Comparative Study on the Chemical Composition and Biological Activity of Polyphenolic Extracts Obtained from *Maclura pomifera* (Raf.) C.K. Schneid Bark and Periderm

Năstaca-Alina Coman,[a,b] Mihai Babotă,[b,e] Ingrid Nădășan,[d,*] Ștefania Dinică,[e] Ruxandra Ștefănescu,[f] Oleg Frumuzachi,[c] Adrian Man,[g] Andrei Mocan,[c,h] and Corneliu Tanase [b,e]

Literature is limited regarding the potential use of the bark of *Maclura pomifera* as a source of bioactive compounds. The influence of different extraction methods was assessed for the chemical and biological properties of extracts obtained from the bark and periderm of *M. pomifera*. The extraction process was completed using three methods: microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), and sonicator-assisted extraction (SAE). The extracts were characterized by total polyphenol content. The antioxidant capacity was evaluated using DPPH and ABTS methods, the antimicrobial effect was tested against Gram-positive and Gram-negative bacterial strains, and the inhibition of enzyme activity was conducted using α-glucosidase, lipase, and α-amylase assays. The extracts obtained from the periderm exhibited a lower total polyphenolic content compared to those obtained from the bark. The highest antioxidant activity using the DPPH method was observed in the MAE bark extracts and when using the ABTS method, and the highest activity was observed in the UAE bark extracts. Enzymatic activity was most strongly inhibited by the SAE bark extract. The most pronounced antibacterial potential was observed in the MAE and UAE bark extracts, with minimum inhibitory concentration values obtained below 1 mg/mL.

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*Contact information: a: Doctoral School of Medicine and Pharmacy, "George Emil Palade" University of Medicine, Pharmacy, Sciences and Technology of Târgu Mures, Târgu Mures, Romania; b: Research Center of Medicinal and Aromatic Plants, "George Emil Palade" University of Medicine, Pharmacy, Sciences and Technology of Târgu Mures, Târgu Mures, Romania; c: Department of Pharmaceutical Botany, "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania; d: Residency Department, "George Emil Palade" University of Medicine, Pharmacy, Sciences and Technology of Târgu Mureș, Târgu Mureș, Romania; e: Department of Pharmaceutical Botany, Faculty of Pharmacy, "George Emil Palade" University of Medicine, Pharmacy, Sciences and Technology of Târgu Mures, Târgu Mures, Romania; f: Department of Pharmacognosy and Phytotherapy, Faculty of Pharmacy, "George Emil Palade" University of Medicine, Pharmacy, Sciences and Technology of Târgu Mures, Târgu Mures, Romania; g: Department of Microbiology, "George Emil Palade" University of Medicine, Pharmacy, Sciences and Technology of Târgu Mureș, Târgu Mureș, Romania; h: Laboratory of Chromatography, Institute of Advanced Horticulture Research of Transylvania, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania; *Corresponding author: ingrid.nadasan@gmail.com*

INTRODUCTION

Lately, interest in researching plant species as sources of natural compounds with therapeutic potential has significantly increased. The number of studies focusing on the assessment of chemical composition and the bioactive potential of secondary products obtained from plant biomass has recorded impressive growth in recent years (Jadoun *et al.* 2021; Salem and Fouda 2021). *Maclura pomifera* (Raf.) C.K. Schneid. (Osage orange) is a tree species belonging to the Moraceae family. It is a deciduous species native to the southwestern United States (Texas and Arkansas); however, it is currently widely spread in Europe as well. Compounds isolated from various parts of the Osage orange belong to different classes, such as triterpenes, xanthones, flavonoids, and stilbenes (Cicco *et al.* 2009; Laczkó-Zöld *et al.* 2018; Ștefănescu *et al.* 2022). These secondary metabolites have been associated with numerous pharmacological actions: antibacterial, anti-inflammatory, antitumor, cardioprotective, and anticholinesterase activities (Polbuppha *et al.* 2017; Khaleghi *et al.* 2019; Saeed *et al.* 2023).

Osajin and pomiferin exhibit antibacterial properties, although their antioxidant properties are more significant (Filip *et al.* 2015). A study examined the anti-aging potential of the methanolic extract from *M. pomifera* (MP) fruits by measuring antioxidant activities and the inhibition of extracellular matrix-degrading enzymes. The analyzed extracts were promising sources for obtaining anti-aging cosmetic products (Barak *et al.* 2022). Scientific evidence has demonstrated that MP fruits can be toxic to certain herbivorous mammals. Macluroxanthone and alvaxanthone, extracted from the root bark, have proven highly detrimental to mosquito larvae and, respectively, to goldfish. Like the antifungal substance in the wood, these pigments likely protect the roots against decay and insects (Smith and Perino 1981).

Various species of *M. pomifera* are used in traditional medicine worldwide, with the fruits and leaves being primarily utilized in this sense. Existing literature to date also describes the potential use of the tree's bark as a source of bioactive chemical compounds, albeit with limited data to verify this aspect (Filip *et al.* 2021). In this context, the aim of this study was to assess the influence of different extraction methods on the chemical and biological properties of extracts obtained from the bark and periderm of *M. pomifera*. As such, this study focused on obtaining extracts through different extraction techniques and evaluating the total polyphenolic content, as well as assessing the antibacterial, antioxidant, and antienzymatic potential of *M. pomifera* bark and periderm extracts.

MATERIALS AND METHODS

Collection and Processing of Plant Samples

The bark and periderm of *Maclura pomifera* (Raf.) C.K. Schneid was collected in 2022 from the Medicinal Plants Garden of "George Emil Palade" University of Medicine, Pharmacy, Science, and Technology in Târgu Mureș, Romania. The sampled tree was aged between 20 and 30 years old. For the drying process of the bark and periderm, a Nahita 631 Plus drying oven (Auxilab S.L., Beriáin, Spain) was used, being set at a temperature of 50 °C for 24 h. Afterward, the dried material was ground using a Pulverisette 15 cutting mill (Fritsch GmbH, Idar-Oberstein, Germany). The biomass was used in its raw form without undergoing any other treatments or further processing.

