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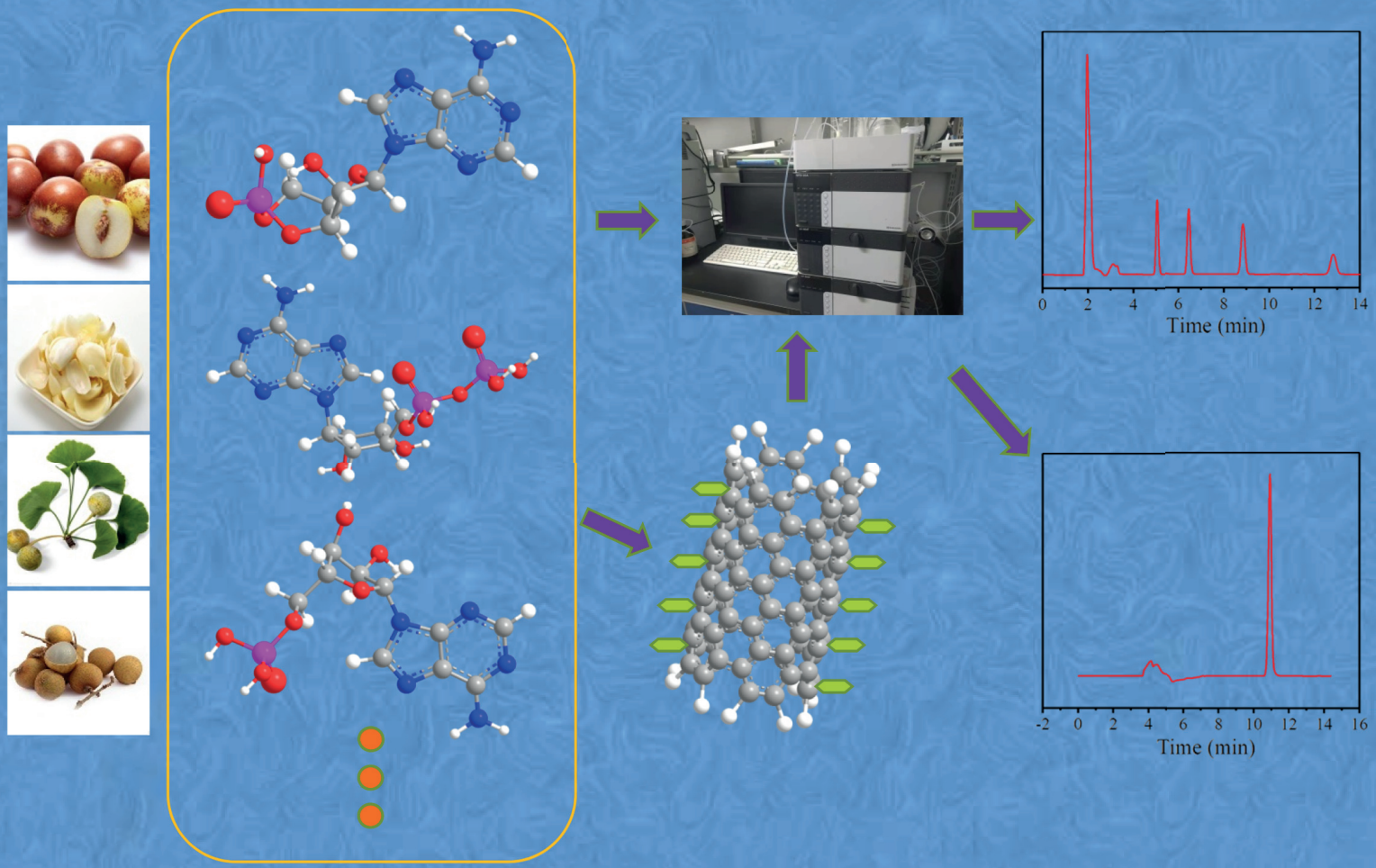

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RESEARCH ARTICLE

A new method of extracting polyphenols from honey using a biosorbent compared to the commercial resin amberlite XAD2

