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ORIGINAL ARTICLE

Phytochemical Content of *Malus floribunda*: In Vitro and Molecular Docking Studies

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Abstract

Malus floribunda Siebold ex Van Houtte is a plant planted for landscaping, and its sour and red fruits have been seen to be frequently used in the treatment of diabetes, making vinegar marmalade, and producing natural food dyes. Apart from these usage areas of this plant, it is aimed at determining the phytochemical content. For this purpose, plant parts (fruit, leaf, and branch) were examined. The antioxidant capacity (vitamins A, E, and C, lycopene, beta-carotene, total phenolic and flavonoid amounts, and DPPH radical scavenging effect), antimicrobial activity (agar well diffusion method, minimum inhibitory concentration-MIC), and GC-MS contents of plant parts were determined. High-performance liquid chromatography (HPLC), spectrophotometers, and gas chromatography/mass spectroscopy (GC-MS) methods were used in the study. It was determined that *M. floribunda* fruit is rich in lycopene, beta-carotene, and antioxidant vitamins and contains many biomolecules. In addition, it was concluded that the extracts of different parts of the plant have antimicrobial activity. This study has revealed the idea that this plant, whose phytochemical, antioxidant, and antimicrobial content has been determined, can be used as a bioactive substance equivalent to antibiotics in medicine, the food industry, and human nutrition. In addition, it is expected that the study will contribute to the plant literature. Molecular docking studies were performed to evaluate the binding interactions between the compound and human peroxiredoxin 5 and *S. aureus*. Both in vitro and in silico results indicated that synthesized extracts could act as potent antioxidant and antimicrobial agents.

Keywords *M. floribunda* · Phytochemicals · Antioxidant capacity · Antimicrobial activity · GC-MS · Molecular docking

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Introduction

Plants have long been the foundation of traditional medicine systems, and they will continue to do so in the future. They will continue to be the best source for the production of medicines used in disease treatment [1]. For years, plants have been used by humans in a wide variety of fields, such as spices, food, beverages, perfumes, medicines, and building materials [2]. *Malus floribunda* belongs to the Rosaceae family of ornamental plants. This plant is known as the Japanese crabapple tree because it originated in Japan [3, 4]. *M. floribunda* is also a decorative plant. This plant's red fleshy fruits are used as a natural food colorant [5]. Furthermore, it has been reported that the fruits of this plant are used to make vinegar and marmalade, as well as in the treatment of diabetes [4, 6]. In a study, the potential use of anthocyanins in crabapple (*M. floribunda*) marmalade as a natural colorant was investigated [7]. In another study, the shell and flesh parts of three different malus species (*M. floribunda*, *M. evereste*, and *M. floribunda coccinella*) were examined in terms of total phenolic, tannin, monomeric anthocyanin, and protein contents. It has been determined that the peel parts of fruits contain higher amounts of these substances than the flesh parts. It was observed that anthocyanin accumulation, especially in fruit flesh, was at the highest level in *M. floribunda* [8]. Raw extracts of medicinal plants' roots, stems, flowers, and fruits are widely used in the treatment of certain diseases [9]. Many diseases occur with the accumulation of free radicals in the human metabolism. Antioxidants can scavenge these free radicals or minimize their effects. In addition to strengthening the immune system, vitamin A plays a role in many biological events such as cell division, skin development, bone growth, and reproduction. It also increases the resistance of the metabolism against infections [10]. Vitamin E functions in the development, reproduction, prevention of various diseases, and protection of the integrity of tissues [11]. Ascorbic acid, known as vitamin C, is an antioxidant vitamin responsible for the detoxification of reactive oxygen species [12]. In addition to being a vitamin A precursor, β-carotene is a lipid antioxidant. It is particularly effective at cleaning reactive oxygen species such as singlet oxygen [13]. Lycopene is a carotenoids derivative that gives it its red color [14]. Lycopene, a member of the carotene family, is a naturally occurring pigment in fruits and vegetables. The human body gets lycopene from the food. Lycopene is found in red grapefruit and fruits such as watermelon and apricots. The highest concentration of lycopene is found in tomatoes and tomato products [15]. Due to their antioxidant properties, phenolic compounds are anticarcinogenic, antimutagenic, and antimicrobial, so it has a positive effect on human health. Flavonoids are polyphenolic antioxidants naturally found in herbal teas, fruits, and vegetables [16]. Antioxidants can scavenge free radicals or minimize their effects. Then, it is important to investigate the natural antioxidants in the structure of the plant. The DPPH (2,2-difenil-1-picrylhydrazyl) radical scavenging test determines the plant's antioxidant capacity [17].

Medicinal plants contain a variety of phytochemicals, such as flavonoids, alkaloids, tannins, and terpenoids, which have antimicrobial and antioxidant properties [18]. In a study in Turkey, they reported that edible wild Japanese crab fruits could be used as a bioactive material in the food industry and human nutrition due to their antimicrobial activities [19].

GC-MS (gas chromatography-mass spectrometry) is an analytical method that is used to reveal the substances in the samples to be analyzed. In particular, it combines the separation properties of gas-liquid chromatography with the detection properties of mass spectrometry [20].

It has been determined that most of the studies on ornamental apples *M. floribunda* are in the fields of genetics and phytopathology. This study aims to elucidate the phytochemical structure of the *M. floribunda* plant by determining its antioxidant capacity, antimicrobial activity, and GC-MS contents. Also, molecular docking studies were carried out to support experimental studies and evaluate the synthesized compound as an antimicrobial and antioxidant agent.

Materials and Methods

Plant Material

In September, plant part samples of *M. floribunda* were collected from trees on the Kirsehir Ahi Evran University campus (Fig. 1). After mixing the samples, they were separated into three parts: fruit, leaf, and branch. Freshly collected samples were first cleaned of impurities by washing them with tap water. It was filtered through the use of distilled water. It was dried in the laboratory for 1 month in the shade without being exposed to sunlight. Dried fruit, leaf, and branch samples were powdered using a mechanical grinder (Fritsch P-15, Germany). UV-visible spectroscopy (Shimadzu 2401PC) device was used to determine the total phenolic and flavonoid amounts and antiradical activities of the samples. In addition, Cecil 1100 series high-performance liquid chromatography (Cotati brand 7125 injection lobe, Cecil 68174 UV detector, and HP 3395 integrator) was used in the analysis of antioxidant vitamins and carotenoids.

