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وزارة التعـلـيـم العـالـي والـبـحـث الـعـلـمي

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THEME

Effect of Honeys from Different Botanical Origins on Xanthine Oxidase Inhibition

Publicly defended on 00/00/2025, before the jury composed of:

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DEDICATION

First and foremost, all praise and thanks are due to Allah, who granted me the strength, patience, and guidance to reach this stage, and without Whose mercy this work would not have been possible.

To my dear parents, the heartbeat of my life and the pillars of my strength, who have always been my light and my guides through the paths of knowledge and life — I offer this work as a token of love and gratitude.

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And to the people of Palestine…

To the hearts that beat under siege, to the souls that breathe dignity despite narrow horizons, to the eyes that shine with hope amid the smoke of war, and to the hands that build life upon the ruins of destruction. You are the steadfast in the face of the storm, the true teachers of endurance, and the dream that refuses to die no matter how long the night.

I dedicate this work to you as a pledge of loyalty, respect, and faith that victory is born.

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List of abbreviations

BSA	: Bovine Serum Albumin
PCA	: Principal Component Analysis
XO	: Xanthine Oxidase
ROS	: Reactive Oxygen Species
NLRP3	: NOD-Like Receptor Family Pyrin Domain Containing 3
UV	: Ultraviolet
TPC	: Total Phenolic Content
GAE	: Gallic Acid Equivalent
PI_{XO}	: Percentage Inhibition of Xanthine Oxidase
AEC	: Allopurinol equivalent capacity
PI_{BSA}	: Percentage Inhibition of Bovine Serum Albumin
A_0	: Absorbance at 0% inhibition
A_1	: Absorbance at sample concentration
DEC	: Diclofenac Equivalent Concentration
HRBCs	: Human Red Blood Cell Stabilization
PBS	: Phosphate Buffered Saline
PH (%)	: Percentage of Hemolysis
pH	: Potential of Hydrogen
ANOVA	: Analysis of Variance
SD	: Standard Deviation
UA	: Uric Acid
AEAXOC	: Allopurinol Equivalent Anti-Xanthine Oxidase Capacity
DEIC	: Diclofenac Equivalent Inhibitory Concentration
AUD	: Absorbance Under Denaturation
AEIC	: Aqueous Extract Inhibitory Capacity
Na	: Sodium
K	: Potassium
Ca	: Calcium

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General introduction

I. General introduction

Honey is a complex natural substance produced by honeybees (Apis mellifera) from the nectar of flowers and other plant secretions. It is composed predominantly of sugars, primarily fructose and glucose, which constitute approximately 70-80% of its content, along with water, organic acids, enzymes, vitamins, minerals, and a wide range of bioactive compounds such as polyphenols and flavonoids **[1,2]**. These bioactive compounds have attracted considerable scientific interest due to their diverse pharmacological properties, including antioxidant, anti-inflammatory, antimicrobial, and enzyme-modulating activities **[3,4].** The chemical composition of honey varies according to its botanical and geographical origins, which significantly influence its therapeutic potential **[5].**

Polyphenolic compounds in honey, such as quercetin, kaempferol, and chrysin, have been identified as key contributors to its biological effects. These compounds act as antioxidants by scavenging free radicals and inhibiting oxidative stress pathways, which are implicated in various chronic diseases **[6,7]**. In addition, certain polyphenols are known to modulate enzymatic activity, including the inhibition of enzymes involved in purine metabolism and inflammatory processes **[8]**. This enzyme inhibition is particularly relevant in the context of gout, a metabolic disorder characterized by elevated serum uric acid levels, leading to the deposition of monosodium urate crystals in joints and tissues, causing acute inflammation and joint damage **[9].**

The enzymatic oxidation of hypoxanthine to xanthine and subsequently to uric acid is catalyzed by xanthine oxidase (XO), a key enzyme in purine metabolism. During these oxidation steps, molecular oxygen (O_2) is reduced, generating reactive oxygen species (ROS) such as the superoxide anion (O_2^-) **(Figure 1)**. This process not only results in to the formation of uric acid but also contributes to oxidative stress, which has been implicated in various pathological conditions including gout, cardiovascular diseases, and renal disorders. Consequently, the inhibition of xanthine oxidase is considered a therapeutic target in the management of hyperuricemia and oxidative damage . **[10-11]**

