Phenolic Profile, Antioxidant and Antimicrobial Activities of Algerian *Maclura Pomifera* (Rafin) Schneider Fruit Extract

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Abstract

The aim of this study is to find out the phenolic profile of ethyl acetate extract of *Maclura pomifera (M. pomifera*) fruit polyphenols (EAEMPFP) using HPLC/MS and to evaluate its antioxidant and antimicrobial activities. Antioxidant activity was assessed with DPPH (2, 2-Diphenyl-1-picrylhydrazyl), β-carotene bleaching method and Ferric Reducing Antioxidant Power (FRAP) assays. The EAEMPFP antimicrobial activity was determined using the agar diffusion and microbroth dilution methods against thirteen different strains. Polyphenol and flavonoid contents of *M. pomifera* were determined using the Folin-Ciocalteu and $AlCl_3$ methods, respectively. EAEMPFP total polyphenol content (103.87 ± 6.35 mg gallic acid equivalent/g) is higher than that of flavonoids (31.33 ± 1.71 mg quercetin equivalent/g). HPLC/MS revealed five phenolic compounds, five flavonoids and coumarin. Ferulic acid and caffeic acid were the most abundant with 1.07 and 1.05 mg/mL of extract, respectively. The ability of EAEMPFP to scavenge DPPH (IC50 = 121.94 µg/mL) and to reduce the ferric iron (IC50 = 2.54 mM Fe^{2+}/mL) is dose-dependent. The IC50 of β-carotene bleaching (380.06 µg/mL of EAEMPFP) is higher than that of the standards (i.e. ascorbic acid and quercetin). The FRAP test has shown that the antioxidant activity of EAEMPFP is lower than that of ascorbic acid but higher than that of quercetin. Inhibition zones diameters generated by EAEMPFP antimicrobial activity towards the different strains ranged from 8.2 ± 0.1 (for the beneficial strain *Lb rhamnosus*) to 27.2 ± 0.1 mm (for the pathogenic strain: *Shigella dysenteriae* CRCT 457). These findings have shown that *M. Pomifera* fruit is a potential source of bioactive substances of interest such as polyphenols.

Keywords: *Maclura pomifera* fruit, Phenolic profile, Antioxidant activity, Antimicrobial activity.

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introduction:

Oxidative stress has actually been described as a crucial etiological factor involved in various chronic human diseases such as cancer, cardiovascular and neurodegenerative diseases, inflammation, diabetes and aging (Uttara et al., 2009). This oxidative damage results from the

attack of free radicals on various biomolecules, especially proteins, lipids, and DNA, ultimately leading to the wasting and death of cells (Moon and Shibamoto, 2009). The use of chemicals to

preserve food against oxidation and bacterial spoilage is increasingly blamed for being responsible for human toxicity (Edziri et al., 2012). Antibiotic resistance is another health challenge facing humans. Plants are a diverse source of bioactive molecules and could represent an alternative to chemicals and antibiotics use. Among these plants is *M. pomifera*, an ornamental plant, as potential source of polyphenols. Several studies have shown the biological properties of *M. pomifera* polyphenols such as antioxidant, antimicrobial, anti-inflammatory, antinociceptive, cardioprotective, anti-tumor, anti-ulcer, and antidiabetic activities (Fatnassi et al., 2009; Zhao et al., 2013; Gruber et al., 2014; Moon, 2014; Florian et al., 2006; Abourashed et al., 2015; Su et al., 2017; Botzkurt et al., 2017; Mendili et al., 2020). Despite the extensive research carried out on the biological properties of secondary metabolites of *M. pomifera* fruit from various regions of the world, there are a very few studies on Osage orange growing in Algeria. The present study aims to complete this lack by providing information on the antioxidant and antimicrobial activities of phenolic extract from the fruit of this plant.

