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Botanical, Physicochemical and Biological Properties of Monofloral Honeys from Mostaganem Province, Northwest Algeria

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ABSTRACT

Background: This study aimed to evaluate the characteristics of monofloral honeys collected from Mostaganem province, based on their melissopalynological, physicochemical and biological properties.

Methods: Eight honey samples were analyzed for pollen profile (quantitative and qualitative) and physicochemical parameters, including moisture, electrical conductivity, pH, hydroxymethylfurfural (HMF), color, sugars (fructose, glucose, sucrose, maltose, turanose, melezitose), as well as their as their antimicrobial and antioxidant activities.

Result: Melissopalynological analysis identified 49 pollen types from 29 botanical families, with *Asteraceae*, *Fabaceae* and *Apiaceae* being the most represented. The pollen types found as dominant were *Eucalyptus* and *Daucus carota*. The number of pollen type identified per sample ranged between 1.900 and 37.700 grains/g. The physicochemical parameters of the studied honeys complied with European and international standards. Among the analyzed samples, *Eucalyptus* honey exhibited the highest polyphenol (56.61 mg GAE/100 g) and flavonoid content (8.54 mg QE/100 g), along with the highest reducing power (55.64%). Correlation analysis revealed significant relationships between, conductivity and color, pH and moisture, as well as polyphenol content with antioxidant, inhibitrice concentration at 50 and antibacterial activity. These findings confirm the high quality of local honeys from the Mostaganem region and their potential applications in alternative therapeutic strategies.

Key words: Antibacterial activity, Honey, Melissopalynological, Mostaganem, Physicochemical.

INTRODUCTION

Honey is a complex sweet natural food produced by honey-bees (*Apis mellifera*) from blossoms nectars or honeydew (Radja *et al.,* 2022), monosaccharides represent a large amount of the carbohydrates in this product, consisting mainly of fructose (38%) and glucose (31%), other carbohydrates are present in low concentration (Homrani *et al.,* 2025). Honey contribute to its diverse biological activities, including antioxidant, anti-inflammatory and wound-healing properties (Damto *et al.,* 2023).

Monofloral honey is derived from the nectar of a single plant species, which gives them distinctive physicochemical, sensory and biological effects (Da Silva *et al.,* 2016).These honeys often exhibit unique color, taste and aroma profiles, as well as specific bioactive compounds linked to their floral origin (Ketfi *et al.,* 2023).

To ensure authenticity and quality, honey is subject to international standards, notably those established by the Codex Alimentarius Commission (2001). Determining its botanical origin is essential for quality control and requires melisso-palynological analysis, which identifies the pollen types present (Belay *et al.,* 2017). This technique, combined with physicochemical and sensory analyses, allows for precise classification and quality assessment of monofloral honeys.

Algeria, with its rich biodiversity and varied climatic conditions, is an important producer of diverse honey types, including several monofloral varieties (Harbane *et al.,* 2024). Mostaganem region also has extensive *Eucalyptus* plantations and forested areas covering 27,451 hectares. Therefore, this study aims to provide a detailed characterization of the most common monofloral honeys from the Mostaganem region by analyzing their melisso-palynological profiles, physicochemical properties and antioxidant activity.

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MATERIALS AND METHODS

Honey samples

A total of eight honey samples were taken from two localities, Hchachta (n= 4) and Khadra (n= 4), in the Mostaganem region, northwest Algeria. The samples were collected from beekeepers under aseptic conditions during the 2024 harvesting season.

Melissopalynological analysis

Quantitative analysis

Ten grams of honey were dissolved in 20 mL of distilled water and the solution was centrifuged. The sediment was deposited over a slide to microscopically examine (Louveaux *et al.,* 1978). After counting the pollen elements, the honeys were classified according to the following classes: Class I <2000 pollen grains per 1 grams of honey (PG/g); Class II: 2000-10,000 pollen grains; Class III: 10,000-50,000 pollen grains; Class IV: between 50,000-100,000; and Class V: >100,000 pollen grains. (Maurizio, 1975).