Analysis of Fruit Samples with Vitamins A, E, and Carotenoids (β-Carotene, Lycopene)

After the fresh fruit samples were washed, they were thoroughly crushed in a homogenizer. One gram of the smashed fruit samples was weighed and put into polyethylene tubes. Each

Fig. 1 *M. floribunda* (Japanese apple)

tube received 5.0 mL of ethanol, which was added and vortexed. For 10 minutes, samples were centrifuged at 4000 rpm. All samples were then given 1.0 mL of n-hexane solvent and shaken. Vitamins (A, E), beta-carotene, and lycopene were extracted into the n-hexane phase in this manner. The n-hexane was obtained by repeating this extraction process twice. The extracts were combined and evaporated to dryness under nitrogen gas. Residues in sample tubes were mixed with 200 µL of methanol and ready for analysis using a high-performance liquid chromatography device (HPLC). Vitamin E was measured at 296 nm, vitamin A was measured at 326 nm, β-carotene was measured at 465 nm, and lycopene was measured at 472 nm [21, 22].

Determination of Vitamin C Amounts in Fruit Samples

The fruit samples, which were thoroughly chopped in the homogenizer, were weighed at about 1 g and taken into polyethylene tubes. All samples were shaken by adding 1 mL of a 0.5 M $HClO_4$ solution. To these samples, 4 mL of distilled water was added. Centrifugation was performed at 4500 rpm for 20 min to precipitate particles from the suspended samples. In the HPLC phase, 3.7 mM KH_2PO_4 is mobile (pH: 4 with H_3PO_4) at a flow rate of 1 mL/min. Vitamin amounts were measured at a wavelength of 246 nm using a C18 column in the HPLC device [23].

Taking Extracts of Plant Samples

Extracts are made from the powdered fruits, leaves, and branches of *M. floribunda*. For this, 10 g of plant parts were weighed and placed in 250-mL bottles. One hundred milliliters of distilled water was added. Plant samples were boiled at 80 °C for 30 min. All samples were allowed to cool to room temperature before being tested. Then, the samples were filtered with Whatman filter paper No. 1 [24].

For the ethanol and acetone extracts, 10 g of powdered fruits, leaves, and branches were weighed. Solvents were placed in a 250-mL Soxhlet flask. For 8 h, Soxhlet instrument with 100 mL capacity extracted 96% ethanol and acetone (Merck, Darmstadt, Germany). Extracts are filtered with Whatman filter paper No. 1. In addition, both aqueous and ethanol and acetone liquid extracts were cooled and concentrated at 30–45 °C in a rotary evaporator. The extracts were then lyophilized in a lyophilizer and stored at −20 °C [25].

Examples: Fruit-water (FW), fruit-acetone (FA), fruit-ethanol (FE), leaf-water (LW), leaf-acetone (LA), leaf-ethanol (LE), branch-water (BW), branch-acetone (BA), and branch-ethanol (BE) were named.

Determination of Total Phenolic Substance of Plant Samples

The total phenolic content of plant part samples from *M. floribunda* was ascertained using the Singleton and Rossi [26] method. For this purpose, samples were prepared from the extracted plant parts with distilled water, acetone, and ethyl alcohol solvents at a concentration of 1 mg/mL. From these samples, 0.1 mL was taken into the test tubes. 0.1 mL of Folin-Ciocalteu reagent and 4.5 mL of bidistilled water were added to the samples, and all were vortexed. After 3 min, 0.3 mL of 2% Na_2CO_3 was added to the tubes and left at room temperature for 2 h. Absorbances were measured against control at a 760-nm wavelength

with a spectrophotometer device. To calculate the concentration of phenolic substances in the samples, gallic acid was used as a control substance. The extracts' total phenolic content was calculated as mg gallic acid equivalent (GAE)/g extract. All parameter analyses were performed three times.

Determination of Total Flavonoid Substances in Plant Samples

The total flavonoid amounts in plant part extracts were determined using the aluminum nitrate method [27]. The method was modified and used. Samples were made from the extracts at a concentration of 1 mg/mL in order to calculate the total flavonoid content of plant part extracts. Approximately 500 µL of samples was collected and 0.1 mL of sodium acetate was added. After 1 min, 0.1 mL of 10% $Al(NO_3)_3$ was added and the mixture was shaken. Then, the volumes of the samples were made up to 5 mL with 96% (v/v) ethanol. For 50 min, the samples were incubated at room temperature. The absorbance values were then measured in the spectrophotometer at 450 nm. The same experimental procedure was applied by adding ethanol instead of the sample blank. The total flavonoid contents of plant part samples were shown as mg quercetin equivalent (QE)/g extract. All parameter analyses were run in triplicate.

Determination of the Antiradical Activity of Plant Samples

The antiradical activity of *M. floribunda* plant part extracts and standard ascorbic acid was determined using the DPPH free radical [28]. The method was modified and used. Standard ascorbic acid was used to compare the results of the samples. First, solutions of plant samples and standard substances at 1 mg/mL concentration were prepared. From this solution, samples at 250, 500, and 1000 µg/mL concentrations were created. One milliliter of each of these solutions was taken and put into tubes. Then, the DPPH solution was prepared using methanol at 0.1 mM concentration. Then, 4 mL of 0.1 mM DPPH solution was added to the samples. All samples were incubated at room temperature and in the dark for 30 min. Then, absorbances were measured against an ethanol blank at 517 nm using a spectrophotometer device. The samples' DPPH radical scavenging activities were calculated using the following equation:

$$Radical\ scavenging\ activity\ (\%inhibition) = \frac{Control\ Absorbance - Sample\ Absorbance}{Control\ Absorbance} \text{ x } 100 \quad (1)$$

The IC_{50} value is the amount of antioxidant substance required to reduce the initial DPPH radical concentration of the reaction by 50% [29, 30]. In addition, the IC_{50} values of the samples prepared at three different concentrations, such as 250, 500, and 1000 µg/mL, were calculated.

Determination of Antimicrobial Activity of Plant Samples

In the study, a total of 10 microorganisms, 9 bacteria and 1 yeast, were used as test microorganisms. These include *S. aureus* ATCC 29213, *B. subtilis* ATCC 6633, *E. coli* ATCC 25922, *B. cereus* 709 Roma, *A. hydrophila* ATCC 7966, *E. faecalis* ATCC 29212, *P. aeruginosa* ATCC 27853, and *K. pneumonia* ATCC 13883, with the bacteria *V. anguillarum* ATCC 43312 and the yeast *C. albicans* ATCC 90028.

