



## Research Article

# Comparison of Antioxidant and Antiacetylcholinesterase Activities of Different Extracts of Tunisia *Maclura pomifera* (Rafin.) Schneid Fruit *In Vitro* and *In Vivo*

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**Abstract**

**Background:** *Maclura pomifera* a member of *Moraceae* family, is a tropical plant with ethnobotanical uses.

**Objectives:** The present study aimed to evaluate bioactive compounds, antioxidants, and acetylcholinesterase (AChE) inhibitory activities of different extracts of Tunisia *M. pomifera* (Rafin.) Schneid fruit *in vitro* and *in vivo*.

**Methods:** Organic extracts in different polarities (chloroform, ethyl acetate, and acetone) were extracted from different parts of the fruit of *M. pomifera* (exocarp, mesocarp, and pips). Phenolic content, antioxidant activity, and anti-AChE activity were determined. The anti-amnesic effects of ethyl acetate extract of the exocarp of *M. pomifera* were measured in galactose-induced memory deficit mice by the Y maze. The levels of biomarkers and AChE activity were determined in brain tissues.

**Results:** The obtained results showed that the ethyl acetate extract of exocarp contains the highest content of flavonoids and polyphenols 22.3 mg quercetin equivalents per g of dry weight and 718.6 mg gallic acid equivalents per g of dry weight. The evaluation of antioxidant activities highlighted that the ethyl acetate extract of exocarp was the most active element. The study of the AChE inhibitory power demonstrated that the ethyl acetate extract of the exocarp had the greatest inhibitory activity. The ethyl acetate extract from the exocarp ameliorated cognitive performance and reversed the oxidative damage as compared to galactose group.

**Conclusion:** *M. pomifera* fruit is a good source of natural antioxidants, which might help prevent oxidative stress-related damage and memory impairment in such mental disorders as Alzheimer's disease (AD).

**Keywords:** *Maclura pomifera*, Phenolic content, Antioxidant activity, Acetylcholinesterase inhibition, *In vivo*, *In vitro*

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## Background

Reactive oxygen species (ROS) are chemically reactive molecules containing oxygen ions and peroxides, which are generated by normal reactive oxygen as a natural substance and play an important role in cell signaling (1). Fresh vegetables and fruits are sources of biological activity (antioxidant, antimicrobial, anticancer) and secondary metabolites (2). In inhibiting and scavenging radicals, antioxidants play an important role in fighting against diverse causes of cancer, a variety of chronic disorders such as neoplasms, cardiovascular problems, inflammation, Alzheimer disease (AD), diabetes, as well as the aging process (3).

The most prevalent form of dementia is AD (4). Inhibition of two important enzymes in the brain, acetylcholinesterase (AChE) and butyrylcholinesterase, is

necessary for the treatment of AD (5).

Therefore, it is important to find new medicines for the treatment of AD. Medicinal plants have long been attractive targets for novel bioactive compounds (6). To discover potential drug candidates with neuroprotective function, several experiments have been performed on plants.

A part of the *Moraceae* family, *Maclura pomifera* (Rafin.) Schneider, a medicinal herb, is commonly referred to as "Osage orange, hedge apple, and horse apple" (7). A fruit from *M. pomifera* is also well known for its rich content of isoflavonoids as well as its xanthone content (8). Many biological activities of the plant, including antimicrobial, estrogenic, anti-inflammatory and antinociceptive activities, have been recorded to date (9).

The current research aimed to examine the *in vitro*

and *in vivo* inhibitory activities of *M. pomifera*, as well as antioxidant and anti-AChE activities of different organic extracts in different polarities (chloroform, ethyl acetate, and acetone) extracted from different parts of the fruit of *M. pomifera* (exocarp, mesocarp and pips) growing in Tunisia.

## Materials and Methods

### Plant Material

Fruit of *M. pomifera* was collected from the trees growing in Bizerte (North Tunisia) in October 2013. A voucher specimen was deposited at the Herbarium of the Faculty of Medicine of Sousse, Sousse University (herbarium number M#251).

### Preparation of Extracts

Chloroform, ethyl acetate, and acetone extracts were obtained by shaker extraction of 100 g of exocarp, mesocarp, and pips of the *M. pomifera* for 72 hours in about 100 mL of each solvent used separately. Extracts were filtered and removed under reduced pressure at 4°C using a rotary evaporator.

### Determination of the Amounts of Phenolic Compounds

#### The Total Content of Polyphenols

Total phenolic compounds were assayed using the Folin–Ciocalteu reagent, following the method by Oktay et al (10), which had been slightly modified by Dewanto et al (11). The concentration of total phenolic compounds in the extracts was determined as mg of gallic acid equivalent using an equation obtained from the standard gallic acid graph and expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g DW).

