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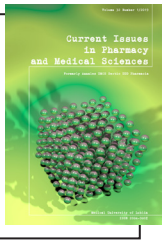

Anticholinesterase and antioxidant activities of foliar extract from a tropical species: *Psidium guajava* L. (Myrtaceae) grown in Algeria

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ABSTRACT

Guava (*Psidium guajava* L.) is a fruit tree largely used in folk medicine in tropical and subtropical areas. This exotic species was introduced in a botanical garden in the northeast of Algeria in the 1950's. The aim of this study is to estimate, for the first time, the antioxidant and anticholinesterase activities of chloroform, ethyl acetate and n-butanol extracts of *P. guajava* growing in Algeria. Six antioxidant assays were tested, results showed very important efficiency in free radical scavenging, reducing power and β-carotene bleaching of tested extracts. Values of IC_{50} or $A_{0.5}$ of some samples were lower than those of standards. With regard to anticholinesterase activity, the inhibitory of both acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) was investigated. The extracts exhibited interesting capacity to inhibit these enzymes with low values of IC_{50} and even less than that of galanthamine. These activities were correlated with total phenolic content which was more important compared to the one found in extracts from trees growing in tropical and subtropical region. This could be due to resistance and adaptation of *P. guajava* grown in Algeria. The data obtained suggest the use of bioactive compounds from *P. guajava* leaves as antioxidant and drugs for symptomatic treatment of Alzheimer disease.

INTRODUCTION

Oxidative stress is defined as a state in which oxidation exceeds the antioxidant systems in the body following a loss in the balance between them [1]. Antioxidants are substances that may protect cells from the damage caused by unstable molecules known as ROS (reactive oxygen species) and by free radicals [2]. The latter are implied in the etiology of a large number of pathologies that are now regarded as major problems of public health. Among others, we can mention arthritis, asthma, rheumatisms, nephritides, cancers, atherosclerosis, sweetened diabetes, inflammatory lesions, diseases with immunosuppression, metabolic disorders and Alzheimer's diseases [3]. In the last 30 years, Alzheimer's disease (AD) has become the most common neurodegenerative disease. It is the cause of 60-70% of all cases of dementia [4]. Acetylcholine (ACh) is the most abundant neurotransmitter in the body and the principal neurotransmitter in the brain that is responsible for cholinergic transmission. Cholinesterases (ChE) constitute a group of esterases that hydrolyse choline esters at a higher rate than other esters, provided that the hydrolysis rates are compared at optimum and controlled conditions [5]. Some active substances are used to stop Alzheimer's development, including galanthamine (Alkaloid). Flavonoids and phenolic acids are the most important groups of secondary metabolites considered as good sources of natural antioxidants and anticholinesterase activities in human diets [6].

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Myrtaceae is one of the plant families that have been widely studied for their antioxidant activity, and many species belonging to this family have strong activity [7]. This family of dicotyledonous plants is composed of 140 genera and 3400 species distributed mainly in the tropical and subtropical region of the world [8]. The genus *Psidium* includes around 70 species distributed in the American and Asian tropics [9]. *Psidium* fruits are a potential source of phytochemicals for which many bioactivities have been proved. Carotenoids, phenolic compounds, and triterpenoids are the main phytochemicals characterized in leaves and fruits of this genus [10]. *Psidium guajava* L., commonly known as guava, is a tropical plant that is native to South America [11]. This fruit tree is an important food crop and medicinal plant in tropical and subtropical countries of Asia, and it is also largely used as food and in traditional medicine in other parts of the world. Many people use leaf decoctions of *P. guajava* for its antimicrobial and antispasmodic properties in the treatment of dysentery and diarrhea [12].

Several exotic species of tropical origin have been introduced into the botanical garden of the Ancient Institute of Agriculture of Skikda in Northeast Algeria and have perfectly been acclimatized. Among these species is *Psidium guajava* L. – introduced in 1952 [13].

Studies on antioxidant activity from the leaves of *P. guajava* remain limited and there are no available data about this species in Algeria. The main aim of the present study is to evaluate the antioxidant activity and inhibition of cholinesterase of leaf extracts from this important species of the family of Myrtaceae.

