Journal of Research in Pharmacy

Research Article

www.jrespharm.com

LC-MS /MS characterization and biological activities of *Morina persica* L. (Caprifoliaceae)

Sefa GÖZCÜ [1] *, Zeynep AKŞİT [2], Samed ŞİMŞEK [3], Ali KANDEMİR [4], Ali AYDIN [5], Mustafa Abdullah YILMAZ [6], Hüseyin AKŞİT [2]

1 Department of Pharmacognosy, Faculty of Pharmacy, Erzincan Binali Yildirim University, 24100, Erzincan, Turkey.
2 Department of Hotel, Restaurant and Service, Tourism and Hospitality Vocational School, Erzincan Binali Yildirim University, 24100, Erzincan, Turkey.
3 Department of Medical Services and Techniques, Çayırlı Vocational School, Erzincan Binali Yildirim University, 24100, Erzincan, Turkey.
4 Department of Biology, Faculty of Arts and Sciences, Erzincan Binali Yildirim University, 24510, Erzincan, Turkey.
5 Department of Basic Medical Science, Faculty of Medicine, Yozgat Bozok University, 66100, Yozgat, Turkey.
6 Department of Analytical Chemistry, Faculty of Pharmacy, Dicle University, 21280, Diyarbakır, Turkey.
7 Department of Analytical Chemistry, Faculty of Pharmacy, Erzincan Binali Yildirim University, 24100, Erzincan, Turkey.
* Corresponding author: Sefa Gözcü, Mail: sgozcu@erzincan.edu.tr (S.G.); Phone: +90-446-226 66 66.

Received: 3 August 2023 / Revised: 6 November 2023 / Accepted: 7 November 2023

ABSTRACT: The study aimed to comprehensively identify the phenolic fingerprint of *Morina persica* L. (Caprifoliaceae) and evaluate its various biological activities. LC-MS/MS analysis of 70% *Morina persica* methanol extract revealed the presence of 27 phytochemicals, with quinic acid, chlorogenic acid, and rutin being the major phenolics. The antioxidant, antibacterial, antiproliferative, and cytotoxic activities were evaluated for biological screening. The results showed that the methanolic extract of *M. persica* has a moderate DPPH radical scavenging and ferric-reducing capacity, indicating antioxidant activity. *M. persica* was observed to have a sufficient antiproliferative effect against cancer cells and low cytotoxicity against normal cells. Moreover, *M. persica* demonstrated good antibacterial activity against *Clostridium perfringens*, *Enterococcus faecalis*, and *Escherichia coli*. These data suggest that the methanolic extract of *M. persica* could be considered both an industrial source of quinic acid and a potential biologically active ingredient for developing drug formulations.

KEYWORDS: *Morina persica* L.; antibacterial; antioxidant; antiproliferative; LC-MS/MS.

1. INTRODUCTION

Morina L. (Caprifoliaceae) includes 14 species that grow in different locations around the world. Throughout history, plants have been a rich source for developing medicines. Nowadays, plants are widely used for human health in all communities and cultures, and are used not only as food supplements but also as medication [1]. The genus is distributed across Anatolia, Southeast Europe, the Himalayas, the Kashmir Mountains, Syria, Lebanon, Afghanistan, and Iran [1, 2]. In Turkey, the genus is represented by two species, *Morina persica* L. and *Morina subinermis* L. [3]. Morina species contain a variety of types of secondary metabolites, including terpenoids [4], saponins [5], flavonoids [6], phenylpropanoids [7], and neolignanes [8]. *Morina persica*, also known as "Merdiven çiçeği," is used to treat colds in Anatolia by preparing an infusion or decoction from the aerial parts of the plant [9]. Due to its melliferous properties, the plant is highly valued in some regions of Turkey [10]. Previous studies have reported the antibacterial [11], antiprotozoal [12], antioxidant [13], antimutagenic [13], and antimycobacterial activities [14] of *M. persica*.

In previous studies, the inhibitory activities of *M. persica* extracts against acetylcholinesterase, *a*-glucosidase, *a*-amylase, and tyrosinase were evaluated [13]. While various biological activity profiles of *M. persica* have been studied before, limited data is available on its chemical profile, antioxidant, antibacterial activity, antiproliferative, and cytotoxicity properties [13]. Therefore, in this study, the phenolic profile of the methanolic extract of *M. persica* was first determined by accurate LC-MS/MS. Additionally, *in vitro* antioxidant, antibacterial, antiproliferative, and cytotoxicity activities were evaluated.

Gözcü S, Akşit Z, Şimşek S, Kandemir A, AydınA, Yılmaz MA, Akşit A. LC-MS MS Characterization and Biological Activities of *Morina persica* L. (Caprifoliaceae) J Res Pharm. 2024; 28(4): 961-973.

ISSN: 2630-6344

http://dx.doi.org/10.29228/jrp.779

2. RESULTS

2.1. Phytochemical analysis by LC-MS/MS

The study used LC-MS/MS system to identify and quantify 56 phenolic acids, organic acids, and flavonoid components in the methanolic extract of *Morina persica* (MP). Out of these, 27 compounds were identified for the first time, providing a more comprehensive chemical profile of the MP extract. The flavonoids detected in the extract included hesperidin, luteolin-7-*O*-glucoside, quercetin-3-*O*-glucuronide, quercetin-3-*O*-glucoside, kaempferol-3-*O*-glucoside, kaempferol-3-*O*-rutinoside, kaempferol, rutin, quercetin, luteolin, naringenin, acacetin, amentoflavone, apigenin-7-*O*-glucoside, apigenin, and chrysin. The extract also contained nine phenolic and organic acids, namely quinic acid, fumaric acid, aconitic acid, gallic acid, protocatechuic acid, chlorogenic acid, caffeic acid, *p*-coumaric acid, and 4-hydroxy benzoic acid. Rutin, kaempferol-3-*O*-rutinoside, and quercetin-3-*O*-glucoside were found to be the major secondary metabolites in the MP extract. The most abundant organic and phenolic acids in the extract were quinic acid and chlorogenic acid, respectively. However, isoflavonoid derivatives daidzin, genistein, and daidzein were not detected in the extract. The quantitative results of the MP methanolic extract are presented in Table 1 and Figure S1.

Table 1. Phytochemicals of methanol extract of Morina persica (mg/g extract) by LC-MS/MS

No	Analytes	mg analyte/g extract	No	Analytes	mg analyte/g extract
1	Quinic acid	42.54	**29**	Salicylic acid	<LOD
2	Fumaric acid	0.23	**30**	Luteolin-7-*O*-glucoside	0.31
3	Aconitic acid	1.57	**31**	Quercetin-3-*O*-glucuronide	0,01
4	Gallic acid	0.01	**32**	Rutin-D3-IS[h]	NA
5	Epigallocatechin	N.D.	**33**	Rutin	8.97
6	Protocatechuic acid	0.13	**34**	Quercetin-3-*O*-glucoside	2.82
7	Catechin	ND	**35**	Hesperidin	3.83
8	Gentisic acid	<LOD	**36**	*o*-Coumaric acid	ND
9	Chlorogenic acid	28.73	**37**	Genistein	ND
10	Protocatechuic aldehyde	0.03	**38**	Rosmarinic acid	<LOD
11	Tannic acid	<LOD	**39**	Ellagic acid	<LOD
12	Epigallocatechin gallate	ND	**40**	Apigenin-7-*O*-glucoside	1.28
13	1,5 -dicaffeoylquinic acid	<LOD	**41**	Quercetin-3-*O*-rhamnoside	<LOD
14	4-hydroxy benzoic acid	0.05	**42**	Kaempferol-3-*O*-glucoside	2.70
15	Epicatechin	ND	**43**	Kaempferol-3-*O*-rutinoside	6.20
16	Vanillic acid	<LOD	**44**	Fisetin	ND
17	Caffeic acid	0.16	**45**	Daidzein	ND
18	Syringic acid	ND	**46**	Quercetin-D3-IS[h]	NA
19	Vanillin	0.03	**47**	Quercetin	0.03
20	Syringic aldehyde	<LOD	**48**	Naringenin	0.08
21	Daidzin	ND	**49**	Hesperetin	ND
22	Epicatechin gallate	ND	**50**	Luteolin	0.08
23	Piceid	ND	**51**	Genistein	ND
24	*p*-Coumaric acid	0.06	**52**	Kaempferol	0.04
25	Ferulic acid-D3-IS[h]	NA	**53**	Apigenin	0.22
26	Ferulic acid	ND	**54**	Amentoflavone	0.81
27	Sinapic acid	ND	**55**	Acacetin	0.10
28	Coumarin	ND	**56**	Chrysin	0.01

