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Research paper

Biological properties of phenolic compound extracts in selected Algerian honeys—The inhibition of acetylcholinesterase and α-glucosidase activities

Hicham Zaidi[a,*], Salim Ouchemoukh[a], Nadia Amessis-Ouchemoukh[b], Nadjet Debbache[a], Rita Pacheco[c,d], M. Luisa Serralheiro[c], M. Eduarda Araujo[e]

[a] Laboratoire de Biochimie Appliquée, Faculté des Sciences de la Nature et de la Vie, Université de Bejaia, Route de Targa-Ouzemour, 06000 Bejaia, Algeria
[b] Laboratoire de Biomathématique, Biochimie, Biophysique et Scientométrie, Faculté des Sciences de la Nature et de la Vie, Université de Bejaia, 06000 Bejaia, Algeria
[c] BioISI – Biosystems & Integrative Sciences Institute, University of Lisboa, Faculty of Sciences, Campo Grande, C8, 1749-016 Lisboa, Portugal
[d] Área Departamental de Engenharia Química, Instituto Superior de Engenharia de Lisboa, Av. Conselheiro Emídio Navarro, 1959-007 Lisbon, Portugal
[e] Centro de Química e Bioquímica, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisbon, Portugal

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ABSTRACT

Introduction: Honey is used in various cultures as a traditional medicine and folkloric treatment. The aim of this study was to determine the antioxidant and the anti-inflammatory activities of 31 Algerian honeys and acetylcholinesterase (AChE) and α-glucosidase inhibitory activities of phenolic compounds extracts of these honeys.
Methods: The anti-inflammatory activity of honey was evaluated by the method of inhibition of BSA (Bovin Serum Albumin) heat-induced denaturation. The inhibition of AChE and α-glucosidase by the honey extracts was evaluated by *in-vitro* methods.
Results: The highest percentage of inhibition (85.33%) of BSA denaturation was obtained with polyfloral honey (H27). Radical scavenging activity of honey samples against 1,1-diphenyl-2-picrilhydrazyl (DPPH), and 2,2′-azinobis^{-3}-ethylbenzothiazoline-6-sulfonic acid (ABTS) varied from 4.41 to 83.93% and from 2.52 to 63.24%, respectively. AChE inhibitory activity is one target to prevent neurodegenerative damage in Alzheimer's disease. AChE inhibition recorded values from 20.69 to 76.04%. α-glucosidase inhibitors such as acarbose is preconized for the control of hyperglycemia and prevent diabetes damages. Honey extracts demonstrated a significant inhibitory effect on α-glucosidase activity. Moreover, the effect of *Fabaceæ* honey (H19) (IC_{50} = 52.20 µg/mL) was found to be more potent than acarbose (IC_{50} = 204.27 µg/mL). Correlations were observed between antioxidants and anti-inflammatory activities, AChE and α-glucosidase inhibitions with total phenolic compounds and flavonoids content in honey samples.
Conclusion: This study showed that honey could be exploited as a potential antioxidant and anti-inflammatory agent within therapeutic medicine.

1. Introduction

As reported by the Codex Alimentarius Commission, honey is:" The natural sweet substance produced by honey bees from nectar of blossoms or from secretions of living parts of plants or excretions of plant sucking insects on the living part of plants". Thus, there are two types of honeys: nectar and honeydew honey [1]. Since antiquity, honey has been known for its biological properties [2]. The virtues of honey are well known in various cultures and used as a traditional medicine and a folkloric treatment, such as ulcer and wound care [3,4]. Sugars represent the major compounds in honey, specially fructose and glucose with 95 and 98% of dry matter. Proteins, amino acids, enzymes, organic acids, minerals, vitamins and pollens represent minority constituents [1,2,3,4,5,1,2–6]. Honey is also a source of several bioactive components Including phenolic compounds and carotenoids. More than 5000 phenolic compounds have been described and they are represented in major part by phenolic acids and flavonoids. These substances are very well known for their antioxidant, antibacterial, antithrombotic and anti-allergic effects [7]. Moreover, research from *in-vitro* and *in-vivo* studies illustrated that honey has antimicrobial, anti-inflammatory and wound dressing properties [5]. Also, it has a neuro-protector effect and reduces anxiety with a positive effect on brain and memory [8]. Inflammatory reactions continue to be the most common human disease. It results from the action of arachidonic acid and prostaglandins derivatives, which are responsible for redness, pain, fever, and edema [9]. The persistence of inflammation is manifested in several diseases such

* Corresponding author.
E-mail address: zaidi-hicham@hotmail.com (H. Zaidi).

