, stearic acid, oleic acid, linolenic acid and nervonic acid were determined in 3 group (Table 5). When palmitic acid (16:00) was examined in fruit extract groups, there was no statistically significant difference in A2 and A3 ($p>0.05$), but decrease in A1 ($p<0.05$). Stearic acid (18:00) showed the highest difference between groups, while A3 had the least A2 ($p<0.05$). The amount of oleic acid (18:1) was determined different in fruit extract groups. The highest effect was found in A3 with the least effect in A1. Linolenic acid (18:3) showed the highest effect in A3, showing the least effect in A2. Nervonic acid (24:1) was determined the highest in A3 (Table 5).

Table 8: Antimicrobial Activities of *M. floribunda* (mm diam.)

Microorganism	Apple juice	Apple juicer + metanol	Apple pulp	Standart
L. monocytogenes	23	15	15	15
S. enterica	20	20	26	20
E. faecium	23	20	25	15
P. mirabilis	20	22	20	20
S. cohnii	20	15	22	10
S. aureus	20	20	25	15
B. megaterium	25	22	25	20
B. subtilis	22	20	20	15
K. pneumoniae	18	16	20	12
E. coli	20	20	20	15
E. aeregenes	22	20	20	15
P. aeroginosa	23	15	18	17
C. albicans	22	17	20	12
C. glabrata	18	15	20	15
C. tropicalis	15	17	22	15
Epidermaphyton spp.	23	20	23	17
Trichophyton spp.	22	18	23	15

Protein Amounts of *M. floribunda*

The highest protein level was determined in A2 see in Table 6. There was no statistically significant difference between these 2 groups ($p>0.05$). The low amount of protein was observed in A1 and decreased in the other 2 groups ($p<0.05$).

Determination of Mineral Element Content of *M. floribunda*

The amount of Ca was detected most in all samples. Mn was found to be at least (see Table 7).

Antimicrobial Activities of *M. floribunda*

It was determined that the extract inhibited the growth of all bacteria, yeast and dermatophyte fungi. The apple juices showed the most antimicrobial effect in *B. megaterium* (25 mm). The fruits juices+methanol showed the most antimicrobial effect to *P. mirabilis* (22 mm) with inhibition zone diam. The maximum effect was shown in *S. enterica typhimirium* (26 mm), *E. faecium* (25 mm), *S. aureus* (25 mm) and *B. megaterium* (25 mm) see in Table 8.

DISCUSSION

The major flavonol glycosides identified in the golden apple bark are quercetin-3-galactoside, quercetin-3-glucoside, quercetin-3-xyloside, quercetin-3-arabinoside, quercetin-3-rhamnoside and the routine.[11] In addition to quercetin glycosides and floridzin and phloretin glycosides was detected in the spartan and greening apple bark.[12,13] Four anthocyanins, three quercetins, two phenolic acids, two flavan 3-ols and one dihydrochalcone glycoside were found in the apple variety of Skugog.[14] During cold storage, after storage, ripening and intermittent heating have been determined to affect the amount of phytosterol.[15] It was determined 7 µg of total vitamin with consist of 0.5 mg vitamin E, 4 µg vitamin K, 11.0 mg vitamin C, 0.2 mg niacin and 6 µg folic acid in 100 gr apple (ww).[16] Fructose, sucrose, galactose, α-glucose, β-glucose and sorbitol were detected in apple fruit samples. Among the existing sugars, fructose was found the most abudance, this was followed by sucrose, β-glucose, α-glucose, galactose and sorbitol.[17] Golden apple was found to be most fructose, it was also contain hight level in the mature apple.[18,19] Highest DPPH activity was determined of apple bark (92%).[20] Oleic acid were detected in the surface wax of apple fruit.[21] Amounts of protein for 100 g apple, it was found 0.3 g in wet apple, 0.3 g in apple juice and 1.8 g in dry apple.[16] The total amount of protein in apple juice was found 0.07 g while the amount of protein in the mature apple was found to be 0.3 g.[3] The minerals found in apple and apple juice are 144-116 g K, 7.0-4.2 g Ca, 6.0-6.9 g Mg and 12.0-7.0 g P, respectively.[3] Seventeen herbal essential oils were used against to *E. coli*, *S. enterica* and apple juice was found most active compound.[22] *M. domestica* has been found to inhibit the growth of *E. coli*, *B. cereus* and *S. aureus* too much.[23] *M. halliana* was found to be effective particularly on *B. subtilus* and *E. coli*.[24] When the results of this study were compared with other studies, similar or different results were obtained (Table 1-8, Figure 1-2). It has been determined that harvesting time, maturation, cold storage, storage conditions affect the amount of phytochemical.[15] It was determined that the environmental parameters can be similar or can change based on the geographical location, the area where the species were collected from the altitude, the time period of measurement and the season. All our studies comply with the observations and results of the research mentioned above, and it is especially important that the nutritive and medicinal characteristics of the *M. floribunda* that the other fruit types grow can vary (Table 1-8, Figure 1-2). In conclusion, it has been determined that *M. floribunda* is rich from the point of view of phytosterol, vitamin (A, D, E, K), protein, unsaturated fatty acids such as linolenic, oleic and palmitic acid, and especially flavonoid such as naringenin, routine, morin and quercetin, and also elements contests (Table 1-7). Edible *M. floribunda* fruits are an excellent food that can be used in a well-balanced diet for their functional compounds, and other nutritional values (Table 1-7) and medicinal effect such as antioxidant and antimicrobial effects (Figure 1-2 and Table 8).

ACKNOWLEDGMENT

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CONFLICT OF INTEREST

None

ABBREVIATION USED

HPLC: High Performance Liquid Chromatography; KOH: Potassium hydroxide; DPPH: free radical scavenging activity; TBA: Thiobarbituric acid; TCA: Trichloroacetic acid; HCI: Hydrochloric acid; TEP: 1,1,3,3-Tetraethoxypropane; HNO_3: Nitric acid; H_2SO_4: Sulfiric acid; H_2O_2: Hydrogen peroxide; dH_2O: Distilled Water; LPO: Lipid Peroxidation; $FeCl_2$: ferrozine, ferrous chloride; SPSS: Statistical Package for the Social Sciences; ANOVA: Analysis of Variance; LSD: Least Significant Difference; SD: standard deviation.

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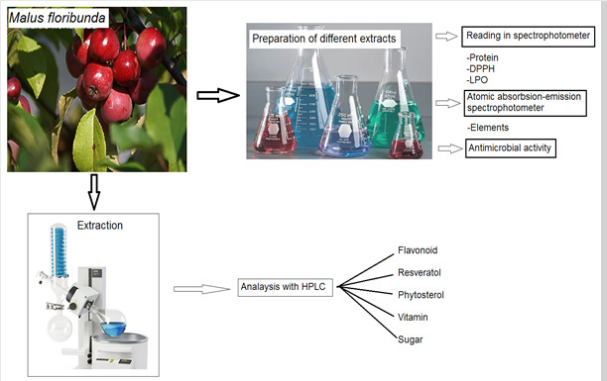
PICTORIAL ABSTRACT



SUMMARY

- The aim of this research is to extend our knowledge on nutritive value and medicinal (antimicrobial and antioxidant) effects of *M. floribunda* within Eastern Turkey.
- It was seen that the flavonoid, sugar, fatty acid, vitamin, protein, element levels can vary. It is rich from the point of view of phytosterol, vitamin (A, D, E, K), protein, unsaturated fatty acids, flavonoid and also elements contests.
- Edible fruits are an excellent food that can be used in a well-balanced diet for their functional compounds, and medicinal effect such as antioxidant and antimicrobial effects.

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