Qualitative analysis

An aliquot of honey was dissolved in 20 mL of distilled water and the solution was centrifuged. The sediment was placed on a slide for microscopic examination. The determination of species, genus or type has been carried out comparison with the pollen grains from the collections of references. Pollen dominance classes were divided into 4 types of pollen: dominant pollen (≥45%), accompanying pollen (from ≥15% to <45%), important pollen (from ≥3% to <15%), rare pollen (from ≥1% to <3%).

Physico-chemical properties

Quality controls

Moisture content, electrical conductivity, pH and hydroxymethylfurfural (HMF) levels were determined according to the International Honey Commission guidelines (Bogdanov, 2009), with all analyses performed in duplicate. Moisture content was measured using refractometer at 20°C. For the measurement of electrical conductivity an aliquot of honey was dissolved in 100 ml of distilled water, the conductivity meter electrodes were introduced into the solution at 20°C, results were expressed in mS/cm. pH was measured using an Inolab pH 7110 meter from honey solution. HMF content was measured according to the White method (AOAC, 2005), by spectrophometry of colored products of HMF reaction.

Color determination

Melanoidin levels were estimated spectrophotometrically, according to the method of Brudzynski and Mitto (2011). The absorbance of 50% (w/v) diluted honey solution was measured at 450 and 720 nm. The results were expressed in absorbance units (AU).

Sugar-composition analysis

The following sugars: glucose, fructose, sucrose, maltose, turanose and Melezitose was mesured by nuclear magnetic resonance (NMR). The principle of the method is to observe the quantum magnetic properties of nuclei in gas, solid or liquid phase. Only atoms whose nucleus has a magnetic moment give rise to the resonance phenomenon. The 1H core is used in our application. On a more practical side, honey samples are dissolved in water and adjusted to a pH defined by our method. The results in spectrum form are automatically compared to a given base, to issue an analysis report with the different parameters analyzed and the results were expressed in %.

Bioactive compound and antioxidant activity

Total polyphenol content

The total polyphenol content of honeys was measured using folin-ciocalteu (Singleton *et al.,* 1999), A volume of 500 µl of honey solution was stirred with 2.5 ml of Folin reagent (0.2 N) after 2 minutes we added 2 ml of sodium bicarbonate (7.5%). The samples were incubated in the dark for 2 hours before reading their absorbances at 760 nm, the results are expressed in mg equivalent of gallic acid per 100 g of honey (mg EAG/100 g).

Total flavonoid content

The determination of total flavonoid was measured by spectrophotometric method (Arvouet-Grand *et al.,* 1994), briefely, 1 ml of honey solution (1mg/ml) was mixed with 0.3 ml NaNO2 (5%) after 5 minutes 0.3 ml of AlCl3 (10%) was added after 6 minutes, 2 ml NaOH (1M) was added. The absorbance was mesured at 510 nm and the results are expressed in mg quercetin equivalent per 100 g of honey (mg EQ/100 g).

Radical scavenging activity

Antioxidant activity was evaluated by DPPH (2,2-difenil-1-picrilhidrazil) free radical scavenging (Brand-Williams, 1995), scavenging activity of the DPPH radical was calculated using following equation:

$$\% \text{ Inhibition} = \frac{\text{Control absorbance - Sample absorbance}}{\text{Control absorbance}} \times 100$$

Inhibitory concentration at 50% (IC50) was calculated graphically by linear regressions of the inhibition percentages as a function of different concentrations of each of the solutions tested.

Antibacterial activity of honey (*In vitro*)

For assessment of antibacterial activity, four (n = 4) pathogenic strains (*Klebsiella pneumonia, Enterobacter sp., Staphylococcus xylosus and Staphylococcus simulans*) isolated from mastitic cow's milk were chosen and identified by standard biochemical test (Bio-Merieux, France), API Staph and API 20E in the Laboratory of Sciences and Technics of Animal Production. According to the agar well diffusion method described by Postali *et al.* (2022), the inhibition zones are expressed as clear halos around the wells and were measured in mm.

Statistical analysis

Using excel and spss Correlations between physicochimical parameters, antioxidant components and antibacterial activities were achieved by Pearson correlation coefficient (r) at a significance level of 99% ($p<0.01$) and ($p<0.05$).