Materials And Methods

Plant material and polyphenol extraction

Fruits of *M. Pomifera* were picked in September 2018 in an orchard in the south-west region of the city of Mostaganem (35°56'52.14''N and 0°5'21.05''E). The fruits were disinfected with a 10% (W/V) sodium hypochlorite solution and the unwanted parts were removed. 500g of the whole cleaned and unpeeled fresh fruit rinsed with distilled water were subsequently cut into cubes of 1.5 to 2.5 cm, and soaked overnight in the darkness in 700mL of ethyl acetate, then left 14h to macerate. The mixture was filtered through Whatmann No1 filter paper (No. Z146374100EA), and the fruit residue was rinsed three times with 300mL of ethyl acetate, to be filtered again, before the extraction solvent was evaporated off using a rotary evaporator at 45°C (Tsao et al., 2003). The gummy greenish-yellow residue obtained was dissolved in 10 mL of methanol and filtered with a 0.45 µm microfilter to remove all impurities and kept at 4°C until analysis.

HPLC/MS analysis of ethyl acetate extract of M. pomifera fruit polyphenols (EAEMPFP)

Chromatographic analysis of EAEMPFP was performed using a Thermo Finnigan Surveyor HPLC instrument equipped with an Advantage Max Ion Trap LCQ mass spectrometer (Thermo Fisher Scientific, Waltham, USA). The separation was carried out on a C18 110 Å column (150 x 2 mm, 5 µm). The mobile phase used consists of water (A) and methanol (B); separately acidified with 0.075% (V/V) formic acid. The composition of the mobile phase varied over time according to the following elution gradient: 2% of solvent B during the first 5 min, then linear gradient of solvent B from 2 to 100% during the following 115 min. The volume of injected sample was 5 µL and the flow rate was 1 mL/min (Belabbas et al., 2020).

Chromatograms were recorded at 270 and 320 nm and spectral data were collected in the range 200-800 nm for all peaks. HPLC/MS data were acquired in positive and negative ionization modes. The quantification of each phenolic compound present in the extract studied was determined using the regression equations of the calibration standard curves.

Colorimetric determination of phenolic compounds

Total Phenolic Content (TPC)

The content of total polyphenols was determined as described by Gutfinger (1981), using Folin Ciocalteau's reagent. 100 µL of EAEMPFP were mixed with 5 mL of distilled water and 500 µL of Folin Ciocalteau's reagent. After 3 min, 1 mL of a 35 % (w/v) sodium carbonate solution was added to the mixture which was shaken and incubated in the dark for 20 min at room temperature; absorbance was measured at 725 nm. The total phenolic content of EAEMPFP was expressed in terms of mg gallic acid equivalent per g extract (mg EGA/g).

Total flavonoid content (TFC)

The determination of the total flavonoid content was carried out according to the method of Arvouet-Grand et al. (1994). 500 µL of EAEMPFP was mixed with 500 µL of 2% (w/v) aluminum trichloride solution. After stirring, the mixture was incubated for 10 min at room temperature in the dark. Absorbance was measured at 415 nm. The total flavonoid content was expressed as quercetin equivalent per g of extract (mg EQ/g).

Evaluation of the Antioxidant Activity of the ethyl acetate extract of *M. pomifera* (MP) fruit polyphenols (EAEMPFP)

DPPH-Scavenging Assay

DPPH-scavenging activity of ethyl acetate extract of *M. pomifera* (MP) fruit polyphenols (EAEMPFP) was carried out using the Sanchez-Moreno (2002) method. A volume of 25 µL of EAEMPFP at different concentrations (from 0.125 to 1.5 mM) was added to 975 µL of 0.06 mM DPPH methanolic solution, and left 30 min in the dark at room temperature. The absorbance was measured at 517 nm against a negative control (methanol without DPPH) and compared to the positive controls prepared with the same concentrations of ascorbic acid and quercetin. The percentage of inhibition was calculated using formula (1) as follows:

$$I\% = 100 \times (A_{control} - A_{sample})/A_{control} \quad (1)$$

Where: I %: Percentage of Inhibition, $A_{control}$: Absorbance of the negative control, A_{sample}: Absorbance of the EAEMPFP (sample) or the positive controls (ascorbic acid and quercetin).

The concentration of EAEMPFP or acid ascorbic insuring 50 % of DPPH-scavenging activity (IC50) was determined graphically from the function: % inhibition = f (antioxidant concentration).

