Turk J Pharm Sci 2022;19(4):400-407
DOI: 10.4274/tjps.galenos.2021.65087

ORIGINAL ARTICLE

In Vitro Anti-Aging Potential Evaluation of *Maclura pomifera* (Rafin.) Schneider 80% Methanol Extract with Quantitative HPTLC Analysis

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ABSTRACT

Objectives: *Maclura pomifera* (Rafin.) Schneider is a widespread species all around the world, which is also frequently cultured for ornamental purposes. Previous studies revealed that *M. pomifera* fruits are rich in prenylated isoflavonoids, exhibit noteworthy biological activities, and have probable benefits, particularly, when applied topically. Considering that phenolic compounds are important sources in the development of anti-aging cosmetic products, this study investigated the anti-aging potential of *M. pomifera* 80% methanolic extract (MPM) by evaluating antioxidant and extracellular matrix degrading enzymes inhibiting activity.

Materials and Methods: For this study, the inhibitory potential of 80% MPM against different enzymes associated with the aging process was evaluated. Given the unequivocal role of oxidative stress in aging, *in vitro* antioxidant tests were employed as well. Moreover, osajin was determined as the major bioactive isoflavonoid of the sample by high performance thin layer chromatography analysis.

Results: Results of the mechanistically different antioxidant assays exhibited notable antioxidant potential of the extract. Inhibition potential of MPM against hyaluronidase, collagenase, and elastase enzymes, which are directly linked to acceleration of the aging process, was investigated and results revealed that MPM inhibited the aforementioned enzymes explicitly. MPM had notable phenolic and flavonoid content; 113.92 ± 2.26 mg gallic acid equivalent/g and 66.41 ± 0.74 mg QE/g, respectively. When total antioxidant capacity assays were considered, it is possible to suggest that MPM is a promising anti-aging agent.

Conclusion: As a result, this study discloses that extracts of fruits of *M. pomifera* have significant anti-aging potential and may be used for this purpose.

Key words: *Maclura pomifera*, anti-aging, antioxidants, HPTLC, osajin

INTRODUCTION

Likewise, all organs, human skin undergoes various physiological changes with advancing age.[1] Two classes of aging exit: intrinsic aging is controlled by genetics and extrinsic aging is the natural result of physiological modifications due to damaging effects of environmental factors like ultraviolet (UV) radiation, chemical toxins, and smoking.[1,2] Vascular and glandular structure degradation, fibrous tissue loss, and decreasing cell regeneration are fundamental factors of aging,[3] which lead to increment in tissue degeneration, wrinkles, and decrement of extracellular matrix (ECM).[4]

As the largest organ, the skin has several roles such as protection, regulation of the body temperature, and detection of senses.[5] The skin consists of epidermis, dermis, and subcutaneous tissue and is the first barrier between human body and outer environment.[6,7] ECM is the largest unit of the dermis and supports growth and elasticity by presenting a structural scaffold.[8] Collagen, elastin, and fibronectin, which are formed by dermal fibroblasts, constitute ECM, are fused with proteoglycans.[5] Collagen is a basic protein comprising approximately 25-35% of all protein content in the body, and is found in the extracellular space of various types of animal

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Received: 07.09.2021, Accepted: 17.09.2021

connective tissues.[3] One of the major causes of skin aging and wrinkle formation is the alteration of collagen structure.[1] Elastin is a protein, which confers a unique physiological elasticity to the skin, and is present in several connective tissues.[6] Skin hydration, keeping the skin smooth, moist and lubricated are important factors that prevent skin aging and a major glycosaminoglycan (GAG), hyaluronic acid, plays a vital role in these activities.[8] This pivotal constituents are degradation by hyaluronidase, collagenase, and elastase enzymes, thus, leading to an acceleration of skin aging. Moreover, exposure to microorganisms, pollution, ionizing radiation, chemicals, and toxins leads to the formation of reactive oxygen species (ROS) and it is harmful consequences accelerate skin aging.[9] ROS can initiate complex molecular pathways and as a result, collagenase, elastase, and hyaluronidase activity may be increased, leading to detectable ECM breakdown and skin texture modifications.[10] For the reasons mentioned, novel natural agents, which decline ROS formation and inhibit ECM degrading proteases, may delay skin-ageing process.[11]

