PRELIMINARY ANALYSIS OF THE CHEMICAL CHARACTERISTICS OF HYDRAU-ACETONIC EXTRACTS FROM ALGERIAN BEE PRODUCTS, THEIR INFLUENCE ON HEXOKINASE ACTIVITY, AND THE ANTIMICROBIAL PROPERTIES OF HONEY

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Abstract. A limited number of studies investigate the chemical composition and effects of Algerian bee products specially propolis, beeswax, bee bread. This study investigated the chemical composition and biological activities of Algerian bee products-propolis, beeswax, bee bread, and honey-focusing on total phenolic and flavonoid contents, antioxidant capacity, anti-diabetic potential, and antimicrobial effects. Extracts obtained using 70% acetone showed varying yields, with honey A and propolis exhibiting the highest (78.7% and 24.26%, respectively). All products demonstrated significant levels of phenolic compounds and flavonoids. Antioxidant activity, measured via DPPH radical-scavenging assay, was strongest in Algerian propolis (IC_{50} = 0.0744 ± 0.0007 mg/mL). LC-ESI-MS/MS analysis identified rutin and 21 phenolic compounds in propolis. While all samples were tested at 126 µg/mL for anti-diabetic activity, hexokinase inhibition was minimal. Disk diffusion assays revealed that Algerian bee honey exhibited notable antimicrobial activity. These findings highlight the rich bioactive profile and therapeutic potential of Algerian bee products.

Key words: Algerian bee products; antioxidant activity; antihexokinase effect; anti-diabetic activity; antimicrobial activity; LC-ESI-MS/MS.

INTRODUCTION

Healthcare professionals manage diabetes mellitus by administering insulin alongside synthetic anti-diabetic drugs such as metformin and glipizide, despite the potential side effects of these medications. To mitigate these undesirable side effects, researchers are actively exploring natural metabolites as potential candidates for new anti-diabetic therapies. Recent studies suggest that abnormal increases in HK2 levels and dysregulated glycolysis play a significant role in impaired glucose metabolism. Elevated levels of G6P and downstream glycolysis intermediates have been identified as key contributors to the tissue-specific, chronic progression of hyperglycemia associated with diabetes [47]. Apitherapy involves using bee products for their natural healing qualities and is a part of alternative and folk medicine. Its origins can be traced back to ancient China or present-day Russia. However, the use of bee products for therapeutic purposes can also be found in ancient Egypt and Greece, where they were used for cosmetics and wound healing preparations. It's important to note that while honey or royal jelly cannot cure conditions like diabetes, they do contain anti-diabetic substances which may help improve glycemic profiles when consumed [53].

Numerous research papers have explored the natural antioxidant substances present in medicinal bee products. These compounds are not only used as food preservatives to replace synthetic antioxidants, but they also play a role in managing hyperglycemia. Therefore, the objective of investigating the extraction of phenolic compounds using 70% acetone is to identify the most effective active molecules with antioxidant and anti-diabetic properties. The increasing ineffectiveness of existing antibiotics, coupled with the emergence of more virulent and resistant microorganisms, has effectively compelled the scientific community to explore therapeutic alternatives from natural sources. As a result, plant- and animal-derived products have regained prominence in the search for natural antimicrobials, with honey and its derivatives standing out as a potential solution [21]. Honey is composed of a variety of chemical and mineral substances, vitamins, carbohydrates, proteins, flavonoids, phenolic acids, and additional components, along with physical and chemical characteristics such as acidity, moisture content, viscosity, and crystallinity, which all contribute to its antioxidant properties and its efficacy in combating bacteria and inflammation [3]. Honey demonstrates significant antimicrobial properties. It effectively inhibits a wide variety of bacterial species. Alcohol extracts from honey show a suppressive effect on numerous bacterial types, including both aerobes and anaerobes, as well as Gram-positive and Gram-negative bacteria. Honey exhibits strong antimicrobial action against both harmful and non-harmful microorganisms, such as yeasts and fungi, including those that have developed resistance to various antibiotics. The antimicrobial effects can be either bacteriostatic or bactericidal, depending on the concentration used [3]. The antibacterial and antifungal activities of honey are influenced by its chemical, physical, and biological characteristics. These factors vary considerably among different honey varieties and play a crucial role in determining the specific properties of each type [36]. This study aimed to evaluate the antimicrobial effects of Algerian bee honey against certain strains of clinically relevant Gram-positive and Gram-negative bacteria as well as fungi, to assess its potential use for microbial sterilization as a natural alternative.

MATERIALS AND METHODS

Sample collection

Table 1. Algerian bee products, sampling area and year of collection.

Algerian bee product	Sampling area	Yaer of collection
Algerian bee honey A.	Djelfa.	2022
Algerian propolis.	El-Medea.	2022
Algerian bee honey B.	Sog-Ahras.	2022
Algerian bee-wax.	Sog-Ahras.	2022
Algerian bee-bread.	Tipaza.	2022
Algerian bee honey S1.	Batna.	2024
Algerian bee honey S2.	El-Bayedhe.	2024
Algerian bee honey S3.	Tizi-Ouzou.	2024
Algerian bee honey S4.	Khanchela.	2024
Algerian bee honey S5.	Barika.	2024
Algerian bee honey S6.	Boussaada.	2024
Algerian bee honey S7.	Djelfa.	2022
Algerian bee honey 8 A mixture of seven honey varieties (S8).	/	/

Extraction

Acetonic extract of samples in room temperature

For each sample, 5 grams of bee product were weighed and combined with 50 milliliters of 70% acetone, stirred continuously for 24 hours. The mixture was then filtered through filter paper. The residue was subjected to three separate extractions. The solvent from the two extracts was evaporated using a rotary evaporator at 40°C, and the extracts were stored for six days at 60°C in a steam room. Thereafter, the extract samples were stored at -20°C.

Determination of the Total Phenol Content

The Folin-Ciocalteu colorimetric method was used to determine the total polyphenol content of the samples [18]. In a 1.5 mL Eppendorf tube, 100 µL of sample extract (1 mg/mL in distilled water) was combined with 500 µL of Folin-Ciocalteu reagent (10%) and 400 µL of Na_2CO_3 (75 mg/mL). After five minutes of incubation at 40°C in the dark, the absorbance was measured at 735 nm. The total polyphenol content was expressed as mg/g of gallic acid equivalents (Fig. 1) using the calibration curve (Y = 0.0122x – 0.0052, R^2 = 0.9995). All measurements were performed in triplicate.

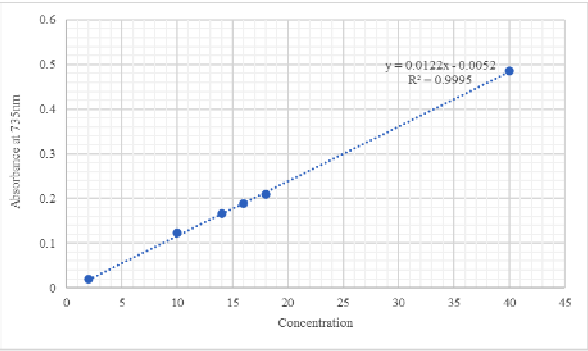

Figure 1. Total polyphenols calibration curve

Total flavonoids content

The method of Zhishen *et al.* (1999), with modifications, was used to determine the total flavonoid content of the samples [60]. In a 1.5 mL Eppendorf tube, 200 µL of sample extract was mixed with 60 µL of 5% $NaNO_2$. After 5 minutes, 60 µL of 10% $AlCl_3$ was added, and the mixture was vigorously mixed. After 6 minutes, 400 µL of 1M NaOH was added. The absorbance was immediately measured at 510 nm against a control. A quercetin solution was prepared, and calibration curves were plotted using standard solutions at concentrations ranging from 0 to 1000 µg/mL (Fig. 2) with the equation (Y = 0.0018x + 0.0357, R^2 = 0.9991). All measurements were performed in triplicate.