ND: Not determined, NA.: Not Applicable, IS: Internal Standard, <LOD: Below limit of detection

2.2. Antioxidant activity of *M. persica* extract

Phenolic compounds, which are a diverse group of phytochemicals found in medicinal plants, exhibit a wide range of biological properties. Among these compounds, flavonoids constitute a significant portion of phenolics and are abundantly present in various foods and medicinal plants, providing not only taste, color, and aroma but also various biological effects [15]. In this study, the total phenolic (TPC) and flavonoid (TFC) contents of the MP extract were determined by spectrophotometric techniques and are presented in Table 2. The TPC and TFC of the MP extract were found to be 39.87 ± 0.98 mg GAE (gallic acid equivalent) and 10.12 ± 0.55 mg QE (quercetin equivalent) per gram extract, respectively. The DPPH test is a commonly used method for evaluating the antioxidant activity of natural or synthetic substances due to their crucial role in scavenging free radicals from biological processes. The present study investigated the dose-dependent DPPH radical scavenging activity of MP and compared it with the positive control Trolox. The results are presented in Table 2 as IC_{50} values. MP demonstrated moderate radical scavenging activity with an IC_{50} value of 87.07 ± 0.74 µg/ml, while Trolox showed an IC_{50} value of 8.25 ± 1.16 µg/ml.

The ability of a compound to reduce ferric ions can be considered a valuable parameter for assessing its antioxidant capacity, as it involves the donation of electrons to radicals, resulting in the formation of stable and less reactive species [16]. In the present study, the ferric ion reducing power of MP was determined and the results were given in terms of Trolox equivalent (TE) in Table 2. The extract has a reducing power of 56.27± 2.01 mg TE, indicating its capacity to reduce Fe^{3+} to Fe^{2+} (Table 2).

Table 2. Total phenolic (TPC), flavonoid (TFC), DPPH, and FRAP values of methanolic extract of *M. persica*

Test sample	[a]TPC	[b]TFC	DPPH IC_{50}(µg/mL)	[c]FRAP
MP	39.87 ± 0,98	10.12 ± 0,55	87.07 ± 0,74	56.27± 2,01
Trolox	-	-	8.25 ± 1,16	-

[a] TPC was expressed as gallic acid equivalents (mg GAE), [b] TFC was expressed as quercetin equivalents (mg QE), [c] DPPH activity was expressed as IC_{50}(µg/mL), [d]FRAP value was expressed as Trolox equivalents (mg TE/g extract)

2.3. Evaluation of the antiproliferative activity of *M. persica* extract

Many anticancer medications in current chemotherapy still do not have the desired therapeutic properties due to cancers' ever-changing mutational burdens [17]. Furthermore, the benefits of usage are diminishing day by day due to mechanisms of resistance and undesirable side effects against chemotherapeutic medicines for a variety of causes [18]. As a reason, research into the discovery of novel anticancer drugs for use in cancer treatment is continuing [19]. To check whether this MP extract was able to affect human cells, the cell proliferation test was studied by MTT dye together with NCI-60 screening methodology. Accordingly, when the lethal concentration (LC_{50}) values of the MP extract on Beas2B, RPE, and HSF control cells were examined, it is seen that the extract has a high LC_{50} value (>1000 µg/mL) than the control anticancer drug, 5FU, indicating that MP extract does not have undesirable toxicity (Table 3-4). As stated in the NCI-60 screening methodology, high LC_{50} values indicate that the cytotoxic effects of the test substances are less, and it is desirable. Considering the Total Growth Inhibition (TGI) and Inhibitory Concentration (IC_{50}) values of the MP extract for control cells, this extract was not toxic against normal cells as it showed lower IC_{50} and TGI values in Beas2B (86.83- 745.01 µg/mL, respectively) normal cells, and RPE (77.21- 78.73 µg/mL, respectively) normal cells than the anticancer control drug, 5FU (Table 3). However, since the IC_{50} and TGI values of MP extract for HSF (75.60- 411.58 µg/mL, respectively) normal cells were slightly higher than the control drug, 5FU, the toxic values of the extract were not within safe limits. Considering the Growth Inhibition (GI_{50}) values, the GI_{50} values of MP extract for Beas2B and RPE control cells (1.12 and 1.28 µg/mL, respectively) were similar to the control anticancer drug 5FU, while it was observed slightly higher GI_{50} (2.27 µg/mL) values for HSF control cells (Table 3-4). Low GI_{50} and TGI values show that the cytostatic effects of the test substances are more, and this is also a desirable situation. These viability indicators, when evaluated together, show that the tested MP extract has a wide range of chemotherapeutic uses that allow safe regulation of their use doses. When the antiproliferative effect of the

MP extract on glioblastoma (A172 and C6), gynecological (HeLa and A2780), and colon (SW620 and HT29) cancer cell lines were examined, it was seen that GI_{50} growth inhibitions (1.06-2.33 µg/mL) were close to the control anticancer drug 5FU (1.27-1.59 µg/mL) (Table 3). The TGI (77.48 – 237.70 µg/mL) values of the MP extract for the HT29, C6, and HeLa cell lines indicated that it shows similar inhibition values as the 5FU control anticancer drug (Table 3-4). However, IC_{50} (83.34 – 105.35 µg/mL) values of the MP extract for the glioma, gynecological, and colon cancer cells implied that it displays the same effect as this, 5FU (Table 3-4). Since the LC_{50} (>1000 µg/mL) values caused by the MP extract on glioma, gynecological, and colon cancer cells were higher than the control anticancer drug 5FU, it can be said that the safety intervals for the use of these extracts were wide (Table 3-4). The emergence of such a result in different cancer cell lines may have been effective in the different cellular mechanisms that cells exhibit against the MP extract.

Table 3. Antiproliferative activity of *Morina persica* extract

Cell lines	*Morina persica* extract			
	GI_{50}	TGI	LC_{50}	IC_{50}
A172[a]	1.11	>1000	>1000	92.70±3.5
A2780 [b]	1.57	>1000	>1000	105.35±2.9
SW620 [c]	2.33	>1000	>1000	101.85±3.3
HT29 [c]	2.27	206.81±5.3	>1000	97.01±3.1
C6 [a]	1.13	77.48±2.7	>1000	91.32±2.8
HeLa [b]	1.06	237.70±3.8	>1000	83.34±3.0
RPE [d]	1.28	78.73±3.4	>1000	77.21±2.9
Beas2B [d]	1.12	745.01±21.6	>1000	86.83±4.0
HSF [d]	2.27	411.58±13.2	>1000	75.60±2.9

[a]A172 and C6 brain cancer cell lines,[b]A2780 and HeLa gynecological cancer cell lines, [c]SW620 and HT29 colon cancer cell lines, [d]Beas2B, RPE, and HSF normal cell lines, respectively lung, retinal, and skin