Maclura pomifera (Rafin.) Schneider belongs to Moraceae or the mulberry family, and is also known as the osage orange tree, cultivated almost all around the world.[12] *M. pomifera* has several biological activities such as antibacterial, antifungal, antiviral, cytotoxic, antitumor, estrogenic, and antimalarial[13] due to its prenylated isoflavones, *i.e.* osajin and pomiferin, which are considered major metabolites of the fruits.[14] In anti-aging cosmetic production, phenolic compounds are significant natural sources. Thus, there is a growing interest in studying phenolic compound rich plants such as *M. pomifera* for such activities. In previous studies, it was found that isoflavones of *Maclura* increase the expression levels of collagen, elastin, and fibrillin comparable or superior to equivalent concentrations of retinol. Hence, it may be assumed that *Maclura* isoflavones are potent ECM protein stimulants.[15] Considering these data, the aim of this study is to investigate the anti-aging potential of *M. pomifera* 80% methanolic extract (MPM) by exploring its potential for antioxidant bioactivity and inhibiting ECM degrading enzymes. Additionally, quantitative analysis of the major bioactive component of the extract, osajin was measured by high performance thin layer chromatography (HPTLC) and total phenolic content and total flavonoid content assays were conducted for more accurate understanding of the phenolic profile. The results show that *M. pomifera* might be a valuable source of anti-aging products.

MATERIALS AND METHODS

Chemicals

All enzymes, chemicals, and references employed in the tests were afforded by Sigma Chemical Co. (St. Louis, MO, USA). The quality of all chemicals was of analytical grade.

Plant material

The fruits of *M. pomifera* were collected from Uşak, Türkiye, in May, 2020. Dr. Hilal Bardakcı carried out the botanical identification procedure of the plant samples. A voucher sample of the plant was deposited at Acıbadem University, Herbarium of Faculty of Pharmacy (ACUPH 00002).

Preparation of extracts

The fruits were separated into small pieces and passed through a blender. The fruits (6.45 kg) were macerated with 3125 mL of 80% methanol (MeOH) by using a shaking device for three days at room temperature in a dark place. The macerate was filtered and this procedure was repeated twice. The filtrates obtained were gathered together and, then, methanol was evaporated in a rotary evaporator. The crude methanolic extract was lyophilized (the yield was 204.37 g, 3.16%) and stored at -20°C (MPM).

Quantification procedure of the major bioactive compounds by HPTLC

All chemicals and reagents used were of analytical grade. Chloroform ($CHCl_3$) and ethyl acetate (EtOAc) were purchased from Sigma-Aldrich. Commercially available standard of osajin was purchased from Sigma-Aldrich (SMB 00092). HPTLC analyses were performed on 20 cm × 10 cm glass HPTLC silica gel 60 F_{254} plates (Merck, Darmstadt, Germany). Osajin content in MPM was determined by a CAMAG HPTLC analytical system. Mobile phase used in the current study was previously described by Bozkurt et al.[16] during the isolation of active principles of *M. pomifera*. 10 mg/mL MeOH extract was used as the analysis test solution. A standard stock solution (0.5 mg/mL) of osajin was prepared using 2 mL of acetone. A working solution with 50 µg/mL concentration of standard compound was prepared by dilution with acetone from the stock solution. Each sample was filtered through a 0.45 µm syringe filter. 10 µL of the extract along with at least five different concentrations of the standard solution (3.3-4.7 µL) were applied in the form of bands of 8 mm length on silica gel glass HPTLC plates 60 F254 with CAMAG Automatic TLC Sampler IV. Developments were carried out in CAMAG Automatic Developing Chamber-2 (ADC-2) and the mobile phase was $CHCl_3$:EtOAc [8:2 (v/v)]. Chamber was saturated for 10 min and the plate was preconditioned for 5 min before the development. Humidity was controlled by ADC-2 using $MgCl_2$ (33% RH) for 10 min. Densitometric evaluation was carried out using a CAMAG TLC Scanner IV in fluorescence mode. The slit dimension was maintained at 5 × 0.2 mm, micro and the scanning speed was set at 20 mm/s. Standard contents were obtained by comparing area under receiver operating characteristic curves (AUCs) with the calibration curve of standards at 280 nm. The presence of standards in the extract was assured by comparison of both retention factors (R*f*) and overlaying UV spectra of each extract and standards. The quantity of osajin was determined by comparison of the intensity of diffusely reflected light from the extract and fractions with the standard compound.

Osajin content in crude plant extract was measured using HPTLC-densitometry. R*f* value of osajin standard was found to be 0.556. The occurrence of osajin in test samples was verified by comparison of their R*f* values and overlapping their UV spectra (Figure 1). Quantification was afforded by

comparing AUCs of samples with the calibration curve obtained using the standard compound osajin. The calibration function was y=2.268*10-8x. The correlation coefficient (R) and the coefficient of variation of the calibration function were 0.998% and 1.06%, respectively. HPTLC analysis showed that MPM contains 0.22% (w/w) osajin. Results of the HPTLC study are given in Table 1.