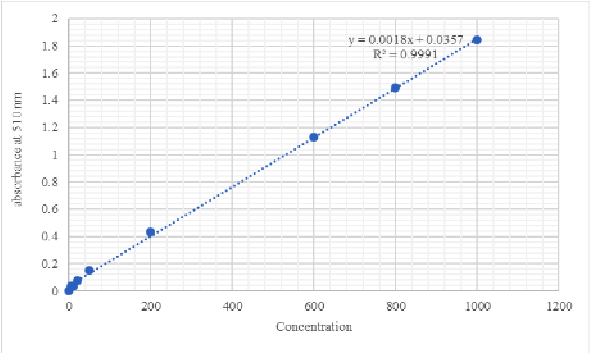

Figure 2. Total flavonoids calibration curve

LC-ESI-MS/MS Analysis

An Agilent Technologies 1260 Infinity II system, coupled with a 6460 Triple Quad Mass Spectrometer, was used for the quantitative analysis of phenolics. A Poroshell 120 SB-C18 column (3.0 × 100 mm, 2.7 µm) was utilized [24]. Samples of 50 mg were dissolved in 1 mL of methanol in a 2 mL Eppendorf tube. One milliliter of hexane was used to extract the end product, followed by centrifugation for 10 minutes at 9,000 rpm. Subsequently, 100 µL of the methanol phase was diluted with 450 µL of water and 450 µL of methanol (900 µL total). After filtering through a 0.22 µm filter, the mixture was injected into the instrument. The injection volume was 5.12 µL, with a flow rate of 0.40 mL/min. The mobile phase consisted of ammonium formate (5.0 mM) and 0.1% formic acid in methanol (B), and 0.1% ammonium formate in water (A). The

gradient program for the B mobile phase was adjusted as follows: 25% for 1-3 minutes, 50% for 4-12 minutes, 90% for 13-21 minutes, and 3% for 22-25 minutes. The column temperature was maintained at 40°C. The nebulizing gas (N_2) flow rate was 11 L/min, with a pressure of 15 psi, a capillary voltage of 4000 V, and a gas temperature of 300°C.

DPPH Test for Scavenging Free Radicals

The DPPH method was used to assess the antioxidant activity of the extracts [43]. To each methanolic extract solution, 50 µL was added to 1.95 mL of DPPH methanolic solution (0.025 g/L) at varying concentrations (from 0.0125 to 5 mg/mL). Similarly, 50 µL of methanol was mixed with 1.95 mL of the DPPH methanolic solution as a negative control. After 30 minutes of incubation at room temperature in the dark, the absorbance was measured at 515 nm against a blank prepared for each concentration. Ascorbic acid was used as the positive control, and its absorbance was measured three times for each concentration under the same conditions as the samples. The inhibition of the DPPH radical was calculated using the following equation:

I%= [(Abs contrôle – Abs test)/ Abs contrôle] x 100

Hexokinase activity

Hexokinase (HK) activity was assayed spectrophotometrically using a coupled reaction with glucose-6-phosphate dehydrogenase (G6PD), monitoring the reduction of NADP at 340 nm [33]. The reaction mixture contained: R1 [MES buffer: 5.0 mmol/L, pH 6.0; Mg^{2+}: 24 mmol/L; ATP: ≥ 4.5 mmol/L; NADP: ≥ 7.0 mmol/L; preservative] and R2 [HEPES buffer: 200 mmol/L, pH 8.0; Mg^{2+}: 4 mmol/L; HK (yeast): ≥ 300 µkat/L; G6PDH (E. coli): ≥ 300 µkat/L; preservative]. The assay was conducted using yeast HK isoforms (GLUC3, Roche/Hitachi Cobas) as well as untreated medium. The enzyme was incubated with the test compounds (126 µg/mL) for 10 minutes prior to the assay.

Antimicrobial properties of Algerian bee Honey

We utilized a modified agar disk diffusion technique for our study [31]. We evaluated the antimicrobial effect of bee honey on various microbial and fungal strains using Mueller-Hinton agar plates that were pre-inoculated with a standardized microbial suspension at 10^8 CFU/mL. Whatman paper disks that were soaked with 10 µL of bee honey were placed on the surface of the agar, with each disk being 6 mm in diameter. After incubating at 37 °C for 24 hours, we analyzed the results by measuring the inhibition zones' diameters in millimeters (mm). All experiments were performed in triplicate.

To assess the antimicrobial activity of honey samples, five clinically identified bacterial strains were selected, and one clinically identified fungus.

Preparation of honey samples

In a tube, we combined 0.2 mL of each honey sample with 1 mL of dimethyl sulfoxide (C_2H_6OS) (DMSO) solvent [44]. The tubes that were prepared were then vortexed thoroughly.

Minimum inhibitory concentration (MIC)

The MIC is defined as the lowest concentration that can inhibit the growth of bacteria [56]. This assay is conducted by performing serial dilutions of honey samples at various concentrations on 96-well microplates.

Initially, 100 µL of MH broth was added to every well of the microplates. Introduce 100 microliters of the prepared honey extract into the first well, mix thoroughly, then transfer 100 microliters from the first well to the second well. Continue this process for the remaining wells to create a series of dilutions ranging from a concentration of 10 mg/mL to 0.020 mg/mL. Next, 50 microliters of the bacterial suspension are added to each well of the microplate. The well designated as the negative control contained solely 100 µL of MH broth, while the positive control well contained 100 µL of MH broth with Honey-free Bacterial Suspension. The microplate was then incubated at a temperature of 37°C for 18 hours. Following incubation, each tube is inspected visually for cloudiness [26].

Statistical analysis

EXCEL 2016 was used to conduct the tests in triplicate and to express the results as mean ± standard error (SE) with $p < 0.05$ serving as the significance level. Using SPSS 17.0, a one-way analysis of variance (ANOVA) was used to examine the impact of the enzyme inhibitor.

RESULTS

Extraction yields

The extraction method for evaluating the phytochemical activities of the samples involves the extraction of polyphenols and flavonoids. Extraction with 70% acetone yielded a higher amount of Algerian bee product extract, as shown in Fig. 3.

Estimation of polyphenol and flavonoid concentration

For Algerian bee products, the total flavonoid (TF) and total phenolic content (TPC) were assessed. The highest TPC and TF concentrations were found in Algerian bee propolis, as shown in Fig. 4, with values of 62.65 (1.43) mg EGA/g and 205.16 (18.26) mg EQ/g, respectively. Other products showed lower concentrations: bee bread had 16.49 (0.45) mg EGA/g and 17.57 (4.19) mg EQ/g; bee wax had 6.16 (0.40) mg EGA/g; and bee honey A and 2 had 2.85 (0.31) mg EGA/g and 2.96 (0.54) mg EGA/g, respectively. Bee wax, honey A, and honey B had a TF concentration of 0 mg EQ/g.

LC-ESI-MS/MS

The LC-ESI-MS/MS method was utilized to identify the primary organic components present in Algerian bee products. A total of 45 phenolic compounds were used as standards (Table 2) (Fig. 5).

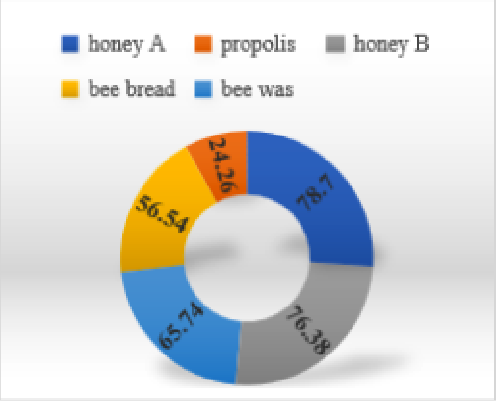

Figure 3. Yields of algerian bee product extracts by aceton 70%

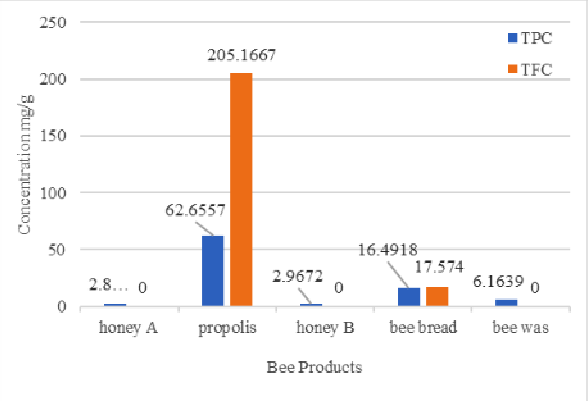

Figure 4. Concentrations of TPC and TFC estimated in acetonic algerian bee product samples.