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A new extraction method of polyphenols from honey using a biodegradable resin was developed and compared with the common commercial resin amberlite XAD2. For this purpose, three honey samples of Algerian origin were selected for the different physicochemical and biochemical parameters study. After extraction of the target compounds by both resins, the polyphenol content was determined, the antioxidant activity was tested, and liquid chromatography–mass spectrometry analyses were performed for identification and quantification. The results showed that physicochemical and biochemical parameters meet the norms of the International Honey Commission, and the H1 sample seemed to be of high quality. The optimal conditions of extraction by biodegradable resin were a pH of 3, an adsorption dose of 40 g/L, a contact time of 50 min, an extraction temperature of 60°C, and no stirring. The regeneration and reuse number of both resins was three cycles. The polyphenol contents demonstrated a higher extraction efficiency of biosorbent than of XAD2, especially in H1. Liquid chromatography–mass spectrometry analyses allowed for the identification and quantification of 15 compounds in the different honey samples extracted using both resins and the most abundant compound was 3,4,5-trimethoxybenzoic acid. In addition, the biosorbent extracts showed stronger antioxidant activities than the XAD2 extracts.

KEYWORDS
antioxidant activity, biosorbents, honey, polyphenols, resin

1 | INTRODUCTION

Honey is one of the oldest foods known to humans because of its high nutritional value and its therapeutic use in traditional medicine [1], produced by bees from the nectar of plants. The latest research has revealed that honey contains several natural compounds. These compounds generally include sugars as the main substance, including monosaccharides (glucose and fructose) and disaccharides (composed of both fructose and glucose linked together). Honey also contains proteins, amino acids, vitamins, minerals, organic acids, alcohols, enzymes, lipids [2], volatiles [3, 4], and other substances with a wide range of biological effects, such as antioxidants, and antibacterial and other therapeutic effects [5].

The honey market has been very active recently due to the strong demand for this product because of its nutritional properties and its curative and therapeutic

Article Related Abbreviation: BR, biodegradable resin

effects, with community exports of honey from Algeria estimated at more than 994 tons in 2017/2018 according to Eurostat Comext. The adulteration of honey has become increasingly popular with the increasing demand for this product. For this purpose, many physical and chemical properties are examined to determine the quality of honey. Compared to multi-floral honeys, mono-floral honeys have high commercial value due to their appreciated flavor and aroma, as well as their unique pharmacological characteristics [6].

Among the thousands of compounds present in honey, phenolic compounds [7] and their derivatives are major potential functional ingredients and play an important role in the therapeutic effects of honey [8].

Currently, the trend toward green chemistry is significant, and for this reason, many studies are based on this idea, particularly in the field of compound extraction and isolation; however, most of the time, the methods of this research are too expensive or only reduce the amount of pollution without devising new ecological and economical extraction methods. To overcome this problem, a new method of polyphenol extraction from honey was developed based on biodegradable synthetic resins.

After optimizing the method using a standard (in Supporting Information Material), three samples of honey from Algeria were selected for polyphenol extraction with this method. To prove the efficiency of our new method, a comparison of several aspects was conducted with the most common and most recent method for the extraction of polyphenols from honey, in which commercial amberlite XAD2 resin is used.

2 | MATERIALS AND METHODS

2.1 | Chemicals and reagents

The chemicals and reagents used in this study are as follows: sodium sulfite (Na_2SO_3), 3,5-dinitrosalicylic acid, potassium sodium tartrate ($C_4H_4O_6KNa$, $4H_2O$), Bradford's reagent, bovine serum albumin (BSA), 1,1-diphenyl-2-picrylhydrazine, and amberlite XAD2 were purchased from Sigma Aldrich (USA). Sodium hydroxide (NaOH), glucose, carboxymethylcellulose, SDS, and gallic acid were purchased from Merck (Germany); sodium chloride (NaCl) was obtained from Carl Roth (Germany); aluminum chloride ($AlCl_3$) and Folin-Ciocalteu reagent were obtained from VWR International (USA); and hydrochloric acid was purchased from Chem-lab (USA).

Analytical-grade organic solvents, namely, methanol, ethanol, and diethyl ether, were from VWR International. HPLC-grade solvents included ultrapure water obtained by a Milli-Q system (Millipore, Bedford, USA) and methanol (VWR International), and acetic acid (Sigma Aldrich, USA).