Phenolic profile assay

Total phenolic content assay

The assay was performed to evaluate total phenolic contents of the samples in accordance with Folin-Ciocalteu's method, as previously used by Kurt-Celep et al.[17] 20 µL of freshly diluted sample solutions were mixed with 75 µL Na_2CO_3 (20%) and 100 µL FCR (Folin-Ciocalteu reagent) diluted with H_2O (1:9). After incubation for 30 min at 45˚C, the absorbance of the mixtures was read spectrophotometrically at 765 nm. The results were expressed as mg gallic acid equivalents (GAE) *per* g of extract.

Total flavonoid content assay

Measurement of total flavonoid contents of the fractions was done as a method previously reported by Bardakci et al.[18] Concisely, freshly prepared 1 M CH_3COONa and 10% $AlCl_3$ were mixed with the samples. Then, 30 min of incubation of the mixtures was performed at room temperature and in the dark. After incubation process, the absorbance was calculated at 415 nm. The results were asserted as mg quercetin equivalents (QE) in 1 g of sample.

Determination of in vitro antioxidant activity

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity test

To determine DPPH radical scavenging activity, a combination of freshly diluted sample solutions (various concentrations prepared from 1 mg/mL stock solution) and methanolic DPPH solution (100 mM) were done. After the incubation interval at room temperature for 45 min, the absorbance was read at 517