Agar Well Diffusion Method

M. floribunda fruit, leaf, and branch extracts were evaluated for their antimicrobial qualities using the agar-well diffusion method [31]. Prior to creating sterile wells in the medium, microbial cultures were first distributed on Sabouraud Dextrose Agar (for yeast) and Trypticase Soy Agar (for bacteria) plates. Using a sterile micropipette, 70 µL of *M. floribunda* fruit, leaf, and branch extracts (clean water, acetone, and ethanol) was added to the wells. Following incubation, the zones that formed around the wells were measured using a ruler, and the results were represented in millimeters (mm). Controls included ethanol, acetone, and purified water.

Minimum Inhibitory Concentrations (MICs) Method

The minimum inhibitory concentrations (MICs) values of the purified water, acetone, and ethanol extracts made from the *M. floribunda* (fruit, leaf, and branch) under study were determined using a microdilution test. Various rates of the stock solution (2, 4, 8, 16, 32, 64, 128, and 256 µg/mL) were prepared to find the amounts that were strongest against the examined microorganisms. Then, 100 µL of fresh cultures of bacteria and yeast was added to a 96-well microplate. According to CLSI [32], MIC values were taken to represent the lowest doses at which microbial growth could not be observed.

Determination of GC-MS Content of *M. floribunda*

The GC-MS method is widely used to identify compounds found in a plant sample [33]. The samples were dissolved in methanol at 50 °C for 6 h in a vibrating mixer. New samples were prepared from these thawed samples by diluting them with methanol at a ratio of 100:1. The obtained samples were cooled and made ready for analysis. In this study, the Shimadzu brand GCMS/QP2010 ultra type device and Rtx-5MS column were used. One microliter of the samples to be examined was injected into the heater section of the instrument at 300 °C at an injection rate of 200 mL/min in splitless mode.

Statistical Analysis

The data were analyzed using the SPSS 29.0 program. The difference between variables with more than two categories was examined with the Kruskal-Wallis H test. In the DPPH analysis, a comparison with the standard value was made, and the Wilcoxon sign test was used for a single sample. The significance level for all tests was taken as sig. $p < 0.05$.

Table 1 A, E, and C vitamins, lycopene, and beta-carotene amounts of the fruits of the plant

4.Parameters	Amount (µg/g)
Vitamin A	4.51 ± 0.74
Vitamin E	6.72 ± 0.95
Vitamin C	29.42 ± 1.06
Lycopene	9.44 ± 0.68
Beta-carotene	29.22 ± 0.96

X ± SD (mean of three measurements ± standard deviation)

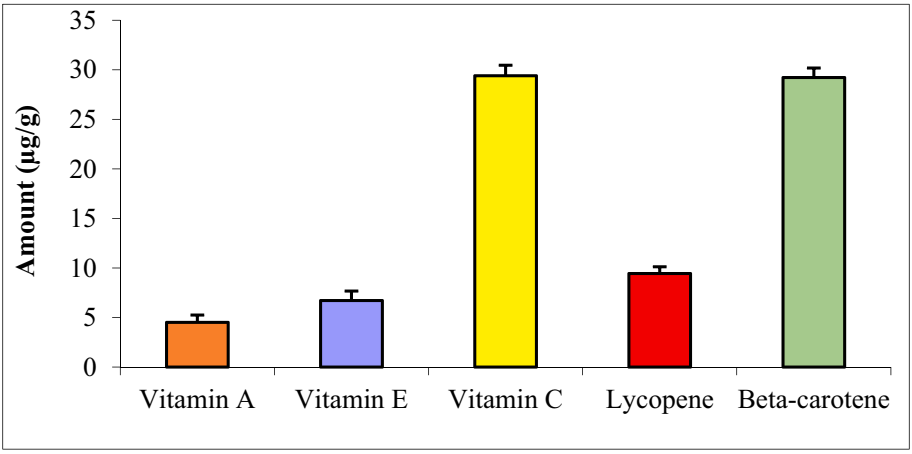

Fig. 2 The amounts of vitamins A, E, C, beta-carotene, and lycopene in *M. floribunda* fruit

Results and Discussion

Antioxidant Capacity

The Amounts of Vitamins A, E, C, Beta-carotene, and Lycopene in *M. floribunda* Fruit

Antioxidants have important functions for human health. Therefore, food scientists have encouraged the search for antioxidant substances in plants [34]. The antioxidant capacity of fruits and vegetables may be due to phytochemicals such as flavonoids, isoflavones, flavones, anthocyanins, catechins, and isocatechins other than beta-carotene and vitamins C and E [35]. Phytochemicals protect the metabolism against oxidative damage. Therefore, these phytochemicals prevent various neurological diseases, cancers, and cardiovascular diseases [36]. The amounts of vitamins A, E, and C, as well as beta-carotene and lycopene of *M. floribunda* fruit samples, are explained in Table 1 and Fig. 2.

Vitamin A is one of the most important vitamins in essential nutrients. Vitamin A is a powerful antioxidant and supports immune function. It also ensures healthy vision, bone development, and healthy skin formation [37]. As can be seen in Table 1, the amount of vitamin A in fresh *M. floribunda* fruit was determined at 4.51±0.74 µg/g. In addition, in

a study conducted with *M. floribunda* fruits, it was reported that the amount of vitamin A varied between 1.73 ± 0.11 and 3.50 ± 0.28 µg/g [4]. The amount of vitamin A in this study was found to be higher.

Vitamin E, known as tocopherols, is a fat-soluble vitamin with four different (α-, β-, γ-, and δ-) forms. In particular, they prevent lipid peroxidation in cell membranes [38]. The amount of vitamin E in the fresh fruit of *M. floribunda* was determined as 6.72 ± 0.95 µg/g (Table 1). In a different study conducted with *M. floribunda* fruits, it was reported that the amounts of vitamin E varied between 2.75 and 2.83 µg/g [4]. *M. floribunda* fruit in this study was found to be rich in vitamin E.

One of the water-soluble vitamins is ascorbic acid (vitamin C). Vitamin C is a vitamin that can scavenge free radicals and plays a protective role in carcinogenesis [39]. The vitamin C content of *M. floribunda* fruits was determined at 29.42 ± 1.06 µg/g (Table 1). Vitamin C amounts in eleven kinds of hawthorn species were measured by the HPLC method, and the results were reported to vary between 15.55 ± 0.91 and 94.18 ± 0.14 µg/g [40]. It can be said that the fresh fruit of *M. floribunda* contains moderate amounts of vitamin C.