Concerning LC-MS standards, 32 phenolic compound standards were obtained: 3,5-dimethoxycinnamic, cinnamic, ferulic, trans-2,4-dimethoxycinnamic, gallic, m-anisic, o-anisic, syringic, iso-vanillic, isoferulic, 3,4,5-trimethoxybenzoic, and 3,4,5-trimethoxycinnamic acids; 3-hydroxyflavone, caffeine, quercetin, and imperatorin were obtained from Sigma-Aldrich (USA); tannic, sinapic, caffeic and ascorbic acids, catechin, orcinol, rutin, coumarin, and taxifolin were obtained from Fluka (Germany), and chlorogenic and vanillic acids, baicalein, daidzein, isorhamnetin, myricetin, and kaempferol were obtained from Extrasynthese (France).

2.2 | Honey samples

In this study, three samples of honey were taken from different regions of Algeria (Table 1). Sample H1 was collected in the desert areas of the Béchar region; of the mono-floral type honey of *Ziziphus-spina-christi* plant. Sample H2 was multi-floral honey from the forests of Djurjura (temperate area). Sample H3 was mono-floral honey of eucalyptus, from the Médéa region (arid climate). All samples were collected in May 2018 and stored in airtight containers at 4°C before analysis.

The study of physical and biochemical analyses such as pH, color intensity, moisture, dry matter, conductivity, and the ash, sugar and protein content are illustrated with detail in the Supporting Information Material.

2.3 | Solid–liquid extraction

The extraction of polyphenols was carried out with two types of resins as the solid phase: biodegradable resin (BR) that was synthesized after optimization and the commercial resin amberlite XAD2.

The BR extraction has been optimized on several factors such as pH, quantity of BR, time, temperature, and stirring. This is clarified in detail in the Supporting Information Material.

2.3.1 | BR synthesis

Following the optimal conditions of synthesis in the works [9, 10], the protocol can be summarized in three steps:

TABLE 1 Information and characteristics of honey samples

Honey	Color	Region	Food (types)	Breed of bees
H1	Hazelnut color	Béchar	Mono-floral (*Ziziphus-spina-christi*)	Apis mellifera sahariensis
H2	Brown	Djurdjura	Multi-floral	Apis mellifera intermissa
H3	Dense brown	Médéa	Mono-floral (*Eucalyptus*)	Apis mellifera intermissa

Step one: Preparation of the stock solution

First, a 3% aqueous solution of NaCl was heated to 50°C, then 1.8% w/v carboxymethylcellulose and SDS surfactant were added; the mixture was left to stir until the anionic surfactant was completely dissolved, and the mixture was decanted for a few minutes to remove any foam that appeared.

Second step: Reticulation

The mixed stock solution was added dropwise into a 5% w/v aqueous solution of $AlCl_3$ (Al^{3+} was the reticulating agent). The beads formed in the $AlCl_3$ solution were left in the $AlCl_3$ solution for 24 h at a temperature of 4°C.

Third step: Drying

The beads were washed with distilled water and ethanol and then introduced into ethanol for 24 h at 4°C. Then, the beads were recovered and left to dry in the oven for 24 h at 50°C.

2.4 | Polyphenol extraction from honey

In this step, two extraction methods were carried out to isolate polyphenols from three honey samples. First, the synthesized resin BR was used under optimal conditions. The second method was based on the commercial amberlite XAD2 resin following the extraction procedure in work [11]. Elution of polyphenols was performed with methanol for both methods, the solvent was evaporated under reduced pressure using a rotary evaporator, and then the total polyphenols and antioxidant activity were determined.

For LC-MS analysis, the residue was dissolved in water, followed by liquid–liquid extraction using diethyl ether three times; thereafter, the organic phase was recovered, and the solvent was evaporated. Then, an amount of the residue was dissolved in a volume of methanol, and the solution was filtered through a 0.45 μm Whatman nylon filter and analyzed by LC-MS.