#### Total Flavonoids Content

The flavonoids content was determined according to the aluminum chloride colorimetric method (12). Total flavonoids were expressed as mg of quercetin equivalent per gram of dry weight (mg EQ/g DW).

#### Flavonols Content

Total flavonols in the *M. pomifera* extracts were estimated using the method reported by Adedapo et al (13). Total flavonols content was expressed mg EQ/g DW.

#### Proanthocyanidins Content

The concentration of proanthocyanidins was determined by butanol–HCl assay (14). The number of proanthocyanidins was expressed as mg (+)-catechin equivalent per gram of dry weight (mg CE/g DW).

#### Total Tannins Content

Total tannins were estimated according to the protocol

developed by Hagerman and Butler (15) based on their precipitation by a protein, bovine serum albumin (BSA). The results were expressed as mg tannic acid equivalent per gram of dry weight (mg TA/g DW).

### Antioxidant Activity

#### DPPH Assay

The electron donation ability of the obtained extracts was measured by bleaching of the purple-colored solution of 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) according to the method of Hatano et al (16). Percentage inhibition (PI %) of free radical DPPH was calculated as follows:

PI (%) =  $(A_{\text{blank}} - A_{\text{sample}}) / A_{\text{blank}} \times 100$ , where  $A_{\text{blank}}$  is the absorbance of the control reaction and  $A_{\text{sample}}$  is the absorbance in the presence of extract. Extract concentration providing 50% inhibition ( $IC_{50}$ ) was calculated from the regression equation prepared from the concentration of the extracts and the inhibition percentage.

#### Iron Chelating

The ferrous ion-chelating ability was determined according to the method introduced by Decker and Welch (17).

#### Reducing Power Assay

The reducing power was determined according to the method introduced by Oyaizu (18). The extract concentration providing 0.5 of absorbance ( $IC_{50}$ ) was calculated from the graph of absorbance at 700 nm against extract concentration.

#### AChE Inhibition

AChE enzymatic activity was measured using an adaptation of Ellman's method described by Ingkaninan et al (19). Extract concentration providing 50% inhibition ( $IC_{50}$ ) was obtained plotting the inhibition percentage against extract solution concentrations.

#### Animals

Male Swiss albino mice (weighing 18–22 g; n = 20) were purchased from SIPHAT, Foundouk Choucha (Ben Arous 2013, Tunisia). They were housed in groups of seven animals per cage under a 12:12 h light-dark period at constant temperature ( $22 \pm 2^\circ\text{C}$ ) and humidity ( $50 \pm 10\%$ ); the animals were housed in groups of seven per enclosure.

The male mice (n = 20, 4 months old) were randomly divided into four groups: a vehicle group (n = 5; control) administered saline water (250  $\mu\text{L}$ ) for 7 days via intraperitoneal (i.p.) injection; the galactose group (n = 5) administered a single galactose dose 10% (gal, i.p.) in distilled water at day 7 of the experiment (D-galactose causes rapid aging at higher doses, causing degradation of cognitive and motor abilities close to signs of normal

aging); exocarp group (n =5) treated with 300 mg/kg ethyl acetate extract of the exocarp without galactose 10%; and an exocarp-gal group (n =5) treated with galactose 10% and 300 mg/kg ethyl acetate extract of the exocarp of *M. pomifera*.

#### Study on Acute Toxicity

Dried supernatant of ethyl acetate extract of the exocarp of *M. pomifera* was injected intraperitoneally in a group of 6 male mice at a dose of 50, 150, 375, 750, 1500, and 3000 mg/kg body weight. The animals were measured every 1 hour up to a period of 4 hours, and rarely, for an additional duration of 8 hours. Mortality was reported after 24 hours. Certain symptoms of toxicity, such as muscle control, corrective reflexes, and respiratory changes, were also found in the mice.

#### Behavioral Assessment: Y Maze

Y maze test was performed according to previously described procedures, using an active avoidance apparatus (20). The Y-Maze consisted of three arms of the same size (13 × 4.5 × 5.5 cm each) and at each extreme of the maze was a mobile box (10 cm × 4.5 cm × 5.5 cm), which allowed the mouse to be transported from the goal alley to the start position without handling. The floor of the start-alley and right arm was made of stainless steel rods (2 mm in diameter) that were spaced by a distance of 2 mm. Intermittent electric shocks (50 Hz, 1s, 2.5 mA intensity) were delivered to the grid floor on the central or right arm by an isolated stimulator (Figure 1).