An important derivative of carotenoids is lycopene, which gives its red color [14]. Studies on lycopene have reported that lycopene is effective in the treatment of cardiovascular diseases and in maintaining bone, skin, and eye health [10, 14]. It is also reported to prevent many types of cancer [15]. It has been reported that rosehip fruit is rich in lycopene, and the total lycopene content is between 129 and 352 µg/g in fresh fruit [41]. In a study conducted on red and dark red fruits, it is reported that lycopene is 8.8–42 µg/g in fresh tomato fruits, 23–72 µg/g in watermelon fruits, and 33.6 µg/g in grapefruit fruits [42]. The lycopene content of *M. floribunda* fruits was found to be 9.44 ± 0.68 µg/g (Table 1). From these results, it can be said that *M. floribunda* fruit contains less lycopene than tomato fruit.

Beta-carotene, the precursor of vitamin A, is a yellow or orange pigment. In addition, beta-carotene, which has a carotenoid structure, is abundant in green leafy plants and carrots [43]. In a study conducted on the fresh fruits of the rosehip plant, it was reported that beta-carotene was found between 12.8 and 37.9 µg/g [41]. The beta-carotene content of *M. floribunda* fruits was found to be 29.22 ± 0.96 µg/g (Table 1). From these results, it was concluded that *M. floribunda* fruits are rich in beta-carotene content.

Table 2 Total phenolic and flavonoid content analysis results of different plant part extracts

	Samples	Total phenolic (mgGAE/g extract)	Total flavonoid (mgQAE/g extract)	sig. (p)
Water extract	Fruit	54.08 ± 2.04	28.55 ± 1.50	
	Leaf	98.24 ± 2.81	69.33 ± 2.97	**< 0.001**
	Branch	28.89 ± 2.07	24.28 ± 1.49	
Acetone extract	Fruit	47.72 ± 2.24	43.54 ± 2.19	
	Leaf	112.07 ± 3.58	93.29 ± 3.50	**< 0.001**
	Branch	40.33 ± 2.46	42.71 ± 1.93	
Ethanol extract	Fruit	63.16 ± 2.46	29.62 ± 1.48	
	Leaf	132.76 ± 3.66	79.14 ± 2.84	**< 0.001**
	Branch	54.25 ± 2.31	26.73 ± 1.41	

Kruskal-Wallis H, sig. ($p < 0.05$). Significant differences were found in terms of phenolic and flavonoid substances between the aqueous extracts of the fruit, leaf, and branch parts of the study (Kruskal-Wallis H, sig. $p < 0.001$). Bonferroni correction post hoc analysis revealed similar differences in all three sections and each plant part for all extracts (adj. sig. $p < 0.001$)

Total Phenolic and Flavonoid Amounts of Plant Part Extracts of *M. floribunda*

Apple fruit contains many important molecules, such as vitamins, sugars, and phenolic compounds, in its structure [44]. According to studies, phenolic and flavonoid compounds play significant roles in neutralizing free radicals [45]. The total phenolic and flavonoid amounts of purified water, acetone, and ethanol extracts of *M. floribunda* fruit, leaf, and branch parts were investigated in this study (Table 2). In addition, the standard gallic acid and quercetin substances were used to calculate the total phenolic and flavonoid substance contents of plant part extracts of *M. floribunda.* When the total phenolic contents of the plant parts were compared, a result of leaf > fruit > branch was obtained ($p < 0.001$). Again, when the total flavonoid amounts of *M. floribunda* plant part extracts were compared, it was determined that leaf > fruit > branch ($p < 0.001$). The highest amount of total phenolic substance was in leaf-ethanol extract (132.76 ± 3.66 mg gallic acid equivalent/g extract), and the highest amount of flavonoid substance was measured as (93.29 ± 3.50 mg quercetin equivalent/g extract) in leaf-acetone extract. In a study investigating the total phenolic amounts of different apple species, it was reported that they ranged from 66.94 ± 1.62 mg gallic acid equivalent/g extract to 71.88 ± 2.30 mg gallic acid equivalent/g extract [46]. The total phenolic content of the extract of firethorn (*Pyracantha coccinea* var. *lalandi)* fruit, which is planted as an ornamental plant and has red-colored fruits, in various solvents was investigated. As a result of the study, the total phenolic content in diethyl ether extraction is 3.80 mg gallic acid equivalent/100 g, in ethanol extract 68.41 mg gallic acid equivalent/100 g, and in methanol extract 114.26 mg gallic acid equivalent/100 g [47]. Recent epidemiological studies have shown that a flavonoid-rich diet significantly reduces cancer and heart disease [48]. Again, the total flavonoid amounts of different apple species were investigated, and it was reported that they ranged from 15.59 ± 0.23 to 21.91 ± 2.55 mg catechin equivalent/g extract [46]. The total flavonoid content of the fruits of the firethorn (*Pyracantha coccinea* var. *lalandi*) plant planted for landscaping was investigated, and it was determined to be 72.15 ± 2.97 mg rutin equivalent/g fresh fruit [49]. It was determined that both total phenolic and flavonoid amounts were higher than the studies in the literature (Table 2 and Figs. 3 and 4).

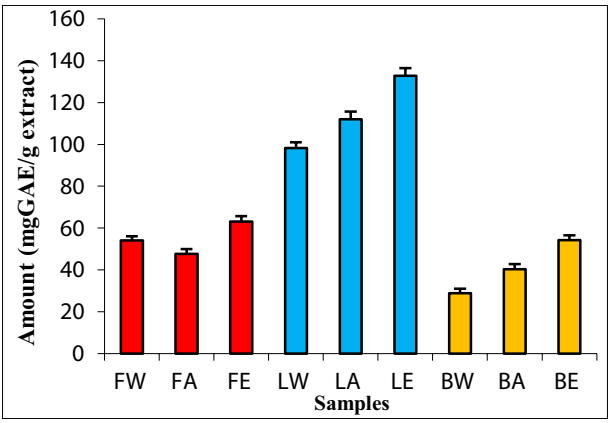

Fig. 3 Total phenolic content of *M. floribunda* plant parts extracted

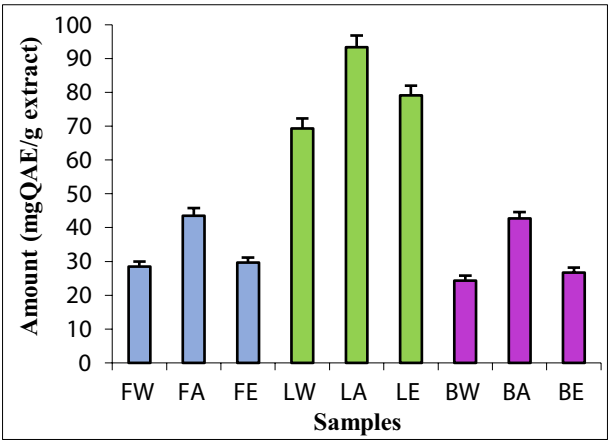

Fig. 4 Total flavonoid amounts of plant part extracts of *M. floribunda*

Table 3 DPPH radical inhibition percentages and IC_{50} values of different plant part extracts