MATERIALS AND METHODS

Plant material

Leaves of *Psidium guajava* L. were collected, in May 2018, from the botanical garden of the Ancient Institute of Agriculture of Skikda in the northeast of Algeria. Species were identified by Chalabi, agronomist from the University of Skikda and an expert in exotic species cultivated and preserved at the botanical garden. A voucher specimen was deposited in our laboratory. Leaves were washed with distilled water and dried at room temperature for twenty days while under shade.

Preparation of extracts

Powdered leaves of *P. guajava* (50g) were macerated with 300 ml of methanol /water (70v/30v) at room temperature for 72 hours (3×24h). After filtration, the solvent was evaporated under reduced pressure and temperature. The residue was dissolved in 200 ml of distilled water and extracted with 200 ml of one of three solvents: Chloroform ($CHCl_3$), Ethyl acetate (C_{4H8O2}) and *n*-butanol (C_{4H10O}), successively. The organic solutions were evaporated under reduced pressure and temperature to obtain the three extracts: Chloroform (3,82 g), Ethyl acetate (20,05 g), n-butanol (32,73 g). Crude extracts were preserved at 4°C.

Total phenolic content

The total phenolic content was determined by using the reagent of Folin-Ciocalteu [14], according to a method of microplate described par Muller and al. [15]. Herein, 20 µl of sample (1 mg extract/1ml methanol) were blended with 100 µl of Folin-Ciocalteu reagent (1:10) and 75 µl of sodium carbonate solution (7,5%). The microplate was incubated two hours at room temperature in darkness. Absorbance at 765 nm was measured by using the microplate reader. The total phenolic content was evaluated as micrograms of gallic acid equivalents per milligrams of extract.

Total flavonoids content

Total flavonoids content was determined by the method of Topçu *et al.* [16] with some modification to adapt it to microplate. Briefly, 130 µl of methanol were added to 50 µl of sample (1mg extract/1ml methanol). Subsequently, 10 µl of 1M potassium acetate (CH_3COOK) and 10 µl of 10% aluminum nitrate (Al $(NO_3)_2$, $9H_2O$) were added and the microplate was incubated at room temperature for 40 minutes. Absorbance was read at 415 nm. Results were expressed as micrograms of quercetin equivalents per milligrams of extract.

Antioxidant activity

1. DPPH free radical-scavenging activity

The free radical-scavenging activity was determined spectrophotometrically by means of DPPH assay [17]. Briefly, 40 µl of MeOH solution of the sample at various concentrations was added to 160 µl DPPH (0.1 mM). The reaction mixture was shaken vigorously and the absorbance of remaining DPPH was measured at 517 nm after 30 min. BHA and BHT were used as antioxidant standards for comparison of the activity. The scavenging capability of DPPH radical was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = \frac{A_{Control} - A_{Sample}}{A_{Control}} \times 100$$

The results were given as IC_{50} value (µg/ml) corresponding to the concentration of 50% inhibition.

2. ABTS radical cation decolorization assay

The spectrophotometric analysis of $ABTS^{\bullet+}$ scavenging activity was determined according to the method of Re *et al.* [18]. After preparation of the oxidation solution of ABTS, the $ABTS^{\bullet+}$ solution was diluted to get an absorbance of 0.700±0.020 at 734 nm with water. Then, 160 µl of ABTS solution was added to 40 µl of sample solution in methanol at different concentrations. After 10 min., the absorbance was measured at 734 nm. Methanol was used as a control. BHA and BHT were used as antioxidant standards for comparison of the activity. The results were given as the IC_{50} (µg/ml), which was calculated by means of the following equation:

$$ABTS^{\bullet+} \text{ Scavenging effect (\%)} = \frac{(A_{Control} - A_{Sample})}{A_{Control}} \times 100$$

3. Reducing power assay

The reducing power of the tested compounds was assessed according to the method of Bouratoua [19]. In order to determine the reducing power activity, 10 µl of serial diluted sample were added into a 96 well round-bottomed plate. Following this, 40 µl of 0.2 M phosphate buffer (pH 6.6)

and 50 µl of potassium ferricyanide (1%), were added to each well and the plate was incubated at 50°C for 20 min. Finally, 50 µl of TCA(10%) and distilled water (40 µl) and 10 µl of ferric chloride (0.1%), was added into each well in order to measure the reducing power activity. The absorbance was measured in a microplate reader at 700 nm. Higher absorbance of the reaction mixture indicates greater reducing power.