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as hepatitis, psoriasis, inflammatory bowel and inflammatory joint disease defined by arthritis [10]. The first line of anti-inflammatory therapy is non-steroid anti-inflammatory drugs (NSAIDs). However, they are usually linked with the undesirable effects exhibited by gastrointestinal and cardiovascular complications [11]. Alzheimer's disease (AD) and diabetes represent a real danger to public health, AD is a neurodegenerative disease. Still, there is no curative treatment, the only alternative is to mitigate symptoms of the disease just as senile dementia [12]. On the other hand, it has been noticed that in patients with AD, there is a factor related to a conformational change of acetylcholinesterase (AChE) and its polymorphism leads to its overactivity and a decrease of neurotransmitter levels in brain [13]. One of the targets of AD treatment is to increase the level of acetylcholine in the brain by inhibiting AChE. Patients with neurodegenerative disease treated with the usual inhibitors present undesirable effects such as gastrointestinal anomalies such as diarrhea, nausea and hepatotoxicity [14]. Diabetes type 2 is characterized by insulin resistance, which may be combined with relatively reduced insulin secretion and leading to hyperglycemia. This metabolic disease can cause many complications, which occur in various organs causing serious health issues, often nephropathy and retinopathy [15]. One of the targets for hyperglycemia reduction, is to reduce the activity of α-glucosidase responsible for carbohydrates hydrolysis [16]. The α-glucosidase inhibitors delay absorption of sugars in the intestinal tract, thus limiting the excursions of postprandial plasma glucose [17]. Acarbose is an oral α-glucosidase inhibitor. It is recommended for the control of hyperglycemia. The application of acarbose in diabetes treatment is no longer without side effects. Indeed, undesirable effects of this synthetic inhibitor are manifested mainly by abdominal distention, diarrhea, nausea and flatulence [18]. Therefore, another approach to treatment applying of inflammation, neuro-degenerative diseases and diabetes from natural products is crucial. Honey is a significant source of biomolecules, such as phenolic compounds which have considerable proprieties [19]. In this regard, within our documentation, there are no reports on anti-inflammatory using inhibition of BSA denaturation model, the acetylcholinesterase and α-glucosidase inhibitions by honeys. The aim of this study was to evaluate the anti-inflammatory and antioxidant effect of 31 Algerian honeys using *in vitro* methods. The honey extracts were tested also in the inhibition of acetylcholinesterase and α-glucosidase activities.

2. Material and methods

2.1. Chemicals and reagents

The bovine serum albumin (BSA), 1,1-diphenyl-2-picrilhydrazyl (DPPH), 2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 5,5′-Dithiobis(2-nitrobenzoic acid) (DTNB), AChE (149 U/mg solid, 241 U/mg protein), tris buffer and α- glucosidase (from *Saccarharomyces cerevisiae*, 10 U/mg) were obtained from Sigma Aldrich (St. Louis, USA). Potassium di-hydrogen phosphate and sodium hydroxide reagent were from Scharlau (Sentmenat, Spain) and Eka Chemicals (St Patrice East Magog, Canada), respectively. The organic solvents were purchased from Carlo Erba (Val de Reuil, France), methanol and diethyl ether from Panreac (Barcelona, Spain). The p-nitrophenyl- α -D-glucopyranoside (PNPG) was obtained from A Johnson Matthay Compagny (Kaflsruche, Germany). All the other chemicals and reagents were from Biochem Chemopharma (Georgia, USA).

2.2. Honey samples

Thirty-one honey samples were obtained from beekeepers in different regions of Algeria. Honey samples were collected during 2015. A pollen analysis was established with method of Louveaux et al. [20] in order to determine the botanical origin of the honey samples (Table 1).

Table 1
Samples of honey analyzed (region, botanical origin).

Samples	Region	Botanical origin
H1	Bejaia	*Myrtaceæ*
H2	Bejaia	*Fabaceæ*
H3	Bejaia	Polyfloral
H4	Bejaia	*Eucalyptus*
H5	Setif	Polyfloral
H6	Setif	*Apiaceæ*
H7	Bourdj bou arreridj	Polyfloral
H8	Boumerdes	Polyfloral
H9	Constantine	Polyfloral
H10	Tebessa	*Fabaceæ*
H11	Bourdj bou arreridj	Polyfloral
H12	Tizi-ouzou	*Fabaceæ*
H13	Tizi-ouzou	*Fabaceæ*
H14	Bouira	Polyfloral
H15	Algiers	Polyfloral
H16	Medea	Polyfloral
H17	Djelfa	Polyfloral
H18	Laghouat	Polyfloral
H19	Mostaganem	*Fabaceæ*
H20	Mostaganem	Polyfloral
H21	Jijel	*Rhamnaceæ*
H22	Laghouat	Polyfloral
H23	Ghardaia	Polyfloral
H24	Blida	Polyfloral
H25	Batna	*Apiaceæ*
H26	Boumerdes	*Fabaceæ*
H27	El bayadh	Polyfloral
H28	Bejaia	Polyfloral
H29	Bejaia	Polyfloral
H30	Bejaia	Polyfloral
H31	Bejaia	*Apiaceæ*

2.3. Determination of total phenolic content

Total phenolic content of honey samples was determined according to the method based on the Folin-Ciocalteu reagent as described by Naithani et al. [21]. A volume of 200 µl of the honey solution (0.1 g/ml, w/v) was added to 200 µl of Folin-Ciocalteu (50%, v/v) reagent and 4 ml of sodium carbonate (2%, v/v). After incubation at room temperature for 30 min in the dark, the absorbance of the reaction mixture was determined at 750 nm. The results of phenolic content were expressed in mg gallic acid equivalent / 100 g of honey (mg GAE / 100 g).

2.4. Determination of total flavonoid content

The total flavonoid content in each honey sample was assessed by using the colorimetric assay developed by Al et al. [22]. One ml of honey solution (0.5 g/mL) was mixed with 300 ml of sodium nitrite (5%, w/v). After 5 min, an equivalent volume of aluminum chloride (10%, w/v) was added and 2 mL of sodium hydroxide (1 mol/L) were added after 6 min. The absorbance of the mixture was read at 510 nm. The results were expressed in mg catechin equivalent /100 g of honey (mg CE / 100 g).