Sample	250 µg/mL	*p*	500 µg/mL	*p*	1000 µg/mL	*p*	IC_{50} (mg/mL)
FW	22.11 ± 1.41	**.008**	30.55 ± 1.12	**.006**	46.65 ± 2.11	**.010**	1.102
FA	23.92 ± 1.07	**.006**	40.22 ± 1.50	**.011**	51.25 ± 1.43	**.007**	0.918
FE	28.43 ± 1.90	**.013**	41.65 ± 1.63	**.013**	55.65 ± 1.23	**.007**	0.813
LW	33.91 ± 1.15	**.009**	46.72 ± 1.40	**.013**	65.42 ± 2.40	**.018**	0.614
LA	45.52 ± 1.32	**.018**	58.93 ± 1.85	**.035**	69.32 ± 1.71	**.015**	0.320
LE	50.12 ± 2.81	**.054**	61.67 ± 2.14	**.053**	72.74 ± 2.94	**.029**	0.186
BW	20.90 ± 1.34	**.007**	29.82 ± 1.27	**.007**	38.44 ± 1.54	**.006**	1.484
BA	21.91 ± 1.47	**.008**	36.73 ± 1.68	**.011**	47.21 ± 1.43	**.007**	1.044
BE	25.13 ± 1.48	**.009**	39.40 ± 1.53	**.011**	50.34 ± 1.48	**.008**	0.945
Standard (ascorbic acid)	61.76 ± 1.98		70.68 ± 2.04		95.53 ± 3.28		0.014

One-sample Wilcoxon signed rank test, sig. ($p < 0.05$) revealed a statistically significant difference between the samples and the standard ascorbic acid results. Significance levels were determined to be $p < 0.05$

DPPH Radical Scavenging Effect and IC_{50} Values of Plant Part Extracts of *M. floribunda*

The DPPH radical capture capacity analysis method for the measurement of antioxidant capacity in extracts is very widely used [50]. The percent inhibition values of the samples prepared at three different concentrations (250, 500, and 1000 µg/mL) and extracted in different solvents were compared with the standard (ascorbic acid) inhibition values. DPPH inhibition percentages and IC_{50} values of purified water, acetone, and ethanol extracts obtained from the plant part of *M. floribunda* are shown (Table 3 and Fig. 5).

In addition, DPPH radical scavenging activities were found to be high in ethanol extracts ($p < 0.05$). When the inhibition percentages of the samples at 1000 µg/mL concentration are examined, ascorbic acid (95.53 ± 3.28) > LE (72.74 ± 2.94) > LA (69.32 ± 1.71) > LW (65.42 ± 2.40) > FE (55.65 ± 1.23) > FA (51.25 ± 1.43) > BE (50.34 ±

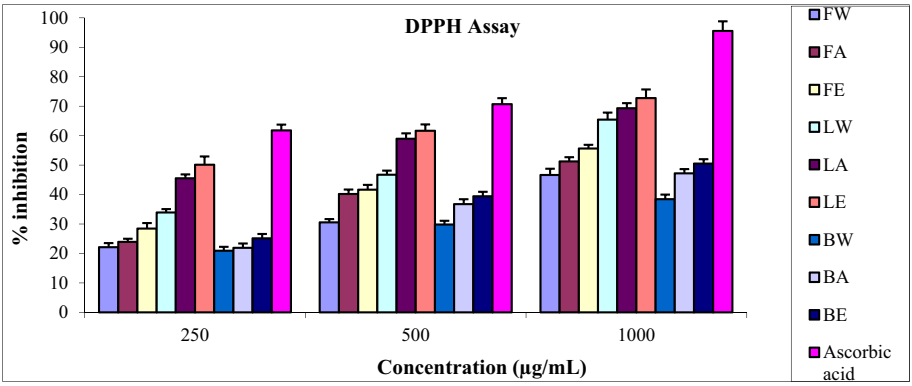

Fig. 5 DPPH radical scavenging activities (% inhibition) of *M. floribunda* plant extracts

1.48) > BA (47.21 ± 1.43) > FW (46.65 ± 2.11) > BW (38.44 ± 1.54). From these results, it was determined that different extracts of leaf samples had high DPPH radical scavenging activities ($p < 0.05$). When the radical removal activities of the plant part extracts were examined, it was determined that there were leaf > fruit > branch parts (Fig. 5).

The smaller the IC_{50} value, the greater the antioxidant activity. That is, the same amount of free radicals at the lowest concentration of substances that can cleanse shows higher antioxidant activity [51]. The IC_{50} values of the samples were compared with the standard ascorbic acid value (Table 3). Statistically significant differences were found ($p < 0.05$). The lowest IC_{50} value belongs to the leaf ethanol extract (0.186 mg/mL), while the highest IC_{50} value belongs to the branch water extract (1.484 mg/mL) sample ($p < 0.05$).

In a study for the determination of the DPPH scavenging activity of firethorn (*Pyracantha coccinea* var. *lalandi*) plant fruits, it has been reported that the percentage of inhibition varies in the range of 7.8–93.43 [49]. In the study in which the DPPH radical scavenging activity of pulp and seed extracts of *M. baccata* fruit was performed, the inhibition percentages were 28.92–77.08, and have been reported to vary between 8.45 and 30.47, respectively. In addition, IC_{50} values were reported as 0.09 and 0.51 mg/mL, respectively [52]. In this study, it was determined that the leaf ethanol extract had the highest percentage of DPPH radical inhibition (72.74 ± 2.94). Therefore, it was determined that the leaf ethanol extract with the lowest IC_{50} value had the highest radical scavenging activity ($p < 0.029$) (Table 3). It is seen that the results obtained are compatible with the literature.