4. Phenanthroline assay

This test was carried out according to the method described by Szydłowska-Czerniak *et al.* [20]. 10 µl of sample solution in methanol at different concentrations was placed into a 96 well round-bottomed plate. 50 µl of $FeCl_3$ (0.2%) and 30 µl of 1, each well was then appended with 10-Phenanthroline (0.5%). Finally, 110 µl of methanol were added. The microplate was incubated 20 minutes at 30°C in a dark. The absorbance of solution was measured at 510 nm. BHT and BHA were used as antioxidant standards.

5. Cupric reducing antioxidant capacity (CUPRAC)

The cupric reducing antioxidant capacity was determined according to the CUPRAC method [21]. In each well the reaction mixture containing 40 µl of sample solution and 50 µl of a copper (II) chloride solution, 50 µl of neocuproine alcoholic solution, and 60 µl of ammonium acetate aqueous buffer at pH 7 was combined to give a final volume of 200 µl. After 30 minutes, the absorbance was measured at 450 nm. Results were recorded as absorbance compared with the absorbance of BHA and BHT, which were used as antioxidant standards.

6. Antioxidant capacity by the β-carotene bleaching assay

The β-carotene bleaching activity of our compounds was evaluated using the β-carotene-linoleic acid system described by Marco [22]. Thus, a solution of β-carotene (0.5 mg) in 1 ml of chloroform is combined with 25 µl of linoleic acid and 200 µl of Tween40. After evaporation in vacuo of the chloroform, 50 ml of Hydrogen peroxide H_2O_2 is added under vigorous agitation. The absorbance of the solution is then adjusted to 0.8-09 nm. Amounts of 160 µl of this solution are added to 40 µl of solution of the studied synthetic compounds at different concentrations. The absorbance was measured at 470 nm, using a 96-well microplate reader. The emulsion system was incubated for 2 h at 50°C. A blank, devoid of β-carotene, was prepared for background subtraction. BHA and BHT were used as standards.

The bleaching rate (R) of β-carotene was calculated according to the following equation:

$$R = \frac{\ln a/b}{t}$$

where: ln = natural log, a = absorbance at time zero, b = absorbance at time t (120 min).

The antioxidant activity (AA) was calculated in terms of percent of inhibition relative to the control, using following the equation:

$$AA\ (\%) = \frac{R_{Control} - R_{Sample}}{R_{Control}} \times 100$$

Anticholinesterase activity

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activity was measured, by the spectrophotometric method developed by Ellman *et al.* [23]. Briefly, 150 µl of 100 mM sodium phosphate buffer (pH 8.0), 10 µl of sample solution dissolved in methanol at different concentrations and 20 µl AChE (5.32×10^{-3} U) or BChE (6.85×10^{-3}U) solution were mixed and incubated for 15 min at 25°C, and 10 µl of 0.5 mM DTNB [5,5′-dithio-bis(2-nitrobenzoic) acid] were added. The reaction was then initiated by the addition of 10 µl of acetylthiocholine iodide (0.71 mM) or butyrylthiocholine chloride (0.2 mM). The hydrolysis of these substrates were monitored spectrophotometrically by the formation of yellow 5-thio-2-nitrobenzoate anion, as the result of the reaction of DTNB with thiocholine, released by the enzymatic hydrolysis of acetylthiocholine iodide or butyrylthiocholine chloride, respectively, at a wavelength of 412 nm, every 5 min for 15 min, utilising a 96-well microplate reader (Perkin Elmer Multimode Plate Reader, EnSpire, USA) in triplicate experiments. Galanthamine was used as reference compound. The results were given as 50% inhibition concentration (IC_{50}) and the percentage of inhibition of AChE or BChE was determined by comparison of reaction rates of samples relative to blank sample (methanol in phosphate buffer, pH 8) using the formula:

$$\text{inhibition of AChE or BChE (\%)} = \frac{E-S}{E} \times 100,$$

where: E is the activity of enzyme without test sample, and S is the activity of enzyme with test sample.