2.5. In-vitro anti-inflammatory activity

The inhibition of BSA denaturation by honey samples was performed as described by Williams et al. [23] with minor modifications. The reaction mixture containing 0.45 mL (0.2%, w/v) of aqueous solution of bovine serum albumin (adjusted to pH 6.3) and 50 µL of aqueous solution of honeys (2.5 mg/mL) or ibuprofen (100 µg/mL) or distilled water were added. Ibuprofen was used as standard and distilled water as negative control. The mixtures were incubated for 15 min in an ambient temperature and then the test tubes were kept at 71 °C in a water bath for 5 min. After that, an amount of 1.5 mL of phosphate buffer saline (0.1 mol/L of potassium di-hydrogen phosphate

and 0.1 mol/L of sodium hydroxide, pH 6.3) was added to the reactional mixture. The turbidity was determined at 660 nm using a UV–vis spectrophotometer. The percentage inhibition of protein denaturation was calculated using the following equation:

$$\% \text{ Inhibition} = ((Abs_{control} - Abs_{sample}) / Abs_{control}) \times 100$$

Where: $Abs_{control}$: Absorbance with BSA solution without honey solution or ibuprofen and Abs_{sample}: Absorbance with honey solution.

2.6. Antioxidant activity

2.6.1. DPPH assay

The DPPH reduction test by the antioxidants present in the honey was carried out by the method described by Meda et al. [24]. A volume of 1 ml of the ethanolic solution of DPPH (6.10^{-5} mole/L) was added to 500 µL of the aqueous solution of honey (2.5%, w/v). The absorbance was read at 517 nm after 15 min of incubation. The reduction percentage was given according to the following formula:

$$\text{Anti-radical activity (\%)} = ((Abs_{control} - Abs_{sample}) / Abs_{control}) \times 100$$

$Abs_{control}$: Absorbance of the control.
Abs_{sample}: absorbance of the sample.

2.6.2. ABTS assay

ABTS radical scavenging activity is another method to evaluate the ability of antioxidant compounds to scavenge free radicals. The ABTS test was performed according to the method reported by Re et al. [25]. A volume of 1 mL of the ABTS solution (7.10^{-3} mole/L) was added to 0.1 mL of the aqueous solution honey (2.5%, w/v). After 7 min, the absorbance was read at 734 nm. The percentage reduction was given according to the following formula:

$$\text{Anti-radical activity (\%)} = ((Abs_{control} - Abs_{sample}) / Abs_{control}) \text{ x } 100$$

$Abs_{control}$: Absorbance of the control.
Abs_{sample}: Absorbance of the sample.

2.7. Extraction and analysis of phenolic compounds in honey

Eighteen samples of honey were used in orderin to extract their phenolic compounds. The phenols extraction was followed according to the method described by Ferreres et al. [26]. A quantity of 50 g of honey was mixed with 125 mL of acidified water with HCl (pH 2). The solution of honey was filtered through a filter paper 70 mm from Filter Lab (Barcelona, Spain). The filtrate was passed through a column (25 × 2 cm) filled with amberlite XAD-2 (mean particle size 663.3 µm, SUPLECO Analytical, Bellfonte, PA, USA) which adsorbs phenolic compounds and eliminates sugars. The column was washed with acidified water (100 mL) then with 300 ml of distilled water. The phenol fraction was eluted with about 400 mL of methanol until the column returns to its whitish origin color. The fraction was collected and evaporated at 40 °C (Büchi Rotavapor R-200). The extract obtained was dissolved in 5 ml of distilled water and extracted with diethyl ether (3 × 5 mL). The whole of the ethyl extracts was concentrated under reduced pressure at 30 °C. Honey extracts were dissolved in methanol and analyzed by thin-layer chromatography (TLC) to obtain the different fractions of phenolic compounds. The eluents used for TLC were first acetone / chloroform (1:9, v/v) followed by chloroform / methanol / water (5:5:1, v/v/v). Flavonoids were visualized by spraying the plate with methanolic solution of aluminum chloride (5%, w/v) and then observing it at UV light 336 nm as described by Amiot et al. [27].

2.8. Inhibition of AChE activity

AChE inhibitory activity by honey phenolic compounds extract was carried out by Elleman's method as described by Ferreira et al. [28]. The reaction mixture consisted of 375 µL Tris buffer (pH 8, 50.10^{-3} mole/L), 50 µL of honey extract diluted in methanol (0.5 mg/mL) and 25 µL of enzyme solution (0.1 U Abs/min). The mixture was incubated for 15 min at ambient temperature and 75 µL of acetylthiocholine iodide (AChI) (15.10^{-3} mole/L) and 475 µL ($3 .10^{-3}$ mole/L) of DTNB were added. The velocity of reaction was read spectrophotometrically at 405 nm during 5 min. Galantamine served as standard inhibitor. The Inhibition of AChE activity was determined as the percentage activity/ inhibition related to the control test with methanol using the relation shown below:

$$\% \text{ inhibition of AChE activity} = 100 - (V_{sample} / V_{control}) \times 100$$

Where: $V_{control}$ is the initial velocity of the control reaction; V_{sample} is the initial velocity of the phenolic compound extract and reaction mixture.

2.9. Inhibition of α- glucosidase activity

α- glucosidase inhibition activity was evaluated by the method of Queiroz et al. [29] with minor modifications. A volume of 150 µL of α-glucosidase enzyme solution from *Saccharomyces cerevisiae* was incubated in 25 µL of methanolic solution of different concentrations of honey phenolic compounds extract during 5 min, at 37 °C. After incubation, a volume of 150 µL of the enzyme substrate PNPG (5.10^{-3} mole/L) and 420 µL of the phosphate buffer (10.10^{-3} mole/L, pH 6.9) was added and then reaction mixture was incubated for 30 min at 37 °C. After that, the reaction was stopped by adding a quantity of 900 µL of sodium carbonate (1 mol/L). The absorbance was determined at 405 nm. Acarbose, a specific inhibitor for α-glucosidase, was used as a positive control. The percentage inhibition calculation (I%) was calculated according to the following formula:

$$\% \text{ inhibition of α glucosidases} = ((Abs_{control} - Abs_{sample}) / Abs_{control}) \times 100$$

Where, $Abs_{control}$: total enzymatic activity without inhibitor and Abs_{sample}: enzyme with honey extract.