Antimicrobial Activity

Alkaloids, lectins, polypeptides, phenolics, terpenoids, and essential oils are polyacetylenes according to the chemical structures of antimicrobial agents in plants. According to Khameneh et al. [53], phenolic compounds include phenolic acids, simple phenols, quinones, flavonoids, flavones, flavonols, tannins, and coumarins. According to the antimicrobial activities of *M. floribunda* gathered from Turkey's eastern area [4], apple juices had the greatest antimicrobial impact in *B. megaterium* (25 mm). In addition to fruit juices, methanol had the greatest antimicrobial activity on *P. mirabilis* (22 mm) with a diameter inhibitory zone. It was determined that *M. domestica* extracts obtained from organically grown plants showed antimicrobial effects against *B. cereus* and *E. coli* strains [54].

These findings suggested that the antimicrobial properties of the extracts and their phenolic components may be related. A second experiment using *M. domestica* found that aqueous extracts made from the plant's fresh fruits were most efficient against the gram-positive bacteria *C. albicans* and ineffective against *E. coli.* The alcohol extract of *M. domestica* exhibited the strongest antimicrobial activity for the gram-positive bacteria *B. subtilis* and no activity against the gram-negative bacterium *P. aeruginosa* [55]. It was reported that the Japanese crab extract tested in a study had the highest antimicrobial activity against *B. cereus* [19]. Like this, our research found that Japanese crabapple extract has an antimicrobial impact on these microorganisms. Antimicrobial substances eliminate microorganisms that cause illness. *M. floribunda* fruit and branch portions (pure water extract) had no inhibitory effect on any of the bacteria tested. The fruit extract extracted with acetone was found to be from bacteria, according to the antimicrobial activity of *M. floribunda* fruit, leaf, and branch extracts prepared with distilled water, acetone, and ethanol by the agar well diffusion method; *A. hydrophila* and *K. pneumoniae* showed an inhibition zone between 17.0 and 18.3 mm, while other bacteria showed a zone of 16.1 mm and below. In the acetone extract, *E. coli* leaf also had the broadest zone, with a diameter of 20.2 mm. Other bacteria have inhibition zones ranging from 10.2 to 18.2 mm. A branching part of the examined plant, extracted with distilled water, showed no inhibition by any of the microorganisms employed. Table 4 shows the antimicrobial activity (zone of inhibition-mm) of *M. floribunda* extracts.

Table 5 displays the outcomes of tests using the minimal inhibitory concentration (MIC-µg/mL) method to determine the antimicrobial activity of *M. floribunda* leaf, fruit, and branch extracts in water, ethanol, and acetone. The ethanol extract of the branch section of the Japanese apple was most effective against *K. pneumoniae* (MIC = 16 µg/mL), whereas it had the least effect on *S. aureus*, *A. hydrophila*, *and V. anguillarum* (MIC= 128 µg /mL). However, acetone fruit extract inhibited *A. hydrophila* the best (MIC = 8 µg/mL). The most successful microorganisms in the leaf (acetone extract) component were *E. coli* and *B. cereus* (MIC = 8 µg/mL), while *C. albicans* (MIC = 256 µg/mL) was the least effective. Pure water leaf extracts inhibited *B. cereus*, *V. anguillarum, C. albicans*, *and E. coli* better (MIC = 16 µg/mL). The acetone-extracted branch showed superior antimicrobial action against *E. coli* and *A. hydrophila* (16 µg/mL) (Table 5).

GC-MS Content of *M. floribunda* Fruit

The GC-MS method has an important place in the phytochemical analysis of plants [56]. Fourteen components were determined in the GC-MS profile of methanol extracts of *M. floribunda* fruit (Fig. 6 and Table 6).

The components with the highest peak areas in the fruit extract were determined to be 5-hydroxymethylfurfural (21.49%), glycerin (17.61%), 1,4-dioxane, 2,3-dimethoxy- (17.59%), and acetic acid, ethyl ester (CAS) (16.91%). Also, furfural (4.21%), 2-furancarboxaldehyde (CAS) (4.19%), 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (3.99%), N-beta-D-ribofuranosyl-pyrimidine-3-methoxy-2,6-dione (2.61%), (R*,S*)-1,2-cyclohexanedimethanol (2.27%), isosorbide (2.20%), 2,5-furandione (2.12%), beta-d-lyxofuranoside, thio-heptyl- (2.12%), 2,4-dihydroxy-2,5-dimethyl-3(2H)-furan-3-one (1.38%), and beta-D-glucopyranose, 1,6-anhydro- (1.31%) compounds were also determined. The substances released by GC-MS from fruit methanol extract have biological and commercial uses. For example, as a result of GC-MS analysis, it was reported that the most abundant 5-hydroxymethylfurfural substance scavenges DPPH and ABTS free

Table 4 Diffusion analysis findings for the inhibition zone diameters (mm) of *M. floribunda* extracts

Diameter of the inhibition zone (mm)[1]												
M. floribunda	Fruit			Leaf			Branch			Control (+)		Control (−)
Microorganisms	Pure water	Ethanol	Acetone	Pure water	Ethanol	Acetone	Pure water	Ethanol	Acetone	Ampicillin (10 µg)	Nystatin (200 µg)	Ethanol
S. aureus ATCC 29213	-	12.4	13.1	-	15.2	16.1	-	12.2	10.2	-	-	-
B. cereus 709 Roma	-	-	16.1	15.2	-	18.2	-	13.2	15.3	16	-	-
B. subtilis ATCC 6633	-	-	-	-	-	-	-	-	-	15		-
E. faecalis ATCC 29212	-	-	-	-	-	-	-	-	-	18	-	-
E. coli ATCC 25922	-	-	12.1	12.4	17.1	20.2	-	13.1	14.3	16	-	-
A. hydrophila ATCC 7966	-	-	18.3	-	12.1	15.2	-	12.1	14.3	-	-	-
P. aeruginosa ATCC 27853	-	-	13.1	-	15.2	12.3	-	-	-	18	-	-
K. pneumoniae ATCC 13883	-	-	17.0	-	13.4	14.5	-	17.3	-	15	-	-
V. anguillarum ATCC 43312	-	11.4	12.2	16.3	-	-	-	11.2	12.3	12	-	-
C. albicans ATCC 90028	-	-	11.2	13.4	-	10.4	-	-	-	-	18.4	-