Table 2. Esmation of chemical composition of Algerian bee products by LC-ESI-MS/MS

Compounds	Analyte	Rt	Phenolics (µg analyte/ g Bee honey A)	Phenolics (µg analyte/ g propolis)	Phenolics (µg analyte/ g Bee honey B)	Phenolics (µg analyte/ g Bee Bread)	Phenolics (µg analyte/ g Bee Wax)
1	Shikimic acid	1.46	ND	ND	ND	ND	ND
2	Gallic acid	3.23	ND	60.79	ND	ND	ND
3	Protocatechuic acid	5.42	ND	50.60	ND	ND	ND
4	Epigallocatechin	6.82	ND	ND	ND	ND	ND
5	Catechin	6.86	ND	ND	ND	ND	ND
6	Chlorogenic acid	7.38	ND	23.01	ND	ND	ND
7	Hydroxyben zaldeyde	7.63	ND	1.93	ND	ND	ND
8	Vanillic acid	7.73	ND	ND	ND	ND	ND
9	Caffeic Acid	7.81	ND	300.08	ND	ND	ND
10	Syringic acid	8.35	ND	ND	ND	ND	ND
11	Caffein	8.34	1.92	3.91	1.11	1.65	1.60
12	Vanillin	8.59	ND	11.75	ND	5.54	4.99
13	o-coumaric acid	9.39	ND	68.45	ND	ND	ND
14	Salicylic Acid	9.73	ND	68.87	ND	ND	ND
15	Taxifolin	9.68	ND	6.00	ND	ND	ND
16	Resveratrol	9.74	ND	ND	ND	ND	ND
17	Polydatine	9.87	ND	ND	ND	ND	ND
18	Trans-ferulic acid	10.05	ND	165.80	ND	ND	ND
19	Sinapic acid	10.33	ND	ND	ND	ND	ND
20	Scutellarin	11.16	ND	2.26	ND	ND	ND
21	p-coumaric acid	11.57	ND	ND	ND	ND	ND
22	Coumarin	11.56	ND	ND	ND	ND	ND
23	Protocatehuic ethyl ester	11.68	ND	ND	ND	ND	ND
24	Hesperidin	11.72	ND	ND	ND	ND	ND
25	Isoquercitrin	11.74	0.69	14.07	ND	ND	ND
26	Rutin	12.21	ND	4489.06	ND	ND	ND
27	Quarcetin-3-Ksilozid	12.41	ND	ND	ND	ND	ND
28	Kaempferol-3-glucoside	13.07	2.21	2.06	ND	ND	ND
29	Fisetin	13.34	ND	ND	ND	ND	ND
30	Baicalin	13.78	ND	ND	ND	ND	ND
31	Chrysin	14.04	ND	ND	ND	ND	ND
32	Daidzein	14.18	ND	ND	ND	ND	ND
33	Trans-cinnamic acid	14.27	ND	23.27	ND	ND	ND
34	Quercetin	14.76	31.18	93.97	ND	ND	ND
35	Naringenin	14.86	9.13	88.82	ND	ND	ND
36	Silibinin	15.60	ND	ND	ND	ND	ND
37	Hesperetin	15.83	ND	12.51	ND	ND	ND
38	Morin	15.70	ND	ND	ND	ND	ND
39	Kaempferol	16.36	268.68	402.56	ND	ND	ND
40	Baicalein	17.07	ND	ND	ND	ND	ND
41	Luteolin	17.78	ND	24.70	ND	ND	ND
42	Biochanin A	17.90	ND	ND	ND	ND	ND
43	Capcaicin	18.17	ND	ND	ND	ND	ND
44	Dihydrocapcaicin	18.58	ND	ND	ND	ND	ND
45	Diosgenin	23.53	ND	ND	ND	ND	ND

Rt: Retention time. ND: not detected

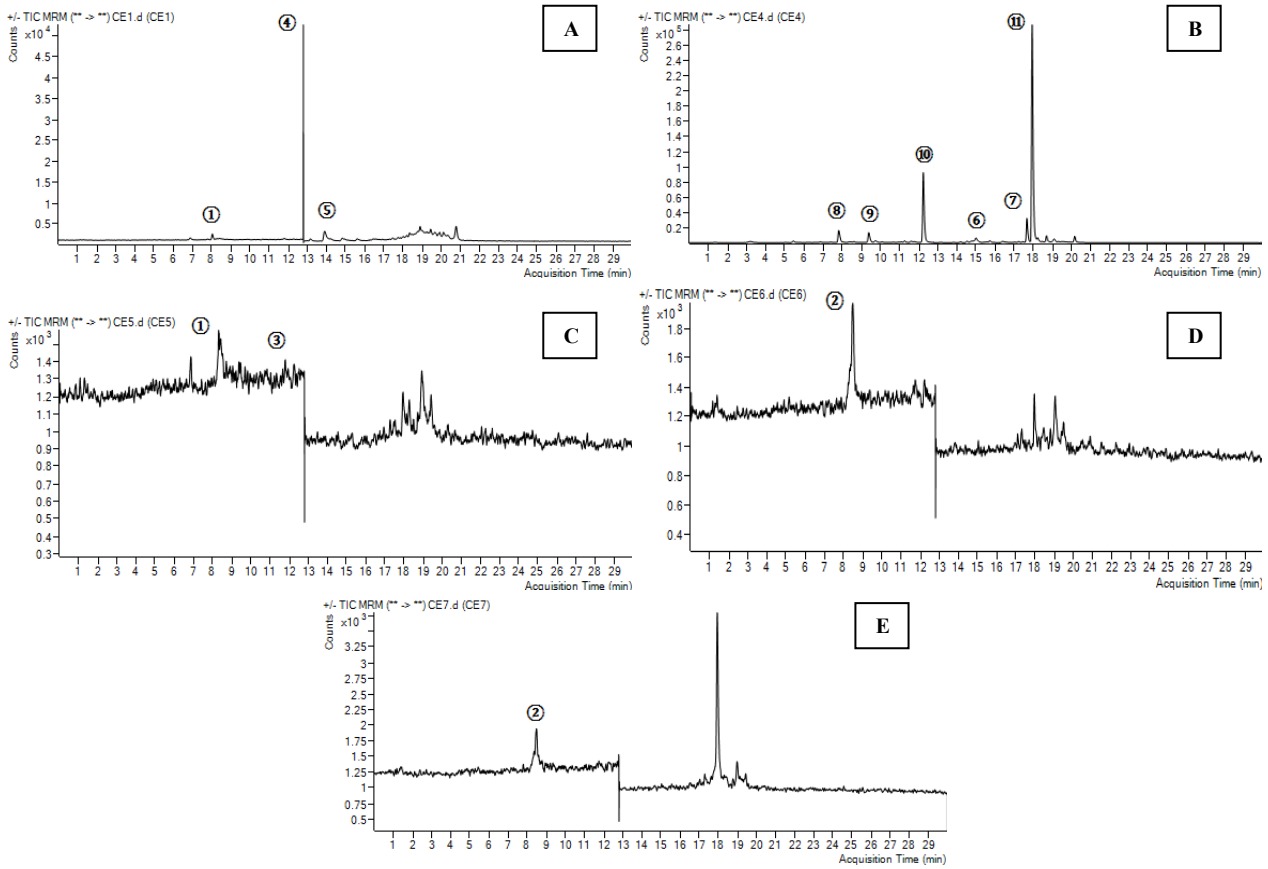

Figure 5. LC-ESI-MS/MS chromatograms of Algerian bee products: A: Honey A; B: Propolis; C: Honey B; D: Bee Bread; E: Bee Wax; ① Caffein; ② Vanillin; ③ Isoquercitrin; ④ Kaempferol-3-glucoside; ⑤ Quercetin; ⑥ Naringenin; ⑦ Kaempferol; ⑧ Caffeic acid; ⑨ Salicylic acid; ⑩ Rutin; ⑪ Luteolin.