Ferric Reducing Antioxidant Power assay (FRAP)

The ferric reducing antioxidant power (FRAP) of ethyl acetate extract of MP fruit polyphenols (EAEMPFP) was run as described by Oyaizu (1986), where ferric iron (Fe^{3+}) of potassium hexacyanoferate (III) $K_3Fe(CN)_6$ is reduced to ferrous iron (Fe^{2+}) in a complex potassium hexacyanoferate (II) $K_4Fe(CN)_6$ of Prussian blue color whose absorbance measured at 700 nm is proportional to the reducing power of the extract. 400 µL of the extract (EAEMPFP) at different

concentrations (from 0.075 to 2 mM) are mixed with 2.5 mL of 0.2M phosphate buffer solution (pH 6.6) and 2.5 mL of a 1 % (w/v) K_3Fe (CN)6 solution, and then incubated 20 min at 50 °C in a water bath before voluntarily stopping the reaction by addition of 2.5 mL of 10 % (w/v) trichloroacetic acid. The mixture was centrifuged (3000 rpm/10 min) and 2.5 mL of the supernatant were mixed with 2.5 mL of distilled water and 0.5 mL of a freshly prepared 0.1% (w/v) ferric chloride solution Absorbance of the sample (EAEMPFP) and the standards (positive controls: ascorbic acid and quercetin) was measured at 700 nm against a negative control (free from EAEMPFP). The results were expressed in mM Fe^{2+}/g of extract.

β-carotene bleaching test

The β-carotene bleaching test was carried out according to the method of Kouamé et al. (2009). An emulsion was prepared as follows: 0.5 mg of β-carotene was dissolved in 1 mL of chloroform and mixed with 25 µL of linoleic acid and 200 µg of Tween-20. The chloroform was removed under vacuum at 50°C, then 100 mL of distilled hydrogen peroxide was slowly added with vigorous stirring to form an emulsion. 5mL of this emulsion was added to 1 mL of the antioxidant solution (EAEMPFP sample or ascorbic acid and quercetin as a positive controls) or to 1mL distilled water (negative control), and an initial absorbance was measured immediately at 470 nm. The tubes were then placed in a water bath at 50°C and the absorbance was measured again at 470 nm after 2h. The antioxidant activity was determined using the following formula (2):

$$AA\ (\%) = [\ 1 - (ASo - A\ S120\ )\ /\ (\ ACo - AC120\ )\ ] \times 100 \qquad (2)$$

Where, AA is the antioxidant activity, Aso is the initial absorbance, AS120 is the absorbance after 2h, Aco is the initial absorbance of the negative control and AC120 is the absorbance of the negative control after 2h.

Antimicrobial activity of the ethyl acetate extract of *M. pomifera* (MP) fruit polyphenols (EAEMPFP)

Microbial strains

A wide range of Gram-positive and Gram-negative bacteria, as well as fungal strains were used to evaluate the antimicrobial activity of ethyl acetate extract of *M. pomifera* (MP) fruit polyphenols (EAEMPFP). These strains mainly belong to the American Type Culture Collection (ATCC) and the Spanish Type culture collection (Colección Española de Cultivos Tipos: CECT) and are represented by: *Staphylococcus aureus* ATCC 49444, *Shigella dysenteriae* CECT 457, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Listeria monocytogenes* ATCC 7644, *Salmonella typhimurium* ATCC 13311, *Bacillus Cereus* ATCC 6633, *Enterococcus hirae* ATCC 10541, *Micrococcus luteus* CECT 457, *Candida albicans* ATCC 10231, *Aspergillus niger* ATCC 1688; while others are from our collection laboratory: *Bifidobacterium animalis* subsp *lactis* and *Lactobacillus rhamnosus* LBRE-LSAS.

Antimicrobial screening

Antimicrobial activity was determined using the agar well diffusion test according to the method of Barefoot and Klaenhammer (1983). Bacterial and fungal strains were incubated at 37°C and 25°C for 24h, respectively; before they were emulsified in sterile physiological water (0.9% NaCl)

and matched with 0.5 Mc Farland turbidity standard corresponding to 10^8 CFU/mL for bacteria and 10^7 CFU /mL for fungal. These young cultures (100 µL) were inoculated in 20 mL of soft Mueller-Hinton; while lactobacilli were inoculated in MRS (De Man et al., 1960) medium on the average agar thickness of 4 ± 0.5 mm. After solidification at room temperature in a sterile area, sterile disks impregnated with 10 µL of the phenolic extract at a concentration of 200 mg/mL of 10% DMSO (dimethylsulfoxide) were placed on the medium. The negative control was a disc soaked in 10% DMSO. 30 µg/mL ampicillin and 40 µg/mL miconazole were used as bacterial positive control and fungal positive control, respectively. The plates were kept at 4°C for 4h to allow enough diffusion of the antimicrobial substance, before incubated at 37°C for 24h for bacteria and at 25°C for 72h for fungi. Inhibition zones diameters (mm) generated by the antimicrobial activity were measured.