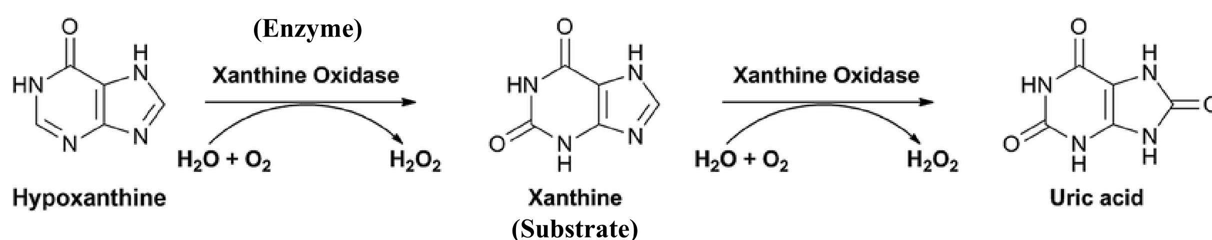

Figure 1 : Enzymatic process catalyzed by xanthine oxidase. **[12]**

Gout is a metabolic disease caused by the accumulation of uric acid, which is the final product of purine degradation. This process is catalyzed by the enzyme xanthine oxidase. When uric acid levels in the blood exceed its solubility threshold, monosodium urate crystals may be deposited in joints, leading to intense inflammation. These crystals trigger immune responses via activation of the NLRP3 inflammasome, resulting in the release of interleukin-1β (IL-1β) and other pro-inflammatory mediators. Clinically, gout is characterized by sudden, painful joint attacks, often affecting the big toe. Management includes dietary control and medications such as xanthine oxidase inhibitors (e.g., allopurinol or febuxostat) to reduce uric acid production. **[13-14]**

Data indicate that, in 2020, the age-standardized prevalence rate of gout was 659.3 cases per 100,000 population globally. This rate was 3.26 times higher in males compared to females, and it increased with age. The number of gout cases is expected to reach 95.8 million by 2050, with population growth and aging significantly contributing to this increasee**.(Figure 02) [15]**