Table 4. Antiproliferative activity of 5-Fluorouracil

Cell lines	5-Fluorouracil			
	GI_{50}	TGI	LC_{50}	IC_{50}
A172[a]	1.38	45.67±2.0	336.86±9.4	45.88±2.1
A2780 [b]	1.29	48.77±2.0	386.42±8.5	49.94±1.9
SW620 [c]	1.59	47.12±1.9	391.24±10.1	53.41±2.7
HT29 [c]	1.29	35.36±1.9	411.54±9.2	43.18±2.1
C6 [a]	1.39	39.87±1.5	348.65±9.1	46.11±2.4
HeLa [b]	1.27	37.18±1.4	393.06±9.5	32.74±1.7
RPE [d]	1.54	55.37±3.6	423.82±8.3	65.30±2.1
Beas2B [d]	1.43	33.08±1.8	417.72±10.8	34.62±1.7
HSF [d]	1.39	39.87±1.5	348.65±9.1	46.11±2.4

[a]A172 and C6 brain cancer cell lines,[b]A2780 and HeLa gynecological cancer cell lines, [c]SW620 and HT29 colon cancer cell lines, [d]Beas2B, RPE, and HSF normal cell lines, respectively lung, retinal, and skin

2.4. Cytotoxic activity of *M. persica* extract

In this study, cytotoxicity induced by the extract was measured using IC_{50} concentrations. Accordingly, the extract that was found to be effective in the MTT proliferation test showed different cytotoxicity values varying between about 9.7-12.4% against normal cell lines (Beas2B, RPE, and HSF) at IC_{50} concentration (Table 5). When the cytotoxic effects of the tested MP extract on cancer cells (A172, C6, HeLa, A2780, SW620, and HT29) were examined, the MP extract displayed different cytotoxicity values, varying in the range of about 10.3-12.4% against cancer cell lines at IC_{50} concentration (Table 5).

2.5. Antibacterial activity of *M. persica* extract

According to the data presented in Table 6, *Clostridium perfringens*, *Enterococcus faecalis*, and *Escherichia coli* displayed the highest sensitivity to the tested microorganisms, with a minimum inhibitory concentration (MIC) value of 7.81 µg/ml, 31.25 µg/ml, and 31.25 µg/ml, respectively. The antimicrobial activity of *M. persica* extract against *Listeria monocytogenes*, *Staphylococcus aureus*, and *Salmonella enteritidis* was moderate, with a MIC value of 250 µg/ml, 125 µg/ml, and 125 µg/ml, respectively. However, the extract showed weak

antibacterial activity against *Bacillus cereus* and *Pseudomonas aeruginosa*, with a MIC value of 500 µg/ml for both.

Table 5. Cytotoxicity of *Morina persica* extract at IC_{50} concentrations against the cell lines

Cell Lines	*Morina persica* extract	5FU
A172[a]	10.3±0.9	10.8±1.0
A2780[b]	11.4±1.1	10.1±1.0
SW620[c]	12.4±1.1	11.0±1.3
HT29[c]	10.3±1.0	10.9±1.2
C6[a]	11.1±1.0	11.5±1.1
HeLa[b]	10.9±1.0	11.9±1.1
RPE[d]	11.4±1.0	10.7±1.0
Beas2B[d]	12.4±1.0	10.1±1.0
HSF[d]	9.7±0.6	10.9±1.1

[a]A172 and C6 brain cancer cell lines,[b]A2780 and HeLa gynecological cancer cell lines, [c]SW620 and HT29 colon cancer cell lines, [d]Beas2B, RPE, and HSF normal cell lines, respectively lung, retinal, and skin

3. DISCUSSION

The methanolic extract of *Morina persica* (MP) was subjected to LC-MS/MS analysis in negative ion mode, which is highly sensitive for the detection of various polyphenols [20]. A previous study, which provided a limited chemical profile, qualitatively identified 15 compounds including organic acids and flavonoids in MP extracts using the HPLC-UV method with different solvents [13]. In this study, the LC-MS/MS system was used to quantitatively analyze 56 phenolic acids, organic acids, and flavonoid components in the MP methanolic extract. Interestingly, 27 of these compounds were identified for the first time. Quinic acid has been shown to have a variety of biological activities, including anti-inflammatory [22], antioxidant [22], antibacterial [23], antiviral [24], antinociceptive [25], antidiabetic [26], and anticancer [27, 28] in the previous study. Similarly, chlorogenic acid has been shown to have a variety of pharmacological properties, including antibacterial [29], anti-hypertension [30], antioxidant [31], antiulcer, hepatoprotective [32], and anti-inflammatory [33] properties. As for flavonoids in the MP extract. Rutin, which has a quercetin core has been showed that anticonvulsant [34], anti-Alzheimer [35], antinociceptive [36], antidiabetic [37], antiarthritic [38], antiplatelet aggregatory [39], antiulcer [40], antiasthmatic [41], anticancer [42], antibacterial [43], antiviral [44], hepatoprotective [45] activity. Being an important flavonoid hesperidin and was reported to display numerous pharmacological actions such as cardioprotective [46], antihyperlipidemic [46], antidiabetic [46], antihypertensive activities [46], anticancer [47], antioxidant [48, 49], anti-Alzheimer's [50] activities. The molecule quercetin-3-*O*-glucoside, which has another quercetin aglycone, has many pharmacological effects such as anticancer [51], antibacterial [52], antiviral [53] activities. Various pharmacological effects of kaempferol-3-*O*-glucoside include antioxidant [54], hepatoprotective [55], antibacterial [54], antiviral [56], anti-inflammatory [57] activities. Kaempferol-3-*O*-rutinoside has been found to have various biological effects, including antidiabetic [58], hepatoprotective [59], antihypertensive [60], antioxidant [61], and anti-inflammatory [62,] activity.

According to the antioxidant activity were compared with the literature, it was determined that there were minor differences. The current study examined the MP's dose-dependent DPPH radical scavenging activity and compared it with the positive control Trolox (Table 2). MP was shown to be a moderate radical scavenger, with an IC_{50} value of 87.07± 0.74 µg/ml. Moreover, the ferric ion reducing power of MP was determined and the results were given in terms of Trolox equivalent (TE) in Table 2. The extract has a reducing power of 56.27± 2.01 mg TE, indicating its capacity to reduce Fe^{3+} to Fe^{2+}. Mocan et al. reported DPPH radical scavenging activity of MP methanolic extract as 48.09 mg TEs/g extract and FRAP experiment as 82.85 mg TEs/g extract [13]. These minor differences in antioxidant activity; can be explained by the fact that they are collected from different locations and their phytochemical contents are different [63]. These results provide confirmatory evidence that MP methanolic extract has moderate antioxidant activity.

In general, when NCI-60 survival parameters were examined, the fact that the MP extract has low GI_{50} values and high LC_{50} ratios against cancer cell lines shows that it was a suitable candidate for advanced pharmacological tests. In previous studies, Depending on the concentration of *M. persica* methanolic extracts, inhibitory activities were increased [13]. Moreover, quinic acid was tested on human oral cancer cells [27] and C6 rat glioma cells [64]. It has been determined that it has cytotoxic activity on both cell lines. In addition, Rutin has been tested on several leukemic cell lines and has been observed to exhibit high antiproliferative activity [65]. All these secondary metabolites have reported effects as chemopreventive agents via several mechanisms [66-68] This finding indicates that the tested MP extract was cancer specific.

By evaluating the effects of the extract on membrane integrity. information about their cytotoxic activities can be obtained. For this, the activity of cytoplasmic lactate dehydrogenase (LDH) leaking from the damaged plasma membrane to the environment is measured with the help of a kit. LDH activity indicates the amount of leakage implied indirect membrane damage. The MP extract displayed different cytotoxicity values, varying in the range of about 10.3-12.4% against cancer cell lines at IC_{50} concentration (Table 5). Thus, the MP extract could potentially be used as a candidate antiproliferative agent due to its optimal cytotoxic activity.