2.10. Statistical analysis

Experimental data obtained were expressed as the mean ± SD (standard deviation). STATISTICA 5.5 software was used to study the variance with a single classification criterion between the different honey samples using LSD Fisher test. Differences were considered significant at $^{*}p < 0.05$. Correlations between the different activities and total phenolic compounds and flavonoids content of honey samples studied were calculated with elementary statistics using the correlation matrix.

3. Results

3.1. Total phenolic compounds content

The results obtained from the total phenolic compounds content of the honey samples showed that the phenolic concentrations varied from 14.50 (H10) to 99.62 (H9) mg GAE / 100 g with an average of 54.55 mg GAE / 100 g (Fig. 1). Monofloral honeys had a content which varied from 14.5 mg GAE / 100 g (H10, *Fabaceae*) to 94.69 mg GAE / 100 g (H19, *Fabaceae*). Polyfloral honey sample H9 recorded the highest value with 99.62 mg GAE / 100 g. These results were slightly inferior compared to the results obtained by Habib et al. [30] on oriental honeys (30.81 to 132.60 mg GAE / 100 g). There was no significant difference

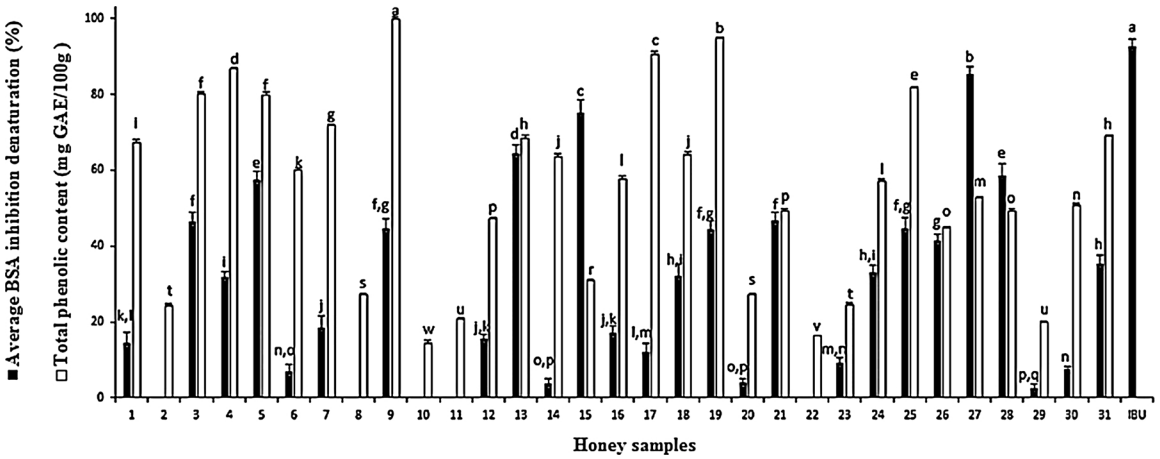

Fig. 1. Total phenolic compound content and BSA anti-denaturation activity of the honeys analyzed. Different letters indicate that the values are significantly different.

with honey samples H21, H28 and H30 concerning the amount of total phenols.

3.2. Flavonoids content

The honey analyzed samples revealed that flavonoids content ranged from 0.29 (H29) to 2.25 (H4) mg CE / 100 g (Table 2). These results were lower than those reported by Escuredo et al. [31] on Iberian Peninsula honeys (2.97 to 9.07 mg CE / 100 g). Honey samples H4 and H9 presented a significant difference in comparison with the other honey samples.

3.3. In-vitro anti-inflammatory study

The inhibition of BSA heat-induced denaturation by honey samples is the method selected to evaluate the anti-inflammatory activity of honey. The results (Fig. 1) showed that honey samples exhibited a significant inhibitory activity of BSA denaturation induced by heat. The inhibition of BSA denaturation varied from 0 (H2, H8, H10, H11, H22) to 85.33% (H27). The highest value presented a significant difference

Table 2
Flavonoids content, BSA denaturation Inhibition (IC_{50}), DPPH and ABTS radical scavenging activity of honey samples analyzed. Different letters indicate that the values are significantly different. ND: not determined.