On day 7, each mouse was gently placed in the active maze and left to habituate to the apparatus. After 100 seconds, the guillotine door was opened and the animal was allowed to leave the start-alley within 5 seconds (temporal component) and to choose the left alley (discrimination component) to avoid an electric shock. Therefore, the mouse could make two types of error within a trial: an active avoidance error when it failed to leave the start alley within 5 seconds, and/or a discrimination error when it chose the wrong alley. Electric shocks were delivered every 7 seconds until the mouse entered the correct alley. The mouse underwent one trial every minute until it reached a criterion of 7 correct out of 8 consecutive trials (Figure 2). After 48 hours of training, a retrieval test (retention trial) was performed with the same criterion and the same individual. Avoidance errors and discrimination errors were recorded in order to evaluate the mouse performance on both temporal and discrimination components of the task, respectively (Figure 3).

#### Preparation of the Tissues

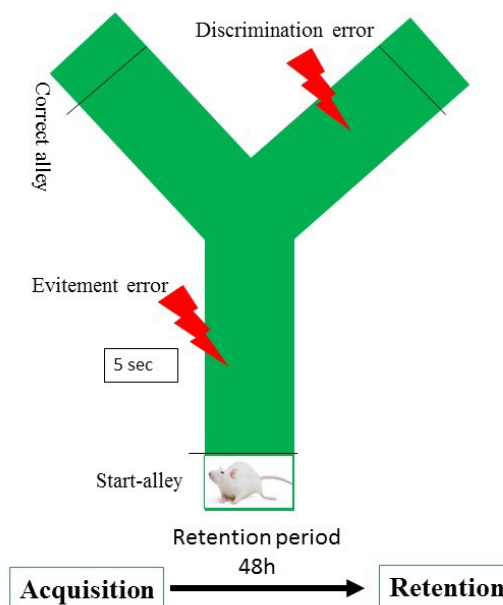
The animals were killed by decapitation on the last day of the trials 60 minutes after the last dose of saline, galactose

(10%), or exocarp extract (300 mg/kg), and the brains were placed in ice-cold saline (w/v: 1/40, 50 mM, pH 7.4). Homogenates were briefly sonicated (15 bursts) on ice and then centrifuged for 15 min at 3000 × g at 4°C. The supernatant was collected and preserved at -20°C before the determination of AChE activity or brain oxidative status.

#### Determination of AChE Ex Vivo

Using Ellman's colorimetric assay, AChE activity was determined (21). The enzyme activity was calculated using the following formula:  $R = 5.74 \times 10^{-4} \times A / CO (4)$  (R: rate in moles of substrate hydrolyzed/minute/mg protein; A: change in absorbance/min; and CO: original concentration of protein (mg/mL)).

#### Determination of Decreased Amounts of Glutathione



The criterion of 7 correct on 8 consecutive

Figure 1. The Y-Maze Test.

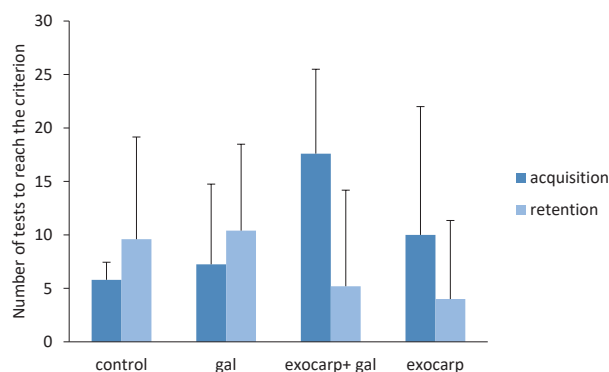
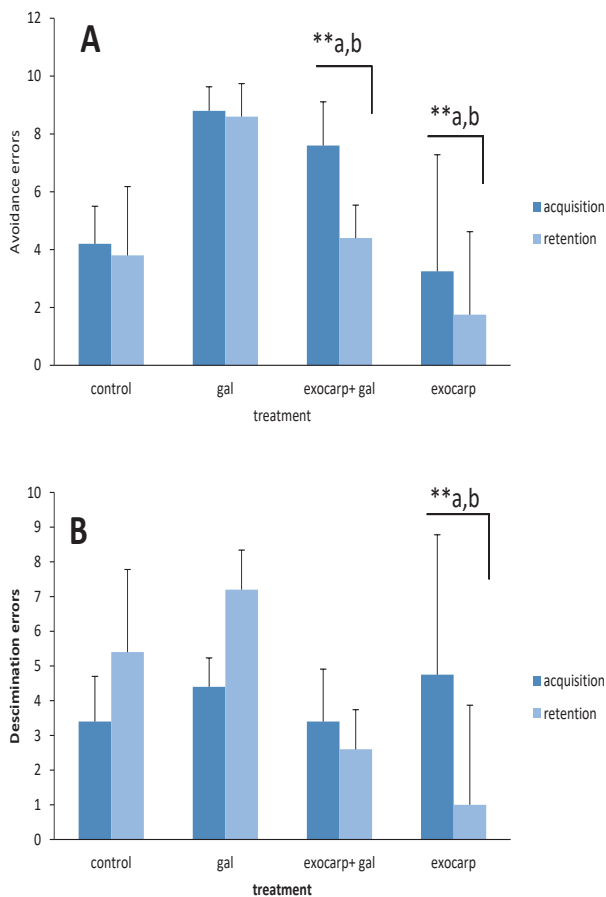


Figure 2. The Effect of Maclura Treatment on the Number of Tests to Reach the Criterion 7/8 Between Acquisition and Retention Session.