RESULTS AND DISCUSSION

Palynological characteristics

Quantitative analysis

The pollen content ranged from 1900 grains/g of honey to 37700 grains/g (mean 26987,5grains/g), 4 samples (S4, S6, S7, S8) samples (50%) were rich in pollen sediment (Classes III of Maurizio) and 4 samples showed a medium pollen content (Classes II of Maurizio) (Table 1).

Qualitative analysis

Total of 49 pollen types were identified, including 39 nectariferous and 10 non-nectariferous species (Table 2). Among the identified pollen types, *Eucalyptus* (*Myrtaceae*) and *Daucus carota* (*Apiaceae*) were present as dominant pollen, confirming their role as major nectar sources in the Mostaganem region. *Thapsia garganica* (*Apiaceae*) and *Silybum marianum* (*Asteraceae*) were classified as accompanying pollen, while 17 taxa, including *Coriandrum sativum*, *Borago officinalis* and *Foeniculum vulgare*, were categorized as important pollen. The presence of 39 taxa classified as rare pollen suggests a broad but uneven distribution of floral sources.

The identified pollen types belonged to 29 botanical families and the most dominant families in the studied honey samples were *Asteraceae* (100%), *Fabaceae* (87.5%), *Apiaceae* (75%), *Myrtaceae* (50%), *Boraginaceae* (50%) and *Papaveraceae* (50%). The dominance of *Asteraceae* and *Fabaceae* in the studied honeys aligns with previous research on Algerian honey composition (Ketfi *et al.,* 2023; Laallam and Chenchouni, 2024). Louveaux and Abed (1984) also emphasized the significant role of *Apiaceae* in North African apiculture. On a global scale, *Fabaceae*, *Asteraceae*, *Apiaceae* and *Lamiaceae* are among the most common pollen families found in honey (Dongock *et al.,* 2017). The high nectariferous potential of *Fabaceae* explains their prevalence in Algerian honeys (Zerrouk and Bahloul, 2020).

Physicochemical analysis

Moisture

The results of the physicochemical analysis were summarized in Table 3, the moisture content in honey samples was within the standards of Codex Alimentarius Commission (2001), the values varied from 15,2% (S1, *Daucus carota*) to 18,4% (S6, S8, *Eucalyptus*), with an average of 17,26±0,93%, This value is similar to the Algerian honey from different geographical regions (Homrani *et al.,* 2020), The variation in the water content of honey is a function of the climatic conditions, the degree of maturity of honey and humidity of botanical origin (Bogdanov *et al.,* 2009).

pH

The studied honey samples presented acidic pH values between 3,60 (S5, *Eucalyptus*) and 3,94 (S3, *Daucus carota*). The Codex Alimentarius recommends a pH of 3.5 to 4.5 for nectar honeys, pH of honey is correlated with the presence and development of microorganisms and a low pH will prevent microbiological spoilage (Ratiu *et al.,* 2020) (Table 3).

Electrical conductivity

Table 3 show that the electrical conductivity values of the honey samples vary between 0,427 mS/cm (S3, *Daucus carota*) and a maximum of 0,863 ìS/cm (S5, *Eucalyptus*). According to the Codex Alimentarius Commission (2001) nectar honey should have a conductivity of no surpass 0.8 mS /cm. Higher values are considered as belonging to honeydew honey or mixtures of honeydew and nectar honey but there are some exceptions as *Eucalyptus*. Our results are similar to those of Cenet *et al.* (2017) for honeys produced in Turkey.

HMF

The HMF for the 8 honey samples analyzed in the present work ranged from 5,2±0,23 to 16,24±0,03 mg/kg these values were within the recommended range set by the Codex Alimentarius at 40 mg/ kg. Makhloufi *et al.* (2021) found HMF values of 3.3 to 15.66 mg/Kg with an average of 8.14±4.8 mg/Kg in Algerian honey (Table 3).

Color

The color of honey is the first physical and sensory property perceived by the consumer, the color of honey varies depending on the botanical source and mineral content (Raziuddin *et al.,* 2021), the color intensity values obtained by measuring the absorbance at 450 nm show a variation between 0,64 (S5, *Eucalyptus*) and 1,35 UA (S8, *Eucalyptus*) (Table 3). The values were similar to other Algerian honey samples (Doukani *et al.,* 2014). The evaluations of melanoidin, which are heterogeneous polymers of high molecular weight of brown color, have a important role in discriminating the botanical origin of honey (Da Silva *et al.,* 2016).