Antioxidant activity

The DPPH scavenging activity method was used to determine the half-maximal inhibition concentration (IC50) values for Algerian bee products, BHA, BHT, and Trolox. The results are presented in Table 3.

Table 3. The DPPH radical-scavenging activity of Algerian bee products.

Antioxidant Activity	DPPH Scavenging IC50 µg/mL	R^2
BHA	4.39	0,04
BHT	10.86	0,06
Trolox	4.95	0,03
Algerian Bee Propolis	74.46	0.78
Algerian Bee Bread	198.66	2.63
Algerian Bee Wax	/	/
Algerian Bee Honey A	/	/
Algerian Bee Honey B	/	/

Hexokinase (HK) Inhibitory Assay

The Algerian bee products (honey A, propolis, honey B, bee bread, and bee wax) were tested for their in vitro anti-diabetic properties. At a concentration of 126 µg/mL, the HK inhibition values were 2.9%, 4.7%, 2.2%, 2.2%, and 1.5%, respectively. The anti-diabetic properties of these bee products were assessed using the HK inhibitory assay, indicating a minimal anti-diabetic effect from the Algerian bee products (Fig. 6).

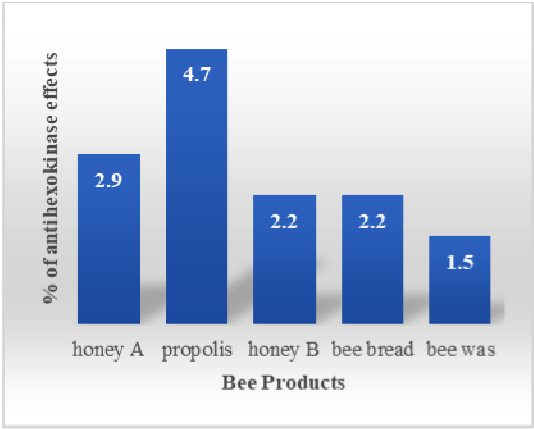

Figure 6. Percentgae of antihexokinase effects of acetonic algerian bee product extracts.

Antimicrobial properties of Algerian bee Honey

Antimicrobial activity results of selected honey samples presented in the table by measuring the inhibition diameter in millimeters for the strains tested (Table 4) (fig. 7-11).

Table 4. Inhibition zone diameters.

Honey samples	*Escherichia coli* Gram -	*Klebsiella pneumoniae* Gram -	*Proteus vulgaris* Gram -	*Staphylococcus aureus* Gram +	*Streptococcus* sp. Gram +	*Candida albicans* Fungi
S1	20.33mm	9.66mm	24.5mm	16mm	-	-
S2	25mm	12.5mm	12.33mm	-	-	-
S3	33.33mm	-	12.66mm	-	-	-
S4	24.66mm	-	17.66mm	-	-	-
S5	22mm	-	15.5mm	-	-	-
S6	20.33mm	-	18mm	-	-	-
S7	22.66mm	-	17.33mm	-	-	-
S8	26.66mm	-	18mm	-	-	-

- : no inhibition zone

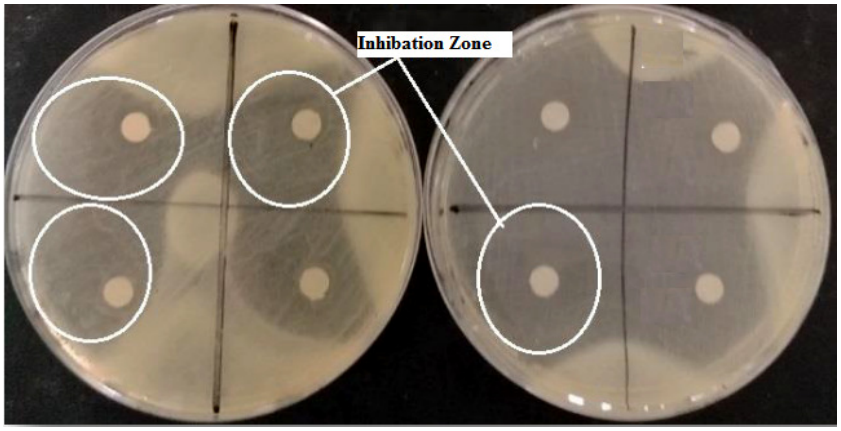

Figure 7. The zone of inhibition in *E.coli* bacteria.

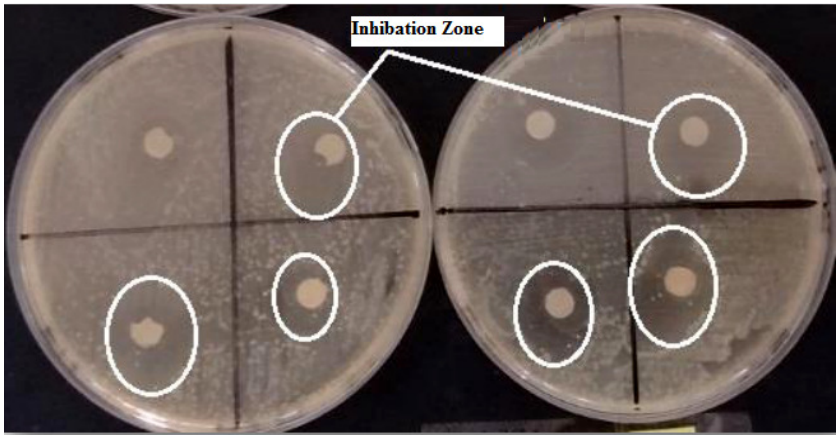

Figure 8. The zone of inhibition in *Proteus vulgaris* bacteria.

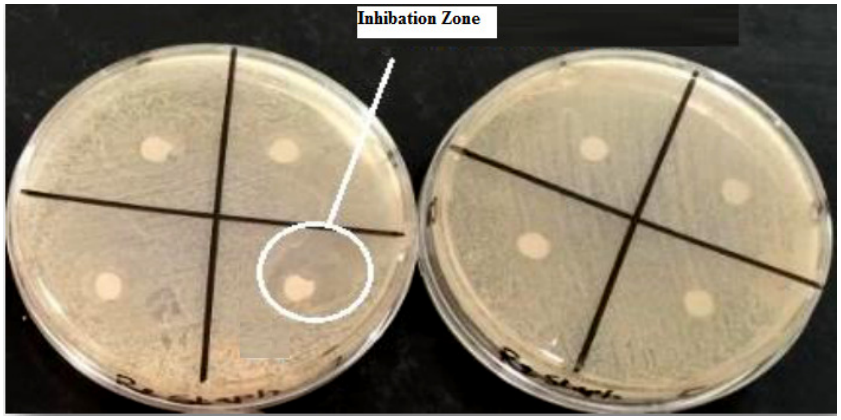

Figure 9. The zone of inhibition in *Staphylococous aureus* bacteria.