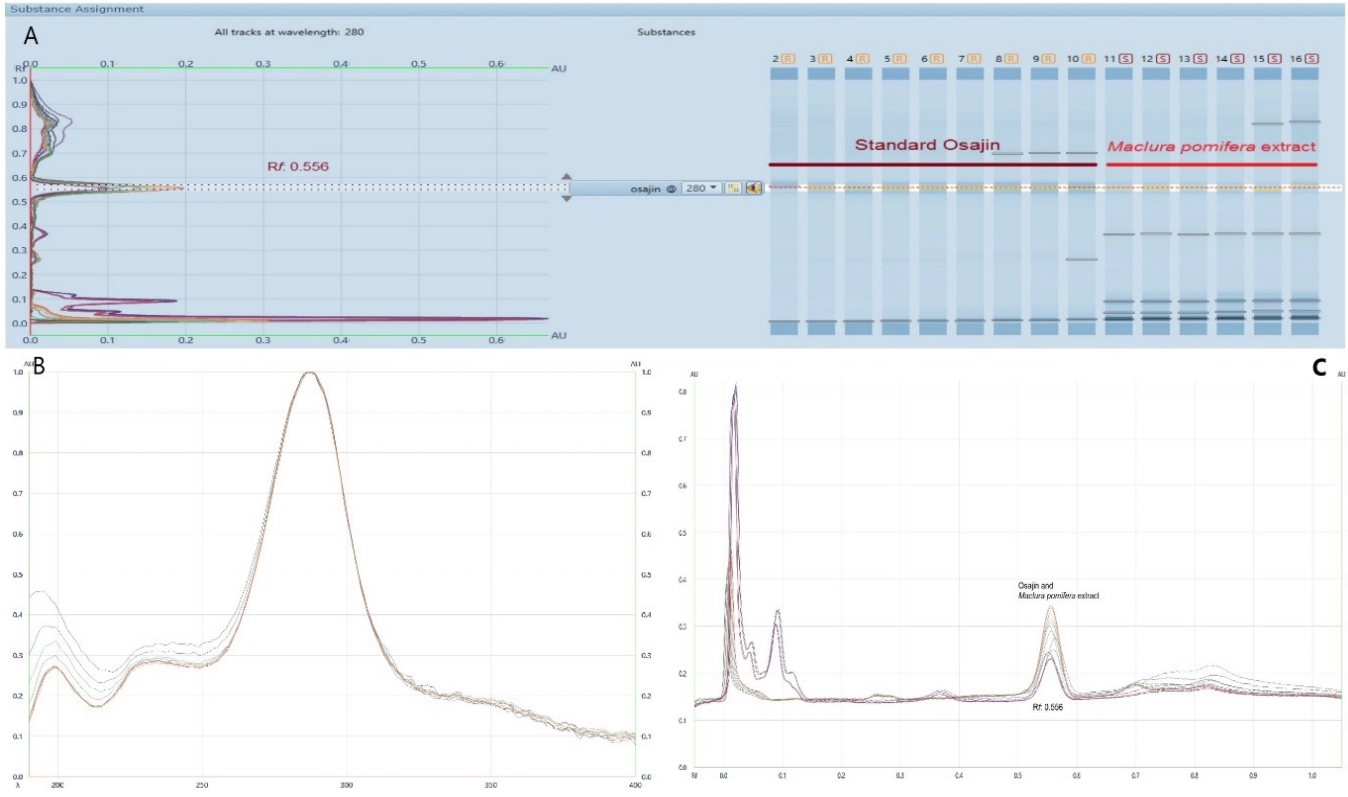

Figure 1. (A) HPTLC chromatogram of standard osajin and test samples at 280 nm. (B) Overlapped UV spectra osajin and *Maclura pomifera* extract. (C) HPTLC chromatogram of standard osajin and test samples at 280 nm

HPTLC: High performance thin layer chromatography, UV: Ultraviolet

Table 1. Spectrophotometric determination of phenolic profile and HPTLC quantification of MPM

	Total phenolic content[A]	Total flavonoid content[B]	Osajin content (%)[C]
MPM[D]	113.92 ± 2.26	66.41 ± 0.74	0.22 ± 0.01

[A]Results were expressed as the mean of triplicates ± standard deviation and as mg gallic acid equivalents in 1 g sample.
[B]Results were expressed as the mean of triplicates ± standard deviation and as mg quercetin equivalents in 1 g sample.
[C]Quantification data of osajin in MPM[D] by HPTLC analysis.
[D]MPM: 80% Methanolic extract of *Maclura pomifera* (Rafin.) Schneider.

nm. Butylated hydroxy toluene (BHT) was used as reference compound to acquire a calibration curve. IC_{50} values of results were stated as µg/mL.[19]

Ferric reducing antioxidant power (FRAP) test

To obtain FRAP reagent, 25 mL of 300 mM acetate buffer (pH 3.6), 2.5 mL TPTZ [2,4,6-tris (2-pyridyl)-s-triazine] and 2.5 mL of $FeCl_3.6H_2O$ (20 mM) were mixed. After that, 10 mL of the sample was added to 260 mL of FRAP reagent and diluted to 300 mL with distilled water in a 96 well-plate. After incubating for 30 min at 37°C, the measurement of absorbance was performed at 593 nm. BHT was used as reference compound. Ferrous chloride solution (0.252 mM) was used to obtain a standard curve and the results were given as mM $FeSO_4$ in 1 g dry extract.[20]

Cupric-reducing antioxidant capacity (CUPRAC) test

CUPRAC test was estimated according to the method described before by Barak et al.[21] Equal volumes of 10 mM $CuSO_4$, neocupraine, and ammonium acetate buffer (85 mL) were mixed in a 96 well plate. After that, 51 mL of distilled water and 43 mL of sample solutions were added to the mixture, respectively. After incubation for 20 min, the absorbance was read at 450 nm. The results were stated as mg ascorbic acid equivalent in 1 g dry extract.

Determination of total antioxidant capacity (TOAC) test

Total antioxidant capacity test was calculated according to the phosphomolybdenum method explained earlier by Barak et al.[22] Firstly, to obtain TOAC solution; 28 mM sodium phosphate monobasic, 4 mM ammonium molybdate and 600 mM H_2SO_4 were mixed. Then, 300 µL of TOAC solution were mixed with 30 µL of sample solutions in a 96 well plate. After the incubation period at 95°C for 90 min, the absorbance was read at 695 nm. Ascorbic acid was used to obtain a standard curve and the results were calculated as mg Trolox equivalents in 1 g dry extract.

Inhibitory activity on skin aging-related enzymes

Anti-collagenase activity

To measure anti-collagenase activity of MPM, 50 mM tricine buffer solution (pH: 7.5) was prepared (400 mM NaCl and 10 mM $CaCl_2$). *Clostridium histolyticum* (ChC - EC. 3.4.23.3) was used as the source of collagenase, which was dissolved in the 50 mM tricine buffer solution to achieve an initial concentration of 0.8 U/mL. 2 mM of *N*-[3-(2-furyl) acryloyl]-Leu-Gly-Pro-Ala (FALGPA) dissolved in tricine buffer was used as substrate. The extracts were incubated with collagenase enzyme in buffer solution for 15 min before adding the substrate to start the reaction. Final reaction mixture comprised a total volume of 150 µL; tricine buffer, 0.8 mM FALGPA, 0.1 units of ChC, and 25 µL MPM. Water was used for the blank results. After addition of the substrate, measurement of absorbance was done immediately. Positive controls performed epigallocatechin gallate (EGCG).[23]