2.5 | Total polyphenol content

The total polyphenol content assay was based on the color reaction of the phenolic compounds with the Folin–Ciocalteu reagent (phosphotungstic acid and phosphomolybdic acid), which was used to quantify the total phenols in the sample, where 500 μL of a 20% honey solution or an extract at a concentration of 2 mg/mL was added to 2.5 mL of Folin–Ciocalteu reagent. After 5 min in the dark, a volume of 2 mL of sodium carbonate (7.5%) was added. After 2 h in the dark, the absorbance was measured at 760 nm [12].

The concentrations of phenolic compounds of honey solutions and extracts were evaluated with reference to a calibration curve obtained under the same experimental conditions using gallic acid at different concentrations between 6.5 and 25 μg/mL.

2.6 | Resin regeneration

The reusability of our resins was tested and compared to that of amberlite XAD2. The experimental extractions of polyphenols in honey were repeated three times using the same beads of either our resin or amberlite XAD2. Adsorption and desorption were carried out under optimal conditions for each resin, and the regenerated resins were collected, washed several times with methanol, dried, and reused for subsequent cycles.

2.7 | Standard solutions

The standard solutions of polyphenols were prepared by dissolving 1 mg of each reference in 1 mL of methanol. Initially, each reference was analyzed individually by LC-MS, and then mixtures of the standards were prepared and injected into the LC-MS. For the quantitative study, different concentrations of the standards were prepared to generate calibration curves for each standard.

2.8 | LC-MS conditions

A Waters Alliance LC-MS instrument was used for the analysis of phenolic compounds in honey. The equipment consisted of a Waters Alliance 2695 HPLC and mass detector (ACQUITY QDa Detector, Waters); the unit was controlled by Masslynx v4.2 software. A C18 XBridge column with a diameter of 4.6 mm, a length of 150 mm, and a particle size of 3.5 μm was used for separation. The mobile phase consisted of 1% acetic acid in ultrapure water (A) and methanol (B). The elution gradient program was as follows: 0 min, 15% B; 10 min, 20% B; 20 min, 40% B; 30 min, 60% B; 45 min, 80% B; and 50 min, 80% B. The flow rate was 0.5 mL/min, the volume of injection was 10 μL, the auto-sampler temperature was 25°C, and the column temperature was 30°C.

The MS (QDa, Waters) was operated in positive scan mode. The capillary voltage was set at 0.8 kV, the cone voltage was 10 V, the source temperature was 600°C, and the desolvation gas flow was 800 L/h. The acquisition mass scan is 100–1000 *m/z* with a sampling rate of 5 scans per second in scan mode. Analyses were carried out using the external standard calibration method.

2.9 | Antioxidant activity

The antioxidant potential of honey samples was determined using the most widely used free radical scavenging assay: the 2,2-diphenyl-picryl-hydrazyl (DPPH) assay. All experiments were performed in triplicate for the different concentrations of each honey extract.

The antioxidant activity assay was performed for each honey extract (using BR and XAD2). The A_0 absorbance of 1.5 mL DPPH solution (60 μmol/L) was measured at 517 nm, after which 750 μL of methanolic extract of different concentrations was added. The resulting mixture was incubated in the dark for 30 min. The final absorbance A was measured at 517 nm, and the IC50 of each extract was determined [13, 14].

A calibration curve was prepared using ascorbic acid as the standard at concentrations ranging from 0.1 to 5 μg/mL to determine IC50 values.

2.10 | Statistical analysis

Experimental results were the mean ± SD of three values by using Microsoft Excel statistical analysis program ($n = 3$).

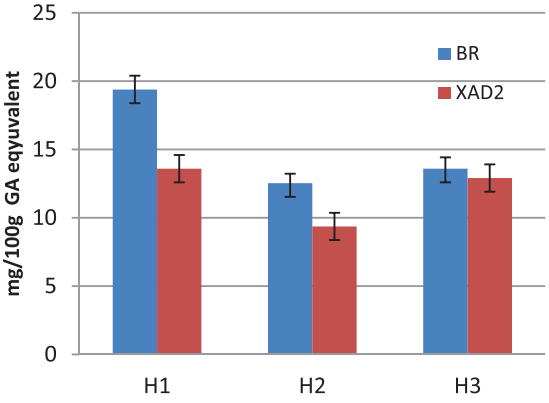

FIGURE 1 Polyphenol content in honey using biodegradable resin (BR) and XAD2 resins

3 | RESULTS AND DISCUSSION

3.1 | Polyphenol content

According to Figure 1, corresponding to the percentage recovery of polyphenols, a significant difference between the extraction carried out by the BRs and those achieved by the XAD2 resin in the three samples of honey was noted.