Sugar

In the analyzed samples, the total content of glucose and fructose was over 60/100 g of honey (mean of 70,43%) (Table 4), in accordance with the European Union Honey

Table 1: Pollen richness of the studied samples.

Samples	S1	S2	S3	S4	S5	S6	S7	S8
Number of pollen grains/g	1990	27220	45350	49350	3750	40700	34900	37700
Class	Class II	ClassII	Class II	Class III	Class II	Class III	Class III	Class III

Table 2: Pollen spectrum of monofloral honeys produced in Mostaganem province.

Family	Pollen types	S1	S2	S3	S4	S5	S6	S7	S8
Amaryllidaceae	*Allium*	-	R	-	-	-	-	-	-
Apiaceae	*Coriondrum sativum*	-	-	-	-	-	-	-	-
	Foenuculum vulgare		I	I	R				
	Daucus carotta	D	D	D	D	I			
	Pimpenella anisum t	R	-	-	-	-	-	-	-
	Thapsia garcanica	A	A	A	A	I		R	
Arecaceae	Chamaerops*	-	-	-	I	-	-	-	-
Asparagaceae	*Asparagus t*		R						
Boraginaceae	*Borago officinalis*	I		I		I			
	Echium	-	-	-	-	-	-	-	R
Brassicaceae	*Brassica napus t*	-	-	-	-	-	-	-	R
Buxaceae	*Buxus sempervirens**	-	-	-	-	-	R	-	R
Cactaceae	*Cirsium arvense*	-	-	R	-	-	-	-	-
Casuarinaceae	Casuarina*	-	-	-	-	-	-	I	-
Chenopodiaceae	Chenopodium	-	-	-	-	-	-	-	R
Astreaceae	Artemisia t	-	-	-	-	-	-	R	-
	Bellis t	-	-	-	-	-	R	-	-
	Centaurea t	R	R				-	-	-
	Echinops	-	-	-	-	R	-	-	-
	Taraxacum officinale	R	R						
	Cichorium t	-	-	-	-	-	-	-	R
	Ambrosia t	-	-	-	-	-	-	-	R
	Silybum marinarum	I	I	A	I		R	R	
Convolvulaceae	Convolvulus	-	-	-	-	R	-	-	
Cyperaceae	*Rhammus alaterna*	-	-	-	-	R			
Fagaceae	Quercus*	-	-	-	-		I	I	I
	Castanea	-	-	-	-	R	-	-	-
Lamiaceae	Lavandula t	R	-	-	-	-	-	-	-
	Mentha t	R	-	R	-	-	-	-	-
	Thymus t	R	-	-	-	-	-	-	-
Fabaceae	Acacia	R	-	-	-	-	R	-	-
	Hydesarum coronarium	-	-	-	-	-	I	I	R
	Melilotus t	-	R	R					
	Ononis natrix	I	-	-	-	-	-	-	-
	Trifolium repenst	R	-	-	R	-	-	-	-
	Vicia t	I				-	-	-	-
Lythraceae	*Punica granatum*	-	-	-	-	-	I	-	-
Myrtaceae	Eucalyptus	-	-	-		D	D	D	D
Nitrariaceae	*Peganum harmala*	-	-	-	R	-	-	-	-
Oleaceae	Olea europea*	-	-	-			-	R	R
Papaveraceae	*Papaver rohaes**	-	-	R		R	R		R
Pinaceae	Pinus*	-					R		R
Plantaginaceae	Plantago	-	R	R	R	-	-	-	-
Poaceae	Poaceae*	-	R			-	-	-	-
Rhamnaceae	*Ziziphus lotus*	-			R	-	-	-	-
Rosaceae	*Eribotrya japonica*	-		R	-	-	-	-	-
	Malus t	-		-	-	-	-	R	-
	Prunus t	-	I	-	-	-	-		-
Smilacaceae	Smilax	-	-	-	R	-	-	R	-
Tamaricaceae	Tamarix	-	-	-		R			-
Urticaceae	Urticaceae*	-	-	-	R				-
Nombre of pollen types		14	12	11	11	10	10	10	11

*: non-nectariferious, A: Accompanying pollen, D: Dominant pollen, I: Important pollen, R: Rare pollen.