Many studies have summarized the correlations between MIC and activity as follows: An extract was considered to have good antibacterial activity if its MIC was less than 100 µg/ml, moderate antibacterial activity if it was between 100 and 500 µg/ml, weak antibacterial activity if it was between 500 and 1000 µg/ml, and inactive antibacterial activity if it was over 1000 µg/ml [69]. Based on the results given in Table 6, the most sensitive tested microorganisms were *Clostridium perfringens, Enterococcus faecalis,* and *Escherichia coli* with a MIC value of 7.81 µg/ml, 31.25 µg/ml, and 31.25 µg/ml MIC value respectively. In a study conducted by Bai et al., it was determined that quinic acid disrupts the oxidative phosphorylation pathway and alters glycerophospholipids and fatty acids to interfere with membrane fluidity [70]. In addition, in a study by Rigano et al., determined that Rutin has high antibacterial activity on both gram positive and gram negative bacteria [71]. Previous studies revealed sufficient data to support the link between certain bacteria and cancer [72]. Various bacteria, including *Escherichia coli* [73] and several *Streptococcus sp*.[74], have been associated to chronic colon infections and an increased risk of colon cancer. In another studies, it has been found that the staphylococcal *a*-toxin can simultaneously activate the production of various cytokines linked to all stages of tumor development and nuclear factor κB, the known tumor promoter, in the progression of hepatocellular carcinoma [75]. Considering all these, MP extract can be used in bacterial infections and cancer prophylaxis due to rich secondary compounds.

Table 6. Antibacterial activity of *Morina persica* extract

Microorganisms	**MIC (µg/mL)**	**Tetracycline (µg/mL)**
Gram positive		
Clostridium perfringens	7.81	7.81
Listeria monocytogenes	250	3.91
Bacillus cereus	500	7.81
Staphylococcus aureus	125	1.95
Enterococcus faecalis	31.25	1.95
Gram negative		
Escherichia coli	31.25	7.81
Pseudomonas aeruginosa	500	7.81
Salmonella enteritidis	125	3.91

4. CONCLUSION

The present study investigated the chemical composition, antioxidant activity, antibacterial activity, and proliferative/cytotoxic activity of *M. persica*. The aerial parts of *M. persica* were found to be a significant source of phenolic compounds, including quinic acid and rutin, which exhibited antioxidant activity. The extract demonstrated antiproliferative effects against cancer cells and low cytotoxicity towards normal cells. Moreover, *M. persica* exhibited potent antibacterial activity against *Clostridium perfringens*, *Enterococcus faecalis*, and *Escherichia coli*. Therefore, *M. persica* holds potential for use as both an industrial source of quinic acid and as a functional food with health-promoting effects. Nonetheless, further research is required to

understand the mechanisms of *in vivo* action, bioavailability, and related metabolic pathways *before M. persica* can be utilized as a functional food.

5. MATERIALS AND METHODS

5.1. Chemicals

For all biological activity studies and LC-MS/MS analyses, including chemicals, solvents, and enzymes, all materials were obtained from Sigma Aldrich, Fluka, and Biological Industries (BI, USA).

5.2. Plant material

Morina persica L. (Caprifoliaceae) was collected from the 8th km of Erzincan-Kelkit Road, Erzincan Turkey in July 2019. The plant material was authenticated by Prof. Dr. Ali KANDEMİR, and a voucher specimen (Kandemir 5089) was deposited at EBYU Herbarium, Erzincan, Turkey.

5.3. Extraction

The aerial parts of *Morina persica* (Herba) were dried at room temperature in a shady area, and then ground into a fine powder using a laboratory mill. 15 grams of the powdered material were macerated in 70% methanol. The resulting extract was then concentrated under vacuum at 40 °C using a rotary evaporator. All samples were stored in the dark at +4 °C until they were used.

5.4. LC-MS/MS Quantification of Phenolic

LC-MS/MS analysis was conducted to quantitatively determine the presence of 56 components in the *Morina persica* extract using a previously validated method developed by a previous study [76]. The extract was first diluted with ethanol to a concentration of 250 mg/L and then filtered through a 0.2 µm microfiber filter prior to injection into the LC-MS/MS. The LC-MS/MS data was processed using Shimadzu Lab Solutions software. More information on the validation method and LC-MS/MS chromatogram can be found in Figure S1-S2 and Tablo S1.

A Shimadzu-Nexera model ultrahigh performance liquid chromatograph (UHPLC) coupled with a tandem mass spectrometer was used to accomplish quantiative evaluation of 56 phytochemicals. The reversed-phase UHPLC was equipped with an autosampler (SIL-30AC model), a column oven (CTO-10ASvp model), binary pumps (LC-30AD model), and a degasser (DGU- 20A3R model). Consequently, the chromatographic separation was performed on a reversed phase Agilent Poroshell 120 EC-C18 model (150 mm×2.1 mm, 2.7 µm) analytical column. The column temperature was set to 40°C. The elution gradient was composed of eluent A (water+5 mM ammonium formate+0.1% formic acid) and eluent B (methanol+5 mM ammonium formate+0.1% formic acid). The following gradient elution profile was used: 20-100% B (0-25 min), 100% B (25-35 min), 20% B (35-45 min). Furthermore, the solvent flow rate and injection volume were settled as 0.5 mL/min and 5 µL, respectively.

The mass spectrometric detection was carried out using a Shimadzu LCMS-8040 model tandem mass spectrometer equipped with an electrospray ionization (ESI) source operating in both negative and positive ionization modes. LC-ESI-MS/MS data were acquired and processed by LabSolutions software (Shimadzu). The MRM (multiple reaction monitoring) mode was used for the quantification of the phytochemicals. The MRM method was optimized to selectively detect and quantify phytochemical compounds based on the screening of specified precursor phytochemical-to-fragment ion transitions. The collision energies (CE) were optimized in order to generate optimal phtochemical fragmentation and maximal transmission of the desired product ions. The MS operatinging conditions were applied as: drying gas (N2) flow, 15 L/min; nebulizing gas (N2) flow, 3 L/min; DL temperature, 250°C; heat block temperature, 400°C, and interface temperature, 350°C.

5.5. Antioxidant activity assays

The antioxidant capacity of *M. persica* extract was evaluated using spectrophotometric techniques to measure the in vitro free radical scavenging activity (DPPH·), ferric ion reducing antioxidant power (FRAP), total phenolic content (TPC), and total flavonoid content (TFC).

5.5.1. Total phenolic content

The total phenol content (TPC) of the extract was measured and the results were reported in milligrams of gallic acid equivalents per gram of extract (mg GAE/g extract), as described by GÖZCÜ et al. [77]. The stock solutions of both gallic acid and extract were prepared (1 mg/mL). 0.1 mL of the stock solution of the extract was diluted with 4.5 mL of distilled water. It was made up to 5 mL with 0.3 mL of Na_2CO_3 (2%) and 0.1 mL of Folin-Ciocalteu reagent. After 10 minutes at room temperature, it was vortexed and kept in the dark for 120 minutes for incubation. The absorbance at 760 nm of the solution was recorded.

5.5.2. Total flavonoid content

Aluminum chloride was used to determine the TFC in the extract with the spectrophotometric method [78]. Quercetin was used as a standard. Stock solutions of the extract and quercetin (1 mg/mL) were prepared in methanol. 0.1 mL of the stock solution of the extract, 4.7 mL of methanol, 0.1 mL of $AlCl_3$ (10%), and 0.1 mL of 1 M ammonium acetate solution were added to a cuvette and vortexed. The mixture was incubated for 45 min. at room temperature. After incubation, the absorbance at 415 nm was recorded using a spectrophotometer. A calibration curve was generated for quercetin using different concentrations (1-800 µg/mL). The total flavonoid content (TFC) of the extract was calculated using the calibration curve and expressed as mg quercetin equivalent (QE) per g extract. The mean and standard deviation of the TFC were determined.