Statistical Analysis

All data on antioxidant and anticholinesterase activities were the average of triplicate analyses. Data were recorded as the mean ± standard deviation. Analysis of variance was executed using ANOVA test. Significant differences between means were determined by Tukey test; p values < 0.05 were regarded as significant. Pearson's correlation was calculated. All these tests were performed using XLSTAT 2014.

RESULTS

Total phenolic and flavonoids content

Results of total phenolic and flavonoids content of extracts are shown in Table 1.

Table 1. Total phenolic content (TPC) and total flavonoids content (TFC) of extracts of P. guajava.

Extract	TPC(µg GAE/mg)[1]	TFC(µg QE/mg)[2]
Chloroform extract	250.47±0.05	112.71±0.09
Ethyl acetate extract	931.15±0.06	269.57±0.10
Butanol extract	808.41±0.04	68.37±0.03

Results are expressed as means ± SD of three measurements
[1] Micrograms of gallic acid equivalents per milligrams of extract
[2] Micrograms of quercetin equivalents per milligrams of extract

The total content of phenols of leave extract of *P. guajava* was estimated by using the Folin-Ciocalteu method. The level of phenolic compounds in each fraction was expressed as micrograms of gallic acid equivalents per milligram

of extract. Results showed that the ethyl acetate fraction contained the highest content (931.15±0.06 µg GAE/mg), followed by the n-butanol fraction with 808.41±0.04 µg GAE/mg. Chloroform extract gave the lowest result (250.47±0.05 µg GAE/mg). The total flavonoids content was determined by the trichloridealumiunm method and results were expressed as micrograms of quercetin equivalents per milligram of extract. Among the three extracts, the ethyl acetate fraction contained the highest amount (269.57±0.10 µg QE/mg). Contrary to total phenolic content, flavonoids content of chloroform fraction was important with a value of 112.71±0.09 µg QE/mg. The minimum level of flavonoids was recorded by the n-butanol fraction (68.37±0.03 µg QE/mg).

Antioxidant properties

The antioxidant activity of the different extracts from the leaves of *Psidium guajava* was evaluated using six different methods: DPPH radical scavenging [17], ABTS cation radicaldecolorization [18], Reducing power assay [19], Phenanthroline assay [20], CUPRAC [21], and β-carotene bleaching [22]. Results are shown in Table 2.

Table 2. DPPH radical scavenging, ABTS cation radical decolorization, Reducing power assay, Phenanthroline assay, CUPRAC, and β-carotene bleaching assays on extracts of *P. guajava*

Samples	DPPH IC_{50} (µg ml^{-1})	ABTS IC_{50} (µg ml^{-1})	Reducing power $A_{0.5}$ (µg ml^{-1})	Phen assay IC_{50} (µg ml^{-1})	CUPRAC $A_{0.5}$ (µg ml^{-1})	β-carotene bleaching IC_{50} (µg ml^{-1})
Chloroform extract	>200	>200	>200	119.20 ±1.85^{c}	>200	9.25 ±1.26^{c}
Ethyl acetate extract	4.26 ±0.05^{a}	5.25 ±0.47^{b}	13.45 ±5.82^{a}	3.25 ±0.11^{b}	3.08 ±0.55^{ab}	3.18 ±0.10^{b}
Butanol extract	5.48 ±1.53^{ab}	1.09 ±0.10^{a}	26.63 ±3.49^{b}	2.62 ±0.01^{ab}	1.72 ±0.06^{a}	3.53 ±0.26^{b}
BHA*	6.82 ±0.49^{b}	1.03 ±0.00^{a}	NT	0.93 ±0.07^{a}	3.64 ±0.19^{b}	0.90 ±0.02^{a}
BHT*	22.32 ±0.02^{c}	1.59 ±0.03^{a}	NT	2.24 ±0.17^{ab}	9.62 ±0.87^{c}	1.05 ±0.01^{a}
Ascorbic acid *	NT	NT	6.77 ±1.15^{a}	NT	NT	NT
Tannic acid*	NT	NT	5.39 ±0.91^{a}	NT	NT	NT

Results are expressed as means ± SD of three measurements;
Analysis of variance (ANOVA) revealed significant effect (p<0,05). The column means that share the same superscript letters are not significantly different; NT – Not tested;
* – Standards

The results revealed that the species *Psidium guajava* possesses important antioxidant activities at various degrees, in particularly the ethyl acetate and n-butanol fractions. Values of IC_{50} and $A_{0.5}$ varied according to extract fraction. The chloroform extract showed weak antioxidant activity.