**Figure 3.** Avoidance Errors (A) and Discrimination Errors (B). Asterisk (\*\*) indicates a significant difference between acquisition and retention trials. Each column represents the mean  $\pm$  SD ( $n = 7$ ) of the mean for seven mice: a: maclura-gal and maclura groups were significantly different from the control group ( $P < 0.05$ ); b: maclura-gal and maclura groups were significantly different from galactose group ( $P < 0.05$ ).

The level of reduced glutathione in the mouse brain was estimated by a specific colorimetric assay of Ellman (21). The method is based on the formation of thionitro benzoic acid (TNB) complex after the reaction of 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB) with glutathione. TNB has a yellow color with a maximum absorption at 412 nm ( $\epsilon = 13\,600 \text{ mol.L}^{-1}.\text{cm}^{-1}$ ). Briefly, 0.2 g of the brain was mixed with 1 mL of TCA 5% and the mixture was centrifuged for 10 minutes to eliminate the protein. Then, 50  $\mu\text{L}$  of supernatant was added to Tris-HCl buffer (0.1 M; pH8) followed by 50  $\mu\text{L}$  of DTNB (50 mM). Finally, the reaction mixture was incubated for 15 minutes and the absorbance was measured at 412 nm. Standard solutions of GSH (Sigma-Aldrich) were prepared in the Tris-HCl buffer (0.1 M; pH8). A good linearity was obtained for GSH in the range of 50-200  $\mu\text{g/mL}$  ( $y = 0.001x$ ,  $R^2 = 0.99$ ). The level of GSH was expressed as mg/g of the brain.

#### Determination of Ascorbic Acid

The ascorbic acid concentration was determined

following the methods reported by Jacota and Dani (22). After precipitation of proteins brain homogenates with trichloroacetic acid (10%) and centrifugation, 1.5 mL of supernatant was added to 200  $\mu\text{L}$  of Folin's reagent (1/10). The reaction solution was incubated for 15 minutes and the absorbance was measured at 769 nm. The reaction mixture without supernatant was used as a blank solution. Measurements were performed in duplicate and results were expressed as mg ascorbic acid/g of the brain. The level of ascorbic acid was calculated from a standard curve (15-125  $\mu\text{g/mL}$ ).

#### Statistical Analysis

Data were analyzed using SPSS software. The one-way analysis of variance was used to evaluate the significance of each independent factor. A Fisher LSD test was used to discriminate the means with a significance level fixed at 5%. Calculations were performed using the SPSS program and the software STATGRAPHICS Centurion XV version 15.2.06.

#### Results

##### Determination of the Amounts of Phenolic Compounds

###### The Total Content of Polyphenols

The results showed that the contents of polyphenols varied significantly between the different extracts depending on the polarity of extraction solvent and organ (Table 1). The ethyl acetate extract of exocarp, which is the richest extract of these compounds, was found as 718.66 mg GAE/g DW followed by acetonic ones of exocarp and mesocarp, that have polyphenol content from 585.29 and 526.05 mg GAE/g DW, respectively. The maximum total phenolic content was represented in the exocarp for all solvent extracts.

###### The Total Content of Flavonoids

The data summarized in Table 1 show that all parties of *M. pomifera* fruit had fairly close contents of flavonoids. Indeed, the total content of flavonoids varied significantly between the various extracts and depended on the polarity of the extraction solvent and the parts of fruit. The highest flavonoids compound was determined in ethyl acetate extract for all parties of fruit (exocarp, mesocarp, pips). They were in the order of 22.3, 18.9, and 22.2 mg EQ/g DW, respectively.

###### Flavonols Content

As shown in Table 1, the flavanols content varied significantly between different extracts. The ethyl acetate extract of pips was the richest extract with a content of about 51.98 mg EQ/g DW followed by exocarp extract. Concerning the chloroform and acetone extracts, the flavanols' contents were similar.