Histological Analysis

The plant material was initially subjected to fixation and preservation by immersion in 70% v/v ethyl alcohol. Subsequently, the samples were processed through sectioning using a microtome blade and a manual microtome, with the sections being retrieved into a container of water. For the clear visualization of the histo-anatomical details, the sections were stained by immersion in an iodine green solution for 10 min, followed by the removal of the excess dye through immersion in water (Tanase *et al.* 2022). The stained sections were mounted on slides using glycerinated albumin as an embedding medium and analyzed using a Motic B Series microscope equipped with a digital camera (Nikon, Tokyo, Japan).

Extraction Procedure

The extraction process was completed using microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), and sonicator-assisted extraction (SAE). The extraction parameters, for the MAE, UAE, and SAE extraction methods, were taken from previously optimized methods on other woody plant matrices. Two solvents with different polarities were used: ethanol-70% which has optimal extraction conditions and water 100% to ensure an economic, sustainable, and ecological extraction with a positive impact on climate change by reducing the use of toxic solvents. A microwave extractor (Ethos X, Milestone, Sorisole, Italy) was used for the MAE. Following that, 10 g of bark/periderm and 200 mL of solvent (water/70% ethanol) were mixed. The aqueous variants were extracted for 29 min and 21 s at a microwave power of 864 W, while ethanol variants were extracted for 17 min and 40 s at a microwave power of 655 W (Nisca *et al.* 2022). For UAE, 2.5 g of plant material was mixed with 100 mL of solvent (water/70% ethanol). The extracts were then placed in an Elma Transsonics ultrasound bath (Elma Schmidbauer GmbH, Singen, Germany) for 15 min at 70 °C with an ultrasound frequency of 40 kHz (Tanase et al. 2018). For SAE, 5 g of plant material were weighed in a round-bottomed flask and mixed with 100 mL of freshly prepared 40% ethyl alcohol by dilution. The mixture was homogenized and subjected to sonication for 15 min at an amplitude of 40% (Hielscher Ultrasonics, UP 200St, 200 W, 26 kHz, Teltow, Germany). The ultrasonic probe was inserted vertically in the middle of the mixture. To counteract the thermal effect induced by ultrasound exposure and possible interference caused by it (*e.g.*, heating of the extraction mixture, solvent evaporation), the extraction was performed on ice and with constant maintenance of the immersion depth of the extractor probe (Coman *et al.* 2023).

All extracts were subjected to the lyophilization process using a lyophilizer model BK-FD12S freeze dryer (Biobase Biodustry Co., Ltd., Shandong, China).

The 10 dried extracts of *M. pomifera* were as follows (Table 1).

Table 1. Characteristics of Solutions Obtained by Various Extraction Methods

Sample Code	Method of Obtaining
MPPE UAE	Ethanol extract of periderm obtained by UAE
MPPA UAE	Aqueous extract of periderm obtained by UAE
MPRA UAE	Aqueous extract of rhytidome obtained by UAE
MPRE UAE	Ethanol extract of rhytidome obtained by UAE
MPPE MAE	Ethanol extract of periderm obtained by MAE
MPPA MAE	Aqueous extract of periderm obtained by MAE
MPRA MAE	Aqueous extract of rhytidome obtained by MAE
MPRE MAE	Ethanol extract of rhytidome obtained by MAE
MPRE SAE	Ethanol extract of rhytidome obtained by SAE
MPPE SAE	Ethanol extract of periderm obtained by SAE

Total Polyphenolic Content

To assess the total polyphenolic content (TPC), the Folin-Ciocâlteu method (Cicco *et al.* 2009) was employed. Gallic acid was used as a reference standard to calculate the polyphenolic content in the samples. Initially, the samples were dissolved in 70% ethanol to obtain working solutions with a concentration of 2 mg/mL. From these working solutions, 400 µL were taken and diluted with 1600 µL of distilled water, resulting in a 1:5 dilution. From the obtained diluted solution, 400 µL were taken and mixed with 400 µL of Folin-Ciocâlteu reagent and 3200 µL of Na_2CO_3 solution (5%). The reaction mixture was stirred and incubated at room temperature in the dark for one hour, and absorbance of the samples was spectroscopically measured at a wavelength of 750 nm using a Specord 200Plus UV-Vis spectrophotometer (Analytik Jena AG, Jena, Germany). To quantify the phenolic compound content, a standard curve was used composed of nine different solutions of gallic acid with concentrations ranging from 0.05 to 0.45 mg/mL. The linear equation for this curve is $y = 11.767\times x + 0.2737$. For each extract, three replicates were performed, and the absorbance of each individual sample was recorded twice, resulting in six absorbance values for each experimental variant. The absorbance of the samples was compared to the absorbance of gallic acid standards, and the total polyphenolic content was expressed in gallic acid equivalents per gram of extract (mg GAE/g) using the calibration curve.

Antioxidant Activity

To evaluate the free radical scavenging activity of the extracts, a microplate reader (Epoch, BioTek, Winooski, VT, USA) was employed. Two *in vitro* assays were conducted for this purpose: the DPPH test and the ABTS test. Inhibition capacity was calculated using the following Eq. 1,

$$\text{Inhibition (\%)} = \frac{A_0 - A_1}{A_0} x100 \quad (1)$$

where A_0 is the absorbance of the DPPH solution and A_1 is the absorbance of the mixture of the sample and the DPPH solution read at 517 nm after an incubation period of 30 min. Half-maximal inhibitory concentration (IC_{50}) was calculated using a dose–response curve and was expressed as µg/mL.