[1]Inactive (-, inhibition zone of 8 mm); weak (8–10 mm); intermediate (10–15 mm); strong (> 15 mm)

The study used ampicillin (10 µg) as a positive control for bacteria and Nystatin (200 µg) as a positive control for yeast

The negative control was ethanol

–: The agar well diffusion method did not show any antimicrobial action

Table 5 The minimum inhibitory concentration (MIC) of *M. floribunda* extracts for antimicrobial properties (µg/mL)

MIC (µg/mL)												
M. floribunda	Fruit			Leaf			Branch			Control (+)		Control (−)
Microorganisms	Pure water	Ethanol	Acetone	Pure water	Ethanol	Acetone	Pure water	Ethanol	Acetone	Ampicillin (10 µg)	Nystatin (200 µg)	Ethanol
S. aureus ATCC 29213	-	128	32	-	16	16	-	128	256	-	-	-
B. cereus 709 Roma	-	-	16	16	-	8	-	64	32	16	-	-
B. subtilis ATCC 6633	-	-	-	-	-	-	-	-	-	15		
E. faecalis ATCC 29212	-	-	-	-	-	-	-	-	-	18	-	-
E. coli ATCC 25922	-	-	32	128	16	8	-	64	16	16	-	-
A. hydrophila ATCC 7966	-	-	8	-	128	16	-	128	16	-	-	-
P. aeruginosa ATCC 27853	-	-	16	-	16	32	-	-	-	18	-	-
K. pneumoniae ATCC 13883	-	-	16	-	32	32	-	16	-	15	-	-
V. anguillarum ATCC 43312	-	128	32	16	-	-	-	128	32	12	-	-
C. albicans ATCC 90028	-	-	64	16	256	256	-	-	-	-	18.4	-

Positive controls included Ampicillin (10 µg) (for bacteria) and Nystatin (200 µg) (for yeast)

The adverse reference tested was ethanol

-: The microdilution technique showed no antimicrobial action

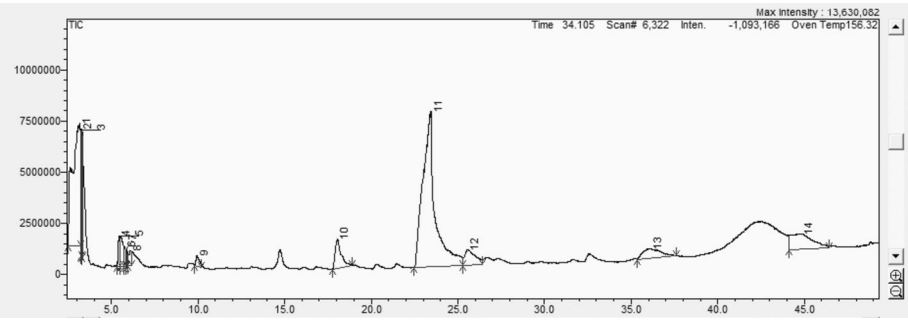

Fig. 6 GC-MS profile of *Malus floribunda* fruit extract

Table 6 GC-MS analysis results of *M. floribunda* fruit

Peak	Ret. time (min)	Name of the compound	Area %	Height %	A/H
1	3.165	Acetic acid, ethyl ester (CAS)	23.16	16.91	31.27
2	3.257	Glycerin	1.84	17.61	2.39
3	3.289	1,4-Dioxane, 2,3-dimethoxy-	1.82	17.59	2.37
4	5.464	Furfural	1.30	4.21	7.07
5	5.539	2-Furancarboxaldehyde (CAS)	2.06	4.19	11.22
6	5.770	N-beta-D-Ribofuranosyl-pyrimidin-3-methoxy-2,6-dione	0.58	2.61	5.08
7	5.855	(R*,S*)-1,2-Cyclohexanedimethanol	0.43	2.27	4.33
8	6.119	2,5-Furandione	0.92	2.12	9.94
9	9.939	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	0.80	1.38	13.18
10	18.041	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	4.17	3.99	23.82
11	23.450	5-Hydroxymethylfurfural	46.39	21.49	49.33
12	25.565	Isosorbide	4.12	2.20	42.75
13	36.115	beta-D-Glucopyranose, 1,6-anhydro-	4.16	1.31	72.74
14	44.827	beta-d-Lyxofuranoside, thio-heptyl-	8.25	2.12	88.70

radicals and has antioxidant properties. In addition, it has also been reported that it shows antiproliferative activity in studies with cell lines [57]. In addition, it has been reported that 1,4-dioxane, 2,3-dimethoxy- substance, which is found in excess, is used for the synthesis of multifunctional chiral compounds [58].

Molecular Docking Study

The molecular docking of the chemical ligand-protein binding site was performed using AutoDock 4.2.6 software [59]. Before docking, the protein structure received a cleaning process. The grid parameter was derived from the active sites of the protein structure. During the docking method, an assessment was made of the binding free energy of the inhibitor within the macromolecule, specifically the target receptor protein. The docked complexes were 2D and 3D visualized using Discovery Studio Software [60] and PyMOL [61], respectively. RCSB, the largest online protein database in the world, is the primary

source for acquiring PDB structures used as target proteins. The RCSB Protein Data Bank(https://www.pdb.org) was used to obtain the antioxidant (human peroxiredoxin 5, PDB ID: 1HD2) and the receptor of gram-positive bacteria (Staphylococcus aureus, PDB ID: 1JIJ). In in vitro studies, M. floribunda extracts have been shown to be a very important intermediate in the development of biologically active antimicrobial and antioxidant compounds. As a result of LC-MS analysis, it was determined that the main compound of M. floribunda extracts is very rich in 5-hydroxymethylfurfural. Thus, the molecular docking interactions of 5-hydroxymethylfurfural, which is the most abundant compound in the extracts, with antioxidant and antimicrobial proteins were evaluated using AutoDock software. The parameters of the molecular docking, the hydrogen bonds with the protein active site residues as a result of the interaction of S. aureus and human peroxiredoxin 5 proteins, the inhibition constant, and binding energy values are given in Table 7. Additionally, the binding modes and nature of the interaction between protein and ligand were investigated, as shown in Fig. 7. The interaction energies of the ligand-protein complexes were calculated as −4.24 and −5.03 kcal/mol, respectively. A protein is where the molecule is bonded when the electrostatic energy is negative.