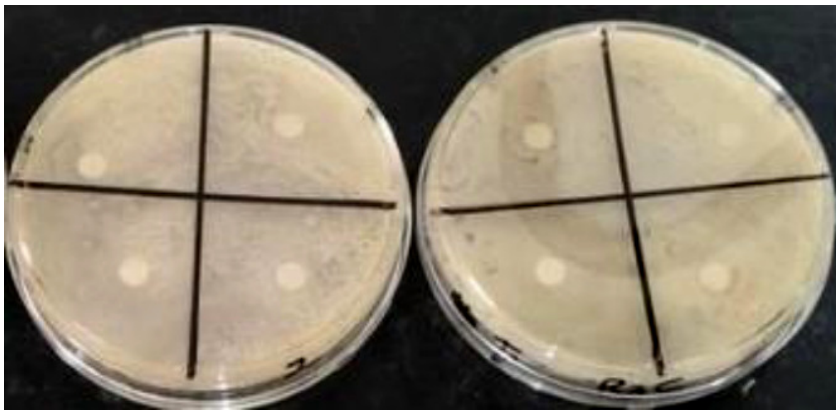

Figure 10. The zone of inhibition in *Streptoccus* sp. bacteria.

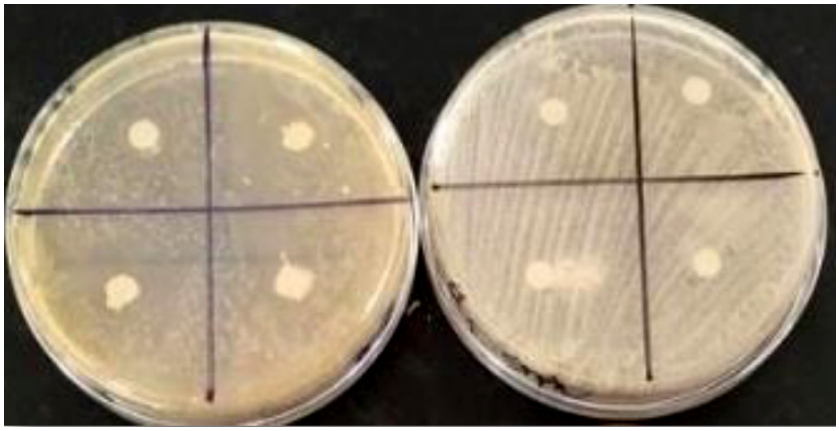

Figure 11. The zone of inhibition in *Candida albicaus* fungi.

Minimum inhibitory concentration (MIC)

The table below displays the minimum inhibitory concentration (MIC) results in mg/mL (Table 5).

Table 5. Result of the minimum inhibitory concentration of honey.

Honey samples	*Escherichia coli* Gram -	*Proteus vulgaris* Gram -	*Klebsiella Pneumoniae* Gram -	*Staphylococcus Aureus* Gram +	*Streptococcus sp.* Gram +	*Candida albicans* Fungi
S1	2.5mg/mL	10mg/mL	5mg/mL	2.5mg/mL	-	-
S2	10mg/mL	10mg/mL	5mg/mL	-	-	-
S3	5mg/mL	10mg/mL	-	-	-	-
S4	10mg/mL	10mg/mL	-	-	-	-
S5	5mg/mL	10mg/mL	-	-	-	-
S6	5mg/mL	10mg/mL	-	-	-	-
S7	5mg/mL	10mg/mL	-	-	-	-
S8	5mg/mL	10mg/mL	-	-	-	-
Cefotaxime Antibiotic	R	R	R	-	S	-

R: Résistance - : no CMI S : sensible

DISCUSSION

Since ancient times, beekeeping products such as honey, bee bread, propolis, and beeswax have been widely used in folk medicine due to their potent medicinal qualities and high concentration of bioactive molecules [38].

In this study, higher extraction yields were observed with 70% acetone compared to methanol and ethanol, except for antioxidant activity, where ethanol performed better [43]. Acetone is known to be more effective at extracting phenolic antioxidants than other organic solvents due to its lower polarity and greater ability to dissolve phenolics [5, 27-29, 31, 40, 46, 48, 54]. The extraction yield is influenced by factors such as the solvent used, temperature, and duration of the extraction process [30, 48, 52].

Bee propolis, bee bread, bee wax, and both varieties of Algerian honey exhibited the highest concentrations of phenolic compounds, with values of 62.65 mg EAG/g, 16.49 mg EAG/g, 6.16 mg EAG/g, 2.96 mg EAG/g, and 2.85 mg EAG/g, respectively. Comparing the Algerian propolis extract to studies by Petar *et al.* (2018) [49] and Nurul Aliah *et al*. (2019) [2], it was

found to have higher concentrations of phenolic compounds and a greater flavonoid content (205.16 mg EQ/g) than reported by Petar *et al.* (2018) [49]. Additionally, the Algerian bee bread extract showed higher levels of phenolics and flavonoids compared to those reported by Carlos *et al.* (2015) [61] and Katarzyna *et al.* (2016) [19]. The Algerian bee wax extract also had higher phenolic content than that reported by Haoan *et al.* (2019) [59]. The Algerian bee honey extracts had higher phenolic levels than those reported by Tania *et al.* (2013) [51], Je-Ruei *et al.* (2013) [35], Ibrahim *et al.* (2018) [8], and Vesna *et al.* (2018) [55]. These variations are likely due to differences in nectar sources and the geographical origin of the samples [6, 11].

There is limited research on the compounds found in Algerian bee products. LC-ESI-MS/MS analyses identified six phenolic compounds in Algerian bee honey sample N°01 (Fig. 5), while only one phenolic compound was identified in sample N°03. In comparison, studies from Cuba identified 16 phenolic compounds using HPLC-DAD-ESI-MS/MS [10], and studies from Brazil identified 20 phenolic compounds by LC-MS and 26 phenolic compounds by HPLC-ESI-MS/MS [15, 16]. Additionally, our analysis determined 21 phenolic compounds in Algerian propolis, while studies from Chile and Portugal reported 29 and 31 phenolic compounds, respectively, using GC-MS and UPLC-DAD-ESI/MS [17, 25]. For Algerian bee bread and bee wax, only two phenolic compounds were identified, whereas Bakour *et al.* (2019) [12] reported 30 phenolic compounds in bee bread. These variations in phenolic compound profiles can be attributed to factors such as the collection time and location of bee pollen (geographic conditions), extraction techniques, extraction solvents, and the methods used to estimate the compounds.

The DPPH test results for scavenging free radicals were expressed as IC50 (mg/mL). The results indicated that the DPPH scavenging capacity of Algerian bee products was lower compared to BHA, BHT, and Trolox (Table 3). However, the bee bread sample exhibited higher antioxidant activity than reported by Bakour *et al.* (2019) [12]. On the other hand, Algerian propolis demonstrated greater antioxidant capacity than other bee product samples and was also superior to the antioxidant capacity reported in Brazil (Abdullah *et al.*, 2019) [2]. This finding may be attributed to the nature, amount, and structure of the synthesized polyphenols, which aligns with previous studies [48]. The mechanism of the reaction between the antioxidant and the DPPH radical is influenced by the spatial structure of the antioxidant [32, 48]. Although ethanol extraction was found to be more effective than acetone extraction in terms of antioxidant activity in some studies [48], our results show that acetone extraction is more potent than ethanol and methanol extraction, consistent with findings from studies in Brazil and Morocco [58]. This may be due to the major compound in our sample, chlorogenic acid, which has known antioxidant effects [4, 23, 42].

For the first time, bee products have been tested for anti-hexokinase activity. The results indicated that our crude acetonic samples did not exhibit significant hexokinase inhibitory activity. This may be due to the presence of kaempferol, a polyphenol known to potentially increase hexokinase activity [7, 23], or the absence of fisetin, as tetrahydroxyflavone (3,7,3',4') can decrease the activity of glucose-6-phosphate dehydrogenase [23, 45]. Additionally, the absence of protocatechuic acid, which has known anti-hexokinase and antihyperglycemic activities [42], could also explain the lack of significant hexokinase inhibition.