The DPPH radical is a stable lipophilic free radical and is always employed for evaluating the free radical scavenging potential of plant extract antioxidants [24]. DPPH radical scavenging, expressed as IC_{50} (µg/ml) values, were compared to those of standards BHAand BHT. *P. guajava* exhibited a high DPPH scavenging ability. Therein, ethyl acetate and n-butanol extracts have greater inhibitory potential than did the standards, with IC_{50} = 4.26±0.05 µg/ml and IC_{50} = 5.48±1.53 µg/ml, respectively.

P. guajava exhibited good activity in ABTS scavenging. Values of IC_{50} are very close to those of standards, especially the n-butanol fraction (IC_{50} = 1.09±0.10 µg/ml), which has an IC_{50} not significantly different to both that of BHA and BHT.

The reducing power activity of the samples was represented as $A_{0.5}$ values. Compared to the standards (ascorbic acid and tannic acid), the best result was given by the Ethyl acetate fraction (IC_{50} = 13.45±5.82 µg/ml). By contrast, the n-butanol fraction showed lower activity, with a IC_{50} value significantly different from that of the standards (26.63±3.49 µg/ml)

Concerning the phenanthroline assay, results showed that ethyl acetate and n-butanol extracts had high activity, with values of IC_{50} close to the standards with a non- significant difference (IC_{50} = 3.25±0.11 µg/ml and IC_{50} = 2.62±0.01 µg/ml, respectively). The reduction of Cu2+ ions was evaluated by Cupric reducing antioxidant capacity (CUPRAC). This test gave very interesting results. Accordingly, ethyl acetate and n-butanol extracts of *P. guajava* showed a higher inhibition than that of BHA ($A_{0.5}$ = 3.08±0.55 µg/ml and $A_{0.5}$ = 1.72±0.06 µg/ml, respectively).

The only antioxidant activity where the chloroform fraction showed efficiency is in β-carotene bleaching inhibition. This fraction exhibited inhibitory activity, but the value of IC_{50} (9.25±1.26 µg/m) was significantly different from standards. Ethyl acetate and n-butanol fractions were more efficient, with similar values of IC_{50} (3.18±0.10 µg/ml and 3.53±0.26 µg/ml, respectively).

The correlation of total phenolic content with antioxidant activities is shown in Table 3. Results reveal high and negative correlation between the abundance of phenolic compounds and IC_{50} or $A_{0.5}$ of several assays. The coefficient of correlation (r) is around -0.900. As regards the total flavonoids content, the correlation is lower and negative.

Anticholinesterase activity

The colorimetric method of Ellman *et al.* [23] is based on determining the amount of thiocholine released when acetylthiocholine or butyrylthiocholine is hydrolysed by Acetylcholinesterase (AChE) or Butyrylcholinesterase (BChE). The thiocholine released is quantified by its reaction with 5,5´-bisdithionitrobenzoic acid (DTNB), which produces a yellow 5-thio-2-nitrobenzoate anion [25].

The capacity of the samples to inhibit the cholinesterase was evaluated by the percentage of inhibition of AChE and BChE at various concentrations. The values of IC_{50} were compared to that of the galanthamine (Table 4).

Table 3. Pearson's correlations between total phenolic content (TPC), total flavonoids content (TFC) and antioxidant activities (DPPH, ABTS, Reducing power, Phen assay, CUPRAC, β-carotene bleaching), anticholinesterase activities (AChE, BChE)

		IC_{50} DPPH	IC_{50} ABTS	$A_{0.5}$ Reducing power	IC_{50} Phen assay	$A_{0.5}$ CUPRAC	IC_{50} β-carotène bleaching	IC_{50} AChE	IC_{50} BChE
TPC	r	-0.986	-0.982	-0.994	-0.985	-0.985	-0.993	-0.933	-0.958
	R^2	0.973	0.965	0.989	0.970	0.969	0.986	0.871	0.919
TFC	r	-0.312	-0.290	-0.367	-0.303	-0.302	-0.356	-0.114	-0.192
	R^2	0.098	0.084	0.135	0.092	0.091	0.127	0.013	0.037

r – Correlation coefficient; R^2 – Correlation of determination

Table 4. Inhibition of acetylcholinesterase (AChE) and (butyrylcholinesterase (BChE) by different extracts