Table 7 also includes the inhibition constant (Ki value), a crucial factor in determining if the molecule will act as a therapeutic candidate [62]. A drug molecule's Ki value should not be greater than the 10 nanomolar range. It can be seen from Table 5 that 5-hydroxymethylfurfural-1JIJ complexes have the lowest Ki value and docking binding energy. As shown in Fig. 7 and reported in Table 7, the compound was involved in hydrogen bonds with the residues of protein active site of 1JIJ protein. The oxygen atom of ligand moiety was hydrogen-bonded with the nitrogen atom of the side chain of GLU'87 (d = 1.77 Å). In addition, the ligand was located in antimicrobial receptor with the help of hydrogen bonds (ARG'158, HIS'161).

Molecular docking studies are a potent theoretical method for studying ligand-protein interactions, which are important in biological processes such as cancer and bacterial illnesses [62]. This approach is crucial for cutting down on the time needed for discovering new drugs [63]. According to the results, our chemical shows potential as a powerful antimicrobial and antioxidant inhibitor. The results suggest that we can conclude that

Table 7 Docking parameters of ligand docked with the protein targets

Receptor	Compound	H-bonded residues	No of hydrogen bonds	Bonded distance (Å)	Binding Energy (kcal/mol)	Inhibition constant K_i (μM)	Reference RMSD (Å)
1HD2	5-Hydroxy-methylfur-fural	GLU16	5	1.94	−4.24	780.03	50.49
		GLU16		2.09			
		GLY 17		2.21			
		THR 81		2.05			
		LEU 96		2.36			
1JIJ		GLU 87	6	1.77	−5.03	1.11	73.96
		ARG 158		2.10			
		ARG 158		2.95			
		HIS 161		2.58			
				2.21			
				2.39			

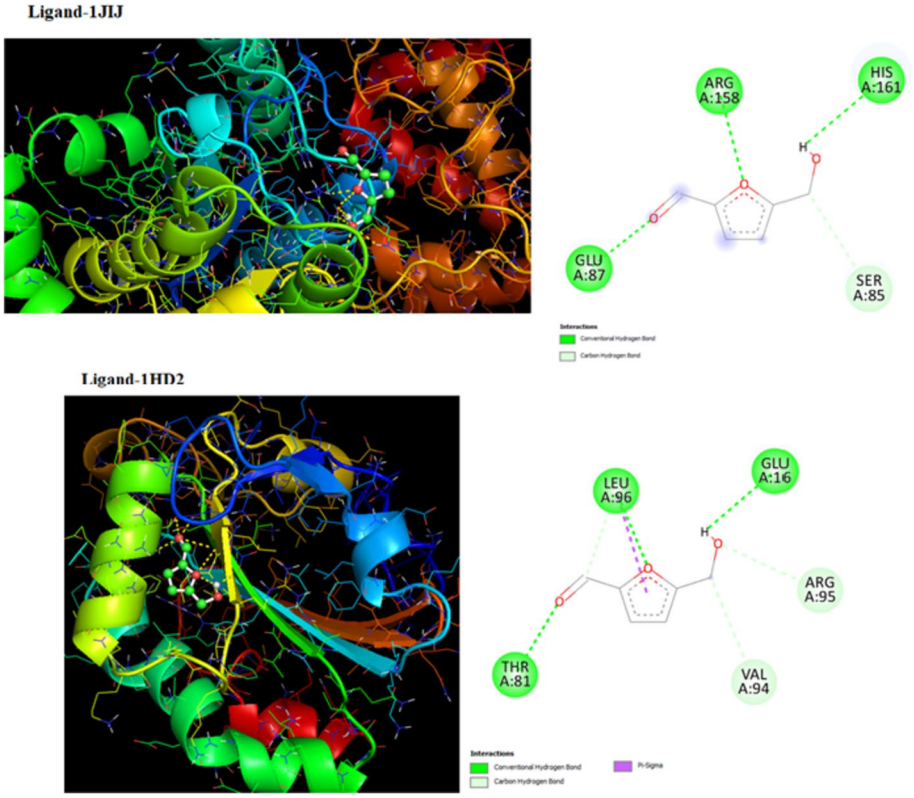

Fig. 7 2D visual representations and 3D docking studies of receptor protein

5-hydroxymethylfurfural compound found in *M. floribunda* extract has the potential to be an important antimicrobial and antioxidant inhibitor.

Conclusion

Recently, studies on the discovery of natural drug substances have increased due to the side effects of synthetic drugs on metabolism. Plants and the raw materials they contain are frequently used in the production of drugs used in disease treatment. The small red fruits of the *M. floribunda* plants are used for ornamental and food purposes. In the antioxidant determinations, it was concluded that *M. floribunda* fruits are rich in A, E, and C vitamins and beta-carotene and lycopene amounts. In addition, plant part extracts were determined to be rich in total phenolics and flavonoids. When the DPPH radical scavenging activities were examined, it was determined that the leaf ethanol extract had the lowest IC_{50} value and had a strong radical scavenging effect. Based on tests for antimicrobial activity, it was determined in this study that *M. floribunda* fruit, leaf, and branch extracts might be employed as antimicrobial agents. In addition, because of the GC-MS analysis of *M. floribunda* fruits, 14 different substances were found in the structure of the fruits. It is thought that this fruit, which has antioxidant and antimicrobial activity and phytochemical

content, may be a good source for newly synthesized antibiotics in medicine, food industry, and human nutrition. In addition, by introducing the biological molecules in the structure of *M. floribunda* fruits, this plant will contribute to the literature. From these results, we believe that consuming *M. floribunda* fruits will be beneficial. The molecular docking study shows that the most abundant molecule in *M. floribunda* extract may be the binding energies of binding to antimicrobial and antioxidant proteins. It is used in pharmaceuticals for the development of new drugs after confirmation by further clinical and in vitro studies.

Code Availability Not applicable

Author Contribution E.C. planning of the study, data collection, determination of antioxidant capacity, GC-MS analysis, statistical analysis**,** interpretation, and writing of the results; B.E. evaluation of molecular docking, interpretation of the results, and antimicrobial capacity analysis; H.C. planning of the study and interpretation of the results. All authors contributed for preparation of the manuscript. All authors read and approved the final manuscript.

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Data Availability All relevant data are within the paper.

Declarations

Ethical Approval The work does not involve human participants and/or animals. Not applicable.

Consent to Participate The authors have agreed to participate in the publication of the paper.

Consent for Publication All authors have agreed to publish the paper.

Conflict of Interest The authors declare no competing interests.

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