The findings indicated that every honey sample evaluated exhibited a substantial inhibitory effect on *Escherichia coli* bacteria. The inhibition diameters varied between 33.33 mm and 20 mm. Sample 03, which was multi-flower honey, had the highest inhibition, followed by sample 08 (a blend of all honey samples) with a diameter of 26.66 mm, while the remaining samples showed similar inhibition diameters. The results obtained surpassed those reported by Ben-Amor (2022) and Alqurashi (2013) for sample 03 and were similar to the findings of our other samples, as well as to Nakib (2020), except for sample M2 in the *Escherichia coli* strain, which yielded the same results since it was sourced from the same region, resulting in almost identical chemical, physical, and biological characteristics. However, results for sample M9 in the *Escherichia coli* strain were superior, and the same trend was observed with samples M4 and M5 in *Staphylococcus aureus*. In the case of the *Klebsiella pneumoniae* strain, our results were more favorable than those found by Nakib (2020). These differences can be attributed to variations in chemical, physical, and biological traits. Such factors vary significantly among different types of honey and play a crucial role in determining the distinctive characteristics of each variety [9, 36, 41, 50]. In his examination of honey samples from Saudi Arabia, the inhibition diameters varied from 17 to 25 mm. Regarding the Gram-negative bacteria *Proteus vulgaris*, Sidr honey sample 01 exhibited the strongest inhibitory effect with a diameter of 24 mm, followed by sample 08 with a diameter of 18 mm. In comparison, samples 02 and 03, with diameters of 12.33 and 12.66 mm respectively, displayed average inhibitory activity relative to the other samples.

These findings surpassed those reported by Mahendran & Kumarasamy (2015) in their analysis of various natural honey samples in India, where the zone of inhibition ranged from 8 to 9.2 mm, indicating a relatively weak effect when compared to our study [37].

The strains of *Escherichia coli* and *Proteus vulgaris* appeared to be more responsive to the honey samples tested compared to the *Klebsiella pneumoniae* strain, which exhibited a low inhibition level in sample 01 with a diameter of 9.66 mm and a moderate inhibition

level in sample 02 with a diameter of 12.50 mm. For the remaining honey samples, there was no observed impact on the activity of these Gram-negative bacteria.

These findings surpass those reported by Zahoor *et al.* (2014) in their investigation of four varieties of Pakistani honey, where the inhibition zones for *Klebsiella pneumoniae* ranged from 6.8 to 9.1 mm [57].

Only Gram-positive *Staphylococcus aureus* bacteria displayed a significant sensitivity to honey sample 01, which had a diameter of 16 mm, while showing no response to the other honey samples, similar to the findings with *Streptococcus* sp. and *Candida albicans*.

Staphylococcus aureus bacteria exhibited strong sensitivity exclusively to honey sample 01, with a 16 mm diameter, and showed no interaction with the other honey samples, mirroring the results seen with *Streptococcus* sp. and *Candida albicans*. These findings contrast with those reported by Abdellah *et al.* (2020) in his research, where a lower zone of inhibition was recorded, falling between 8 and 9.5 mm for *Staphylococcus* bacteria and measuring at 6 mm in diameter for *Candida albicans* [1].

The findings indicate that bacterial and fungal strains exhibited varied interactions across different samples. The honey samples displayed a pronounced zone of inhibition against Gram-negative bacteria, especially *Escherichia coli* and *Proteus vulgaris*.

According to Merah (2010), this suggests that certain honey samples might influence Gram-negative bacteria while having no impact on Gram-positive bacteria or fungi, potentially clarifying the observed ineffectiveness on *Streptococcus* bacteria and *Candida albicans* fungi [39].

According to Cilia *et al.* (2020), the antibacterial properties of honey are influenced by its botanical source, quality, extraction methods, and shelf life, all of which impact its physicochemical characteristics, enzymatic activities, and effectiveness [22].

Belhaj *et al.* (2016) explained honey's antibacterial effects by noting the presence of the enzyme glucose oxidase, which facilitates the transformation of glucose into gluconic acid and hydrogen peroxide [14].

In the antibiotic testing results, all tested bacteria, including *Escherichia coli, Proteus vulgaris*, and *Klebsiella pneumoniae*, exhibited resistance to the antibiotic Cefotaxime. *Escherichia coli* demonstrated the highest sensitivity to the eight honey samples, with a concentration of 2.5 mg/mL in sample 01 effectively preventing the growth of *Escherichia coli*, and a concentration of 5 mg/mL in all other samples 3.5.6.7.8.

In other words, honey's efficacy against *Escherichia coli* surpassed that of Ukrainian honey in the research conducted by Cilia *et al.* (2020), where the minimum inhibitory concentration (MIC) values ranged from 188 to 375 mg/ml [22].

Proteus vulgaris showed less sensitivity compared to *Escherichia coli*, with a minimum inhibition concentration of 10 mg/ml across all honey samples. These findings were more favorable than those presented by Bouhlali *et al.* (2020) in his MIC evaluations of various plant-origin samples (orange, lavender, eucalyptus), which yielded MIC results of 10.33 - 11.50 - 12.50 mg/mL [20].

Regarding *Klebsiella pneumoniae* bacteria, the concentration of 5 mg/mL in honey sample 01 and honey sample 02 was adequate to inhibit them. The other honey varieties showed no inhibitory effect on these bacteria.

The *Staphylococcus aureus* bacterium exhibited sensitivity only to sample 01, with an MIC of 2.5 mg/mL, while other honey samples did not demonstrate any sensitivity. This finding surpasses the results reported by Bazaid *et al.* (2023), which indicated the lowest concentration needed to inhibit this bacterium was 400 mg/mL in their study on Saudi Sidr honey [13].

In the case of *Streptococcus* and *Candida albicans*, these bacteria did not show sensitivity or reactivity to any of the tested honey samples, differing from the results of Bazaid *et al.* (2023), which recorded an MIC of 700 mg/mL [13].

In conclusion, LC-ESI-MS/MS analysis identified caffeine as a common component in Algerian bee products, including propolis, beeswax, bee bread, and honey. Algerian propolis contained 21 phenolic compounds, with rutin as the most abundant. The phenolic content may be influenced by the bees' diet. Although these products do not appear to act as anti-hexokinase agents, they may have potential as natural inhibitors of alpha-amylase and beta-glucosidase, relevant for diabetes management. Antimicrobial testing revealed variability in bacterial susceptibility among honey types, with *Escherichia coli* and *Proteus vulgaris* being the most sensitive. Notably, sample (08), a combination of seven honeys, showed strong synergistic inhibition against these strains « synergy ». Further research is needed to validate these findings and explore their therapeutic potential.

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REFERENCES

[1] Abdellah, F., Makhloufi, C., Boukraa, L., Hammoudi, S.M., Safa, A., Dellel, N., Benamara, A., Benhadiri, M., Marouf, N., Benaraba, R., (2020): Physico-chemical properties and antibacterial and antioxidant activity of two varieties of honey from Algerian steppe. Journal of Apitherapy and Nature, 3(2): 59-74.

[2] Abdullah, N.A., Ja'afar, F., Yasin, H.M., Taha, H., Petalcorin, M.I., Mamit, M.H., Kusrani, E., Usman, A., (2019): Physicochemical analyses, antioxidant, antibacterial, and toxicity of propolis particles produced by stingless bee *Heterotrigona itama* found in Brunei Darussalam. Heliyon, 5(9): e02476.

[3] Adimasu Abeshu, M., Bekesho, G., (2015): Medicinal uses of honey. Biology and Medicine, 08(2): 1000276.

[4] Agrawal, P.K., Agrawal, C., Blunden, G., (2021): Rutin: A potential antiviral for repurposing as a SARS-CoV-2 main protease (Mpro) inhibitor. Natural Product Communications, 16(4): 1934578X21991723.

[5] Alasalvar, C., Karamać, M., Amarowikz, R., Shahidi, F., (2006): Antioxidant and antiradical activities in extracts of hazelnut kernel (*Corylus avellana* L.) and hazelnut green leafy cover. Journal of Agricultural and Food Chemistry, 54(13): 4826-4832.

[6] Alfarisi, H.A.H., Ibrahim, M.B., Mohamed, Z.B.H., Hamdan, A.H.Bt., Mohamad, C.A.C., (2021): Honey and its role in medical disorders. Bulletin of Environment, Pharmacology and Life Sciences, 10(3): 250-256.