Anti-elastase activity

Evaluation of MPM for anti-elastase activity was carried out using 0.2 mM tris-HCl buffer solution (pH: 8.0). A stock solution of elastase (P.E., E.C. 3.4.21.36) obtained from porcine pancreas was prepared with distilled water at a concentration of 3.33 mg/mL. *N*-Succinyl-Ala-Ala-*p*-nitroanilide (AAAPVN) to used as substrate was dissolved in a buffer solution (1.6 mM). MPM extract was incubated with 1 µg/mL of PE for 15 min at 37°C before substrate addition. At the end of 15 min pre-incubation, 0.8 mM AAAPVN substrate was added to the enzyme mixture containing 1 mg/mL plant extract and incubation was performed again for 15 min at 37°C. While using 0.25 mg/mL EGCG as positive control, this test sample contains the same volume of EGCG instead of MPM, and the test setup was repeated. Following the incubation periods, measurements were taken at 4 different time points for 5 to 30 minutes by Thermo Scientific Multiskan SkyHigh Microplate Spectrophotometer at 365 nm excitation and 410 nm emission.[24, 25]

Anti-hyaluronidase activity

Anti-hyaluronidase activity was performed by modifying the method described by Kolayli et al.[26] and Lee et al.[3] Firstly, the commercially-purchased hyaluronidase (EC 3.2.1.35, Sigma-Aldrich) was dissolved in 0.02 M phosphate buffer (pH: 3.5) containing NaCl and bovine serum albumin. Then, hyaluronic acid, an appropriate substrate of the enzyme, was prepared in acetate buffer (0.1 M, pH: 3.5) and made ready for use. The assay mixture consisting of 20 µL of MPM at a concentration of 1 mg/mL, 10 µL of hyaluronidase and 60 µL of 0.1 M acetate buffer was pre-incubated for 20 min at 37°C. After the incubation time, 10 µL of hyaluronic acid was added to the mixture and incubated again at 37°C for 20 min. At the end of the total incubation time, measurements were made at different time points by Thermo Scientific Multiskan SkyHigh Microplate Spectrophotometer at 600 nm. The blank group did not contain enzyme in the experimental setup, while the control group did not contain the extract. The percent of anti-hyaluronidase activity was calculated using the following equation:

Anti-aging activities (%)= [(Abs of control - Abs of sample)/ Abs of control] × 100

Statistical analysis

Anti-elastase, anti-collagenase, and anti-hyaluronidase activity experiments included in this study were repeated three times independently. The statistical difference was analyzed using the *t*-test of the GraphPad Prism 8 software ($p \leq 0.05$).

RESULTS AND DISCUSSION

Determination of anti-aging potential

Elastin, collagen, and hyaluronic acid are known contents of ECM, which have pivotal roles for young appearance of the skin. Elastin is a vital protein for maintaining the elastic properties of the skin, consequently decrement of elastin in ECM leads to acceleration in the aging process.[27] Previous literature clearly indicates the direct link between wrinkling and aging of skin with lowered amounts of elastin.[28] Hyaluronic acid is a hydrophobic GAG molecule, which is depolymerized *via* hyaluronidase. Hyaluronic acid is crucial to keep the skin smoothness and moisture stable; hence, it has been shown that excessive

breakdown leads to drying and wrinkling to the skin.[29] Through aging process with time, decreased level of collagen causes thinning on dermis, which is considered a distinctive indication under microscopic examination.[30] It was precisely indicated that delaying breakdown of collagen *via* collagenase inhibitors consequently intervals the wrinkling and aging of skin structure.[5] Considering this information, substances that inhibit elastase, collagenase, and hyaluronidase have noteworthy potential for anti-aging products. Previous studies have demonstrated that various isoflavonoids exhibit significant inhibitory bioactivity against aforementioned enzymes. Addotey et al.[31] showed that four different isoflavonoids inhibited hyaluronidase up to 61.2%. Kim et al.[32] have shown that an isolated isoflavonoid from *Glycyrrhiza uralensis* Fisch., licoricidine, has significant elastase inhibitory activity. IC_{50} value of licoridine was calculated as 61.2 ± 4.2 µM while oleanolic acid was calculated 131.4 ± 11.4 as reference compound. Results indicated that isoflavonoids might inhibit elastase enzyme. Consistent with aforementioned study, Kim et al.[33] studied nine different prenylated isoflavonoids, exceedingly related structures with osajin, isolated from roots of *Flemingia philippinensis* Merr. & Rolfe. Researchers reported that five of the prenylated isoflavones had potent inhibition activity against neutrophil elastase, IC_{50} values diversified between 1.9-12.0 µM, while IC_{50} value of oleanolic acid was 28.4 µM. In another study, Ergene Öz et al.[34] investigated *in vitro* inhibitory activity of five isoflavonoids isolated from roots of *Ononis spinoza* L. against hyaluronidase, collagenase, and elastase. Hyaluronidase inhibitory activity of the isoflavones was reported to be between 22.08-45.58%, while, at the same concentration, tannic acid showed 88.32% inhibition. Collagenase inhibition results were calculated between 20.41-28.49% and elastase inhibition was measured as 20.47-46.88%. EGCG was used as reference for both assays and the inhibition activities at the same concentration were measured as 41.18% and 84.64%, respectively. Another study investigated topical treatments of pomiferin directly isolated from *M. pomifera*.[15] Pomiferin is a prenylated isoflavonoid, which can be found in *M. pomifera* fruits and its molecular structure exceedingly resembles that of osajin. Investigators reported that pomiferin exhibited potent ECM protein stimulation activity by increasing collagen and elastin that is superior or equivalent to the reference compound, retinol. All mentioned studies revealed that isoflavones are moderate to potent inhibitors of these enzymes and have significant potential as natural anti-aging materials.