Directive for blossom honey (European Commission, 2002). Fructose content varied between 37,2 and 41,5% (mean value of 39,15%) and glucose content exhibited a variation between 28,6 and 33,4% (mean value of 31,28%), Zerrouk and Bahloul (2020) found similar glucose and fructose contents in Algerian honey.

Saccharose content in honey is considered an important sugar from the legislative point. (Zerrouk and Bahloul, 2020). Saccharose content of the samples was <1,9 all values are within 5% range which indicates that these honeys are ripe. Melezitose content in the studied honeys was <3,7%, The presence of melezitose has been advanced as useful indicator of contribution of honeydew (Wackers, 2001).

It is noteworthy the content of maltose in honey samples ranged from 1.3±11% to 2.2±11% with a mean value of 1.92±0.41% for *Daucus carota* honeys and 1.3±11 to 2.1% with a mean value of 1.62±0.42% for *Eucalyptus* honeys, similar result to those reported by Ouchemoukh *et al.* (2010) on Algerian *Eucalyptus* honeys (1.62±0.84%), but lower values were reported by Homrani *et al.* (2020) for *Apiaceae* honeys produced in Algeria. The turanose content of the investigated honey samples varied from 1.4±8 to 1.9±8% (mean of 1,52%) and 1.6±8 to 2±8% (mean of 1,82%) for wild carrot and *Eucalyptus* honeys. On the contrary, Chettoum *et al.* (2023) mentionned lower turanose content for Algerian honey.

Bioactive compound and antioxidant activity

Polyphenol content

The polyphenol content of *Eucalyptus* honey (mean of 56,61 mg AG/100 g) could be considered high when compared with *Daucus carota* honey (mean of 47,22 mg AG/100 g) (Table 5). Some *Apiaceae* from Algeria were studied regarding polyphenol content (Ouchemoukh *et al.,* 2010).

Flavonoid content

The mean flavonoid content of the studied honeys was 8,54 mgEQ/100 g, 6,29 mg EQ/100 g for *Eucalyptus* and *Daucus carota* respectively (Table 5), was greater than that found by Homrani *et al.* (2020).

Table 3: Results of physicochemical parameters studied.

Sample code	Color (UA)	HMF (mg/kg)	pH	Water content (%)	CE (mS/cm)
S1	1,07±0,38	7,85±0,23	3,93±0,00	15,20	0,574±0,007
S2	0,89±0.002	11,64±0,41	3,86±0,007	16,90±0,14	0,497±0,014
S3	0,81±0.002	8,13±0,37	3,94±0.007	16,20±0,14	0,427
S4	0,87±0.01	6,29±0,91	3,61±0,00	17±0,00	0,526±0,001
S5	1,35±0.14	9,58±0,45	3.60±0,00	18±0,00	0,863±0,04
S6	1,009±0.07	13,96±0,48	3.72±0,003	18,40±0,00	0,566±0,01
S7	0,77±0.008	5,2±0,23	3.62±0,00	18±0,00	0,473±0,008
S8	0,64±0.005	16,24±0,03	3.69±0,006	18,40±0,00	0,465±0,01

Table 4: Results of sugar analysis.

Sugar (%)	Fuctose	Glucose	F + G	F/G	G/moisture	Saccharose	Turanose	Maltose	Mélizitose
S1	41,5	33,4	74,9	1,24	2,19	<1,9	1,4	1,3	<3,7
S2	40,1	30,9	71	1,29	1,82	<1,9	1,4	3,1	<3,7
S3	39,9	32,3	72,2	1,23	1,99	<1,9	1,9	2,1	<3,7
S4	37,9	31,6	69,5	1,19	1,85	<1,9	1,4	2,2	<3,7
S5	38,6	28,6	67,2	1,34	1,58	<1,9	1,6	1,8	<3,7
S6	40,3	29,1	69,4	1,38	1,58	<1,9	1,8	2,1	<3,7
S7	37,2	33,3	70,5	1,11	1,85	<1,9	1,9	1,1	<3,7
S8	37,7	31,1	68,8	1,21	1,69	<1,9	2	1,5	<3,7

Table 5: Polyphenols, favonoids, RSA content and IC50.