Similarly, the assessment of the ABTS free radical neutralization capacity (Ștefănescu *et al.* 2022) was conducted. Ninety-six-well microplates were used, with each well containing 50 µL of extract at various concentrations along with 200 µL of ABTS methanolic solution. The reaction mixture was then incubated at room temperature for 30 min. Following incubation, the absorbance of the samples was measured at 734 nm. To evaluate the inhibition capacity of the extracts and to calculate the IC_{50} (the concentration at which 50% of the free radical activity is inhibited), the formula (Eq. 1) described above was utilized.

Enzyme Inhibitory Activity

The potential enzymatic inhibition was tested on three enzymes: α-glucosidase, α-amylase, and lipase.

To determine the α-glucosidase inhibitory capacity (Tanase *et al.* 2022), the dried extract was first dissolved in phosphate buffer (100 mM, pH = 6.8) supplemented with 5% DMSO (concentrations ranging from 0.002 to 4 mg/mL). Subsequently, 50 µL of the extract was pipetted along with 50 µL of enzyme solution (0.75 U/mL in potassium

phosphate buffer, pH = 6.8) and 50 µL of substrate solution (pNPG). After an incubation period of 15 min at 37 °C, the absorbance of the sample was measured at a wavelength of 405 nm. The positive control used in the α-glucosidase inhibition test was acarbose (3.33 to 333.33 µg/mL). The inhibition percentage (%I) of each extract was calculated according to Eq. 2, with the final results being expressed as IC_{50} values (µg/mL),

$$Inhibition(\%) = \frac{(A_b - A_c) - (A_d - A_e)}{(A_b - A_c)} x100 \quad (2)$$

where A_b is the absorbance of the control blank, A_c is the absorbance of the control, A_d is the absorbance of the sample blank, and A_e is the absorbance of the sample.

To assess the inhibitory capacity of the extract's α-amylase inhibition, the Caraway-Somogyi method utilizing iodine/potassium iodide (IKI) reagent was employed. In a 96-well plate, 25 µL of the extract (previously dissolved in 20 mM phosphate buffer, pH = 6.9, supplemented with 5% DMSO, concentrations ranging from 0.002 to 4 mg/mL) were homogenized with 50 µL of enzyme solution (0.05 mg/mL in phosphate buffer), and the mixtures were pre-incubated for 10 min at 37 °C in darkness. Subsequently, 50 µL of 0.05% starch solution was pipetted into the reaction mixture, and the plate was further incubated for 10 min at 37 °C in darkness. Finally, the catalytic reaction was halted by adding 25 µL of 100 mM hydrochloric acid, followed immediately by the addition of 100 µL of 5 mM IKI solution. The absorbance of the samples was determined at a wavelength of 615 nm. The positive control used in the α-amylase inhibition test was acarbose (3.12 to 200 µg/mL). The inhibition percentage of the samples was calculated according to Eq. 2.

The lipase inhibition assay was based on a previously described protocol using a 96-microplate reader (Moldovan *et al.* 2023). Each well contained 40 µL of tested sample and 40 µL of type II lipase (L3126, Sigma-Aldrich, Saint Louis, MO, USA) from porcine pancreas (2.5 mg/mL prepared in Tris-Buffer (100 mM Tris–HCl and 5 mM $CaCl_2$, pH 7.0)). After preincubation of 15 min at 37 °C, 20 µL of 10 mM pNPB solution was added to each well and samples were incubated for another 10 min, at 37 °C. Finally, the absorbance of the samples was measured at 405 nm. Orlistat was used as positive inhibitor, while the results were calculated using Eq. 2.

Antimicrobial Activity

Tests were conducted to evaluate the antibacterial activity of extracts derived from *M. pomifera* against a broad spectrum of bacteria, including Gram-positive bacteria, such as *Staphylococcus aureus* ATCC 25923, methicillin-resistant *Staphylococcus aureus* ATCC 43300, and *Enterococcus faecalis* ATCC 29212, as well as Gram-negative bacteria, such as *Escherichia coli* ATCC 25925, *Klebsiella pneumoniae* ATCC 3883, and *Pseudomonas aeruginosa* ATCC 27853.

To evaluate the antibacterial activity of MP extracts, the microdilution method in sterile 96-well plates was used. After dissolving the extracts, the authors prepared a series of binary dilutions of the MP extracts in sterile water by sequential transferring of 100 µL of the MP extracts solution from the first column to the subsequent columns of the plate. Simultaneously, a 0.5 McFarland bacterial inoculum using sterile saline solution and freshly obtained cultures of human pathogenic bacteria was prepared. The microorganisms used for antimicrobial evaluations were supplied through the Microbiology Department of the George Emil Palade University of Medicine, Pharmacy, Sciences, and Technology from Târgu-Mureș. From this, 10 µL were mixed with 9990 µL of 2X Mueller Hinton Broth (MHB) and 100 µL of this mixture were transferred into the MP extract dilutions contained

in each column of the plate. To ensure proper control, a positive control without the addition of the TS was prepared. Additionally, a negative control consisting of water and 2X MHB to verify sterility was used.

To facilitate result interpretation, 3 µL of a 0.015% resazurin solution was added to each well (Tanase *et al.* 2019). The plate was then incubated at 37 °C for 2 to 4 h to allow bacterial growth. Colors of resazurin in each well were observed. The last well where the resazurin did not change its color was recorded as the Minimum Inhibitory Concentration (MIC). Before adding the 0.015% resazurin, the wells without visible bacterial growth were identified and 3 µL from these wells were inoculated onto a Sheep Blood Agar culture medium (Oxoid Ltd., Hampshire, United Kingdom). The plates were then incubated at 35 °C for 18 h. The position where no bacterial growth was observed on the Sheep Blood Agar plate was considered the Minimum Bactericidal Concentration (MBC).