5.5.3. DPPH· Free Radical Scavenging Ability

The free radical scavenging activity was tested using a modified version of the procedure described by Liyana et al. technique [79]. In this experiment, a 0.26 mM DPPH· solution was prepared along with stock solutions of the extracts at 1 mg/mL. The stock solutions were then taken at various amounts ranging from 20-400 µL and the final volume was adjusted to 3 mL with MeOH. Next, 1 mL of the DPPH· solution was added to each solution, which was then vortexed and incubated at room temperature for 30 minutes. At the end of the incubation period, the absorbance of each reaction mixture was measured at 517 nm. This procedure was repeated six times, and the mean±standard deviation of the results was calculated. The absorbance values were then converted to % DPPH· scavenging activity, and the IC_{50} (µg/mL) for each extract was determined.

5.5.4. FRAP· Free Radical Scavenging Ability

Ferric reducing antioxidant power (FRAP) study was conducted by Oyaizu et al. with a few minor changes [80]. To measure the total antioxidant capacity of the MP, 250 µL of the MP was mixed with 1.25 mL of 0.2 M phosphate buffer (pH 6.6) and then combined with 1.25 mL of $K_3Fe(CN)_6$ solution. The resulting mixture was incubated at 50 °C in a water bath for 20 min and allowed to cool to room temperature. After adding 1.25 mL of TCA (10 %) and 0.25 mL of $FeCl_3$ (0.1 %) to the cooled mixture, it was mixed, and the absorbance was measured at 700 nm. The absorbance values were then converted to mmol Trolox equivalent (TE) activity/g using a calibration curve consisting of various Trolox concentrations ranging from 10–100 µmol/L. All measurements were performed in triplicate, and mean values and standard deviations were calculated.

5.6. Antiproliferative activity and Cytotoxicity test

The inhibition of cell proliferation and cytotoxicity was evaluated in various cancer cell lines, including A172 (ATCC, CRL-1620) and C6 (ATCC, CCL-107) brain cancer cell lines, HeLa (ATCC, CCL-2) and A2780 (RRID, CVCL-0134) gynecological cancer cell lines, SW620 (ATCC, CCL-227) and HT29 (ATCC, HTB-38) colon cancer cell lines, and normal cell lines, including Beas2B (ATCC, CRL-9609), RPE (ATCC, CRL-4000), and HSF (ATCC, CRL-7449) skin, lung, and retinal cell lines, respectively. The MTT assay was used to evaluate cell proliferation, while the LDH cytotoxicity assay was used to evaluate cytotoxicity. The *M. persica* extract was used in these evaluations [81]. Cell preparation procedures were carried out in a sterile environment in a laminar cabinet. The cell lines were used after reaching confluence in DMEM or RPMI1640 medium containing 10% FBS and 2% PenStrep solution, under 37ºC and 5% CO_2 conditions. Measuring plates were seeded with 10,000 cells per well for the MTT cell proliferation assay and 5,000 cells per well for the LDH cytotoxicity assay. After approximately 16 hours of pre-incubation, test extracts were added and

measurements were performed after 24 hours of incubation. The MTT test results were reported as % cell inhibition, with the optical density of the solvent (DMSO) treated cells assumed to be 100%. The measurement of NCI-60 survival parameters (GI50, TGI, and LC50) was analyzed using a logarithmic function on the logarithmic curve prepared from the absorbance values obtained after the following formulas: Cell proliferation: [(Ti-Tz)/(C-Tz)]x100 if Ti>/=Tz (cytocytic effect) or [(Ti-Tz)/Tz]x100 if Ti<Tz (cytocidal or cytotoxic effect) (Tz; zero point, C; control growth, Ti; inhibition by test substance). GI50: Concentration value that reduces growth by 50% ([(Ti-Tz)/(C-Tz)]x100=50), TGI: Concentration value that reduces growth by 100% (Ti=Tz), LC50: concentration value that by 50% kill cells in the medium ([(Ti-Tz)/Tz]x100=-50) [81, 82]. The LDH test results determined the change in the amount of formazan formed as a result of LDH enzyme activity was measured and evaluated according to the formula below; % Cytotoxicity = [(Substance Absorbance - Low Control / High Control - Low Control) x 100] [81, 82].

5.7. Antibacterial activity

To determine the antibacterial properties of *Morina persica* extract, a microdilution technique was used as described by Elshikh et al. [83]. The test was conducted on five gram-positive bacteria including *Staphylococcus aureus* (ATCC 6538), *Listeria monocytogenes* (ATCC 51774), *Bacillus cereus* (ATCC 10876), *Clostridium perfringens* (ATCC 13124), *Enterococcus faecalis* (ATCC 8459), and three gram-negative bacteria; *Pseudomonas aeruginosa* (ATCC 15442), *Salmonella enteritidis* (ATCC 15442), *Escherichia coli* (ATCC 25922). Test material was serially diluted (from 1000 to 1.95 µg/ml) in 10% w/v DMSO and added to the first row of a 96-well plate. The other wells were filled with 50 µL of sterile Muller Hinton broth (MHB), then 50 µL of the first well was transferred to the latter wells. 10 µL of bacterial suspension (1x108 CFU/ml) was added to the corresponding well and incubated at 37°C for 18 hours. Then, 30 µL of resazurin solution (0.02%) was added to each well, and the plates were incubated for 6 hours. A sterile control well was set up by adding 50 µL of DMSO solution (10% w/v), 10 µL of MHB, 30 µL of indicator solution, and 10 µL of bacterial solution for each bacterium. Negative control was prepared by adding 30 µL of indicator solution and 60 µL of MHB. The minimum inhibitory concentration (MIC) values in µg/mL for each bacterial strain were noted as the concentration level at which a color change from purple to pink was observed, indicating a non-inhibited bacterial action.

Acknowledgements: -

Author contributions: Concept – S.G., Z.A., S.S., H.A.; Design – S.G., Z.A., S.S., H.A.; Supervision – S.G..; Resources – S.G., Z.A., S.S., A.A. A.A., M.A.Y., H.A.; Materials – S.G., Z.A., S.S., H.A.; Data Collection and/or Processing – S.G., Z.A., S.S., A.A. A.A., M.A.Y., H.A.; Analysis and/or Interpretation – S.G., Z.A., S.S., A.A. A.A., M.A.Y., H.A.; Literature Search – S.G., Z.A., H.A.; Writing – S.G., H.A.; Critical Reviews – S.G., H.A.

Conflict of interest statement: The authors declared no conflict of interest in the manuscript.

REFERENCES

[1] Safaeian R, Ghareghan F, Ghanbarian G. The evaluation of essential oil composition of *Morina Persica* L. As an endemic ethnoveterinary plant in Iran. Heliyon 2021; 1: 1-5. http://dx.doi.org/10.2139/ssrn.3984076

[2] Kumar A, Varshney VK, Rawat MSM, Martinez JR, Stashenko EE. Chemical composition of the essential oil of Morina longifolia Wall. leaves. J Herbs Spices Med Plants. 2013; 19(4): 348-356. http://dx.doi.org/10.1080/10496475.2013.800624

[3] Güner A, Aslan S, Ekim T, Vural M, Babaç MT. Türkiye bitkileri listesi:(Vascular Plants) first ed., Nezahat Gökyiğit Botanik Bahçesi Yayınları, İstanbul, Türkiye, 2012.

[4] Bodakhe SH, Ram A, Pandey DP. A new aromatic glycoside from *Morina longifolia* Wall. Asian J Chem. 2010; 22(4): 2789-2793.