Sample code	Polyphenol mg/100 g	Flavonoid mg/100 g	RSA %	IC50
S1	85,11±0,52	5,5±0,08	50,37±0,7	14,13±0,31
S2	41,69±0,26	5,71±0,83	47,02±0,54	14,67±0,12
S3	36,83±0,26	6,28±0,3	25,95±0,3	25,25±0,19
S4	25,28±0,42	7,68±0,08	18,8±0,3	36,43±0,21
S5	60,39±0,37	10,81±0,35	69,72±0,42	8,27±0,29
S6	49,16±0,42	6,06±7,51	43,69±2,33	15,3±0,11
S7	68,58±0,26	7,37±1,23	57,08±1,06	9,61±0,31
S8	56,34±0,37	9,93±0,88	52,08±0,36	12,9±0,13

Table 6: Antibacterial activity honeys.

Honey samples	Diameter of the inhibition zone (mm)			
	Klebsiella pneumonae	*Enterobacter* sp.	*S. simulans*	*S. xylosus*
S1	12,5±0,71	16±0,71	21,5±2,12	19±1,41
S2	12±1,41	13,5±2,17	15±0,00	15,5±071
S3	11,5±0,31	11±1,41	14±0,00	15±1,41
S4	14,5±0,71	12,5±0,71	14,5±0,71	19,5±0,71
S5	13,5±0,71	14,5±0,71	17±1,41	19±1,41
S6	11,5±0,71	13±1,41	15,5±0,71	15±1,41
S7	12,5±0,71	13,5±0,71	16,5±2,12	21±1,41
S8	13,5±0,71	14,5±0,71	17±1,41	14,5±0,71

Radical scavenging activity

The mean antioxidant capacity of the *Eucalyptus* and *Daucus carota* honeys was 55,64%, 35,53% respectively, similar results to those reported by Nakib *et al.* (2021). The lowest IC50 was observed in (S5, *Eucalyptus*, 8,27 mg/ml). (Table 5). The botanical origin of the honey is considered the major role in the variations of phenolic content and the antioxidant activity (Perna *et al.,* 2013).

Antibacterial activity (*In vitro*)

Zones of inhibition were produced on all strains, the highest value of inhibitory activity observed in *Staphylococcus simulans* 21,5±2,12 mm with *Daucus carota* honey, *Klebsiella pneumonae* and *Enterobacter sp* were the most resistant (Table 6). *Eucalyptus* honey exhibited the highest antibacterial effect, the variation of antibacterial activity depends on bacterial strain and botanical origin honey, geoclimatic conditions and the harvest season (Azonwadé *et al.,* 2017).

Results of stastistical analysis

The Pearson correlation analysis revealed several significant relationships among the physicochemical and bioactive properties of the honey samples. Conductivity showed a strong correlation with color ($p<0.01$), consistent with previous findings that mineral content plays a key role in determining honey color intensity, same observation was noted by Makhloufi *et al.* (2021). Total polyphenol content with antioxidant capacity, inhibitrice concentration at 50 and antibacterial activity against *Staphylococcus simulans* and *Enterobacter sp.* showed a highly significant correlation ($p<0.01$), reinforcing the role of phenolic compounds in antioxidant activity of honey. The antibacterial activity of the analyzed honeys against *S xylosus* was negatively correlated with the HMF content ($p<0.01$). A significant correlation was observed between pH and moisture content ($p<0.05$), suggesting a relationship between acidity and water content in honey degradation. Further studies are needed to explore the mechanisms underlying these relationships and their implications for honey classification and quality control.

CONCLUSION

This study confirms the high quality of monofloral honeys from Mostaganem, meeting international standards. *Eucalyptus* and *Daucus carota* were identified as dominant pollen types, with *Eucalyptus* honey showing the highest polyphenol and flavonoid content. Correlation analysis highlighted key relationships between physicochemical and bioactive properties. These findings suggest the potential of these honeys as natural therapeutic agents, warranting further research.

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Disclaimers

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Conflict of interest

The authors declare no conflict of interest.

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