**Table 1.** Total Polyphenol, Flavonoids Total, Tannins, Flavonols and Proanthocyanidins Content in Organic Extracts of *Maclura Pomifera* Fruit

	Total polyphenol (mg GAE/g DW)			Flavonoids total (mg QE/g DW)			Total content of tannins (mg ETA/g DW)			Flavonols (mg QE/g DW)			Proanthocyanidins (mg CE/g DW)		
	E	M	P	E	M	P	E	M	P	E	M	P	E	M	
Chloroform	248.3±0.35 <sup>a</sup>	202.2±0.2 <sup>c</sup>	219.9±0.15 <sup>b</sup>	8.9±0.25 <sup>c</sup>	9.6±0.2 <sup>b</sup>	11.1±0.05 <sup>a</sup>	56.6±0.3 <sup>c</sup>	77.1±0.1 <sup>a</sup>	66.2±0.4 <sup>b</sup>	34.0±0.0 <sup>a</sup>	26.2±0.15 <sup>b</sup>	32.1±0.3 <sup>a</sup>	97.3±0.15 <sup>a</sup>	87.3±0.05 <sup>b</sup>	
Ethyl acetate	718.6±0.4 <sup>a</sup>	361.1±0.1 <sup>b</sup>	221.7±0.1 <sup>b</sup>	22.3±0.35 <sup>a</sup>	18.8±0.25 <sup>b</sup>	22.2±0.25 <sup>a</sup>	57.2±0.02 <sup>b</sup>	57.8±0.2 <sup>b</sup>	61.1±0.1 <sup>a</sup>	47.8±0.05 <sup>b</sup>	23.1±0.05 <sup>c</sup>	51.9±0.0 <sup>a</sup>	77.0±0.0 <sup>a</sup>	73.5±0.1 <sup>b</sup>	
Acetone	585.3±0.79 <sup>a</sup>	526.0±0.0 <sup>a</sup>	304.7±0.37 <sup>b</sup>	15.7±0.30 <sup>a</sup>	15.3±0.03 <sup>a</sup>	15.5±0.0 <sup>a</sup>	89.3±0.3 <sup>b</sup>	106.1±0.1 <sup>a</sup>	79.1±0.05 <sup>c</sup>	41.1±0.1 <sup>a</sup>	31.7±0.2 <sup>b</sup>	33.3±0.01 <sup>b</sup>	80.6±0.1 <sup>a</sup>	76.2±0.02 <sup>b</sup>	

E: exocarp; M: mesocarp; P: pips.  
GAE, gallic acid equivalents; DW, dry weight; QE, quercetin equivalents; CE, (+)-catechin equivalent; ETA, equivalent of tannic acid. Mean values ± SD (n = 3), with superscript letters indicating homogenous sub-classes resulting from ANOVA ( $P < 0.05$ ).

### Proanthocyanidins Content

All extracts recorded an important proanthocyanidin content, essentially the chloroform extract from different parts of the fruit of *Maclura pomifera* (exocarp, mesocarp, pips) with contents equal to 97.27, 87.32, and 82.35 mg CE/g DW, respectively (Table 1).

### The Total Content of Condensed Tannins

The extract of the fruit of *M. pomifera* had the highest tannins content. Indeed, the acetonic extract from three parts of *M. pomifera* fruit showed strong tannins content. Also, both extracts of the chloroform and ethyl acetate had almost the same value (Table 1).

### Antioxidant Activity

#### DPPH Assay

All extracts saved an important antioxidant activity (Table 2). The best results were observed in the acetone extracts of the exocarp parts with  $IC_{50}$  about 0.09 mg/mL. Also, the antioxidant activity of other extracts varied from 0.23 to 0.85 mg/mL, which was less significant when compared with the acetone extract of the exocarp.

#### Metal Chelating Activity

The capacity of metal chelating activity from different extracts of the three parts of *M. pomifera* fruit is presented in Table 2. The ethyl acetate extract from exocarp saves the best capacity to metal chelating with  $IC_{50} = 0.015$  mg/mL, followed by the acetone extract of exocarp with  $IC_{50}$  equal to 0.03 mg/mL. Other extracts showed a low antioxidant activity.

#### Reducing Power Assay

According to the results shown in Table 2, it can be deduced that the exocarp extract for all solvents (chloroform, ethyl acetate, acetone) exhibited the high FRAP antioxidant capacity with  $IC_{50}$  equal to 0.06, 0.02, and 0.03 mg/mL, respectively. However, the rate of FRAP capacity was found to vary with the polarity of extracting solvent and of the organ of the fruit.

#### AChE Inhibition

Analysis of the results showed that the fruit of *M. pomifera* gives a good inhibition of AChE (Table 3). Indeed, the highest inhibitory activity is saved in ethyl acetate of exocarp extract with  $IC_{50} = 0.010$  mg/mL. Thus, the exocarp extracts indicated the elevated capacity of AChE ( $IC_{50}$  varied to 0.010 at 0.017 mg/mL).