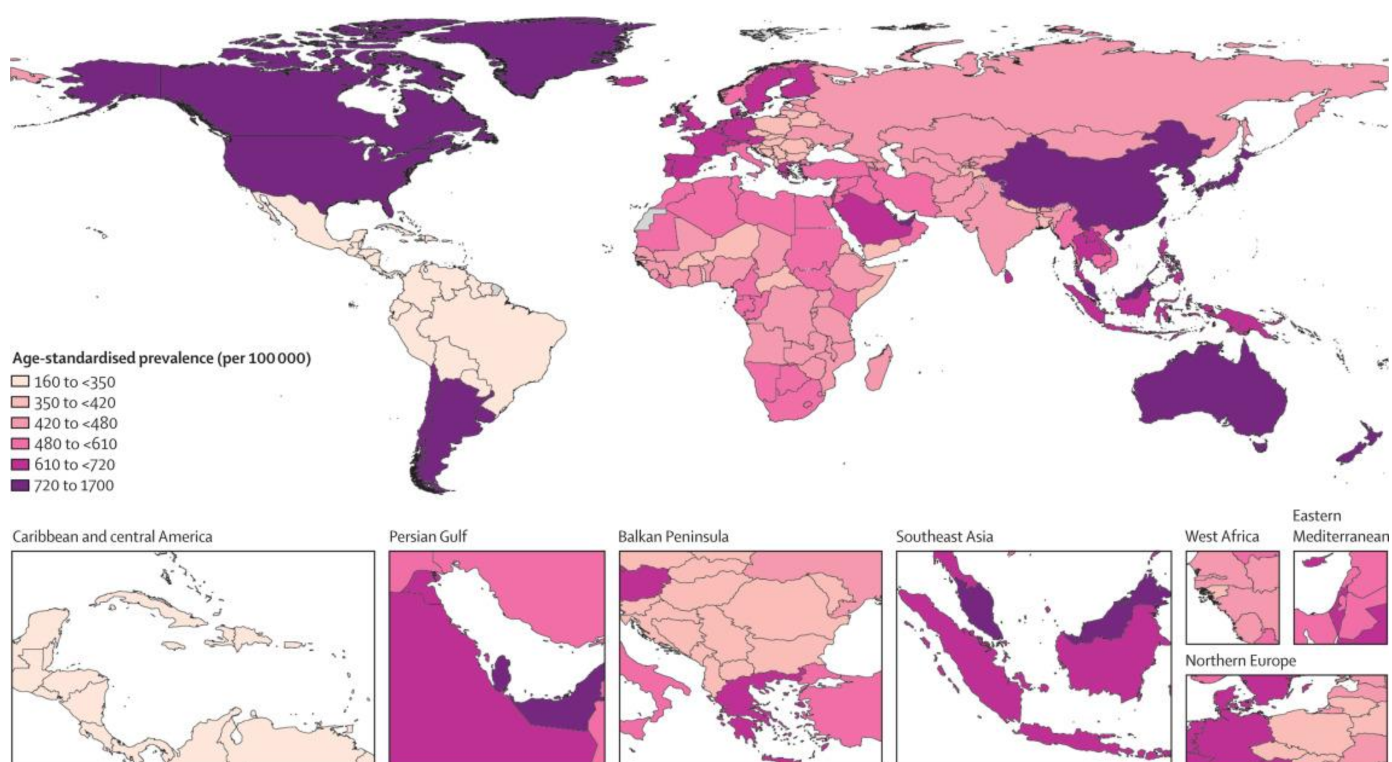

Figure 2: Age-standardised prevalence of gout by country for male and female sexes combined and all ages in 2020 **[15]**

Accordingly, regulation of xanthine oxidase activity represents a key therapeutic strategy in managing hyperuricemia and gout, as inhibiting this enzyme contributes to reducing uric acid production and mitigating the associated oxidative stress. Despite the proven efficacy of some synthetic inhibitors such as allopurinol and febuxostat, their use can lead to undesirable side

effects, including skin eruptions, hepatic dysfunction, and severe hypersensitivity reactions in certain individuals. These limitations have prompted researchers to explore natural, effective, and safer alternatives that could modulate uric acid levels without the side effects associated with pharmaceutical agents.

In this context, honey has emerged as a promising natural substance, not only for its nutritional value but also for its pharmacological potential, owing to its richness in polyphenolic and flavonoid compounds that have demonstrated inhibitory effects on several enzymes involved in inflammatory and metabolic processes.

Based on this rationale, the present dissertation aims to investigate the relationship between the botanical origin of honey and its potential inhibitory effect on xanthine oxidase activity. This investigation will be carried out through the study of six different types of Algerian honey, each derived from a distinct botanical source, by conducting the following laboratory analyses:

- ✓ quantification of total polyphenols
- ✓ metal content analysis (Na^+, K^+, Ca^{2+})
- ✓ uric acid dissolution test.
- ✓ xanthine oxidase inhibition test.
- ✓ anti-inflammatory activity assay using (BSA).
- ✓ hemolysis test.

Our manuscript begins with a general introduction that discusses general information about honey and the enzyme xanthine oxidase. The second part presents the experimental section, including materials and methods, as well as results and discussion. Finally, the thesis concludes with a general conclusion that summarizes the key findings obtained during this work and provides recommendations for its continuation in the future.

Materials and methods

II. Materials and methods

Our research work was conducted within the laboratory of fundamental sciences and the pedagogical laboratory of the department of fundamental sciences at Amar Telidji University of Laghouat, as well as the laboratory of applied sciences and didactics at the higher normal school of Laghouat.