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of the EAEMPFP was determined by the broth microdilution method (CLSI, 2009), using sterile 96-well polystyrene round-bottom microtiter plates. Each well received 100µL of strain suspension (10^5 CFU/mL) and 100 µL of EAEMPFP at different concentrations (from 0.08 to 20 mg/mL). Thus, control wells with the culture medium alone (negative control) or inoculated with the strains to be tested without the extract (positive control) were prepared. All tests were performed in triplicate and the MIC was defined as the lowest concentration at which no bacterial or fungal growth was observed after incubation at 37°C for 24h for bacteria and at 25°C for 72h for fungi.

Statistical analysis

The set of experiments was repeated three times and the data obtained were analyzed using IBM SPSS statistics, version 26.0. Results were reported as mean ± SD.

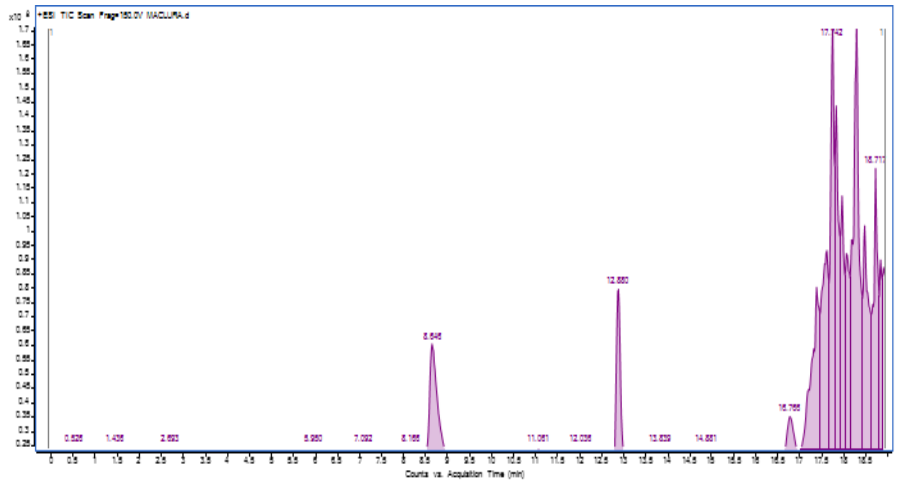
The Pearson correlation coefficients made it possible to characterize the relationship between the antioxidant capacities detected by the various tests and the total content of phenols and flavonoids. The level of statistical significance was set at $p < 0.05$, for a two-tailed test.

One-way ANOVA followed by Tukey's test was used to compare the antioxidant activity results of EAEMPFP obtained in the three tests (FRAP, DPPH and β-carotene bleaching inhibition test) with those of ascorbic acid and quercetin. The multidirectional MANOVA followed by Tukey's test was used to compare the results of the antimicrobial activity of the EAEMPFP obtained in the test of inhibition of the growth zone of the strains with those of reference antimicrobials.