Monofloral honey Samples	Flavonoids content (mg CE / 100 g)	BSA denaturation inhibition (IC_{50}, mg/ml)	DPPH Assay (% I)	ABTS Assay (% I)
H1	1.46 ± 0.19^{c}	6.18 ± 1.44^{a}	59.96 ± 1.79^{e}	37.67 ± 1.00^{d}
H2	0.32 ± 0.05^{e}	ND	4.41 ± 1.43^{h}	2.52 ± 0.59^{h}
H4	2.25 ± 0.16^{a}	$3.53 \pm 1.13^{b,c,d}$	83.93 ± 1.27^{a}	63.24 ± 0.54^{a}
H6	$2.05 \pm 0.11^{a,b}$	ND	55.74 ± 1.11^{f}	43.95 ± 1.15^{c}
H10	0.50 ± 0.08^{e}	ND	9.68 ± 2.44^{g}	12.24 ± 1.04^{g}
H12	0.90 ± 0.18^{d}	$5.73 \pm 0.95^{a,b}$	56.29 ± 1.19^{f}	45.33 ± 1.49^{c}
H13	1.35 ± 0.09^{c}	2.18 ± 0.84^{d}	59.14 ± 1.07^{e}	34.95 ± 1.05^{e}
H19	1.44 ± 0.08^{c}	$3.20 \pm 0.53^{c,d}$	55.55 ± 1.61^{f}	45.57 ± 0.86^{c}
H21	1.40 ± 0.24^{c}	$2.75 \pm 0.74^{c,d}$	63.73 ± 0.64^{d}	39.38 ± 1.33^{d}
H25	1.37 ± 0.16^{c}	$2.69 \pm 0.16^{c,d}$	74.01 ± 0.32^{b}	30.86 ± 1.17^{d}
H26	1.89 ± 0.09^{b}	$3.68 \pm 0.38^{b,c}$	71.99 ± 1.24^{b}	29.33 ± 1.05^{d}
H31	1.37 ± 0.14^{c}	$3.48 \pm 0.90^{b,c,d}$	69.37 ± 0.87^{c}	51.95 ± 1.37^{b}
Polyfloral honey Samples	**Flavonoids content (mg CE / 100 g)**	**BSA denaturation inhibition (IC_{50}, mg/ml)**	**DPPH Assay (% I)**	**ABTS Assay (% I)**
H3	$1.82 \pm 0.08^{b,c}$	$3.64 \pm 1.03^{c,d}$	64.46 ± 0.48^{a}	48.38 ± 2.50^{a}
H5	2.00 ± 0.19^{b}	$2.48 \pm 0.58^{d,e}$	54.73 ± 1.27^{d}	$41.24 \pm 0.79^{b,c,d}$
H7	$1.42 \pm 0.13^{d,e}$	$4.81 \pm 0.37^{b,c}$	57.67 ± 0.65^{c}	30.29 ± 1.25^{g}
H8	0.36 ± 0.17^{i}	ND	6.43 ± 1.11^{k}	2.93 ± 0.91^{k}
H9	2.28 ± 0.11^{a}	$2.60 \pm 0.42^{d,e}$	$55.98 \pm 0.16^{c,d}$	$40.57 \pm 0.71^{c,d}$
H11	$0.65 \pm 0.19^{g,h}$	ND	9.68 ± 2.16^{i}	16.38 ± 1.66^{j}
H14	$1.38 \pm 0.35^{d,e}$	ND	60.51 ± 1.75^{b}	$38.81 \pm 2.29^{d,e}$
H15	0.81 ± 0.05^{g}	$2.23 \pm 0.75^{d,e}$	47.57 ± 1.95^{e}	23.48 ± 1.08^{h}
H16	1.46 ± 0.16^{d}	7.43 ± 1.72^{a}	54.27 ± 1.72^{d}	36.19 ± 2.08^{f}
H17	1.10 ± 0.22^{f}	$6.71 \pm 1.63^{a,b}$	64.00 ± 0.64^{a}	43.43 ± 1.48^{b}
H18	$1.58 \pm 0.06^{c,d}$	$3.95 \pm 0.83^{c,d}$	60.33 ± 1.81^{b}	46.81 ± 1.64^{a}
H20	$0.74 \pm 0.08^{g,h}$	ND	17.78 ± 1.85^{i}	$17.71 \pm 1.17^{i,j}$
H22	$0.49 \pm 0.29^{h,I}$	ND	11.48 ± 0.80^{j}	$17.57 \pm 1.97^{i,j}$
H23	$0.65 \pm 0.31^{g,h}$	ND	28.37 ± 1.43^{h}	19.67 ± 1.84^{i}
H24	$1.58 \pm 0.20^{c,d}$	$3.59 \pm 0.51^{c,d}$	43.43 ± 1.75^{f}	43.67 ± 1.00^{b}
H27	$1.64 \pm 0.14^{c,d}$	1.72 ± 0.22^{e}	$55.93 \pm 1.58^{c,d}$	$42.10 \pm 1.53^{b,c}$
H28	$1.15 \pm 0.29^{e,f}$	$2.14 \pm 0.43^{d,e}$	49.40 ± 2.07^{e}	$37.67 \pm 1.47^{e,f}$
H29	0.29 ± 0.06^{I}	ND	17.08 ± 1.91^{i}	16.14 ± 1.14^{j}
H30	$0.88 \pm 0.09^{f,g}$	ND	33.61 ± 1.43^{g}	29.57 ± 1.68^{g}

compared with the standard ibuprofen which has a potential of 92.76% at a concentration of 25 µg/mL. Honey samples H3, H9, H19, H21, H25 and H26 revealed a comparable effect on BSA denaturation with inhibitory activity of 44.84%; all the other honey samples showed no significant activity. The honey samples which presented a significant anti-inflammatory activity have been selected to determine their IC_{50} Values which varied from 1.72 (H27) to 7.43 (H16) mg/mL (Table 2). There was not a significant difference among the monofloral honey samples except samples H1 and H12. IC_{50} values of the polyfloral honey samples had no significant difference. The results revealed that the honey samples have anti-inflammatory properties, which support previous findings.

3.4. Honey antioxidant activity

3.4.1. DPPH radical assay

DPPH radical scavenging activity is a good *in vitro* model to investigate the antioxidant activity of compounds. The honey samples analyzed for their DPPH scavenging activity revealed a significant radical scavenging activity (Table 2). These results were close to those reported by Liu et al. [7] (15.2–84.9 %). Sample H2 had the lowest antiradical activity and it was characterized by a low total phenols content.

3.4.2. ABTS radical assay

As shown in Table 2, a significant difference between the samples was recorded and the highest ABTS scavenging activity was displayed by H4 sample (*Eucalyptus* honey) (63.24%). *Apiaceæ* honey (H31) presented a value equal to 51.95%; our results corroborate the investigations of Perna et al. [32] on *Eucalyptus* (58.40%) and chestnut (60.42%) honeys. This similarity was probably due to the common plant diversification and climate between Algeria and Italy, which benefit both from a Mediterranean climate. The anti-radical ABTS activity of the polyfloral honey samples ranged from 2.93 (H8) to 48.38% (H3). These results were superior to those obtained by Wilczyńska [33] who studied the antioxidant activity of Polish honeys (from 2.29 to 31.51%).