Samples	AChE			BChE		
	% inhibition		IC_{50} (µg/ml)	% inhibition		IC_{50} (µg/mL)
	100 µg/ml*	200 µg/ml*		100 µg/ml*	200 µg/ml*	
Chloroform extract	36.42 ±2.43	53.52 ±0.81	177.11 ±2.30^{d}	NA	NA	>200
Ethyl acetate extract	82.42 ±1.39	96.99 ±0.85	56.11 ±4.04^{c}	61.65 ±0.91	95.65 ±4.78	44.95 ±2.67^{b}
n-butanol extract	90.78 ±5.32	94.44 ±3.10	24.44 ±3.45^{b}	91.01 ±3.78	94.88 ±1.81	21.87 ±10.48^{a}
Galanthamine**	91.80 ±0.20	94.77 ±0.34	6.27 ±1.15^{a}	73.57 ±0.77	78.95 ±0.58	34.75 ±1.99^{ab}

Results are expressed as means ± SD of three measurements;
Analysis of variance (ANOVA) revealed significant effect ($p<0,05$). The column means that share same superscript letters are not significantly different;
NA – No activity;
* – Concentration is expressed as micrograms of extract per milliliter of methanol;
** – Standard

P. guajava seems to have important anticholinesterase activity. The results showed a high percentage of inhibition of AChE at 100 µg/ml and 200 µg/ml. The ethyl acetate and *n*-butanol extracts especially stood out, with values ranging up to 96.99% of inhibition (Fig. 1). Therein, IC_{50} of the two fractions was estimated at 56.11±4.04 µg/ml and 24.44±3.4 µg/ml, respectively. The chloroform fraction had the highest IC_{50} (177.11±2.30 µg/ml). This same species showed a very interesting power to inhibit the enzyme BChE at a rate going up to 95% (Fig. 2). The n-butanol extract gave an IC_{50} (21.87±10.48 µg/ml) that was lower than that of galanthamine. Moreover, the difference between means was not significant. In contrast, the ethyl acetate fraction had

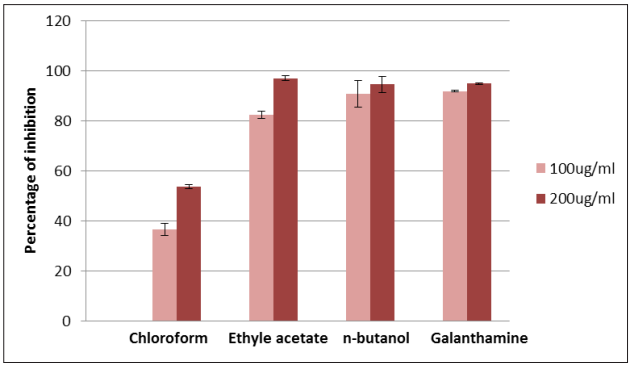

Figure 1. Percentage of inhibition of Acetylcholinesterase (AChE) at different concentrations of extract fractions of *P. guajava* and standard

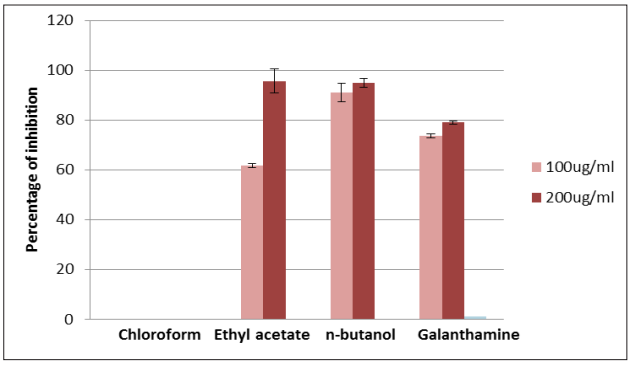

Figure 2. Percentage of inhibition of Butyrylcholinesterase (BChE) at different concentrations of extract fractions of *P. guajava* and standard

an activity close to that of the standard (IC_{50} = 44.95±2.67 µg/ml).