The first sample (H1) exhibited the highest polyphenol content compared to that of the other two samples, yielding 19.38 mg/100 g gallic acid equivalent extracted by BRs and 13.59 mg/100 g extracted by the XAD2 resin. Sample H3 presented 13.59 mg/100 g for BRs and 12.91 mg/100 g for XAD2, and the lowest contents were found in H2 (BR: 12.53 mg/100 g; XAD2: 9.37 mg/100 g). These differences in polyphenol recovery between the two resins were due to the size of the pores, the specific surface, and the polarity of the resin sites, which influenced the adsorption process of polyphenols [15, 16].

3.2 | Resin regeneration

In this work, the reuse (regeneration) of BR and XAD2 resin was tested. According to Figure 2, we noted a decrease of approximately 50% of the polyphenols extracted during the second use compared to the first in both resins and for all three honey samples. Then a significant increase in the third cycle compared to the second was noted as well as a significant decrease of up to 80% in the fourth cycle compared to the first and third cycles was also observed.

For instance, H1 exhibited a polyphenol yield of 19.38 mg/100 g gallic acid equivalent after the first use of BR, then followed by a decrease to 11.36 mg/100 g in the second cycle, increase to 13.59 mg/100 g in the third cycle and

TABLE 2 Identification and quantification of polyphenols honey extracts using biodegradable resin (BR) and XAD2 resins

Standards	Concentration (μg/g of dry matter)					
	XAD2			BR		
	H1	H2	H3	H1	H2	H3
2,5-Dimothoxycinnamic acid	3.25 ± 0.71	0.21 ± 0.05	0.44 ± 0.08	0.38 ± 0.05	0.52 ± 0.07	–
Syringic acid	–	–	–	1.19 ± 0.32	–	–
3,4,5-Trimethoxybenzoic acid	21.61 ± 2.44	24.05 ± 2.89	82.18 ± 4.24	8.84 ± 1.67	8.23 ± 1.81	52.80 ± 3.48
Tannic acid	–	–	2.28 ± 0.33	–	–	–
Caffeine	–	3.08 ± 0.65	2.25 ± 0.48	0.04 ± 0.003	0.68 ± 0.09	–
Catechin	0.81 ± 0.19	–	5.69 ± 0.81	–	–	–
3-Hydroxyflavone	1.19 ± 0.23	–	2.93 ± 0.46	0.77 ± 0.13	–	5.80 ± 0.98
Coumarin	–	4.20 ± 0.93	–	–	–	–
Baicalein	–	0.11 ± 0.02	1.12 ± 0.19	–	1.29 ± 0.27	–
Imperatorin	1.70 ± 0.34	–	–	–	–	–
Vanillic acid	5.40 ± 1.06	–	–	–	–	–
Trans-2,4-Dimethoxycinnamic acid	3.59 ± 0.63	5.10 ± 0.87	15.45 ± 2.54	–	–	10.70 ± 1.73
Quercetin	4.84 ± 0.78	–	1.36 ±	–	–	–
m-Anisic acid	3.87 ± 0.59	–	6.81 ± 0.96	–	–	–
Isoferulic acid	–	–	–	1.36 ± 0.51	–	–

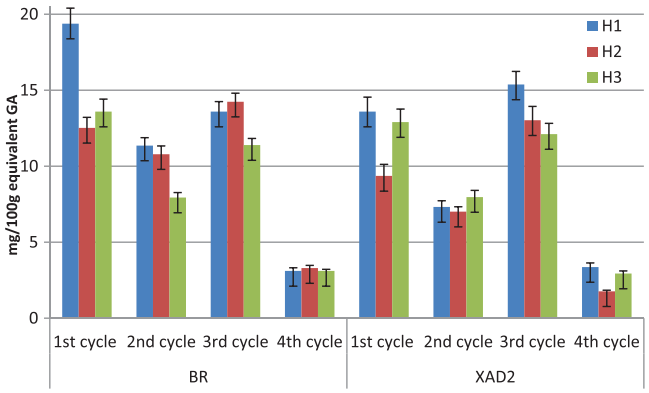

FIGURE 2 Polyphenol content of the different regeneration cycles of the biodegradable resin (BR) and XAD2

finally a decrease to 3.11 mg/100 g in the fourth cycle. When using XAD2, H1 yielded 13.59 mg/100 g polyphenols after the first use, 7.32 mg/100 g in the second, 15.38 mg/100 g in the third, and 3.37 mg/100 g in the fourth cycle.