[5] Zhu Y, Lü ZP, Xue CB, Wu WS. New triterpenoid saponins and neolignans from *Morina kokonorica*. Helv Chim Acta. 2009; 92(3): 536-545. https://doi.org/10.1002/hlca.200800216

[6] Teng R, Xie H, Liu X, Wang D, Yang C. A novel acylated flavonol glycoside from *Morina nepalensis* var. *alba*. Fitoterapia. 2002; 73(1): 95-96. https://doi.org/10.1016/S0367-326X(01)00324-0

[7] Su BN, Takaishi Y. Morinins HK. Four novel phenylpropanol ester lipid metabolites from *Morina chinensis*. J Nat Prod. 1999; 62(9): 1325-1327. https://doi.org/10.1021/np990145n

[8] Su BN, Takaishi Y, Kusumi T, Morinols AL. Twelve novel sesquineolignans and neolignans with a new carbon skeleton from *Morina chinensis*. Tetrahedron. 1999; 55(51): 14571-14586. https://doi.org/10.1016/S0040-4020(99)00933-3

[9] Baser K, Kürkçüoglu M. Composition of the essential oil of *Morina persica* L. flowers. J Essent Oil Res. 1998; 10(1): 117-118. https://doi.org/10.1080/10412905.1998.9700856

[10] Tashev A, Pancheva E. The Melliferous plants of the Bulgarian flora—Conservation importance. Forestry. 2011; 17(2): 228-237.

[11] Tasdemir D, Dönmez A, Çalıs I, Rüedi P. Evaluation of biological activity of Turkish plants. Rapid screening for the antimicrobial, antioxidant, and acetylcholinesterase inhibitory potential by TLC bioautographic methods. Pharm Biol. 2004; 42(4-5): 374-383. https://doi.org/10.1080/13880200490519695

[12] Tasdemir D, Brun R, Perozzo R, Dönmez A. Evaluation of antiprotozoal and plasmodial enoyl-ACP reductase inhibition potential of turkish medicinal plants. Phytother Res. 2005; 19(2): 162-166. https://doi.org/10.1002/ptr.1648

[13] Mocan A, Zengin G, Uysal A, Gunes E, Mollica A, Degirmenci NS, Alpsoy L, Aktumsek A. Biological and chemical insights of *Morina persica* L.: A source of bioactive compounds with multifunctional properties. J Funct Foods. 2016; 25: 94-109. https://doi.org/10.1016/j.jff.2016.05.010

[14] Tosun F, Akyüz Kızılay Ç, Şener B, Vural M. The evaluation of plants from Turkey for *in vitro* antimycobacterial activity. Pharm Biol. 2005; 43(1): 58-63. https://doi.org/10.1080/13880200590903372

[15] Sadi G, Kaya A, Yalcin HA, Emsen B, Kocabas A, Kartal DI, Altay A. Wild edible mushrooms from Turkey as possible anticancer agents on HepG2 cells together with their antioxidant and antimicrobial properties. Int J Med Mushrooms. 2016; 18(1): 83-95. https://doi.org/10.1615/IntJMedMushrooms.v18.i1.100

[16] Córdoba E, Muñoz J, Blázquez M, González F, Ballester A. Leaching of chalcopyrite with ferric ion. Part II: Effect of redox potential. Hydrometallurgy. 2008; 93(3-4): 88-96. https://doi.org/10.1016/j.hydromet.2008.04.016

[17] Nurgali K, Jagoe RT, Abalo R. Adverse effects of cancer chemotherapy: Anything new to improve tolerance and reduce sequelae?. Front. Pharmacol. 2018; 9: 245-249. https://doi.org/10.3389/fphar.2018.00245

[18] Powers MP. The ever-changing world of gene fusions in cancer: a secondary gene fusion and progression. Oncogen. 2019; 38(47): 7197-7199. https://doi.org/10.1038/s41388-019-1057-2

[19] Conti L, Macedi E, Giorgi C, Valtancoli B, Fusi V. Combination of light and Ru (II) polypyridyl complexes: Recent advances in the development of new anticancer drugs. Coord Chem Rev. 2022; 469: 214-256. https://doi.org/10.1016/j.ccr.2022.214656

[20] Cuyckens F, Claeys M. Mass spectrometry in the structural analysis of flavonoids. J Mass Spectrom. 2004; 39(1): 1-15. https://doi.org/10.1002/jms.585

[21] Mocan A, Zengin G, Uysal A, Gunes E, Mollica A, Degirmenci NS, Alpsoy L, Aktumsek A. Biological and chemical insights of *Morina persica* L.: A source of bioactive compounds with multifunctional properties. J Funct Foods. 2016; 25: 94-109. https://doi.org/10.1016/j.jff.2016.05.010

[22] Pero RW, Lund H, Leanderson T. Antioxidant metabolism induced by quinic acid. Increased urinary excretion of tryptophan and nicotinamide. Phytother Res. 2009 ;23(3): 335-346. https://doi.org/10.1002/ptr.2628

[23] Özbek H. *Cydonia oblonga* Mill. In: Dereli Gürağaç FT, Ilhan M, Belwal T. (Eds). Novel Drug Targets With Traditional Herbal Medicines. Springer, Sweden, 2022. Pp. 209-228.

[24] Wang GF, Shi LP, Ren YD, Liu QF, Liu HF, Zhang RJ, Li Z, Zhu FH, He PL, Tang W, Tao PZ, Li C, Zhao WM, Zuo JP. Anti-hepatitis B virus activity of chlorogenic acid, quinic acid and caffeic acid in vivo and in vitro. Antivir Res. 2009; 83(2): 186-190. https://doi.org/10.1016/j.antiviral.2009.05.002

[25] Toghyani Khorasgani A, Amini Khoei H, Shadkhast M, Salimian S, Majidian M, Habibian Dehkordi S. Quinic acid through mitigation of oxidative stress in the hippocampus exerts analgesic effect in male mice. Adv Herb Med 2021; 7(2): 1-11.

[26] Arya A, Al Obaidi MMJ, Shahid N, Noordin MIB, Looi CY, Wong WF, Khaing SL, Mustafa MR. Synergistic effect of quercetin and quinic acid by alleviating structural degeneration in the liver, kidney and pancreas tissues of STZ-induced diabetic rats: a mechanistic study. Food Chem Toxicol. 2014; 71: 183-196. https://doi.org/10.1016/j.fct.2014.06.010

[27] Samimi S, Ardestani MS, Dorkoosh FA. Preparation of carbon quantum dots-quinic acid for drug delivery of gemcitabine to breast cancer cells. J Drug Deliv Sci Technol. 2021; 61: 102-187. https://doi.org/10.1016/j.jddst.2020.102287

[28] Singh A, Chauhan SS, Tripathi V. Quinic acid attenuates oral cancer cell proliferation by downregulating cyclin D1 expression and Akt signaling. Pharmacogn Mag. 2018; 14(55): 14-19.

[29] Ayseli MT, Ayseli Yİ. Flavors of the future: Health benefits of flavor precursors and volatile compounds in plant foods. Trends Food Sci Technol. 2016; 48: 69-77. https://doi.org/10.1016/j.tifs.2015.11.005

[30] Onakpoya I, Spencer E, Thompson M, Heneghan C. The effect of chlorogenic acid on blood pressure: a systematic review and meta-analysis of randomized clinical trials. J Hum Hypertens. 2015; 29(2): 77-81. https://doi.org/10.1038/jhh.2014.46

[31] Santana Gálvez J, Cisneros Zevallos L, Jacobo Velázquez DA. Chlorogenic acid: Recent advances on its dual role as a food additive and a nutraceutical against metabolic syndrome. Molecules. 2017; 22(3): 358-379. https://doi.org/10.3390/molecules22030358

[32] Xu D, Hu L, Xia X, Song J, Li L, Song E, Song Y. Tetrachlorobenzoquinone induces acute liver injury, up-regulates HO-1 and NQO1 expression in mice model: the protective role of chlorogenic acid. Environ Toxicol Pharmacol. 2014; 37(3): 1212-1220. https://doi.org/10.1016/j.etap.2014.04.022

[33] Liu CC, Zhang Y, Dai BL, Ma YJ, Zhang Q, Wang Y, Yang H. Chlorogenic acid prevents inflammatory responses in IL-1β-stimulated human SW-1353 chondrocytes, a model for osteoarthritis. Mol Med Rep. 2017; 16(2): 1369-1675. https://doi.org/10.3892/mmr.2017.6698