#### The Correlation Coefficient

Table 4 showed a positive correlation between the phenolic compound and the antioxidant capacity; the

**Table 2.** Antioxidant Activity Represented by Three Tests and Exprimed in IC<sub>50</sub> (mg/mL)

	Radical Scavenging			Reducing Power Assay			Metal Chelating Power		
	Exocarp	Mesocarp	Pips	Exocarp	Mesocarp	Pips	Exocarp	Mesocarp	Pips
Chloroform	0.77±0.001 <sup>a</sup>	0.29±0.05 <sup>c</sup>	0.33±0.002 <sup>b</sup>	0.06±0.0 <sup>b</sup>	0.1±0.002 <sup>b</sup>	0.11±0.005 <sup>b</sup>	0.47±0.01 <sup>a</sup>	0.57±0.05 <sup>b</sup>	1.8±0 <sup>b</sup>
Ethyl acetate	0.23±0.007 <sup>b</sup>	0.85±0.01 <sup>a</sup>	0.34 <sup>b</sup>	0.02±0.0005 <sup>b</sup>	5.7±0.1 <sup>a</sup>	1.87±0.2 <sup>a</sup>	0.015±0.0 <sup>b</sup>	0.9±0.075 <sup>a</sup>	3.8±0.02 <sup>a</sup>
Acetone	0.09±0.005 <sup>c</sup>	0.51±0.004 <sup>b</sup>	0.51 <sup>a</sup>	0.03±0.0 <sup>b</sup>	0.85±0.05 <sup>b</sup>	1.84±0.09 <sup>a</sup>	0.03±0.0 <sup>b</sup>	0.22±0.004 <sup>c</sup>	0.32±0.001 <sup>c</sup>

Mean values ± SD (n = 3), with superscript letters indicating homogenous sub-classes resulting from ANOVA ( $P < 0.05$ ).

**Table 3.** Acetylcholinesterase Inhibition Represented by IC<sub>50</sub> (mg/mL)

Extracts	Inhibition d'AChE		
	Exocarp	Mesocarp	Pips
Chloroform	0.014±0.00	0.17±0.02	0.023±0.00
Ethyl acetate	0.010±0.00	0.11±0.01	0.058±0.01
Acetone	0.017±0.00	0.12±0.01	0.042±0.00

proanthocyanidins content showed a positive correlation with the antioxidant capacity (DPPH) with  $r = 0.154$ . The flavonoids content suggests a positive correlation with the chelating and reducing power ( $r = 0.407$  and  $r = 0.292$ ).

Hence, the tannin content indicates a positive correlation with the AChE inhibitory effect ( $r = 0.345$ ). DPPH and reducing power assay also had a positive correlation with the AChE activity (Table 4).

#### Ex Vivo

The results of antioxidant activities and the inhibitory effect of AChE *in vitro* showed that ethyl acetate extract of the exocarp had the best activities. Hence, we conducted an *ex vivo* study to work on this extract.

#### Behavioral Testing: A Step Through Active Avoidance Task

During the task acquisition session, all groups of the mice were presented with several tests to reach the criterion of 7 correct on 8 consecutive tests. After 48 hours, we noticed that the control and galactose groups had been put in more trials to reach the criterion during the retention session. On the other hand, the groups treated with the ethyl acetate extract of the exocarp had been put in fewer trials

to meet the criterion (Figure 2).

We found that the number of avoidance and discriminative errors (Figure 3A,B) increased for control and galactose groups after the retention test and we found a reduction in the number of the avoidance and discriminative errors in the group treated with the ethyl acetate extract of the exocarp.

#### The Level of Reduced Glutathione

In this study, brain GSH levels in the group treated with *M. pomifera* extract (0.536 mg/g Brain) were higher compared to the control group (Table 5, Figure 4).

#### The Ascorbic Acid

Figure 5 and Table 5 shows the amount of vitamin C in the brain in the control and treated groups (treated with ethyl acetate extract of the exocarp). It can be noted that the content of vitamin C was close to each other in both groups.

#### AChE Inhibition

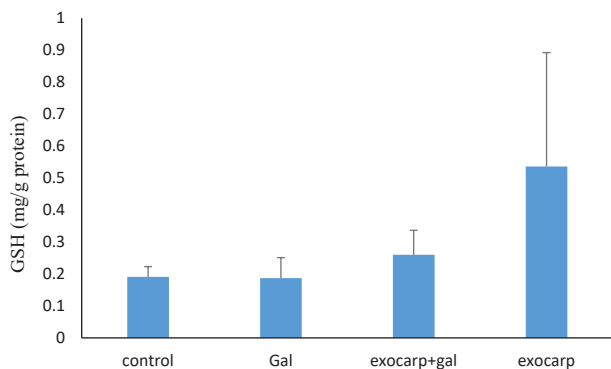
The treatment with ethyl acetate extract of the exocarp showed a decreased AChE activity (14.19 UI/mg) compared to the control group. This reduction is about 25% (Figure 6).