3.5. Extraction and analysis of phenolic compounds in honey

3.5.1. TLC of phenolic compound of honey extract

TLC Analysis confirmed that the honey extracts are a complex mixture of phenolic compounds (Fig. 2), at UV light 254 nm, the chromatography showed various fractions of phenolic compounds (Fig. 2A1). Flavonoids present in the honey extract were visualized by aluminum chloride methanolic solution and then the plate was observed at UV light 336 nm (Fig. 2B1). The results showed various fluorescent yellowish spots, indicating the presence of flavonoids. The retention factor (RF) of quercetin was identical to one of the major spots in the sample, suggesting the presence of this flavonoid (Fraction F1).

3.6. Anti-acetylcholinesterase activity

Anti-acetylcholinesterase activity was carried out on all the different extracts of honey. Inhibition of AChE activity revealed that all of them significantly inhibited the enzyme with an average of 51.25% as showed in Fig. 3. For the polyfloral honeys, the inhibition of AChE ranged from 20.69 (H20) to 76.04% (H9) and from 39.89 (H6) to 67.15% (H19) for monofloral honeys. Sample H9 had the best AChE inhibition and the results obtained was significantly different from the others. AChE Inhibition values of samples H4, H17 and H19 had no significant difference. The honey extract concentration able to inhibit 50% of the AChE and α-glucosidase activities were evaluated from the regression equations obtained from the activity of samples at different concentrations. Samples that showed significant inhibition potential were selected to determine IC_{50} values. In Table 3, IC_{50} values of eight honey samples extracts were presented for AChE inhibition effect. The results obtained revealed that honey extracts H4 and H9 presented the best AChE inhibition and had a significant difference with the results of the other samples. Galantamine used as an inhibitor control, recorded the lowest IC_{50} value and presented a significant difference with the data of honey extracts. Monofloral honey extracts had IC_{50} values with this order: H6 > H31 > H1 > H4.

3.7. α- glucosidase inhibition assay

Due to insufficient quantities, only seven phenolic compound extracts of honey could be used to evaluate α-glucosidase inhibition activity from *Saccharomyces cerevisiae.* The results of this *in vitro* study

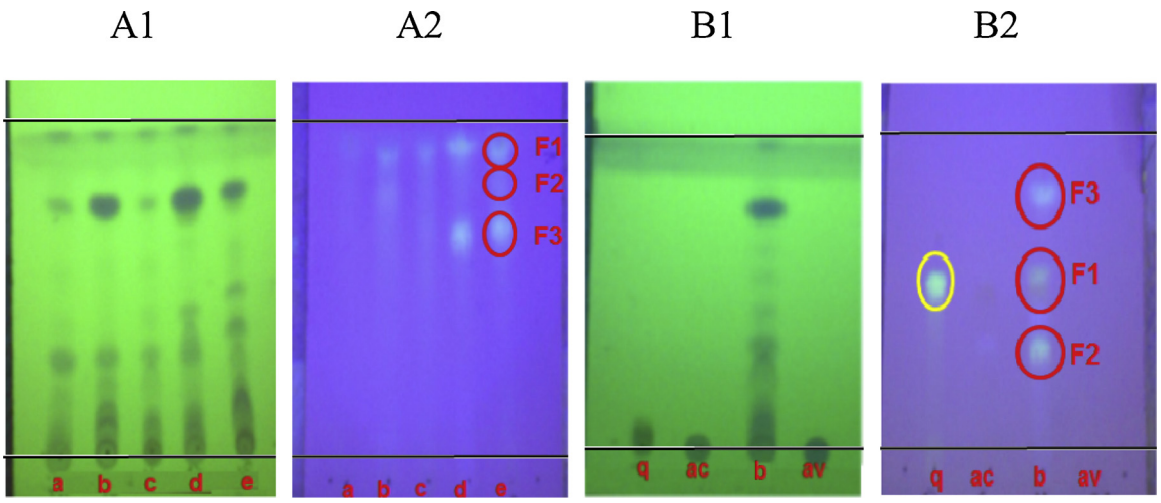

Fig. 2. TLC of phenolic compound extract from some samples of honey and revelation of flavonoids.
A1. TLC of some honey samples: a: H20, b: H30, c: H16, d: H17, e: H18. First revelation: acetone/chloroform (1:9); 254 nm.
A2. Flavonoids fraction at second revelation: chloroform/methanol/water (5:5:1); 336 nm. F1, F2, F3: flavonoid fractions.
B1. TLC of phenolic compounds extract from honey sample (H17) and comparison with phenolic compounds standards (q: quercetin, ac: caffeic acid, b: H17, av: vanillic acid). Acetone/chloroform (1:9); 254 nm.
B2. Revelation of flavonoids fraction in sample H17 at second revelation.