[34] Nieoczym D, Socała K, Raszewski G, Wlaź P. Effect of quercetin and rutin in some acute seizure models in mice. Prog Neuro-Psychopharmacol. Biol Psychiatry. 2014; 54: 50-58. https://doi.org/10.1016/j.pnpbp.2014.05.007

[35] Javed H, Khan M, Ahmad A, Vaibhav K, Ahmad M, Khan A, Ashafaq M, Islam F, Siddiqui MS, Safhi MM, Islam F. Rutin prevents cognitive impairments by ameliorating oxidative stress and neuroinflammation in rat model of sporadic dementia of Alzheimer type. Neuroscience. 2012; 210: 340-352. https://doi.org/10.1016/j.neuroscience.2012.02.046

[36] Selvaraj G, Kaliamurthi S, Thirungnasambandam R, Vivekanandan L, Balasubramanian T. Anti-nociceptive effect in mice of thillai flavonoid rutin. Biomed Environ Sci. 2014; 27(4): 295-259. https://doi.org/10.3967/bes2014.052

[37] Srinivasan K, Kaul C, Ramarao P. Partial protective effect of rutin on multiple low dose streptozotocin-induced diabetes in mice. Indian J Pharmacol. 2005; 37(5): 327

[38] Guardia T, Rotelli AE, Juarez AO, Pelzer LE. Anti-inflammatory properties of plant flavonoids. Effects of rutin, quercetin and hesperidin on adjuvant arthritis in rat. Farmaco. 2001; 56(9): 683-687. https://doi.org/10.1016/S0014-827X(01)01111-9

[39] Chen W, Jin M, Wu W. Experimental study on inhibitory effect of rutin against platelet activation induced by platelet activating factor in rabbits. Zhongguo Zhong Xi Yi Jie He Za Zhi. 2002; 22(4): 283-285. PMID: 12584792

[40] Dubey S, Ganeshpurkar A, Bansal D, Dubey N. Experimental studies on bioactive potential of rutin. Chron Young Sci. 2013; 4(2): 153-163. https://doi.org/10.4103/2229-5186.115556

[41] Jung CH, Lee JY, Cho CH, Kim CJ. Anti-asthmatic action of quercetin and rutin in conscious guinea-pigs challenged with aerosolized ovalbumin. Arch Pharm Res. 2007; 30(12): 1599-1607. https://doi.org/10.1007/BF02977330

[42] Alonso Castro AJ, Domínguez F, García Carrancá A. Rutin exerts antitumor effects on nude mice bearing SW480 tumor. Arch Med Res. 2013; 44(5): 346-351. https://doi.org/10.1016/j.arcmed.2013.06.002

[43] Araruna MK, Brito SA, Morais Braga MF, Santos KK, Souza TM, Leite TR. Evaluation of antibiotic & antibiotic modifying activity of pilocarpine & rutin. Indian J Med Res. 2012; 135(2) :252-254.

[44] De Clercq E, Field HJ. Antiviral prodrug the development of successful prodrug strategies for antiviral chemotherapy. Br J Pharmacol. 2006; 147(1): 1-11. https://doi.org/10.1038/sj.bjp.0706446

[45] Khan RA, Khan MR, Sahreen S. CCl_4-induced hepatotoxicity: protective effect of rutin on p53, CYP2E1 and the antioxidative status in rat. Complement Altern Med. 2012; 12(1) :1-6. https://doi.org/10.1186/1472-6882-12-178

[46] Zanvar AA, Badole SL, Shende PS, Hegde MV, Bodhankar SL. Cardiovascular effects of hesperidin: A flavanone glycoside. In: Watson RR, Preedy VR, Zibadi. (Eds). Polyphenols in human health and disease: Elsevier, Sweden, 2014. pp. 989-992.

[47] Lee J, Kim DH, Kim JH. Combined administration of naringenin and hesperetin with optimal ratio maximizes the anticancer effect in human pancreatic cancer via down regulation of FAK and p38 signaling pathway. Phytomedicine. 2019; 58: 152-162. https://doi.org/10.1016/j.phymed.2018.11.022

[48] Wilmsen PK, Spada DS, Salvador M. Antioxidant activity of the flavonoid hesperidin in chemical and biological systems. J Agric Food Chem. 2005; 53(12) :4757-4761. https://doi.org/10.1021/jf0502000

[49] Panda S, Kar A. Antidiabetic and antioxidative effects of *Annona squamosa* leaves are possibly mediated through quercetin-3-*O*-glucoside. Biofactors. 2007; 31(3-4): 201-210. https://doi.org/10.1002/biof.5520310307