#### Discussion

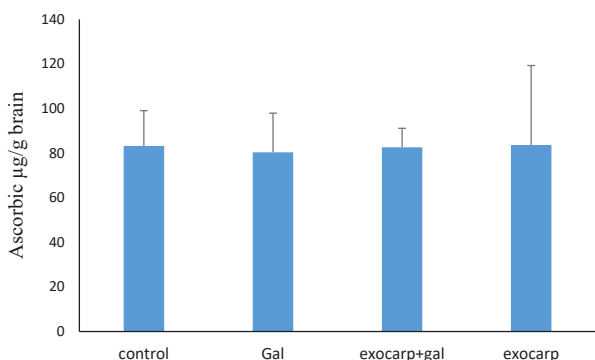
We quantified 5 phenolic compounds of 3 parts of *M. pomifera* fruit. Phenolic compounds are an important family of secondary metabolites produced by plants. In this context, all extracts showed higher contents of their compounds. Indeed, the ethyl acetate extract of the exocarp includes the best content of the polyphenols compounds followed by acetone extract of the exocarp parts. A solvent's

**Table 4.** The Correlation Coefficient Between Phenolic Compound, Antioxidant Capacity and Antiacetylcholinesterase Activity of *Maclura pomifera*

	Total Polyphenol	Flavonoids's Total	Flavonols	Total content of Tannins	Proanthocyanidins
Radical scavenging	-0.344	-0.159	-0.546	-0.293	0.154
Reducing power assay	-0.147	0.407	-0.352	-0.247	-0.537
Metal chelating power	-0.557	0.292	0.393	-0.367	-0.271
Acetylcholinesterase inhibition	-0.275	-0.171	-0.578	0.345	-0.133



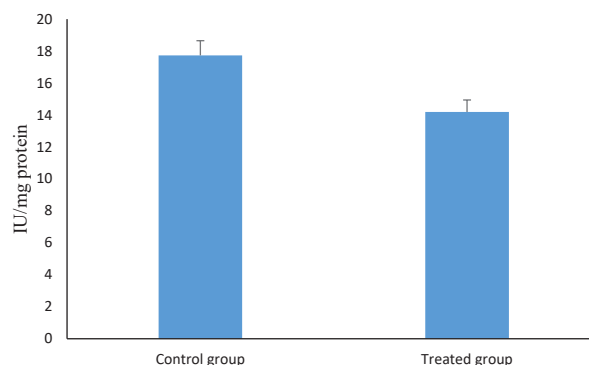
**Figure 4.** Effect of Exocarp Extract on GSH Concentration in Mice Brain.



**Figure 5.** Effect of Exocarp Extract on Ascorbic Concentration in Mice Brain.

total extraction potential depends mainly on its polarity. Many phenolic compounds are very susceptible to different factors of degradation, such as temperature, presence of oxygen, and light (23). As a result, when opposed to the shaking process, the soxhlet extraction method extracted a lower number of phenolic compounds, as in some other conventional extraction techniques that reveal elevated temperature and oxygen to phenolic compounds (24,25). Furthermore, there was a significant difference between the polyphenolic contents of different parts of the fruit; the difference depends on the polarity of the extraction solvents and the parts of studied fruit. This is in agreement with the results of Orhan et al (26) that reported *M. pomifera* as rich in phenolic compounds. They also provided that these compounds were the major bioactive molecules found in the fruits (27).

It can be suggested that the *M. pomifera* fruit is rich in flavonoids and flavanols. The richness in their compounds gives a broad range of many biological activities such as antioxidant, antimicrobial, and antitumor. These results are in agreement with a study done on numerous fruits like grape, orange, kiwi, and apple, indicating that the essential compounds of these fruits are flavonoids contents (28). All the extracts of exocarp, mesocarp, and pips parts of *M. pomifera* presented higher values of the polyphenolic compound than those obtained by Omena et al (29) in



**Figure 6.** Acetylcholinesterase Activity (IU/mg Protein).

genipap, siriguela, and umbu fruits. Hence, this compound can play an important role in the prevention and treatment of the same neurodegenerative disease such as AD (30-31). In this context, few studies have been carried out to evaluate the flavonoids contents and the antioxidant activities (32).

Moreover, in other medicinal species, the flavonoid content achieved 12.44 mg GAE/g DW in the fruit extract of *Pistacia atlantica*, 21 mg GAE/g DW in the fruit of *Persea americana*, and 110 mg GAE/g DW in the fruit of *Psidium guajava* (33). The phenolic compounds have benefits for human health since they can inhibit the cell viability of glioma stem cells in tumors (34).