II.1 Materials

II.1.1 Honeys

Our study was conducted on six honey samples collected from different regions (**Table 01)**:

Table 01: Presentation of the different samples of honey

Sample Code	Name of the samples	Harvest date	Harvesting region
M_1	Bunium mauritanium (تالغودة)	June 2022	Sougger - Tiaret
M_2	Ziziphus lotus + Euphobia guyoniana Boiss. (لبينة +سدر)	June 2022	Oued Mzi - Laghouat
M_3	Mountain Honey(الجبلي)	June 2024	Aflou
M_4	Eruca vesicaria ssp. الجرجير	January 2022	Illizi
M_5	Euphobia guyoniana Boiss. + Retama raetam (الرتم + اللبينة)	June 2022	Oued Twil - Laghouat
M_6	Ortica dioica (الشوكي)	June 2022	Mssila

II.1.2 Materials and chemicals

This work was supported by a number of material resources listed in **Table 02**. All chemical products are of purity grade.

Table 02: Product, apparatus and equipment used in this work

Chemicals used
Sodium hydroxide (NaOH), Monobasic potassium phosphate (KH_2PO_4), Dibasic potassium phosphate (K_2HPO_4), Hydrochloric acid (HCl), Xanthine Oxidase, Xanthine ($C_5H_4N_4O_2$), Uric acid ($C_5H_4N_4O_3$), Uric acid reagent, Bovine serum albumin (BSA), Sodium carbonates (Na_2CO_3), Folin-Ciocalteu ($H_3PW_{12}O_{40}$-$H_3PMo_{12}O_{40}$), Gallic acid ($C_7H_6O_5$), Potassium Chloride (KCl), Calcium chloride ($CaCl_2$), Sodium chloride (NaCl), Red blood cell (RBC), Allopurinol ($C_5H_4N_4O$), Febuxostat ($C_{16}H_{16}N_2O_3S$), Diclofenac ($C_{14}H_{11}Cl_2NO_2$), Dimethyl sulfoxide (DMSO), Trizma Base.
Apparatus and other equipment
Analytical balance (KERN, ABS 220-4), UV/Visible Spectrophotometer (Shimadzu 1800), pH-meter (WTW : inoLab® pH 7310), Micropipette 10-100µL (ISOLAB), Micropipette 100-1000µL (ISOLAB), Multichannel pipette 10-100µL (ISOLAB), Multichannel pipette 10-200µL (ISOLAB), Plastic UV/visible cuvette, Front (Memmert), Water bath (Memmert), beakers, Volumetric flasks (5, 10, 25, 100, 250, 500, 1000 mL), Glass test tube (5ml, 10ml, 20ml), Pissettes, Tube holder, Spatula, Watch glass, Centrifuge (Nahita Model 2650), Glass bottles, funnels, Ultrasound (5l, Joident), Agitator, 96-well microplates, Plate reader (96 and 384 wells MULTISKAN FC) with incubator Thermo, vortex.

II.2 Methods

II.2.1 Preparation of extracts

Test tubes are filled with 0.5 g of honey, followed by the addition of 20 ml of distilled water. The tubes are then placed in a water bath at 37°C for 20 minutes, followed by 3 to 4 minutes agitation using a vortex.

II.2.2 Phytochemical quantification

II.2.2.1 Determination of total phenolic content

The total phenolic content (TPC) of the samples was determined using the method described by Singleton and Rossi **[16]**, which is based on the reaction with Folin–Ciocalteu reagent. This method relies on a colorimetric change measured using a UV-Visible spectrophotometer. The assay is based on the chemical interaction between the hydroxyl groups

in phenolic compounds and the Folin–Ciocalteu reagent, resulting in the formation of a blue-colored complex that exhibits absorbance at 760 nm.