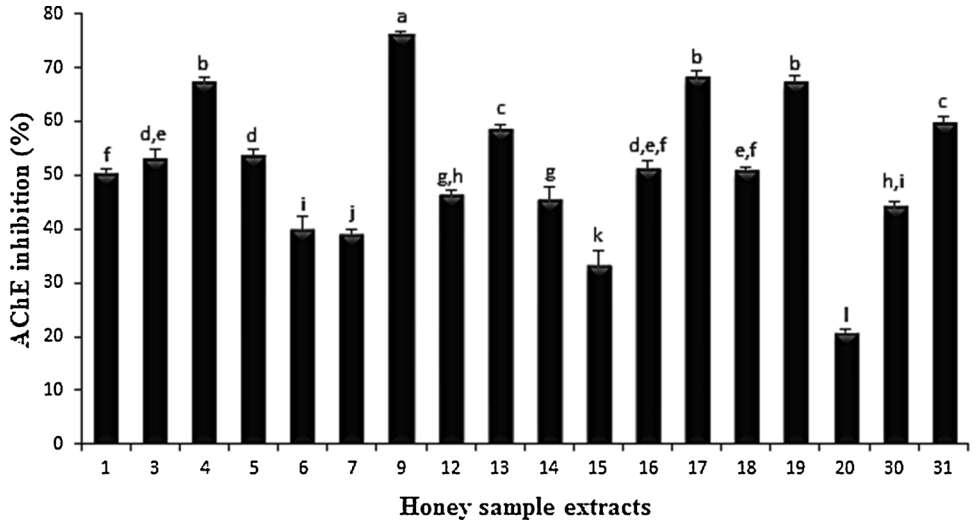

Fig. 3. AChE activity inhibition by phenolic compound extracts. Same letters indicate that the values are not significantly different.

Table 3
IC_{50} values of Acetylcholinesterase and α-glucosidase activities. Same letters indicate that the values are not significantly different. ND: not determined. NA: not analyzed.

Samples	AChE (IC_{50}, mg/ml)	α-glucosidase (IC_{50}, μg/ml)
H1	0.459 ± 0.039^{d}	NA
H3	ND	109.82 ± 1.54^{f}
H4	0.374 ± 0.002^{e}	NA
H5	ND	97.71 ± 1.62^{g}
H6	0.617 ± 0.038^{a}	NA
H7	0.629 ± 0.021^{a}	NA
H9	0.367 ± 0.025^{e}	NA
H12	ND	141.81 ± 0.87^{c}
H13	ND	123.31 ± 1.19^{e}
H14	0.557 ± 0.010^{b}	NA
H15	ND	153.36 ± 0.94^{b}
H16	ND	136.15 ± 1.22^{d}
H18	0.506 ± 0.015^{c}	NA
H19	ND	52.20 ± 0.72^{h}
H31	0.497 ± 0.008^{c,d}	NA
Galantamine	0.210 ± 0.020^{f}	/
Acarbose	/	204.27 ± 1.56^{a}

demonstrated that the honey extracts had α-glucosidase inhibitory activity. IC_{50} values of phenolic compound extracts were presented in Table 3. The average value of IC_{50} polyfloral honeys was 124.26 μg/mL. Their values were classified as follows: H15 > H16 > H3 > H5. H19 sample extract (IC_{50} = 52.20 μg/mL) presented a better α-glucosidase activity inhibition than the standard acarbose (IC_{50} = 204.27 μg/mL) with a significant difference. These results showed that phenolic compound extracts in honeys used to test this activity contain active components acting as inhibitor α-glucosidase of yeast.

4. Discussion

The variation in total phenol content of honey is due to the botanical origin. Indeed, the main source of polyphenols brought by the bee comes from nectar and plant secretions [34]. This variation in concentration of polyphenols in honey depend not only on the floral source but also on the geographical area, and even seasonal and environmental factors may be responsible for the differences detected. Honey is a source of phenolic compounds and flavonoids and seems to have a close correlation with different biological activities such as the antioxidant one [19]. The anti-denaturation effect of samples may be explained by the different floral source, total phenolic compounds and flavonoids content. Our results corroborated those reported by Liu et al. [7]. Protein denaturation can be manifested in the inflammatory reaction such as rheumatoid arthritis. Protection against protein denaturation was the main mechanism of action of NSAIDs [35]. The ability of honey to inhibit thermal protein denaturation may contribute to its anti-inflammatory properties and could have an important effect on the anti-arthritic activity. The results in this study showed that the different honey samples inhibited the BSA denaturation induced by heat. As reported by Williams et al. [23], the BSA anti-denaturation activity of NSAIDs drugs was due to the interaction with BSA tyrosine or threonine in envelope structure and lysine residue regions thus preventing protein denaturation. We can suggest that there was the same interaction between phenolic compounds like phenolic acids and flavonoids with different BSA sites, thus avoiding its denaturation. Kassim et al. [36] demonstrated that gallic acid, chrysin, quercetin, the phenethyl ester, caffeic acid, luteolin, kaempferol and hesperetin present in honey have anti-inflammatory and immunomodulatory effects, consolidating therefore our findings.

The differences in the radical scavenging activity with DPPH and ABTS can be explained by the content variation of the active biomolecules present in mono and polyfloral honeys [37]. The diversity of the botanical and geographical origin, the nature and the contents of the phenolic compounds in honey. Van den Berg et al. [38] with the thin layer chromatographic analysis revealed that honey extracts contain various phenolic compounds. It was reported in the same study that those active molecules in honey are responsible for the antioxidant activity. Several other compounds contribute to the antioxidant capacity of honeys, such as organic acids, ascorbic acid and enzymes [39].

The common approach to treating AD is to inhibit the activity of AChE in order to increase the level of cholinergic neurotransmitter in the brain. Anti-acetylcholinesterase assay was used to determine the neuro-protector effect of honey extracts. In fact, AChE inhibition can reduce neurodegenerative damage of AD. This assay is based on the capacity of molecules to inhibit the active site thus reducing its activity. AChE inhibition activity has been reported previously from various plant extracts [14]. Inhibition of AChE was described as being an inhibition by a hydrophobic ligand [40]. Neuroprotective effect has long been attributed to polyphenols. The antioxidant activity has also been previously correlated with AChE inhibition and associated with the neuroprotective effect of phenolic compounds [41]. As reported by Szwajgier [42], luteolin, myricetin and kaempferol were strong inhibitors against AChE and it has been described that there is an interaction between central heterocyclic ring of flavonoids and the active site of the enzyme. These flavonoids such as luteolin, myrcetin and kaempferol were already reported in Algerian honeys [43]. In this

study, the statistical analysis showed a very high significant correlation between the AChE inhibition activity and total phenols content in honey. Therefore, phenols present in honey extract might act as potent inhibitors against AChE and could be used as an alternative therapy, probably in association with other AChE inhibitors.