The correlation of total phenolic content with anticholinesterase activity is shown in Table 3. Results revealed high and negative correlation between the abundance of phenolic compounds and the IC_{50} of both AChE and BChE tests, with ($r = -0.933$, $R^2 = 0.871$) and ($r = -0.958$, $R^2 = 0.919$), respectively. As regards the total flavonoids content, the correlation was lower and negative ($r = -0.114$, $R^2 = 0.013$) and ($r = -0.192$, $R^2 = 0.037$), respectively.

DISCUSSION

Our work shows elevated levels of phenolic compounds (TPC) and flavonoids (TFC), especially in the ethyl acetate fraction, followed by the n-butanol fraction. Chloroform contained the lowest rate. The results we attained are consistent with those of Mouffouk *et al.*[26] who found that the highest phenolic and flavonoids content were recorded in the ethyl acetate extract, followed by n-butanolic extract. Saeed *et al.* [27] have also noted similar results. The study of Babbar *et al.* revealed that ethyl acetate was better than the dichloromethane in extracting phenolic compounds and that polarity of extracting solvent might influence the TPC and TPC of the extracts [28]. The total phenolic and flavonoids content of *P. guajava* has been reported in many previous studies. Chang [29] recorded values of TPC = 229 µg/mg and TFC = 208 µg/mg in aqueous guava budding leaf extract; these results are close to ours. In Brazil, a study on *P. guajava* leaves indicated a similar level in the ethanolic extract (TPC = 766.08 µg/mg, TFC = 118.90 µg/mg) [30]. Another Brazilian study showed total flavonoid and polyphenol content of concentrated and spray-dried leaf extracts of *P. guajava* to be (TPC = 25.93 µg/mg, TFC = 23.48 µg/mg) [31]. Several other authors have confirmed the high level of phenol and flavonoids content in this species when grown in the Asian area [32-34].

Our results reveal that antioxidant activity and values of IC_{50} of some samples were lower than those of standards. This was the case for the DPPH radical scavenging assay and CUPRAC assay. These two tests are based on the capacity of antioxidant to transfer electrons. ABTS scavenging, Reducing power activity, Phenanthroline and β-carotene bleaching assays revealed results close to standards.

The β-carotene bleaching assay showed the activity of chloroform extract to be contrary to the other assays. This result is consistent with that of Hacibekiroglu and Kolak who found that chloroform extract induced high inhibition of lipid peroxidation as indicated by β-carotene bleaching assay, the effect being due to the presence of the phenolic and flavonoid compounds [35]. This antioxidant activity could be related to the abundance of phenolic compound in extracts. Extracts with a high level of total phenolic content showed best antioxidant capacities. Statistical results of correlation confirm this hypothesis with a very high coefficient of correlation between total phenolic content and all antioxidant assays.

Previous studies on leaf extracts of *P. guajava* reported a high degree of antioxidant activity that was due to richness in phenolic compounds. Qian *et al.* [36] found that different

extracts from guava leaf showed good free radical-scavenging activity depending on the concentration used. Seo *et al.* [37] studied the antioxidant ability of water, ethanol and methanol extracts of *P. guajava* leaves, and their results demonstrated superior antioxidant ability of guava leaf extracts, albeit due more to phenolic compound content rather than flavonoid content. Another study on *P. guajava* leaves extract showed heightened antioxidant activity by way of using Ferum Reducing Power (FRAP) [38]. The aim of a recent Indonesian research was to evaluate antioxidant activity from ten species of Myrtaceae, including *P. guajava*. Herein, DPPH assay of ethyl acetate extract of *P. guajava* leaves gave IC_{50} = 53.54±2.23 µg/ml – which was lower than that of Ascorbic acid [39]. These results are in accordance with ours, but the level of phenolic compounds was higher, The IC_{50} values we obtained were lower than most of the studies mentioned above and we also found greater antioxidant activities through all our assays. This difference could be explained by the fact that our plant material consisted of leaves of *P. guajava* which had acclimated and adapted to the local conditions of Algeria, hence, this differed to other studies on the same species grown in its natural environment (mainly in the Asian area, and within tropical and subtropical climates). Plant phenolics are well known to play crucial roles in plant ecology and physiology [40]. Studies that had gone before ours had investigated the role of phenolic compounds in the interactions of plants with various stress factors, with special attention to their antioxidant properties. Phenolic compounds play important roles in plant growth and development, particularly in defense mechanisms and adaptation to climatic conditions [41]. There is a potential, therefore, to use polyphenols as indicators of abiotic stress resistance [42].