From the above, it can be deduced that the BR is reusable several times, similar to the commercial XAD2 resin. Both resins can be used three times for the extraction of polyphenols from honey.

3.3 | LC-MS analyses of the polyphenols from the spent resins of different honey samples

To extract a maximum amount of information on the phenolic composition of our honey samples, two resins, XAD2 and BR, were tested, and the isolated substances were analyzed and identified by LC-MS.

For the identification of the compounds, the retention time and mass spectra of chromatographic peaks were compared to those of the standard used. As illustrated in Table 2, among the 34 standard compounds analyzed, 15 compounds were identified in the samples. Phenolic acids that were identified as vanillic acid (21.91 min) and syringic acid (21.94 min) were identified in 32 samples of Algerian honey [17] and in linden and heather honey [18]. The 3,4,5-trimethoxybenzoic acid (eudesmic acid) (28.47 min) and anisic acid (31.31 min) are considered to be characteristic compounds of manuka (*Leptospermum scoparium*) honey from New Zealand [19]; isoferulic acid (28.6 min) has been detected in propolis [20]; 3,5-dimethoxycinnamic acid (36.22 min) and its isomer 3,4-dimethoxycinnamic acid have been identified in acacia honey [21] and a study [22] confirmed the presence of hydroxy and methoxy derivatives of benzoic and cinnamic acids in honey. Regarding flavonoids, catechin (18.1 min), baicalein (41.4 min), quercetin (41.71 min), and 3-hydroxyflavone (47.4 min) were detected.

The extraction of polyphenols with XAD2 allowed us to identify nine compounds in H1, six compounds in H2, and 10 compounds in H3 from the list of external standards injected. 3,4,5-Trimethoxybenzoic acid (eudesmic acid) was the most abundant phenolic compound among the compounds identified in the three honey samples, particularly in H3 at 82,18 μg/g of dry matter, followed by H1 (21,61 μg/g) and H2 (24,05 μg/g). Smaller

amounts of 3,5-dimethoxycinnamic acid were present in H2 (0.21 μg/g) and H3 (0.44 μg/g) compared to H1 (3.25 μg/g).

However, trans-2,4-dimethoxycinnamic acid was more abundant in H3 (15.45 μg/g) than in H2 (5.10 μg/g) and H1 (3,59 μg/g). Other important compounds were exclusively detected in H1 and H3, such as quercetin (H1: 4.84 μg/g; H3: 1.36 μg/g), 3-hydroxyflavone (H1: 1.19 μg/g; H3: 2.93 μg/g), catechin (H1: 0.81 μg/g; H3: 5.69 μg/g), and m-anisic acid (H1: 0.81 μg/g; H3: 5.69 μg/g).

Caffeine (H2: 3.08 μg/g; H3: 2.25 μg/g) and baicalein (H2: 0.11 μg/g; H3: 1.12 μg/g) were also isolated in nonnegligible amounts from our honey samples, except H1. While imperatorin (3.59 μg/g) and vanillic acid (5.40 μg/g) were exclusively detected in H1, coumarin (4.20 μg/g) and tannic acid (2.28 μg/g) were detected only in H2 and H3, respectively.

Overall, according to the results of the extraction of polyphenols by XAD2, each honey sample was characterized by the presence of a specific compound (H1: vanillic acid, H2: coumarin, and H3: a high amount of trans-2,4-dimethoxycinnamic acid).

Clear differences were thus seen between the two resins used, with the XAD2 extracts consistently having a higher number of compounds identified than that in the BR extracts.