A micropipette was used to transfer 100 µL of each extract into test tubes, followed by the addition of 500 µL of Folin–Ciocalteu reagent at a concentration of 10%. After a 2-minute incubation period, 2 mL of sodium carbonate solution (Na_2CO_3) at a concentration of 2% was added to each tube, followed by immediate shaking to ensure proper mixing of the components. The tubes were then kept in the dark for 30 minutes at room temperature. After the reaction was complete, the absorbance was measured at 760 nm using a UV-Visible spectrophotometer (Shimadzu 1800), with a blank sample used as a reference. The total phenolic content of each extract was calculated using a previously prepared calibration curve of gallic acid, and the results were expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g extract).

II.2.2.2 Determination of sodium, potassium, and calcium in extracts

Sodium, potassium, and calcium concentrations were determined using flame photometry, based on the method described by Knight et al**. [17]**

This technique relies on the application of heat energy to excite electrons within the atoms of the elements, causing them to move to higher energy levels. When the electrons return to their ground states, they emit energy in the form of photons (light). Flame photometry compares the emission intensity of the sample solution containing the target element with that of a standard solution. A Sherwood® M410 flame photometer was used for this purpose, with an air-butane flame serving as the excitation source. Accordingly, the emission intensities of Na, K, and Ca were measured from various extracts diluted in distilled water, at their respective optimal wavelengths. The concentrations of sodium, potassium, and calcium in each extract were calculated using standard calibration curves prepared from sodium chloride (NaCl), potassium chloride (KCl), and calcium chloride ($CaCl_2$), respectively. The results were expressed in milligrams equivalent of sodium, potassium, and calcium per gram of extract, i.e., (mg Na/g extract), (mg K/g extract), and (mg Ca/g extract), respectively.

II.2.3 Evaluation of anti-gout activity in vitro

II.2.3.1 Evaluation of anti-xanthine oxidase activity (XO)

Total xanthine oxidase (XO) activity (µmol) was determined by measuring the increase in uric acid production at a wavelength of 550 nm in the presence of xanthine as the enzyme substrate. The inhibitory effect of the extracts on XO activity was assessed

spectrophotometrically, based on the amount of uric acid formed during xanthine oxidation, according to the method of Fridovich and Handler **[18].** For inhibition measurements, a 96-well microplate was used: 80 µL of sodium phosphate buffer (0.2 M, pH 7.8) was mixed with 20 µL of extract and 20 µL of XO solution (0.006 units in 2.5 mL phosphate buffer, pH 7.8). The mixture was pre-incubated at 37°C for 5 minutes in a Multiskan™ FC Microplate Photometer. Subsequently, 20 µL of xanthine solution (0.08 g/L, pH adjusted to 7.8 with HCl) was added, and the reaction mixture was incubated for 30 minutes at 37°C. The enzymatic reaction was then carried out using 100 µL of uric acid reagent, with the final mixture incubated for an additional 10 minutes at 37°C. Optical density was measured immediately at 550 nm. The anti-xanthine oxidase activity of the extracts was expressed as the percentage inhibition of XO activity (PI_{XO}%), calculated using the following equation:

$$\mathbf{PI_{XO}}(\%) = \frac{(\boldsymbol{A_0} - \boldsymbol{A_1})}{\boldsymbol{A_0}} \times \mathbf{100}$$

Where: A_0 is the absorbance in the absence of inhibitor.

A_1 is the absorbance in the presence of the inhibitor (extract or standard).

Anti-XO activity was also evaluated by comparison to standard calibration curves of allopurinol and febuxostat, and results were expressed as mg allopurinol equivalent capacity (mg AEC/g extract) or µg febuxostat equivalent capacity (µg FEC/g extract), respectively.

II.2.3.2 Evaluation of the uric acid dissolution effect

The anti-gout activity of the extracts was evaluated using the uric acid dissolving method, by quantifying the mass of uric acid dissolved in each extract solution. This experimental model was newly developed and applied for the first time. **[19]**

In this procedure, 5 mg of uric acid was added to 5 mL of each extract (or distilled water, as a control) in test tubes. The mixtures were centrifuged at 2000 rpm for 10 minutes, and the supernatants were collected for analysis. To determine the concentration of dissolved uric acid, 20 µL of each supernatant was mixed with 200 µL of uric acid reagent, and the mixtures were incubated in the dark at 37°C for 10 minutes. The optical density of each solution was then measured at 550 nm using a Multiskan™ FC Microplate Photometer. The amount of uric acid dissolved in each extract was calculated using a standard calibration curve for uric acid, and the

results were expressed as milligrams of soluble uric acid per liter of extract, assuming an extract concentration of 1 mg/mL.