Some studies associated antioxidant properties of *P. gujava* leaves with their phenolic compounds. Isolated compounds included ascorbic acid, gallic acid and caffeic acid [43], protocatechunic acid, ferulic acid, quercetin and guavin B [44], gallic acid, catechin, epicatechin, rutin, quercetin, naringenin and kaempherol [45]. The methanolic extract of Thai guava leaves has high antioxidant activity and three flavonoids with different levels of antioxidant power were isolated, quercetin, quercetin-3-O-glucopyranoside and morin [46].

The current study demonstrates the heightened inhibitory activity of ethyl acetate and n-butaol exacts of *P. gujava* leaves against both acetylcholinestrase (AChE) and butyrylcholinesterase (BChE). Herein, the chloroform extract showed interesting inhibition against AChE, despite its weak antioxidant activity. These findings can be explained by the abundance in the chloroform extract of other bioactive compounds that exhibit inhibition of cholinesterase. Udoidong *et al.* [47] undertook a phytochemical screening of the chloroform extract of a medicinal species; the results of this revealed the presence of alkaloids, steroids, and flavonoids.

Studies analyzing the anticholinesterase activity of *P. guajava* species or even the genus *Psidium* are rare. Some research, however, has been done on other species of Myrtaceae. Gasca *et al.* [48] isolated two flavonoids, quercitin and catechin, from *Eugenia dysenterica* leaves. These compounds exhibited superior acetylcholinesterase inhibitory effects, with IC_{50} = 46.59±0.49 µg/ml and 42.39±0.67 µg/ml respectively. With regard to *P. guajava*, a previous study on thirteen Tai medicinal plants, including *P. guajava*, revealed that the latter's essential oils have no inhibitory effect on AChE [49]. In contrast, Indian research on *P. guajava* fruit showed that it produced significant reduction of brain acetylcholinesterase activity in young and aged mice. In the study, pretreatment of 15 days with *P. guajava* fruit protected the animals from developing memory deficits, and *P. guajava* fruit induced elevation of the brain acetylcholine level by significantly reducing acetylcholinesterase activity [50]. Our results on *P. guajava* leaves show the efficiency of this species as inhibitor of cholinesterase (AChE and BChE). This effect could be related to its contained phenolic compounds and the resulting antioxidant activity.

The antioxidant effect of *P. guajava* fruit might also be beneficial in protecting the brains of rodents against oxidative stress [50]. A previous study confirmed the effect of quercetin on the acquisition and retention of spatial memory in a rat model of Alzheimer's disease and concluded that there is convincing evidence on the neuroprotective effects of flavonoids against Alzheimer's disease [51]. According to the studies cited above, *P. guajava* leaves contain quercitin.

CONCLUSION

Psidium guajava is a tree grown in tropical and subtropical regions where it is known for its medicinal properties. Our pioneering study focuses on *P. guajava* which has grown in a botanical garden in Algeria. A high level of total phenolic and flavonoid content was found. Therefore, extracts manifested antioxidant activities which were greater than the standards in some assays, notably DPPH scavenging and CUPRAC assays. The efficiency of *P. guajava* leaf extracts against Alzheimer's disease was also investigated by testing the inhibitory effect of the two enzymes acetylcholinesterase and butyrylcholinesterase. Results revealed that *P. guajava* extracts had values of IC_{50} close and even lower than the standard. We suppose that this important antioxidant activity is caused by the abundance of phenolic compounds produced unduly by leaves of our *P. guajava* trees through its acclimation and developed resistance to abiotic stress by way of manufacturing secondary metabolites. It should be noted that extracts with a high level of total phenolic content showed best antioxidant capacities.

As a perspective, ulterior study for isolation of those compounds could be realized. Chromatographic analysis would be necessary to identify these molecules. This species must undergo greater cultivation in Algeria as its bioactive molecules can be used as drugs and as an anti-Alzheimer remedy, though necessarily with the testing of other effects such as toxicity for safe use.

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