The BRs used permitted the identification of six compounds in H1, four in H2, and three in H3. On the other hand, 3,4,5-trimethoxybenzoic acid was the most common and most abundant compound in the three honey samples (H1: 8.23 μg/g H2: 8.84 μg/g, and H3: 52.80 μg/g). Caffeine (H1: 0.04 μg/g; H2: 0.68 μg/g) and 2,5-dimethoxycinnamic acid were exclusively detected in H1 (0.38 μg/g) and H2 (0.52 μg/g), whereas 3-hydroxyflavone was detected in H1 (0.77 μg/g) and H3 (5.80 μg/g).

Other compounds as the syringic acid (1.19 μg/g) and isoferulic acid (1.36 μg/g) were also detected in H1, baicalein in H2 (1.29 μg/g), and trans-2,4-dimethoxycinnamic acid in H3 (10.70 μg/g). In addition, it was noted that some characteristic compounds were detected only in samples treated with BR, such as syringic and isoferulic acids (H1), baicalein (H2), 3,4,5-trimethoxybenzoic acid (in high content), and trans-2,4-dimethoxycinnamic acid (H3).

3,4,5-Trimethoxybenzoic acid was most abundant in all three honey samples regardless of the extraction method used (BR or XAD2), from which it can be said that the three Algerian honey samples used in this study were similar to manuka (*Leptospermum scoparium*) honey from New Zealand [19]. Indeed, in addition to the effect of resin used, there was a significant effect of origin on the phytochemical composition of the honey samples.

According to the two extraction techniques used, Table 2 illustrates that compounds such as imperatorin, vanillic acid, quercetin, trans-2,4-dimethoxycinnamic acid, catechin, and m-anisic acid were not isolated in H1 by BR, unlike XAD2. However, we noted the presence of caffeine, syringic acid, and isoferulic acid exclusively in the BR-extracted sample. 3,4,5-Trimethoxy benzoic acid (XAD2: 21.61 μg/g; BR: 8.84 μg/g), 2,5-dimethoxycinnamic acid (XAD2: 3.25 μg/g; BR: 0.38 μg/g), and 3-hydroxyflavone (XAD2: 1.19 μg/g; BR: 0.77 μg/g) were isolated by both resins with lower recoveries for BR compared to XAD2.

For the H2 sample, of the six compounds extracted by XAD2, four compounds were detected when the extraction was performed with BR, including 3,4,5-trimethoxybenzoic acid (XAD2: 24.05 μg/g; BR: 8.23 μg/g), caffeine (XAD2: 3.08 μg/g; BR: 0.68 μg/g) with a low yield in BR, 2,5-dimethoxycinnamic acid (XAD2: 0.21 μg/g; BR: 0.58 μg/g), and baicalein with a high yield in BR (XAD2: 0.11 μg/g; BR: 1.29 μg/g). Moreover, coumarin and trans-2,4-dimethoxycinnamic acid were not identified when BR was used.

Concerning the H3 sample, among the 10 compounds isolated with XAD2 extraction, only 3,4,5-trimethoxybenzoic acid (XAD2: 82.18 μg/g; BR: 52.80 μg/g), 3-hydroxyflavone (XAD2: 2.93 μg/g; BR: 5.80 μg/g), and trans-2,4-dimethoxycinnamic acid (XAD2: 15.45 μg/g; BR: 10.70 μg/g) were present when using BR.

On the whole, we noted that 13 compounds were extracted and identified with XAD2, and only seven compounds were extracted and identified with BR. Six constituents were common between the two methods, seven compounds were exclusively isolated by XAD2 and two compounds were isolated by BR. When including the polyphenol content data (Supporting Information Table S2), it can be seen that the polyphenol and flavonoid contents in BR-based extracts were higher than those in XAD2-based extracts, underlining that the standards selected for this study were chosen based on the literature of polyphenols extracted by XAD2. All of these data confirm the potential presence of new phenolic compounds in honey treated with BR.

3.4 | Antioxidant activity

Several works have proven the relationship between polyphenols and antioxidant activity [23]. Indeed, the best antioxidant activity (Figure 3) depended on the polyphenol content of each extract (Figure 1). As illustrated in Figure 3, the best results were obtained for H1 honey (BR: IC50 = 19.24 μg/mL; XAD2: IC50 = 32.18 μg/mL) followed by H3 (BR: IC50 = 34.91 μg/mL and XAD2: IC50 = 72.47 μg/mL), whereas the lowest activity was noted for H2 (BR: IC50 = 57.72 μg/mL, XAD2: IC50 = 103.86 μg/mL).