[50] JiménezAliaga K, BermejoBescós P, Benedí J, MartínAragón S. Quercetin and rutin exhibit antiamyloidogenic and fibril disaggregating effects in vitro and potent antioxidant activity in APPswe cells. Life Sci. 2011; 89(25-26): 939-945. https://doi.org/10.1016/j.lfs.2011.09.023
[51] Sudan S, Rupasinghe HV. Quercetin-3-O-glucoside induces human DNA topoisomerase II inhibition, cell cycle arrest and apoptosis in hepatocellular carcinoma cells. Anticancer Res. 2014;34(4):1691-1699.
[52] Sholkamy EN, Ahmed MS, Yasser MM, Mostafa AA. Antimicrobial quercetin 3-O-glucoside derivative isolated from Streptomyces antibioticus strain ess_amA8. J King Saud Univ Sci. 2020; 32(3): 1838-1844. https://doi.org/10.1016/j.jksus.2020.01.026
[53] Bilginer S, Gözcü S, Güvenalp Z. Molecular docking study of several seconder metabolites from medicinal plants as potential ınhibitors of COVID-19 main protease. Turk J Pharm Sci. 2022;19(4): 431-441. https://doi.org/10.4274/tjps.galenos.2021.83548
[54] Taiwo FO, Oyedeji O, Osundahunsi MT. Antimicrobial and antioxidant properties of kaempferol-3-O-glucoside and 1-(4-hydroxyphenyl)-3-phenylpropan-1-one ısolated from the leaves of *Annona muricata* (Linn.). J Pharm Res Int. 2019; 26: 1-13. https://doi.org/10.9734/JPRI/2019/v26i330138
[55] Zang Y, Zhang D, Yu C, Jin C, Igarashi K. Antioxidant and hepatoprotective activity of kaempferol 3-*O*-*β*-D-(2, 6-di-*O*-*a*-L-rhamnopyranosyl) galactopyronoside against carbon tetrachloride-induced liver injury in mice. Food Sci Biotechnol. 2017; 26(4): 1071-1076. https://doi.org/10.1007/s10068-017-0170-7
[56] Zarei A, Ramazani A, Pourmand S, Sattari A, Rezaei A, Moradi S. In silico evaluation of COVID-19 main protease interactions with honeybee natural products for discovery of high potential antiviral compounds. Nat Prod Res. 2022; 36(16): 4254-4260. https://doi.org/10.1080/14786419.2021.1974435
[57] Parveen Z, Deng Y, Saeed MK, Dai R, Ahamad W, Yu YH. Antiinflammatory and analgesic activities of Thesium chinense Turcz extracts and its major flavonoids, kaempferol and kaempferol-3-O-glucoside. J Pharm Soc Jpn. 2007; 127(8): 1275-1279. https://doi.org/10.1248/yakushi.127.1275
[58] Gözcü S, Ugan RA, Özbek H, Gündoğdu B, Guvenalp Z. Antidiabetic and antioxidant properties of *Paeonia mascula* L.: *In vitro* and *in vivo* studies, and phytochemical analysis. Fitoterapia. 2023; 170: 105658. https://doi.org/10.1016/j.fitote.2023.105658
[59] Wang Y, Tang C, Zhang H. Hepatoprotective effects of kaempferol 3-*O*-rutinoside and kaempferol 3-*O*-glucoside from *Carthamus tinctorius* L. on CCl_4-induced oxidative liver injury in mice. J Food Drug Anal. 2015; 23(2): 310-317. https://doi.org/10.1016/j.jfda.2014.10.002
[60] Ahmad M, Gilani AUH, Aftab K, Ahmad VU. Effects of kaempferol-3-*O*-rutinoside on rat blood pressure. Phytother Res. 1993; 7(4): 314-316. https://doi.org/10.1002/ptr.2650070411
[61] Liana L, Rizal R, Widowati W, Fioni F, Akbar K, Fachrial E, Ehrich Lister N. Antioxidant and anti-hyaluronidase activities of dragon fruit peel extract and kaempferol-3-O-rutinoside. J Kedokteran Brawijaya. 2019; 30(4): 247-252. https://doi.org/10.21776/ub.jkb.2019.030.04.3
[62] Hu WH, Dai DK, Zheng BZY, Duan R, Chan GKL, Dong TTX, Qin QW, Wah-Keung Tsim K. The binding of kaempferol-3-O-rutinoside to vascular endothelial growth factor potentiates anti-inflammatory efficiencies in lipopolysaccharide-treated mouse macrophage RAW264. 7 cells. Phytomedicine. 2021; 80: 153400. https://doi.org/10.1016/j.phymed.2020.153400
[63] Iqbal S, Bhanger M. Effect of season and production location on antioxidant activity of *Moringa oleifera* leaves grown in Pakistan. J Food Compost Anal. 2006; 19(6-7): 544-551. https://doi.org/10.1016/j.jfca.2005.05.001
[64] Soh Y, Kim JA, Sohn NW, Lee KR, Kim SY. Protective effects of quinic acid derivatives on tetrahydropapaveroline-induced cell death in C6 glioma cells. Biol Pharm Bull. 2003; 26(6): 803-807. https://doi.org/10.1248/bpb.26.803
[65] Samanta SK, Bhattacharya K, Mandal C, Pal BC. Identification and quantification of the active component quercetin 3-*O*-rutinoside from *Barringtonia racemosa*, targets mitochondrial apoptotic pathway in acute lymphoblastic leukemia. J Asian Nat Prod Res. 2010; 12(8): 639-648. https://doi.org/10.1080/10286020.2010.489040
[66] Feng R, Lu Y, Bowman LL, Qian Y, Castranova V, Ding M. Inhibition of activator protein-1, NF-κB, and MAPKs and induction of phase 2 detoxifying enzyme activity by chlorogenic acid. J Biol Chem. 2005; 280(30): 2788-2795. https://doi.org/10.1074/jbc.M503347200
[67] Gözcü S, Polat KH. Thermosensitive ın situ gelling system for dermal drug delivery of rutin. Turk J Pharm Sci. 2023; 20(2): 78-83. https://doi.org/10.4274/tjps.galenos.2022.00334
[68] Benali T, Bakrim S, Ghchime R, Benkhaira N, El Omari N, Balahbib A. Taha D, Zengin G, Hasan MM, Bibi S, Bouyahya A Pharmacological insights into the multifaceted biological properties of quinic acid. Biotechnol Genet Eng Rev. 2022; 1: 1-30. https://doi.org/10.1080/02648725.2022.2122303
[69] Holetz FB, Pessini GL, Sanches NR, Cortez DAG, Nakamura CV, Dias Filho BP. Screening of some plants used in the Brazilian folk medicine for the treatment of infectious diseases. Mem Inst Oswaldo Cruz. 2002; 97: 1027-1031. https://doi.org/10.1590/S0074-02762002000700017
[70] Bai J, Wu Y, Bu Q, Zhong K, Gao H. Comparative study on antibacterial mechanism of shikimic acid and quinic acid against *Staphylococcus aureus* through transcriptomic and metabolomic approaches. LWT. 2022; 153: 112441. https://doi.org/10.1016/j.lwt.2021.112441
[71] Rigano D, Formisano C, Basile A, Lavitola A, Senatore F, Rosselli S, Bruno M. Antibacterial activity of flavonoids and phenylpropanoids from *Marrubium globosum* ssp. *libanoticum*. Phytother Res. 2007; 21(4): 395-397. https://doi.org/10.1002/ptr.2061

[72] Mager D. Bacteria and cancer: cause, coincidence or cure? A review. J Transl Med. 2006; 4(1): 1-18. https://doi.org/10.1186/1479-5876-4-14
[73] Martin HM, Campbell BJ, Hart CA, Mpofu C, Nayar M, Singh R, Englyst H, Williams HF, Rhodes JM. Enhanced *Escherichia coli* adherence and invasion in Crohn's disease and colon cancer. Gastroenterology. 2004; 127(1): 80-93. https://doi.org/10.1053/j.gastro.2004.03.054
[74] Kim NH, Park JP, Jeon SH, Lee YJ, Choi HJ, Jeong KM, Lee JG, Choi SP, Lim JH, Kim YH, Kim YS, Kim YM, Hwang MH, Cho JW, Moon Y, Oh SK, Jeong JW. Purulent pericarditis caused by group G *streptococcus* as an initial presentation of colon cancer. J Korean Med Sci. 2002; 17(4): 571-573. https://doi.org/10.3346/jkms.2002.17.4.571
[75] Kullander J, Forslund O, Dillner J. *Staphylococcus aureus* and squamous cell carcinoma of the skin. Cancer Epidemiol Biomarkers Prev. 2009; 18(2): 472-478. https://doi.org/10.1158/1055-9965.EPI-08-0905
[76] Yilmaz MA. Simultaneous quantitative screening of 53 phytochemicals in 33 species of medicinal and aromatic plants: A detailed, robust and comprehensive LC–MS/MS method validation. Ind Crops Prod. 2020; 149: 112-147. https://doi.org/10.1016/j.indcrop.2020.112347
[77] Zengin G, Sarikurkcu C, Aktumsek A, Ceylan R. *Sideritis galatica* Bornm.: a source of multifunctional agents for the management of oxidative damage, Alzheimer's's and diabetes mellitus. J Funct Foods. 2014; 11: 538-547. https://doi.org/10.1016/j.jff.2014.08.011
[78] Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal. 2002; 10(3) :178-182. https://doi.org/10.38212/2224-6614.2748
[79] Liyana Pathirana CM, Shahidi F. Antioxidant activity of commercial soft and hard wheat (*Triticum aestivum* L.) as affected by gastric pH conditions. J Agric Food Chem. 2005; 53(7): 2433-2440. https://doi.org/10.1021/jf049320i
[80] Oyaizu M. Studies on products of browning reaction antioxidative activities of products of browning reaction prepared from glucosamine. Jpn J Nutr Diet. 1986; 44(6): 307-315. https://doi.org/10.5264/eiyogakuzashi.44.307
[81] Aydın A, Ökten S, Erkan S, Bulut M, Özcan E, Tutar A, Eren T. *In vitro* anticancer and antibacterial activities of brominated ındeno [1, 2-b] qinoline amines supported with molecular docking and MCDM. ChemistrySelect. 2021; 6(13): 3286-3295. https://doi.org/10.1002/slct.202004753
[82] Aydin A, Karadağ A, Tekin Ş, Korkmaz N, Özdemir A. Two new coordination polymers containing dicyanidoargentate (I) and dicyanidoaurate (I): synthesis and characterization, and a detailed in vitro investigation of their anticancer activities on some cancer cell lines. Turk J Chem. 2015; 39(3): 532-549. https://doi.org/10.3906/kim-1412-13
[83] Elshikh M, Ahmed S, Funston S, Dunlop P, McGaw M, Marchant R, Banat IM. Resazurin-based 96-well plate microdilution method for the determination of minimum inhibitory concentration of biosurfactants. Biotechnol Lett. 2016; 38(6): 1015-1019. https://doi.org/10.1007/s10529-016-2079-2