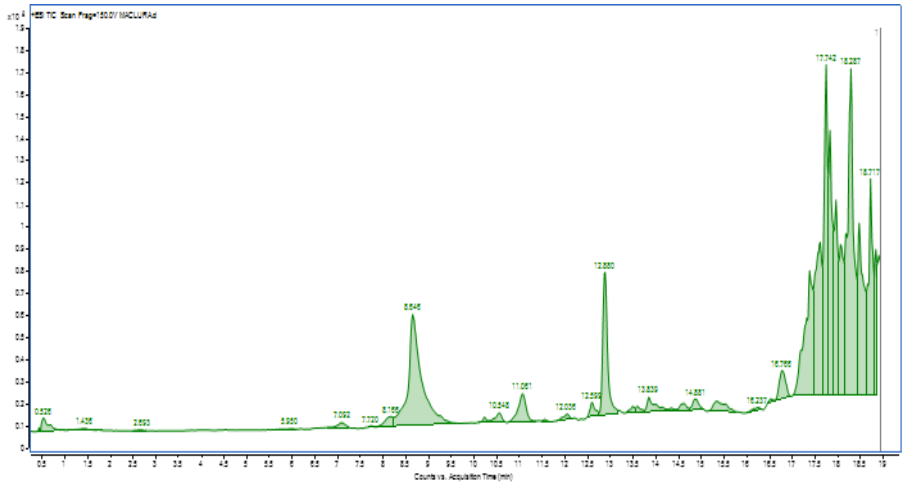
Result And Discussion

Maclura pomifera HPLC/MS phenolic profile

Polyphenols present in EAEMPFP were identified using PDA spectra and MS data. The typical UV chromatographic profile of the extracted polyphenols at 270 and 320 nm is shown in figure 1. Eleven of the thirteen identified compounds are phenols which were numbered and quantified according to their elution order and the interpolation of the calibration curves (Table 1).



(A)



(B)

Figure 1: HPLC profile of ethyl acetate extract of *Maclura pomifera* fruit polyphenols (EAEMPFP) recorded at 270 nm (A) and 320 nm (B)

The five identified phenolic acids were the major phenols (59.34%) present in *M. pomifera* fruit, and they were followed by five flavonoids which represent 32.76%; while coumarins were represented by one single compound, 4-hydroxycoumarin (7.62%). The minor phenol found was thymol (0.23%). Acid ascorbic was also detected in the extract at a level of 3.03%.

The two major phenolic acids found in the extract were hydroxycinnamic acids and gallic acid. The first acids are almost equally represented by 20.54% of ferulic acid (1.072 mg/mL of extract) and 20.11% of caffeic acid (1.050 mg/mL of extract); while gallic acid level is about 16.55% (0.864 mg/mL of extract).

EAEMPFP flavonoids are mainly in unconjugated form, namely Kaempferol (0.518 mg/mL of extract, i.e. 9.91%), quercetin (0.388 mg/mL, i.e. 7.43%), rutin (0.348 mg/mL, i.e. 6.67%), hespertin (0.250 mg/mL, i.e. 4.79%) and apigenin (0.206 mg/mL, i.e. 3.95%).

Table 1: Identification of ethyl acetate extract of *Maclura pomifera* fruit polyphenols (EAEMPFP) by HPLC/MS.

N	Retention time (min)	Molecular formula	m/z	Compounds	Concentration (µg/mL)
1	0.67	$C_6H_8O_6$	117.039	Ascorbic acid	158.24 ±0.005
2	1.41	$C_7H_6O_5$	171.028	Gallic acid	864.36 ± 0.07
3	7.09	$C_9H_8O_4$	181.049	Caffeic acid	1050.11 ± 0.477
4	8.64	$C_8H_8O_3$	153.054	Vanillin	83.22 ± 0.003
5	11.06	$C_{10}H_{10}O_4$	195.064	Ferulic acid	1072.55 ± 0.049
6	12.59	$C_{27}H_{30}O_{16}$	611.160	Rutin	348.53 ± 0.038
7	12.88	$C_9H_6O_3$	163.038	4 hydroxycoumarin	398.06 ± 0.147
8	13.84	$C_{16}H_{14}O_6$	611.197	Hespertin	250.49 ± 0.039
9	14.56	$C_9H_8O_2$	149.09	Cinnamic acid	28.10 ± 0.004
10	14.88	$C_{15}H_{10}O_7$	303.049	Quercetin	387.95 ± 0.120
11	16.51	$C_{15}H_{10}O_5$	271.059	Apigenin	206.14 ± 0.005
12	16.76	$C_{15}H_{10}O_6$	237.055	Kaempferol	517.68 ± 0.036
13	17.74	$C_{10}H_{14}O$	151.110	Thymol	13.98 ±0.001

To our knowledge, this study is the first report on the identification of different classes of polyphenols from the whole fruit of *M. pomifera*; since previous investigations targeted the extraction of the pharmacologically interesting class of isoflavones, in particular osajin and pomiferin (Ribaudo et al., 2017; Darji et al., 2013; Taso et al., 2003). It has been specified that ethyl acetate or diethyl ether extractions will yield osajin and pomiferin (Tsao et al., 2003; Ribaudo et al., 2015), while chloroform extracts will be rich in scandenone and auriculasin (Kupeli et al., 2006). Zushang et al. (2017) reported that maclurin from the xanthone class was identified in an ethanolic extract of dried M. pomifera fruits collected in Texas (USA).