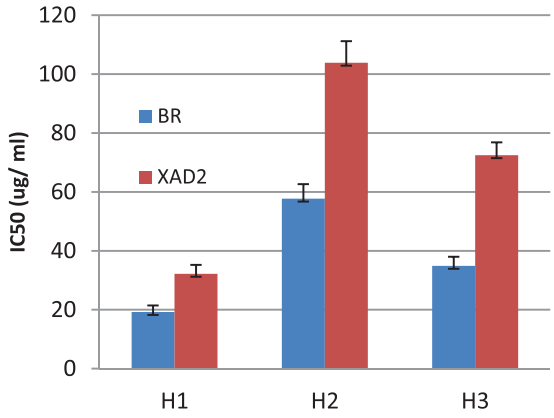

FIGURE 3 Antioxidant activity of honey extracts using BR and XAD2 resins

From these results, it was clear that honey polyphenolic extracts separated by BR had higher activity than that of XAD2 extracts because of the qualitative and quantitative profile of phenolic compounds extracted by BR compared to XAD2. In the first sample (H1), the high antioxidant activity could be explained by the appearance of syringic acid (1.19 μg/mL) and isoferulic acid (1.38 μg/mL) in BR, which have been shown to have strong antioxidant power [24, 25]. For H2, the difference in antioxidant power could be due to the high level of baicalein in the BR extract (1.29 μg/mL) compared to that in the XAD2 extract (0.11 μg/mL), which presented strong antioxidant activity [26]. For sample H3, the difference in antioxidant activity may be due to the composition of the BR extract. Consequently, it is possible that some compounds not identified in the BR extract presented a strong antioxidant activity; a hypothesis that could also be applied to H1 and H2.

In addition, we noted that our honey extracts had slightly lower antioxidant activities compared to that of ascorbic acid (0.60 μg/mL), which was used as a reference.

4 | CONCLUDING REMARKS

The physicochemical and biochemical properties of the honey samples studied in this work allowed us to evaluate the quality of Algerian honey.

The parameters studied (Supporting Information Tables S1 and S2) meet the standards proposed by the International Honey Commission CIM. Indeed, the water content of the H1 sample was lower than 13%, indicating that it was of good quality. The pH of our honey samples was between 3.94 and 6.21, suggesting that samples H2 (Djurdjura) and H3 (Médéa) were of nectar origin, while honey sample H1 from Béchar was of honeydew origin. Ash content and electrical conductivity values confirm that sample H3 was rich in phenolic acids, as confirmed by LC-MS analysis, where we noted that 3,4,5-trimethoxybenzoic acid and trans-2,4-dimethoxycinnamic acid were more abundant in H3 than in H1 and H2.

After optimization of the extraction conditions for the BR, it was found that at pH 3, the BR adsorption efficiency was best, the optimal amount of BR in the medium was 40 g/L, the adsorption equilibrium between BR and gallic acid was reached at 50 min, the best extraction temperature was 60°C, and stirring was not recommended during the extraction process.

High polyphenol yields were observed in sample H1, followed by H3 and H2 (the lowest yields were obtained in these samples for both BR and XAD2). BR was considered the most efficient resin for the extraction of polyphenols from honey compared to the commercial resin amberlite XAD2. In conclusion, the number of reuses was the same for both resins (three times), but the reuse efficiency of BR was better than that of XAD2.

In total, 13 compounds were identified in the three honey samples treated with XAD2, and only eight compounds were identified using BR. Moreover, the best polyphenol contents were noted with BR for extraction. In addition, the choice of standards in our work was based on the phenolic composition of honey from the literature data (using XAD2 resin for extraction), so it is possible that many phenolic compounds were extracted for the first time in honey by BR and were not identified. The antioxidant activity was directly related to the polyphenol content extracted by BR and XAD2. Consequently, BR extracts had stronger activities than those of XAD2 extracts. This was mainly due to the qualitative and quantitative properties of the phenolic compounds isolated by BR compared to XAD2 resin.

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CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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