The antioxidant potential of the phenolic compound in *M. pomifera* requires further study since previous studies have revealed that its fruit is rich in this compound (35).

DPPH, chelating, and reducing power have been frequently used to estimate the antioxidant properties in fruit and vegetables. Hence, all the studied extracts of the fruits have important antioxidant activities. Therefore, the acetone extract of the exocarp is more effective in trapping free radicals of DPPH than the other extracts, since the fruit of *Maclura pomifera* has shown a good chelating and reducing power. These results indicate that the exocarp of the fruit may be an appropriate source of natural antioxidants. The same results of the reduction power indicate that the biggest reduction power was seen in ethyl acetate and acetate extract of exocarp. Our results of DPPH demonstrated an important activity than the *Sorbus terminalis* fruit (36).

Concerning the correlation coefficient between the phenolic compound and the antioxidant capacity, the proanthocyanidin and the flavonoids contents showed a positive correlation with the antioxidant capacity. This agrees with various studies that have demonstrated

**Table 5.** The level of reduced glutathione and ascorbic acid

	Treated group	Control group
GSH mg/g brain	0.536±0.005	0.19±0.09
Ascorbic acid (µg/g brain)	83.71±0.1	83.63±0.5

the relationship between the phenolic content and their antioxidant capacities (37).

*Maclura pomifera* makes many bioactive compounds as important therapeutic targets with the capacity to be used for the treatment of several diseases such as AD. Indeed, AD is a progressive neurodegenerative disorder associated with a shortfall of memory characterized by the low level of acetylcholine in the brain. Murray et al (38) showed that the disease corresponds to a progressive loss of neurons. The principal biological role of AChE is the regulation of transmission of the nerve impulses by the rapid hydrolysis of acetylcholine at the level of the synapses. The inhibitors of this enzyme are used mainly for the treatment of AD. However, herbal medicines represent a good potential in the prevention and treatment of several diseases. In this context, *M. pomifera* fruit has been reported to exhibit various bioactivities such as antioxidant and anti-AChE activities.

The obtained results demonstrated that all ethyl acetate extracts from three parts of the fruit had the best AChE activity. Indeed, they had AChE proprieties; a few experimental studies showed that the fruit has significant AChE activities (30,31). Also, another study developed by Murray et al (38) suggested that the ethanol extract of *Terminalia bellirica* and *Piper nigrum* have an activity similar to that of exocarp and pips of *M. pomifera* fruit. Hence, our results show that the exocarp presents high tannin contents. This indicates a positive correlation with the AChE inhibitory activity ( $r = 0.345$ ) (Table 5). Also, the antioxidant activities (DPPH and reducing power assay) had a positive correlation with the inhibitory potential of acetylcholine.

The good results of the ethyl acetate extract of the exocarp allowed us to continue to work on this extract in the second part of the work. In this context, we used the active avoidance task, in which animals learn to connect an aversive stimulus with a venue. The number of tests to obtain the right 7/8 consecutive criteria (must be moved to the central arm during a preset period of <5 seconds and join the left arm to prevent mild electrical shock) and the number of avoidance and discrimination errors during the acquisition and retention trial were assessed. On day 9, the mean right trial of mice treated with a high exocarp extract concentration (300 mg/kg) was substantially higher than that of the other groups. Compared to the control group and other groups, the group treated also demonstrated a substantial decrease in the amount of avoidance and prejudice errors during the retention experiment. The rich extract of a phenolic compound tends to have a beneficial impact on enhancing memory capacity.

The results of this study showed that the exocarp exhibits antioxidant activities and excellent inhibitory potential of AChE. The fruit is used for the development of the same

natural products and could be used for future therapeutic medicine. Further research is needed to establish which chemical constituents in this extract are relevant to memory enhancement and AChE inhibition.

### Conclusion

*Maclura pomifera* fruit is a good source of natural antioxidant, which might be helpful for further studies to unravel novel treatment strategies for diseases associated with free radical-induced tissue damage.

Higher antioxidant ability and mild inhibitory activity of AChE were seen in all extracts. This dual activity of exocarp encourages the medicinal use of this plant in preventing and alleviating symptoms of certain neurological disorders. The fruit is used for the development of the same natural products and can be used for future therapeutic medicine.

### Authors' Contributions

AK designed the experiments and supervised the work. MM, MAS, and AK carried out the experimental bench work and analyzed and interpreted the data. MM, MAS, NH, and AK wrote the article. All authors approved the final version of the manuscript.

### Conflict of Interest Disclosures

The authors declare no potential conflict of interests.

### Ethical Issues

The protocol was duly approved by local ethics Committee of Tunisia University for use and care of animals in conformity with the National Institutes of Health (NIH) recommendations.

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