In this study, *in vitro* hyaluronidase, collagenase, elastase inhibitory activities of MPM were investigated for determination of antiaging potential. A comparative assay was conducted for collagenase inhibition assay at two time points, *e.g.* 20 and 40 min, for both MPM and the reference compound, EGCG. The results demonstrated that collagenase inhibition activity increased with time. 1 mg/mL MPM showed 84.55 ± 1.99% inhibition, while 250 µg/mL EGCG showed 84.66 ± 1.83% after 20 min of incubation. Inhibitory bioactivity was amplified through

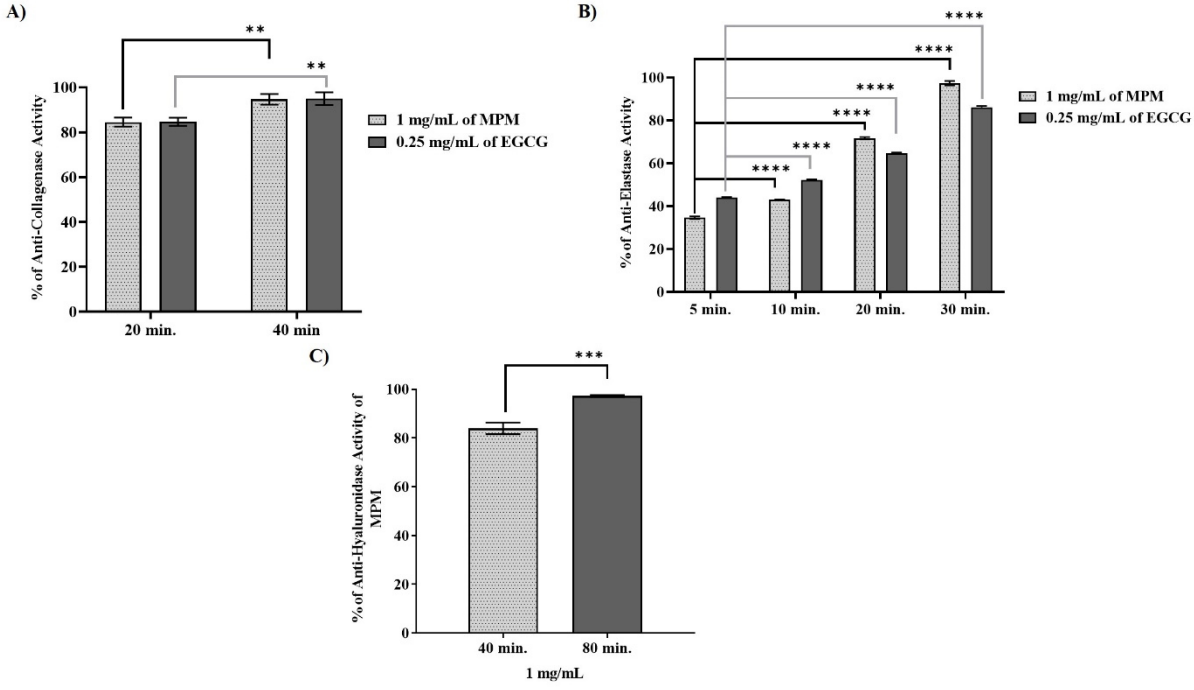

Figure 2. (A) The percent anti-collagenase activity of MPM at a 1 mg/mL concentration at the end of 20 and 40 minutes is presented. In the statistical analysis, the $p \leq 0.01$ value was symbolized with **. % of Anti-elastase activity of 1 mg/mL MPM was shown in (B), and **** was meant $p \leq 0.0001$. EGCG was used as positive control in anti-collagenase and anti-elastase assays (A and B). (C) was shown that % of the anti-hyaluronidase activity of 1 mg/mL MPM at two different time points (at the end of the 40 and 80 minutes). $p \leq 0.001$ value was symbolized with ***