Statistical Analysis

The data were presented as mean ± standard deviation. The statistical analysis was performed using the GraphPad Prism 9.4.1 software (GraphPad Software, San Diego, CA, USA). The normality of the sample data was assessed by conducting the Shapiro-Wilk test and visually examining quantile-quantile (Q-Q) plots. Within the analysis, the data underwent a one-way multivariate analysis of variance (ANOVA), followed by the examination of mean group differences using the Tukey *post hoc* test. Spearman's correlation coefficients were employed to assess the relationships between total phenolic content (TPC), antioxidant capacity, and enzymatic inhibition. For the chord diagram, the ORIGIN 9 software (OriginLab Corporation, Northampton, MA, USA) was used. Statistical significance was considered at $p < 0.05$.

RESULTS

Histo-anatomical Analysis of *Maclura pomifera* Bark

The obtained cross-sectional sections allowed the visualization of the histo-anatomical details of two distinct components of the MP bark (Fig. 1).

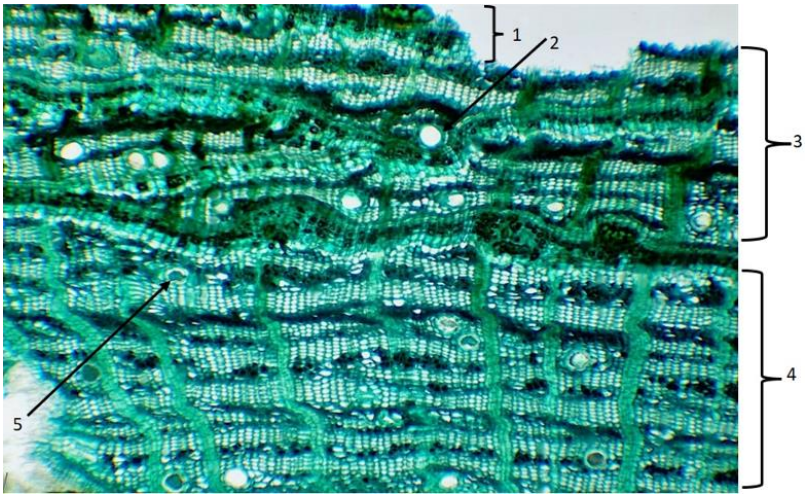

Fig. 1. The rhytidome of *Maclura pomifera*: 1-periderm; 2-sclerenchyma fibers; 3-primary bark; 4-secondary bark; 5-calcium oxalate druses

The well-represented rhytidome can be observed, which includes the outer periderm that has been noticed to be thin and serves to protect the stem externally, and it is composed of 3 to 6 layers of cork (tabular, flattened cells containing tannin), phellogen, and collenchymatous phelloderm. Immediately below the periderm, the primary cortex becomes apparent. It is characterized by multiple layers, rich in strongly lignified sclerenchymatic fibers. The secondary cortex appears as a region radially marked by the medullary rays (lightly stained in green due to its low lignin content); it is also rich in groups of sclerenchymatic fibers, whose cells are highlighted by the darker coloring (most likely due to the contained tannins), and presents numerous large, irregularly shaped druse-type oxalate crystals.

Total Phenolic Content (TPC) and Antioxidant Activity

The total phenolic content (Table 2) varied based on the type of matrix used for the extract preparation, the extraction method employed, and the type of solvent used ($p < 0.0001$). Regarding the matrix that was used, the extracts obtained from the periderm had a lower total phenolic content compared to those obtained from the rhytidome. It is worth mentioning that the ethanolic extract of periderm obtained through microwave-assisted extraction exhibited a higher phenolic content compared to the aqueous extract obtained from rhytidome using ultrasound. This highlights the enhanced efficiency of microwave-assisted alcoholic extraction compared to aqueous ultrasound extraction, a trend that was also observed for the rhytidome extracts.

Furthermore, the solvents used were also determinants in obtaining polyphenol-rich extracts, although this has not been shown to be statistically significant. Regardless of the applied extraction method, the total amount of polyphenols in aqueous extracts was lower than that of the ethanolic extracts. The use of ethanol also increased the efficiency of the extraction methods used, which was noticeable in both periderm and rhytidome extracts. This effect is due to the intermediate polarity of the ethanol-water mixture, which usually allows the extraction of a wider range of polyphenolic compounds compared to water-only extract.

Table 2. TPC and Antioxidant Activity of *Maclura pomifera*

Code Sample	TPC (mg GAE/g DW)	IC_{50} DPPH (µg/mL)	IC_{50} ABTS (µg/mL)
MPRA UAE	69.94 ± 4.82^{b}	$10.24 \pm 0.69^{a,b}$	1.03 ± 0.002^{b}
MPRE UAE	233.57 ± 11.39^{a}	2.42 ± 0.07^{a}	0.12 ± 0.05^{a}
MPPA UAE	13.33 ± 4.64^{d}	156.70 ± 16.23^{e}	7.37 ± 0.17^{f}
MPPE UAE	22.46 ± 1.11^{a}	$21.87 \pm 0.64^{b,c}$	$1.96 \pm 0.07^{b,c}$
MPRA MAE	107.95 ± 4.41^{c}	$9.81 \pm 0.43^{a,b}$	1.09 ± 0.006^{a}
MPRE MAE	264.33 ± 2.65^{e}	1.31 ± 0.03^{a}	0.231 ± 0.004^{a}
MPPA MAE	18.12 ± 3.33^{a}	108.23 ± 3.27^{d}	14.13 ± 0.50^{e}
MPPE MAE	74.08 ± 11.13^{b}	$17.75 \pm 0.20^{a,b}$	$2.23 \pm 0.04^{a,b}$
MPRE SAE	424.50 ± 15.11^{f}	3.20 ± 0.15^{c}	0.28 ± 0.02^{d}
MPPE SAE	20.99 ± 2.17^{a}	$50.92 \pm 2.71^{a,b}$	$3.30 \pm 0.15^{a,b,c}$