[7] Alkhalidy, H., Moore, W., Wang, Y., Luo, J., McMillan, R.P., Zhen, W., Zhou, K., Liu, D., (2018): The flavonoid kaempferol ameliorates streptozotocin-induced diabetes by suppressing hepatic glucose production. Molecules, 23: 2338.

[8] Alotibi, I.A., Harakeh, S.M., Al-Mamary, M., Mariod, A.A., Al-Jaouni, S.K., Al-Masaud, S., Alharbi, M.G., Al-Hindi, R.R., (2018): Floral markers and biological activity of Saudi honey. Saudi Journal of Biological Sciences, 25(7): 1369-1374.

[9] Alqurashi, A.M., Masoud, E.A., Alamin, M.A., (2013): Antibacterial activity of Saudi honey against gram negative bacteria. Journal of Microbiology and Antimicrobials, 5(1): 1-5.

[10] Alvarez-Suarez, J.M., Giampieri, F., Brenciani, A., Mazzoni, L., Gasparrini, M., González-Paramás, A.M., Santos-Buelga, C., Morroni, G., Forbes-Hernández, T.Y., Simoni, S., Afrin, S., Giovanetti, E., Battino, M., (2018): *Apis mellifera* vs *Melipona beecheii* Cuban polifloral honeys: A comparison based on their physicochemical parameters, chemical composition and biological properties. LWT Food Science and Technology, 87: 272-279.

[11] Alzahrani, H.A., Alsabehi, R., Boukraâ, L., Abde-llah, F., Bellik, Y., Bakhotmah, B.A., (2012): Antibacterial and antioxidant potency of floral honeys from different botanical and geographical origins. Molecules, 17(9): 10540-10549.

[12] Bakour, M., Fernandes, Â., Barros, L., Sokovic, M., Ferreira, I.C., Lyoussi, B., (2019): Bee bread as a functional product: Chemical composition and bioactive properties. Lwt, 109: 276-282.

[13] Bazaid, A.S., Alsolami, A., Patel, M., Khateb, A.M., Aldarhami, A., Snoussi, M., Almusheet, S.M., Qanash, H., (2023): Antibiofilm, antimicrobial, anti-quorum sensing, and antioxidant activities of Saudi Sidr honey: *In vitro* and molecular docking studies. Pharmaceutics, 15(9): 2177.

[14] Belhaj, O., Abbadi, I.E., Ouchbani, T., (2016): Contribution à l'étude de l'activité antibactérienne du miel naturel d'origine marocaine. Revue Marocaine des Sciences Agronomiques et Vétérinaires, 4(3): 12-22.

[15] Biluca, F.C., da Silva, B., Caon, T., Mohr, E.T.B., Vieira, G.N., Gonzaga, L.V., Vitali, L., Micke, G., Fett, R., Dalmarco, E.M., Costa, A.C.O., (2020): Investigation of phenolic compounds, antioxidant and anti-inflammatory activities in stingless bee honey (Meliponinae). Food Research International, 129: 108756.

[16] Biluca, F.C., de Gois, J.S., Schulz, M., Braghini, F., Gonzaga, L.V., Maltez, H.F., Rodrigues, E., Vitali, L., Micke, G.A., Borges, D.L.G., Costa, A.C.O., Fett, R., (2017): Phenolic compounds, antioxidant capacity and bioaccessibility of minerals of stingless bee honey (Meliponinae). Journal of Food Composition and Analysis, 63: 89-97.

[17] Bittencourt, M.L.F., Ribeiro, P.R., Franco, R.L.P., Hilhorst, H.W.M., de Castro, R.D., Fernandez, L.G., (2015): Metabolite profiling, antioxidant and antibacterial activities of Brazilian propolis: Use of correlation and multivariate analyses to identify potential bioactive compounds. Food Research International, 76: 449-457.

[18] Boizot, N., Charpentier, J.P., (2006): Méthode rapide d'évaluation du contenu en composés phénoliques des organes d'un arbre forestier. Le Cahier des Techniques de l'INRA, In: Numéro spécial: Observation et évaluation, pp. 79-82.

[19] Borycka, K., Grabek-Lejko, D., Kasprzyk, I., (2015): Antioxidant and antibacterial properties of commercial bee pollen products. Journal of Apicultural Research, 54(5): 491-502.

[20] Bouhlali, E.D.T., Hmidani, A., Bourkhis, B., Khouya, T., Ramchoun, M., Filali-Zegzouti, Y., Alem, C., (2020): Phenolic profile and anti-inflammatory activity of four Moroccan date (*Phoenix dactylifera* L.) seed varieties. Heliyon, 6(2): e03436.

[21] Cabezas-Mera, F., Cedeño-Pinargote, A.C., Tejera, E., Álvarez-Suarez, J.M., Machado, A., (2024): Antimicrobial activity of stingless bee honey (Tribe: Meliponini) onclinical and foodbornepathogens: Asystematicreviewand meta-analysis. Food Frontiers, 5, 964–993.

[22] Cilia, G., Fratini, F., Marchi, M., Sagona, S., Turchi, B., Adamchuk, L., Felicioli, A., Kačániová, M., (2020): Antibacterial activity of honey samples from Ukraine. Veterinary Sciences, 7(4): 181.

[23] El Ghouizi, A., Bakour, M., Laaroussi, H., Ousaaid, D., El Menyiy, N., Hano, C., Lyoussi, B., (2023): Bee pollen as functional food: Insights into its composition and therapeutic properties. Antioxidants, 12(3): 557.

[24] Erenler, R., Atalar, M.N., Yildiz, İ., Geçer, E.N., Yildirim, A., Demirtas, İ., Alma, M.H., (2023): Quantitative analysis of bioactive compounds by LC-MS/MS from *Inula graveolens*. Bütünleyici ve Anadolu Tıbbı Dergisi, 4(3): 3-10.

[25] Ferreira, B.L., Gonzaga, L.V., Vitali, L., Micke, G.A., Maltez, H.F., Ressureição, C., Costa, A.C.O., Fett, R., (2019): Southern-Brazilian geopropolis: A potential source of polyphenolic compounds and assessment of mineral composition. Food Research International, 126: 108683.

[26] Ganfon, H., Houvohessou, J.P., Assanhou, A.G., Bankole, H.S., Gbenou, J., (2019): Activité antibactérienne de l'extrait éthanolique et des fractions de anogeissus leiocarpa (DC) Guill. Et Perr. (Combretaceae). International Journal of Biological and Chemical Sciences, 13(2): 643-651.

[27] Gonzàlez-Montelongo, R., Lobo, M.G., Gonzàlez, M., (2010): Antioxidant activity in banana peel extracts: testing extraction conditions and relative bioactive compounds. Food Chemistry, 119(3): 1030-1039.

[28] Gülcin, İ., (2011): Antioxidant activity of eugenol: a stucture-activity relationship study. Journal of Medicinal Food, 14(9): 975-985.

[29] Kajdzanoska, M., Petreska, J.P., Stefova, M., (2011): Comparision of different extraction solvent mixtures for characterization of phenolic compounds in strawberries.

Journal of Agricultural and Food Chemistry, 59(10): 5272-5278.

[30] Kavita, S., Eun, Y.K., Awraris, D.A., Soyoung, H.A., Shivraj, H.N., Eul, T.L., Se, W.P., (2014): Temperature-dependent studies on the total phenolics, flavonoids, antioxidant activities, and sugar content in six onion varieties. Journal of Food and Drug Analysis, 23: 243-252.

[31] Klancnik, A., Piskernik, S., Jersek, B., Mozina, S.S., (2010): Evaluation of diffusion and dilution methods to determine the antibacterial activity of plant extracts. Journal Microbiol Methods, 81(2): 121-126.

[32] Kouri, G., Tsimogiannis, D., Bardouki, H., Oreopoulou, V., (2007): Extraction and analysis of antioxidant components from *Origanum dictamnus*. Innovative of Food Science and Emerging Technolgy, 8: 155-168.