MPM: *Maclura pomifera* 80% methanolic extract, EGCG: Epigallocatechin gallate

time, after 40 min MPM and EGCG inhibited collagenase 94.68 ± 2.42% and 94.98 ± 2.81%, respectively. Consistent with the literature, MPM exhibited significant inhibition activity against elastase. Results were measured for four time points (5, 10, 20, and 30 min) and demonstrated increment over time (Figure 2). EGCG (250 µg/mL) was used as reference and inhibition activity increased at every time point (44.07 ± 0.00%, 52.19 ± 0.00%, 64.69 ± 0.00% and 86.21 ± 0.00%, respectively). Meanwhile MPM in 1 mg/mL concentration exhibited higher enhancement and inhibition activity, which raised from 34.70 ± 0.57% to 97.40 ± 1.04% from 5 min to 30 min. Similarly, 1 mg/mL MPM inhibited hyaluronidase enzyme significantly after 40 min incubation. 83.91 ± 2.36% inhibition was measured following 40 min, after that 80 min of incubation, inhibition rate amplified to 97.19 ± 0.45%. When entire enzyme inhibition assays were considered, results noticeably demonstrated that MPM may be a valuable natural anti-aging agent and may be used in the contents of various anti-aging products; thus, *M. pomifera* may gain extra economic importance.

Determination of antioxidant potential

Numerous exogenous and endogenous factors lead to skin aging *via* various mechanisms. A majority of these factors are directly or indirectly affected by ROS formation in the ECM of the skin.[35] Since the skin covers the outer part of our body, it encounters significant amounts of UV irradiation in daily life. Thus, majority of the skin problems such as sunburn, hyperpigmentation, and skin carcinogenesis originate or are related to direct effects of UV radiation. Likewise, photoaging is an additional consequence of its hazardous properties.[36] Moreover, UV light generates ROS formation and, consequently, oxidative stress in skin tissue, which is one of the most important mechanisms that leads to photoaging.[37] It was hypothesized that since excessive ROS formation causes premature skin aging, agents with a significant antioxidative capacity may be a valuable tool against the perilous effects of UV radiation. Accordingly, clinical studies show that topical antioxidant usage has a protective effect on the skin.[38]

After the interlink between topical antioxidant usage and postponement of skin aging, which is well established in previous literature, examining *in vitro* antioxidant potential of MPM provides valuable information for it is anti-aging potential, when applied topically. Earlier literature clearly demonstrated that extracts with a high number of isoflavonoids might be valuable antioxidants. In a previous study, isoflavonoid-rich extract of *F. macrophylla* extract reduced UVB-induced skin damage by scavenging ROS.[39] Santos and Silva[40] indicated that prenylated isoflavonoids have important antioxidant potential due to their flavonoid moiety and an additive effect of prenyl sidechain. Antioxidant potential of extracts and isoflavonoids of *M. pomifera* was evaluated in a previous study. Results demonstrated that the hydroalcoholic extract and pure osajin showed significant activity on DPPH, FRAP, and TOAC assays, eventhough pomiferin and ethyl acetate fractions showed higher activity.[41] For this study, DPPH radical scavenging activity, FRAP, CUPRAC, and TOAC assays were conducted for comprehensive determination of *in vitro* antioxidant potential of MPM (Table 2). MPM exhibited significant DPPH radical scavenging activity, where IC_{50} value was measured 1998.86 ± 0.02. FRAP and TOAC assays also resulted with notable metal-reducing activity, 0.191 ± 0.01 mM $FeSO_4$/DE and 114.43 ± 0.02 AAE/g DE, respectively. These findings were consistent with the previous study published by Orhan et al.[41] CUPRAC assay was conducted on *M. pomifera* fruit extracts for the first time to our knowledge. Correspondingly, MPM showed noteworthy copper-reducing activity in the CUPRAC assay, where results were measured as 73.928 ± 0.01 AAE/g DE. When total antioxidant capacity assays were considered, it is possible to suggest that MPM is a promising anti-aging agent.