Note: dw = dry weight. Different superscript letters (a through f) in the same column mean statistically significant differences at $p < 0.05$ and the same alphabetical superscript in a column indicates no statistically significant difference; ± standard deviation

Regarding the action of MP rhytidome and periderm extracts on the DPPH radical (Table 2), it was observed that the lowest IC_{50} values were obtained in ethanolic rhytidome extracts. It is worth mentioning that ethanolic periderm extracts exhibited IC_{50} values close

to those of rhytidome extracts, indicating that polyphenols in these extracts are not the sole responsible metabolites for their antioxidant activity. Therefore, ethanolic extracts obtained through ultrasound-assisted and microwave-assisted extraction proved the most active among the rhytidome extracts. Similar results were obtained for the samples extracted with a sonicator, where the highest IC_{50} (the inhibition concentration needed to reduce the DPPH absorbance by half) was achieved among rhytidome extracts.

Furthermore, the antioxidant potential of MP rhytidome and periderm extracts was also evaluated using the ABTS method (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)). The results obtained indicate a much better ABTS radical neutralization capability for all extracts compared to their activity against the DPPH radical. The variation trend of the IC_{50} values was similar to the one observed in the complementary test employed; the lowest values were obtained for rhytidome extracts, particularly the ethanolic ones, whereas the inhibitory activity significantly decreased in the series of periderm extracts.

Additionally, as can be observed in Table 2, there were differences of approximately an order of magnitude in antioxidant activity determined against two different radicals (DPPH and ABTS). This is an interesting finding and may indicate various aspects related to the antioxidant behavior of the tested compounds. One of these differences could be antioxidant selectivity. This significant difference in antioxidant activity may suggest that the tested compounds have a strong selectivity for a particular type of free radical. This could be useful in developing specific antioxidants to combat certain types of oxidative stress. Another difference in antioxidant activity may be related to the chemical structure of the tested compounds. Certain functional groups or molecular configurations may be more efficient in neutralizing certain types of free radicals. Additionally, the free radicals used in experiments can have different characteristics and may interact differently with the tested compounds. This can lead to significant variations in antioxidant activity. If the compounds exhibit significantly higher antioxidant activity against a specific type of free radical, this could have implications for the development of treatments or dietary supplements for specific conditions or diseases related to oxidative stress.

Inhibitory Potential Against α-Glucosidase, α-Amylase, and Lipase

The assessment of enzymatic inhibition capacity was tested using an *in vitro* model of α-glucosidase inhibition as a primary study model. The obtained values highlighted a significantly higher inhibition capacity for rhytidome extracts compared to that of periderm extracts. The effects of the extraction method and solvent were also reflected in relation to the α-glucosidase inhibition, with the most active extracts obtained from rhytidome using MAE and sonicator extraction with ethanol as the extraction solvent. The enzymatic activity was most strongly inhibited by the ethanolic extract obtained from rhytidome through sonicator extraction (IC_{50} = 2.47 μg/mL), which also had the highest determined TPC. It is worth mentioning that at the opposite end, the aqueous extracts of periderm obtained through ultrasonic and sonicator extraction exhibited the least inhibition of α-glucosidase, which is most likely due to their low polyphenol content (Table 3).

The inhibition of α-amylase was significantly reduced compared to α-glucosidase, with IC_{50} values for the tested extracts being up to 100 times higher. Furthermore, three of the samples under investigation did not exhibit any inhibition of the enzyme. While the results suggest a lower anti-amylase capacity of the extracts obtained from both MP rhytidome and periderm, it is important to note that the trend of variation described for this activity is similar to that observed in the α-glucosidase inhibition test. Thus, it can be

observed that overall, the lowest IC_{50} values were obtained for the ethanolic extracts of rhytidome processed through MAE and sonication.

Table 3. Enzyme-inhibitory Potential of *Maclura pomifera* Extracts

Code Sample	α-Glucosidase IC_{50} (µg/mL)	α-Amylase IC_{50} (µg/mL)	Lipase IC_{50} (µg/mL)
MPRA UAE	$7.29 \pm 0.42^{a,b,c}$	$1397.41 \pm 324.14^{a,b,c}$	2137.33 ± 216.26^{b}
MPRE UAE	$3.93 \pm 0.27^{a,b}$	$1715.55 \pm 103.56^{b,c,d}$	1363.32 ± 532.87^{a}
MPPA UAE	531.11 ± 32.12^{e}	-	-
MPPE UAE	$32.44 \pm 3.29^{a,b,c,d}$	$2353.77 \pm 471.04^{c,d}$	-
MPRA MAE	$9.52 \pm 0.28^{a,b,c,d}$	-	841.58 ± 200.23^{b}
MPRE MAE	$4.76 \pm 0.06^{a,b,c,e}$	$881.75 \pm 68.64^{a,b}$	-
MPPA MAE	$35.38 \pm 0.64^{b,d,e}$	-	-
MPPE MAE	$23.82 \pm 1.67^{a,b,c,d}$	-	1674.35 ± 288.04^{a}
MPRE SAE	2.47 ± 0.08^{b}	$911.62 \pm 54.18^{a,b}$	1954.93 ± 365.06^{b}
MPPE SAE	56.96 ± 1.50^{d}	$2204.75 \pm 9.65^{c,d}$	-

Note: dw = dry weight. Different superscript letters (a through e) in the same column mean statistically significant differences at $p < 0.05$ and the same alphabetical superscript in a column indicates no statistically significant difference; ± standard deviation

Likewise, with respect to the inhibition of α-glucosidase and α-amylase, the effects of the extraction method and used solvent influenced the lipase inhibition potential of tested extracts. Aqueous extract of rhytidome obtained by MAE had the highest potential to inhibit porcine pancreatic lipase, with an IC_{50} value of 842 µg/mL, while the lowest noticed potential was exhibited by aqueous extract of rhytidome obtained by UAE, with an IC_{50} value of 2140 µg/mL. While some of the tested extracts exhibited a low-to-moderate lipase inhibition potential, the lipase-inhibition capacity of five extracts could not be established.