Although other classes of polyphenols have been previously identified in M. pomifera fruit extracts, they were not recorded in our sample. Plant polyphenols composition vary considerably depending on different intrinsic and extrinsic factors, such as plant genetics and varieties, soil composition, phenological stages, and others (Farag et al., 2013)

Results of the present study reiterate the richness of *M. pomifera* extract in ferulic acid, caffeic acid, gallic acid, Kaempferol, quercetin, hespertin, apigenin and rutin; compounds with antioxidant and antimicrobial activities (Al Maruf et al., 2015, Vajic et al., 2018).

Evaluation of total phenols, flavonoids and antioxidant activity of ethyl acetate extract of *Maclura pomifera* fruit polyphenols (EAEMPFP)

The results of the total polyphenol (TPC) and flavonoid (TFC) contents are presented in Table 2. The richness of EAEMPFP in phenolic compounds (103.87 ± 6.35 mg EGA/g of extract) is similar to that reported by Barak et al. (2022) (113.92 mg EGA/g) for a methanolic extract of Turkish *M. pomifera* fruit, and is lower than that recorded in the work of Altuner et al. (2012) (316.87 mg EGA/g) in a Soxhlet methanolic extract of *M. pomifera* fruit polyphenols.

Table 2: Results of total polyphenol (TPC) and total flavonoid content (TFC)

	TPC (mg EGA/g E)	TFC (mg EQ/g E)
Ethyl acetate extract of *Maclura pomifera* fruit polyphenols (EAEMPFP)	103.87 ± 6.35	31.33 ± 1.71

P<0,05.

Flavonoid content found herein (31.33 ± 1.71 mg EQ/g of extract) represents almost half that of the content determined in the methanolic extract (66.41 mg EQ/g of extract) by Barak et al. (2022). Such results for total polyphenol and flavonoid contents are consistent with those obtained by HPLC (Table 1).

The results reported in table 3 show antioxidant activity of EAEMPFP determined by different tests (DPPH, FRAP and β-carotene bleaching inhibition) in comparison with two standard antioxidants (i.e. ascorbic acid and quercetin). According to the ANOVA test, the effect of the antioxidants used (EAEMPFP, ascorbic acid, quercetin) in the three tests used is very highly significant ($p < 0.001$) on the resulting antioxidant activity. The Tukey test made it possible to determine the homogeneous groups, and thus for the DPPH test, there is no significant difference between the activity recorded for the extract and that of quercetin which exerts strong antioxidant activity (Cai et al., 2004).

Table 3: In vitro antioxidant activity of ethyl acetate extract of *M. pomifera* fruit polyphenols, ascorbic acid and quercetin

	DPPH scavenging activity (IC_{50} µg/mL)	Inhibition of β-carotene bleaching (IC_{50} µg/mL)	FRAP mmol Fe^{2+}/g
EAEMPFP	121.94 ± 1.84^{b}	380.06 ± 8.41^{c}	2.54 ±0,39^{b}
Quercetin	120.54 ± 1,05^{b}	236.73 ± 4.45^{b}	1.36 ± 0.29^{c}
Ascorbic acid	83.19 ± 2.15^{a}	53.77 ± 1.3^{a}	6.63 ± 0.41^{a}

P<0,001. Each value represents mean ± S.D. Statistical analysis was performed using one-way ANOVA followed by Tukey's test.

The β-carotene bleaching test is based on the loss of the yellow color of β-carotene due to its reaction with radicals formed by the oxidation of linoleic acid in an emulsion (Silvestre et al., 2012). This test evaluates the inhibitory power which represents the antioxidant potential of the sample by measuring the amount of remaining β-carotene (Bamoniri et al., 2010). The IC50 of EAEMPFP in this test (380.06 µg /mL) is 7.06 and 1.6 times higher than those of ascorbic acid (53.77µg/mL) and quercetin (236.73 µg/ mL), respectively.

Moreover, the ferric reducing antioxidant power (FRAP) of the *M. pomifera* extract is significantly higher than that of quercetin, but 2.6 times lower than that of ascorbic acid.