Ademiluyi and Oboh [16] reported that α-glucosidase is responsible for hydrolyzing polysaccharides and disaccharides containing an α-glucose unit. They indicated that the enzyme inhibition was mainly attributed to polyphenols. Ramkumar et al. [44] reported that numerous studies showed the close correlation between polyphenols and α-glucosidase inhibition. Other studies conducted on the characterization of α-glucosidase inhibitors, purified phenolic compounds such as gallic acid, methyl gallate and propyl gallate manifested an inhibitory effect on the enzyme [45]. It was reported that glycosylated flavonoids have an inhibitory effect on α-glucosidase by the interaction of the glucosidic fragment and the α-glucosidase's active site [46]. Phenols extracts from honey have been suggested to reduce hyperglycemia in diabetes by inhibiting digestive enzymes, α-glucosidases. Indrianingsih et al. [47] described the molecular mechanism of α-glucosidase inhibition by the binding of hydrogen to the active site of the enzyme. Honey phenolic compounds could have this property through their hydroxyl group to inhibit the catalytic activity of the enzyme. Li et al. [48] showed that flavonoids such as quercetin, isoquercetin and rutin have powerful inhibitory effect on the activity of this enzyme. In addition, Kim et al. [49] demonstrated that luteolin and luteolin 7-O-glucoside are strong inhibitors of yeast α-glucosidase. Regarding the work done in this study, honey phenolics are strong inhibitors of α-glucosidase. Therefore, the results obtained in this present study on α-glucosidase inhibition by phenolic compounds in Algerian honeys may represent a real alternative to synthetic molecules to diabetes treatments. These results could be improved by the purification of the active phenolic compounds of the honey extracts and the specific interactions between honey components and the investigated enzymes determined.

5. Correlation analysis

A correlation matrix was performed between the total phenols and flavonoids content, anti-denaturation BSA and antioxidant activity of honeys analyzed (Table 4). The level of phenolic compounds and flavonoids in the honey samples indicated the existence of a very high significant correlation. This correlation between these two parameters was observed also in the work of Al et al. [22]. Total phenols and flavonoids content showed also a very high significant correlation with BSA anti-denaturation results. This close correlation suggests that phenols in honey contribute to the anti-inflammatory activity, as has already been pointed out by Kassim et al. [36]. The data also showed a very high significant correlation between BSA anti-denaturation and radical scavenging activity on one hand with DPPH and on the other hand with ABTS radical. As expected the total phenol compounds content showed a very high significant correlations with the antioxidant activities: DPPH and ABTS. This data was in agreement with the results reported by Saxena et al. [50] and Alvarez-Suarez et al. [5]. Furthermore, a very high significant correlation was observed between flavonoids and DPPH also with ABTS radical. Other author [51] also found a close correlation between flavonoids and DPPH radical inhibition. This data showed once more the important relationship between the total phenols content, flavonoids and the antioxidant potential. Our results confirm that polyphenols are the main compounds responsible for this biological property and are comparable with results of Perna et al. [32] which found very high significant correlations between the antioxidant effects (DPPH, ABTS scavenging) and phenolic, flavonoid content. The data presented a very high significant correlation between the AChE inhibition activity with total phenols content also with flavonoids. Total phenols and flavonoids content revealed a very high significant correlation with the α-glucosidase inhibition results. This data confirmed that phenols and flavonoids can be potential inhibitors of the activity of AChE and α- glucosidase. This observation was reported by numerous authors where the phenols and flavonoids have the capacity to link the active site of AChE and α- glucosidase so to inhibit these enzymes [40–42,1–16].

6. Conclusion

In this work, anti-inflammatory and antioxidant activities of 31 Algerian honeys were evaluated. The results showed that honey had an effect on BSA anti-denaturation and radical scavenging activity, indicating that the studied honeys have anti-inflammatory and antioxidant properties. The phenolic compounds extraction confirmed that honey is a source of these biological substances. The phenolic compounds extract obtained from Algerian honeys studied had a significant effect on the activity of AChE and α-glucosidase. These biological proprieties were correlated with the total phenolic compounds and flavonoids content. The variation found between samples can be associated with its botanical origin. Further study is now needed at the molecular scale to explore the reaction between the active molecules of honey and these biological properties. In the light of the results obtained, honey could be a real source of treatment for rheumatoid arthritis, AD and diabetes, indeed potentially as an alternative or as a nutritional complement to other type of treatment. Therefore, further studies are necessary for the determination and the characterization of honey biomolecules involved in these activities and the elucidation of their mechanism of action.

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Conflicts of interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Table 4
Correlation matrix between antioxidants (total polyphenols, flavonoids) and biological activities. *** ($p < 0.001$) indicate that the correlation is very high significant, NS: not significant.

Variables	Total Phenols	Flavonoids	BSA	DPPH	ABTS	AChE	α-glucosidase
α-glucosidase	0.83***	0.68***	0.21^{NS}	0.25^{NS}	0.42^{NS}	0.70***	–
AChE	0.89***	0.55***	0.27^{NS}	0.67***	0.70***	–	
ABTS	0.81***	0.79***	0.49***	0.89***	–		
DPPH	0.81***	0.80***	0.57***	–			
BSA	0.44***	0.51***	–				
Flavonoids	0.78***	–					
Total Phenols	–						

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