[33] Lamprecht, W., Trautschold, I., (1974): Determination with hexokinase and glucose-6-phosphate dehydrogenase. In: Bergmeyer HU, ed. Methods of enzymatic analysis. New York: Academic press, pp. 1127-1131.

[34] Lien, D.T.P., Tram, P.T.B., Toman, H.T., (2015): Effect of extraction process on phenolic content and antioxidant activity of soybean. Journal of Food and Nutrition Sciences, 3(1-2): 33-38.

[35] Liu, J.R., Ye, Y.L., Lin, T.Y., Wang, Y.W., Peng, C.C., (2013): Effect of floral sources on the antioxidant, antimicrobial, and anti-inflammatory activities of honeys in Taiwan. Food Chemistry, 139(1-4): 938-943.

[36] Magdalena, K., Anna, R., Adrian, W., Marcin, G., Mariola, A., Michał, B., Maciej, S.B., Marcin S., Michał, T., Robert, R., Marek, G., Aneta, A.P., (2023): Chemical Composition and Antimicrobial Activity of New Honey Varietals. International Journal of Environmental Research and Public Health, 20, 2458.

[37] Mahendran, S., Kumarasamy, D., (2015): Antimicrobial activity of some honey samples against pathogenic bacteria. International Letters of Natural Sciences, 34: 15-20.

[38] Martinello, M., Mutinelli, F., (2021): Antioxidant activity in bee products: A review. Antioxidants, 10(1): 71.

[39] Merah, M., Bachagha, M.B., Bouderhem, A., (2010): Etude de l'effet antimicrobien de trois echentillons du miel naturel recoltes du territoire Algerien. Annales des Sciences et Technologie, 2(2): 115-125.

[40] Mokrani, A., Madani, K., (2016): Effect of solvent, time and temperature on the extraction of phenolic compounds and antioxidant capacity of peach (*Prunus persica* L.) fruit. Separation and Purification Technology, 162: 68-76.

[41] Nakib, R., Ouelhadj, A., Seijo-Coello, M.C., (2022): Assessment of Physicochemical, Antimicrobial and Antiradical Characteristics of Some Algerian Honeys from Different Floral and Geographical Origins. Phytothérapie, 20 (4), 230-240.

[42] Nisa, N., Rasmita, B., Arati, C., Uditraj, C., Siddhartha, R., Dinata, R., Bhanushree, B., Bidanchi, R.M., Manikandan, B., Laskar S.A., Abinash, G., Pori, B., Roy, V.K., Gurusubramanian, G., (2023): Repurposing of phyto-ligand molecules from the honey bee products for Alzheimer's disease as novel inhibitors of BACE-1: Small molecule bioinformatics strategies as amyloid-based therapy. Environmental Science and Pollution Research, 30(17): 51143-51169.

[43] Nusret, G.E.N.Ç., Elmastaş, M., Telci, İ., Erenler, R., (2020): Quantitative analysis of phenolic compounds of commercial basil cultivars (*Ocimum basilicum* L.) by LC-TOF-MS and their antioxidant effects. International Journal of Chemistry and Technology, 4(2): 179-184.

[44] Omari, N.E., (2021): Étude phytochimique et toxicologique et évaluation de l'activité antioxydante, antidiabétique et antibactérienne des racines d'*Aristolochia longa* L. PhD Thesis, Université Mohammed V-Rabat, Morocco.

[45] Prasath, G.S., Pillai, S.I., Subramanian, S.P., (2014): Fisetin improves glucose homeostasis through the inhibition of gluconeogenic enzymes in hepatic tissues of streptozotocin induced diabetic rats. European Journal of Pharmacology, 740: 248-254.

[46] Quy, D.D., Artik, E.A., Phuong, L.T.N., Lien, H.H., Felycia, E.S., Suryadi, I., Yi-H, J., (2013): Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatic*. Journal of Food and Drug Analysis, 22: 296-302.

[47] Rabbani, N., Thornalley, P.J., (2019): Hexokinase-2 glycolytic overload in diabetes and ischemia–reperfusion injury. Trends in Endocrinology and Metabolism, 30(7): 419-431.

[48] Radjah, A., Chorfi, H., Bouatrous, Y., (2019): Stage of development and solvent effects on phytochemistry and antioxidant activity of three algerian plants. Analele Universităţii din Oradea, Fascicula Biologie, 26(2): 91-99.

[49] Ristivojević, P., Dimkić, I., Guzelmeric, E., Trifković, J., Knežević, M., Berić, T., Yesilada, E., Milojković-Opsenica, D., Stanković, S., (2018): Profiling of Turkish propolis subtypes: Comparative evaluation of their phytochemical compositions, antioxidant and antimicrobial activities. LWT, 95: 367-379.

[50] Safia, B.A., Scherazad, M., Walid, B., Leila, A.B., Faisal, A., Taibi, B.H., Magda, H.A., Sarkar, M.A.K., (2022): Phytochemical Characterization and Bioactivity of Different Honey Samples Collected in the Pre-Saharan Region in Algeria. Life, 12, 927.

[51] Silva, T.M.S., dos Santos, F.P., Evangelista-Rodrigues, A., da Silva, E.M.S., da Silva, G.S., de Novais, J.S., dos Santos, F.A.R., Camara, C.A., (2013): Phenolic compounds, melissopalynological, physicochemical analysis and antioxidant activity of jandaíra (*Melipona subnitida*) honey. Journal of Food Composition and analysis, 29(1): 10-18.

[52] Su, X., Duan, J., Jiang, Y., Shi, J., Kakuda, Y., (2006): Effect of soaking conditions on the antioxidant potenials of oolong tea. Journal of Food Composition and Analysis, 19(4): 348-353.

[53] Szabat, P., Poleszak, J., Szabat, M., Boreński, G., Wójcik, M., Milanowska, J., (2019): Apitherapy – the medical use of bee products. Journal of Education, Health and Sport, 9(8): 384-396.

[54] Uma, D.B., Ho, C.W., Wan Aida, W.M., (2010): Optimization of extraction parameters of total phenolic compounds from Henna (*Lawsonia inermis*) leaves. Sains Malaysiana, 39(1): 119-128.

[55] Vasić, V., Gašić, U., Stanković, D., Lušić, D., Vukić-Lušić, D., Milojković-Opsenica, D., Tešić, Ž., Trifković, J., (2019): Towards better quality criteria of European honeydew honey: Phenolic profile and antioxidant capacity. Food Chemistry, 274: 629-641.

[56] Voidarou, C., Alexopoulos, A., Plessas, S., Karapanou, A., Mantzourani, I., Stavropoulou, E., Fotou, K., Tzora, A., Skoufos, I., Bezirtzoglou, E., (2011): Antibacterial

activity of different honeys against pathogenic bacteria. Anaerobe, 17(6): 375-379.

[57] Zahoor, M., Naz, S., Sangeen, M., (2014): Antibacterial, antifungal and antioxidant activities of honey collected from Timergara (Dir, Pakistan). Pakistan Journal of Pharmaceutical Sciences, 27(1): 45-50.

[58] Zhang, H., Wang, X., Wang, K., Li, C., (2015): Antioxidant and tyrosinase inhibitory properties of aqueous ethanol extracts from monofloral bee pollen. Journal of Apicultural Science, 59(1): 119-128.

[59] Zhao, H., Zhu, M., Wang, K., Yang, E., Su, J., Wang, Q., Cheng, N., Xue, X., Wu, L., Cao, W., (2020): Identification and quantitation of bioactive components from honeycomb (Nidus Vespae). Food Chemistry, 314: 126052.

[60] Zhishen, J., Mengcheng, T., Jianming, W., (1999): The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry, 64(4): 555-559.

[61] Zuluaga, C.M., Serratob, J.C., Quicazana, M.C., (2015): Chemical, nutritional and bioactive characterization of Colombian bee-bread. Chemical Engineering, 43: 175-180.

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