The previously obtained results were used to estimate the correlations based on the Pearson coefficients to evaluate the relationships between total phenol (TPC) and flavonoid (TFC) contents, and the antioxidant activity of the extract. Statistical analysis (Table 4) has shown a strong significant correlation between TPC and TFC (r = 0.903, P < 0.05), indicating the likelihood that TFC is partially responsible for the antioxidant/radical scavenging activities of EAEMPFP.

Spearman's ranking analysis showed a strong correlation between TPC on one hand, and DPPH, FRAP and β-carotene bleaching inhibition tests on the other hand; suggesting that the phenolic compounds present in the extract are responsible for both free radical scavenging and antioxidant activities. Another important finding is the strong significant negative correlation between TPC and FRAP (r= - 0.950, P<0.05). This correlation indicates the likelihood that the compounds present in the extract exhibit a high capacity to reduce heavy metals.

Finally, the good correlation between the antiradical and antioxidant activities supports the reported results of Table 4. Thus, the ethyl acetate extract of *M. pomifera* fruit polyphenols studied exerts both an antiradical activity and an antioxidant activity; which is in line with the results reported by other authors (Barak et al., 2022; Taso et al., 2003) for *M. pomifera* fruit extract.

Table 4: Pearson's correlations between antioxidant activity parameters and total phenolic and flavonoid contents.

		TPC	TFC	FRAP	DPPH	IbβC
TPC	Correlation r	1	0,903*	-0,950*	0,827*	0,998*
	P-value	-	0,032	0,014	0,046	0,039
TFC	Correlation r		1	-0,892*	0,941*	0,875
	P-value		-	0,049	0,034	0,321
FRAP	Correlation r			1	-0,766*	-0,929*
	P-value			-	0,044	0,021
DPPH	Correlation r				1	0,885**

	P-value	-	0.008
IbCB	Correlation r		1
	P-value		-

Antimicrobial activity

Antimicrobial activity of EAEMPFP against several pathogens is reported as inhibition zone diameters (mm) in Table 5. The results of the MANOVA test reveal a significant difference between the antimicrobial activity of the sample (EAEMPFP) and the standards (i.e. ampicillin and miconazole) resulting in different levels of growth inhibition of bacteria and fungi tested. The Tukey test was undertaken to better appreciate the comparison between the efficacy of the extract and the standards used, and has shown that there is no significant difference between the antibacterial effect of the extract and ampicillin against *Listeria monocytogenes*. Antibacterial activity of the extract is significantly higher than that of ampicillin against *Bacillus cereus*.

In contrast, the growth of the two beneficial bacteria, namely Bifidobacterium animalis subsp lactis and Lactobacillus rhamnosus was not significantly inhibited by the M. pomifera fruit polyphenol extract (less than 8 mm, which represents the limit near the halo limit).

Table 5: Antimicrobial activity of ethyl acetate extract of M. pomifera fruit polyphenols

Strains	Phenolic extract (mm)	Ampicillin (mm)	miconazole (mm)	P value (MANOVA test)
Micrococcus luteus	17,23 ± 0.25^{b}	19,57 ± 0,4^{a}	-	0,000
Staphylococcus aureus	25,17 ± 0,3^{b}	37,9 ± 0,26^{a}	-	0,000
Listeria monocytogenes	16,3 ± 0,26^{a}	17,19 ± 1,07^{a}	-	0,000
Bacillus cereus	25,19 ± 0,66^{a}	22,4 ± 0,53^{b}	-	0,001
Enterococcushirae	19,16 ± 0,29^{b}	21,2 ± 0,26^{a}	-	0,000
Shigelladysenteriae	27,3 ± 0,56^{b}	30,6 ± 1,22^{a}	-	0,002
Escherichia coli	14,33 ± 0,5^{b}	17,47 ± 0,45^{a}	-	0,002
Pseudomonas aeruginosa	21,1± 0,28^{b}	25,64 ± 1,18^{a}	-	0,008
Salmonella typhimurium	14,5 ± 0,5^{b}	17,06 ± 1,01^{a}	-	0,000
Candida albicans	12,67 ± 0,42^{b}	-	15,74 ± 0,6^{a}	0,000
Aspergillus niger	23,94 ± 0,21^{a}	-	20,67 ± 0,42^{b}	0,003