From Table 4, a strong and statistically significant correlation ($p < 0.05$) was apparent between TPC-DPPH, TPC-ABTS, and DPPH-ABTS for all three extraction methods. Between ABTS and α-amylase, as well as between DPPH and α-amylase, a negative correlation was found for extracts obtained through UAE and SAE. Conversely, for extracts obtained through MAE, a strong positive correlation between DPPH and α-amylase was observed, but it did not reach statistical significance. Regarding the relationship between α-amylase and α-glucosidase, as well as between lipase and α-glucosidase, or between lipase and ABTS, a significant correlation was noted, but without reaching statistical significance for extracts obtained through UAE and SAE. Conversely, for extracts obtained through MAE, a negative correlation between these elements was identified.

Evidence for all of these explanations provided in Table 4 can also be observed in Fig. 2. In this figure, the concept of chord diagrams refers to a graphical method used to visualize and illustrate correlations or relationships between different categories. The analyzed categories include TPC, DPPH, ABTS, α-Glucosidases, α-Amylase, and lipase. Additionally, in the figure, one can observe the lines connecting these categories. These lines are drawn between two points to indicate a link or correlation between them. The thickness or intensity of these chord lines may vary to reflect the degree of correlation or connection between categories. Thicker or more intense lines may indicate a stronger correlation.

Therefore, for extracts obtained through UAE and MAE, the thickest line (indicating the strongest correlation) is between DPPH and lipase, whereas for extracts

obtained through SAE, the strongest correlation is between ABTS and lipase. However, it is important to mention that the absence of a line indicates that there is no correlation between the respective categories. These diagrams provide a visual and intuitive way to interpret and communicate complex chemistry-related data, thus facilitating the understanding and analysis of these relationships.

Table 4. Spearman's Rank Correlation Coefficient ρ (p-value) Between TPC, DPPH, ABTS, α-Glucosidases, α-Amylase, Lipase Among Tested Extracts

	TPC	DPPH	ABTS	α-Glucosidases	α-Amylase	Lipase
Extracts obtained by ultrasounds assisted extraction						
TPC	1.00	0.62 (0.034*)	0.89 (0.0003*)	-0.63 (0.033)	-0.74 (0.028*)	0.99 (0.006*)
DPPH	0.62 (0.034*)	1.00	0.71 (0.012*)	-0.03 (0.939)	-0.25 (0.521)	0.89 (0.033*)
ABTS	0.89 (< 0.0001*)	0.71 (0.012*)	1.00	-0.56 (0.063)	-0.85 (0.006*)	0.77 (0.103)
α-Glucosidases	-0.63 (0.033*)	-0.03 (0.939)	-0.56 (0.063)	1.00	0.53 (0.148)	0.66 (0.175)
α-Amylase	-0.74 (0.028*)	-0.25 (0.521)	-0.85 (0.006*)	0.53 (0.148)	1.00	-0.37 (0.497)
lipase	0.99 (0.006*)	0.89 (0.033)	0.77 (0.1028)	0.66 (0.175)	-0.37 (0.497)	1.00
Extracts obtained by microwave assisted extraction						
TPC	1.00	0.64 (0.029*)	0.77 (0.005*)	-0.62 (0.033*)	-0.70 (0.167)	-0.94 (0.017*)
DPPH	0.64 (0.029*)	1.00	0.74 (0.008*)	-0.92 (< 0.0001*)	0.60 (0.242)	0.83 (0.058)
ABTS	0.77 (0.005*)	0.74 (0.008*)	1.00	-0.76 (0.006*)	-0.83 (0.058)	-0.60 (0.242)
α-Glucosidases	-0.62 (0.033*)	-0.92 (< 0.0001*)	-0.76 (0.006*)	1.00	-0.94 (0.017)	-0.71 (0.136)
α-Amylase	-0.70 (0.167)	0.60 (0.242)	-0.83 (0.058)	-0.94 (0.017*)	1.00	0.77 (0.103)
lipase	-0.941 (0.017*)	0.83 (0.058)	-0.60 (0.242)	-0.71 (0.136)	0.77 (0.103)	1.00
Extracts obtained by sonication assisted extraction						
TPC	1.00	0.62 (0.214)	0.93 (0.022*)	-0.81 (0.072)	-0.93 (0.022)	0.87 (0.667)
DPPH	0.62 (0.214)	1.00	0.73 (0.032)	-0.75 (0.106)	-0.73 (0.122)	-0.50 (1.000)
ABTS	0.93 (0.022*)	0.73 (0.122)	1.00	-0.71 (0.136)	-1.00 (0.003*)	1.00 (0.333)
α-Glucosidases	-0.81 (0.072)	-0.75 (0.106)	-0.71 (0.136)	1.00	0.71 (0.136)	1.00 (0.333)
α-Amylase	-0.93 (0.022)	-0.73 (0.122)	-1.00 (0.003*)	0.71 (0.136)	1.00	-1.00 (0.333)
lipase	0.87 (0.667)	-0.50 (1.000)	1.00 (0.333)	1.00 (0.333)	-1.00 (0.333)	1.00