Phenolic profile and HPTLC analysis

Isoflavonoids are phenolic substances, which are known as plant constituents responsible for various noteworthy biological activities such as antioxidant, anticancer, and against gynecological problems.[42] Previous studies evidently demonstrated that prenylated isoflavonoids are major phenolic compounds in *M. pomifera* fruits.[43] Numerous studies identified osajin and pomiferin as the major ingredients of *M. pomifera* fruits, which are primarily accountable for it is biological activities.[12] Osajin and pomiferin are highly similar prenylated isoflavonoids that only differ with one hydroxyl group.[44] Previous reports demonstrated conflictual results for osajin and pomiferin contents of *M. pomifera* fruits. Kartal et al.[45] developed an LC-MS method for determination of osajin and pomiferin in *M. pomifera,* which were collected from Ankara province of Türkiye. The results demonstrated that pomiferin content was slightly higher than osajin content in different parts of the fruit samples. In another study, *M. pomifera* fruit samples were collected from different regions of Midwest and Southern United States and osajin and pomiferin contents were measured *via* a novel HPLC analysis method. Results showed that geographic differences lead to significant alterations of isoflavonoid amounts in samples.[46] Tsao et al.[47] determined osajin and pomiferin content of the fruits collected from Canada

Table 2. *In vitro* antioxidant activity of MPM

	DPPH scavenging activity[A]	FRAP[B]	CUPRAC[C]	Total antioxidant capacity[C]
MPM[D]	1998.86 ± 8.02	0.191 ± 0.01	73.928 ± 0.004	114.43 ± 0.02

[A]Results were expressed as the mean of triplicates ± standard deviation and IC_{50} value of the reference compound "butylated hydroxytoluene" in DPPH scavenging activity is found to be 975.11 ± 4.16.
[B]Results were expressed as the mean of triplicates ± standard deviation and as Mm $FeSO_4$ equivalents in 1 g sample.
[C]Result was expressed as the mean of triplicates ± standard deviation and as ascorbic acid equivalents in 1 g sample.
[D]MPM: 80% methanolic extract of *Maclura pomifera* (Rafin.) Schneider.
DPPH: 2,2-Diphenyl-1-picrylhydrazyl, FRAP: Ferric-reducing antioxidant power, CUPRAC: Cupric-reducing antioxidant capacity

and found that pomiferin content was slightly higher than osajin content. In contrast, Hwang et al.[48] summarized several studies that found osajin amount higher than pomiferin amount in various extracts. It can be claimed that osajin and pomiferin contents of *M. pomifera* fruits are exceptionally variable with geographic differences and extraction techniques due to their decidedly analogous chemical structure. For this study, the amount of MPM was measured *via* HPTLC analysis, for the first time to our knowledge. Results of the analysis showed that osajin is the predominant ingredient of MPM which were collected from Uşak province, 0.22% of the sample consisted of osajin (Table 1). Furthermore, to achieve further assessment of phenolic profile of MPM, total phenolic and total flavonoid content assays were conducted. Results showed that MPM had notable phenolic and flavonoid content as follows; 113.92 ± 2.26 mg GAE/g and 66.41 ± 0.74 mg QE/g, respectively. Results of phenolic profile evaluation showed that MPM might be a prominent candidate as a novel natural anti-aging agent.

CONCLUSION

Even though studies investigating the topical implementation of *M. pomifera* fruits are relatively new, attention in this manner is increasing with encouraging reports. Therefore, this study was aimed to describe a comprehensive evaluation of possible anti-aging potential of *M. pomifera* fruit extract. HPTLC analysis used for *M. pomifera* fruits for determination of isoflavonoid content for the first time to our knowledge, along with *in vitro* studies for determining total phenolic profile. Results showed that osajin is the major ingredient of the samples. Additionally, *in vitro* antioxidant potential of the extract was assessed with four different assays and results demonstrated a significant antioxidant potential of MPM. Additionally, inhibitory activity against the enzymes, which are related to the aging process, was measured and it was seen that MPM had notable enzyme inhibition activity. In conclusion, this study provides information, which may lead to production of novel skincare products.

Ethics

Ethics Committee Approval: Ethics committee approval is not required for the study.

Informed Consent: Not necessary.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: T.H.B., T.B.Ş., H.B., Design: T.H.B., İ.K.C., E.C., Data Collection or Processing: T.H.B., İ.K.C., H.B., Analysis or Interpretation: T.H.B., İ.K.C., Literature Search: T.H.B., T.B.Ş., Writing: T.H.B., E.C.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: This study was supported by Acıbadem Mehmet Ali Aydınlar University Commision of Scientific Projects (no: 2020/02/07).

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