Bifidobacteriumanimalissubsp lactis	6,5 ± 0,5[b]	11,7 ± 1,25[a]	-	0,000
Lactobacillus rhamnosus	7,07 ± 0,9[b]	9,9 ± 0,26[a]	-	0,05

Such insensitivity of intestinal bacteria and generally probiotics to natural growth-inhibiting substances was also previously reported by other authors (Cueva et al., 2010; Lee et al., 2008). In revenge, ampicillin induced a significantly higher zones of inhibition than EAEMPFP. This suggests that the extract studied does not have a negative impact on the bacteria of intestinal origin.

Gram-positive bacteria tested were sensitive to *M. pomifera* fruit extract with inhibition zone diameter ranging from 17.19 mm for *Listeria monocytogenes* to 37.9 mm for one of the most common bacteria responsible for food poisoning, *Staphylococcus aureus* (Rauha et al., 2000). Gram-negative bacteria were also sensitive to the extract with inhibition zone diameter ranging from 14.33 mm for E. coli to 27.3 mm for *Salmonella dysenteriae*.

Candida albicans is responsible for most clinical mycoses; it was sensitive to the *M. pomifera* fruit polyphenol extract with an inhibition zone diameter of 12.67 mm. The effect of miconazole was significantly lower than that of EAEMPFP against *Aspergillus niger*.

The antibacterial potential of the ethyl acetate extract of *M. pomifera* fruit polyphenols recorded in this work would be linked to its richness in hydroxycinnamic acids such as ferulic acid and caffeic acid which have broad-spectrum antibacterial activity against gram-negative and positive bacteria (Borges et al., 2013; Naz et al., 2006); and in flavonoids like kaempferol, quercetin and rutin which have antimicrobial activity against *S. aureus, E. coli, P. aeruginosa, B. cereus and C. albicans* (Soni, 2013, Araruna et al., 2012).

Minimal inhibitory concentration (MIC) was determined for bacteria in which zones of inhibition greater than 8 mm were recorded; the results are presented in Table 6. The lowest MICs were recorded with *B. cereus* (1.25mg/mL) and *S. aureus* (2.5mg/mL); while *S. typhimurium* and *C. albicans* are more resistant with MIC value above 20 mg/mL. Findings of the present work have shown that antimicrobial activity of the ethyl acetate extract of *M. pomifera* fruit polyphenols is stronger than that of whole ethanolic extract of the same fruit reported by Canli et al. (2017); as well as that of phenolic extract of *M. pomifera* fruit peel as found by Fatnassi et al. (2009); while Azizian-Shermeh et al. (2017) had found similar results when they used a whole aqueous extract of the same fruit.

Table 6: Minimum inhibitory concentration (CMI) of ethyl acetate extract of *M. pomifera* fruit polyphenols.

Strains	MIC ($mg.mL^{-1}$)
Micrococcus luteus	5
Staphylococcus aureus	2.5

Listeria monocytogenes	10
Bacillus cereus	1.25
Enterococcus hirae	5
Shigella dysenteriae	5
Escherichia coli	10
Pseudomonas aeruginosa	10
Salmonella typhimurium	>20
Candida albicans	>20
Aspergillus niger	5

Conclusion

In the present study, HPLC/MS analysis of *M. pomifera* fruit extract evidenced the presence of thirteen different phenolic compounds, with a high content of hydroxycinnamic acids, mainly represented by ferulic acid and caffeic acid, and, to a lesser extent, by an hydroxybenzoic acid (i.e. gallic acid); as well as by flavonoids with the predominance of kaempferol. Strong correlation between the polyphenol content of *M. pomifera* fruit extract and the antioxidant and antimicrobial activities was observed. These findings clearly show that the fruit of *M. pomifera* grown in Algeria is a good source of polyphenols that can be used as food biopreservative and for pharmaceutical formulations.

Authors contribution

The first author carried out the ethyl acetate extraction of polyphenols from the fruit of *Maclura pomifera* and their HPLC/MS analysis to determine the phenolic profile. He also wrote the manuscript. The second author contributed to the determination of the antimicrobial activity. The third author carried out the antioxidant activity and supervised all the study and the manuscript writing.

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