*Significant result: $p < 0.05$; ρ = Spearman's rank correlation coefficient

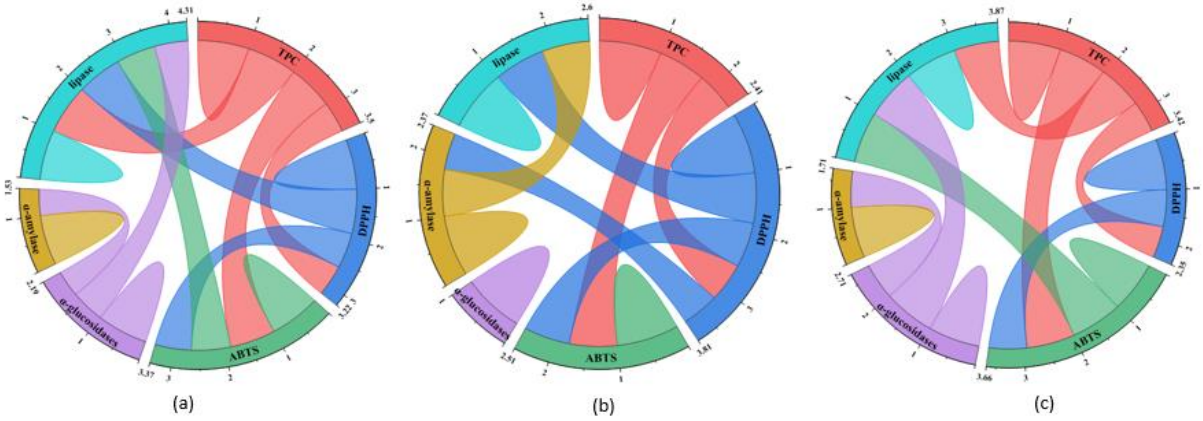

Fig. 2. Chord diagram presenting the correlation between TPC, DPPH, ABTS, α-glucosidase, α-amylase, and lipase for extracts obtained by: ultrasound-assisted extraction (a), microwave-assisted extraction (b), and sonication-assisted extraction (c)

Antibacterial Activity

The authors tested the antibacterial activity *of M. pomifera* extracts against Gram-positive bacteria and Gram-negative bacteria. All the results obtained are represented in Table 5. The findings showed that periderm extracts were generally inactive against all tested strains, with only a very weak inhibitory activity against bacterial growth observed for ethanol extract of periderm obtained *via* UAE. In contrast, rhytidome extracts exhibited slightly increased activity, with the lowest MIC and MBC values observed among ethanol extracts. The most pronounced antibacterial potential was observed for ethanol extracts of rhytidome obtained through MAE and UAE, with MIC values below 1 mg/mL for these extracts.

Table 5. Antibacterial Potential of the Tested Extracts Against Bacterial Strains Evaluated Through MIC and MBC Values

	S. aureus ATCC 25923	*MRSA* ATCC 43300	*E. faecalis* ATCC 29212	*E. coli* ATCC 25922	*K. pneumoniae* ATCC 13883	*P. aeruginosa* ATCC 27853
	MIC / MBC	MIC / MBC	MIC / MBC	MIC / MBC	MIC / MBC	MIC / MBC
Extracts obtained by ultrasounds assisted extraction						
MPPA UAE	>5 / >5	>5 / >5	>5 / >5	>5 / >5	>5 / >5	>5 / >5
MPRA UAE	2.5 / >5	2.5 / 5	>5 / >5	>5 / >5	2.5 / 2.5	>5 / >5
MPPE UAE	5.03 / >5	5.03 / 5.03	>5 / >5	>5 / >5	>5 / >5	>5 / >5
MPRE UAE	0.32 / 3.79	0.97 / 3.79	2.59 / >5	>5 / >5	>5 / >5	>5 / >5
Extracts obtained by microwave assisted extraction						
MPPA MAE	>5 / >5	>5 / >5	>5 / >5	>5 / >5	>5 / >5	>5 / >5
MPRA MAE	1.87 / 3.76	2.5 / 3.76	>5 / >5	>5 / >5	1.87 / 1.87	>5 / >5
MPPE MAE	>5 / >5	>5 / >5	>5 / >5	>5 / >5	>5 / >5	>5 / >5
MPRE MAE	0.23 / 2.48	0.62 / 3.72	2.48 / >5	>5 / >5	>5 / >5	>5 / >5
Extracts obtained by sonication assisted extraction						
MPPE SAE	>5 / >5	>5 / >5	>5 / >5	5.11 / 5.11	>5 / >5	>5 / >5
MPRE SAE	1.29 / 5.18	1.29 / 2.59	1.29 / >5	5.18 / 5.18	>5 / >5	>5 / >5

Expressed in mg of lyophilized extract per mL of solution

Staphylococcus aureus and *Methicillin-Resistant Staphylococcus aureus* (MRSA) were the most sensitive to the tested extracts, as most of the extracts inhibited the growth of these strains, except for the MPPA UAE, MPPA MAE, MPPE MAE, and MPP SAE extracts. The MPRE MAE extract was the most effective, inhibiting bacterial growth at a concentration of 0.23 mg/mL (MIC) for *Staphylococcus aureus* and 0.62 mg/mL for MRSA. For *Enterococcus faecalis*, the extracts MPRE UAE, MPRE MAE, and MPR SAE only had an inhibitory effect without being bactericidal, as the minimum bactericidal concentration (MBC) was not reached for these extracts.

Regarding the Gram-negative bacterial strains, the authors observed that the antibacterial activity was significantly lower compared to the antibacterial activity against Gram-positive bacteria. For the *Escherichia coli* ATCC 25922, resistance to almost all tested extracts was observed (MIC and MBC values higher than 5 mg/mL indicate lack of sensitivity), except for the extracts obtained *via* sonication (MPP-SAE and MPR-SAE). A similar situation was observed for the *Klebsiella pneumoniae* ATCC 13883, which showed resistance to most of the tested extracts, except for the MPRA UAE and MPRA MAE extracts. None of the tested extracts demonstrated efficiency against the *Pseudomonas aeruginosa* ATCC 27853, either at equal concentrations or at concentrations lower than 5 mg/mL.

DISCUSSION

Histo-anatomical evaluation of the stem structure of *M. pomifera* details aspects specific to woody plants belonging to the Moraceae family. From the specialized literature searches, no information was found about the description of the histo-anatomical structure of the bark and periderm. *M. pomifera* has been used in the past for various purposes, including obtaining natural pigments. Regarding the antioxidant activity of *M. pomifera*, there is some research suggesting that this plant may contain compounds with antioxidant properties. Antioxidants are substances that help protect cells against oxidative stress, which can contribute to aging processes (Barak *et al.* 2022) and the development of various conditions, such as cardiovascular diseases, cancer, and neurodegenerative disorders (Alhilal *et al.* 2023).

Although research on *M. pomifera* is limited, it was found that MP contains certain classes of compounds that may have antioxidant potential. Among these compounds are osajin and pomiferin, which are known for their antioxidant properties. It is also believed that certain chemicals found in the fruits and leaves of the plant could contribute to its antioxidant effects (Saeed *et al.* 2023). A study in which osajin (42.9%) and pomiferin (30%) extracted from MP fruits were tested on soybean and fish oils, from which antioxidants and polar components were removed, showed higher antioxidant activity in both oils compared to other commercially available natural antioxidants. Therefore, this study suggests that *M. pomifera* extracts could be used as an antioxidant for edible oils, though additional safety tests are necessary before implementation (Hwang *et al.* 2021). Antioxidant activity has also been demonstrated by isolating pomiferin from MP fruits, this molecule being often isolated together with a small amount of osajin, which structurally resembles pomiferin but lacks an aromatic hydroxyl group. Experiments *ex vivo* on hair follicles showed that doses of pomiferin between 0.05 and 5 ppm had equivalent effects on collagen and elastin expression. Pomiferin could be a chemical substance with potential uses in topical therapies for skin and scalp (Gruber *et al.* 2014).

Regarding the antibacterial activity of *M. pomifera* extracts, there are several studies suggesting that certain chemical components found in this plant could have antibacterial potential. Mahmoud (1981) examined the antibacterial activity of the alcoholic fraction of *M. pomifera* fruit extract using a variation of the microdilution method in an agar medium. It was concluded that two previously isolated isoflavones from this plant, osajin and pomiferin, are responsible for the antibacterial activity of the fruit extract (Mahmoud 1981). Filip *et al.* (2021) demonstrated that Gram-positive bacteria are often more sensitive to extracts obtained from MP fruits compared to Gram-negative strains. The highest antimicrobial activity was highlighted against the species *Listeria monocytogenes* and *Enterococcus faecalis* (Filip *et al.* 2021). Another study showed that the ethanolic extract from *M. pomifera* fruits exhibits antibacterial activity against all investigated microbial strains (Canli *et al.* 2017). The intermediate efficiency of microwave-assisted extraction compared to sonicator-based extraction can be explained by the more intense thermal effects induced by microwave exposure. Although temperature elevation is generally linked to increased extraction yields of bioactive compounds from plant matrices, it can also secondarily induce degradation phenomena, particularly for thermolabile compounds. This can potentially impact the quality of the final extracts (Sridhar *et al.* 2021).

The description of the α-amylase and α-glucosidase inhibitory capacity of the extracts from *M. pomifera* bark and periderm represents a novel and original aspect of the present study. The current literature provides information on a moderate potential for α-glucosidase inhibition in extracts obtained through maceration in ethyl acetate from *M. fruticosa* branches, as well as the efficiency of the flavonoid fraction isolated from immature fruits of *M. tricuspida* in inhibiting the activity of this enzyme (Polbuppha *et al.* 2017; Jo *et al.* 2022). Another study demonstrated that pomiferin significantly stimulated blood insulin levels while simultaneously reducing serum glucose and triglyceride levels in normal rats over a 14-day period following administration of doses at 100 and 300 mg/kg. In comparison to osajin, pomiferin exhibited a higher potential for antidiabetic action (Moon 2014).

Research on *M. pomifera* continues to evolve, and further investigations are needed to definitively confirm the potential and level of antioxidant activity, antimicrobial activity, and antidiabetic activity of this plant, as well as to gain a deeper understanding of the compounds responsible for these effects.

CONCLUSIONS

1. Sonicator-assisted extraction (SAE) and microwave-assisted extraction (MAE) ensured maximum recovery of total polyphenols from both analyzed matrices compared to ultrasound bath extraction. The choice of solvent was also highlighted as a crucial factor in the quality of the extracts, with ethanol proving suitable for the optimal recovery of polyphenolic phytoconstituents from the matrices under investigation. Regarding the matrix used, rhytidome was found to be much richer in polyphenols, which is associated with the intense metabolic activity present in this tissue compared to periderm, which comprises tissues with low or nonexistent metabolic activity.
2. Tests using the 2,2-dephenyl-1-picrylhydrazyl (DPPH) radical revealed that the lowest IC_{50} values were recorded for ethanol extracts of rhytidome. Ethanolic extracts of

periderm showed IC_{50} values close to those of rhytidome extracts, indicating that polyphenols in these extracts are not the sole compounds responsible for their antioxidant activity. Furthermore, the results have highlighted a significantly higher 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical neutralization capacity for all extracts compared to the DPPH radical.

3. Regarding the three enzymes analyzed: Ethanol extract of rhytidome exhibited the highest inhibition capacity against α-glucosidase, correlated with its polyphenol content. Lipase inhibition was weaker than that of α-amylase. Aqueous periderm extracts showed the lowest inhibition capacity against both enzymes, likely due to their low polyphenol content.
4. The observed antibacterial effects were moderate to low against the tested bacterial strains.
5. The obtained results support the potential use of *M. pomifera* rhytidome as a source of polyphenols with biological activity and provide a preliminary basis for further in-depth studies needed to elucidate the mechanisms involved in the biological activity of the phytopreparations derived from these matrices.

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