II.2.4 Anti-inflammatory activity by inhibition of protein denaturation

To assess the anti-inflammatory activity of the extracts, 500 µL of each extract was mixed with 500 µL of bovine serum albumin (BSA; 0.1% w/v prepared in 0.5 M Tris-HCl buffer, pH 6.3). The mixtures were incubated at 37°C for 20 minutes, followed by heating at 72°C for 5 minutes. After rapid cooling in cold water for 2 minutes, the absorbance was measured at 600 nm using a UV-Visible spectrophotometer (SHIMADZU 1800), with a blank used as the reference. The anti-inflammatory effect of each extract was expressed as the percentage inhibition of BSA denaturation (PI_{BSA}%), calculated using the following equation: **[20]**

$$PI_{(BSA)}(\%) = \frac{(A_0 - A_1)}{A_0} X100$$

Where: A_0: absorbance of BSA in the absence of inhibitor,

A_1: absorbance of BSA in the presence of the inhibitor (either extract or standard).

The anti-BSA denaturation activity was further quantified using a diclofenac calibration curve, and results were expressed as milligrams of diclofenac equivalent capacity par 1 g of extract (mg DEC/g extract).

II.2.5 Evaluation of the cytotoxicity by human red blood cell hemolysis test

The cytotoxicity of the extracts was assessed by evaluating their in vitro hemolytic activity on human red blood cells (HRBCs), following the method described by Li and Liu (2008) with minor modifications. Fresh human blood was collected from a healthy volunteer into a heparinized tube and centrifuged at 2000 rpm for 10 minutes to separate the plasma. The resulting red blood cell (RBC) pellets were washed five times with phosphate-buffered saline (PBS) and subsequently diluted with PBS to yield 1:10, 1:50, and 1:100 suspensions. For the hemolysis assay, 500 µL of each erythrocyte suspension was mixed with 500 µL of extract, and the mixtures were incubated at 37°C for 60 minutes. After incubation, the tubes were centrifuged at 2000 rpm for 10 minutes, and the absorbance of the supernatant was measured at 500 nm using a UV-Vis spectrophotometer, with PBS used as the blank. **[21]**

$$\mathbf{PH}\ (\%) = \frac{\boldsymbol{A}_{\mathbf{extract\ 60\ min}}}{\boldsymbol{A}_{\mathbf{total\ hemolysis\ 60\ min}}} \mathbf{X100}$$

As a positive control for total hemolysis, 500 µL of RBC suspension was incubated with 500 µL of distilled water (no extract). The percentage hemolysis (PH%) induced by each extract was then calculated relative to total hemolysis using the following formula:

Where: A_{sample}: is the absorbance of the extract-treated sample

A_{total}: is the absorbance of the total hemolysis control (distilled water-treated RBCs)

II.2.6 Statistical analysis

All test results are presented as mean ± standard deviation (SD) based on three replicates. Data analysis was performed using Excel 2018. Descriptive statistics were carried out using XLSTAT 2018.5.03, with a tolerance level of 0.00001 and a 95% confidence interval, to calculate the Pearson correlation coefficients and conduct a principal component analysis (PCA). To compare group means, one-way analysis of variance (ANOVA) followed by Tukey's post hoc test was applied.

Results and discussion

III. Results and discussion

III.1 Phytoconstituents quantification

III.1.1 Total phenolic content

The total phenolic content (TPC) of the honey samples was determined using the Folin–Ciocalteu reagent method and expressed as mg GAE/g extract. The calibration curve of gallic acid **(Figure 1)** displayed excellent linearity ($R^2 = 0.9999$), validating the accuracy of the measurement method.

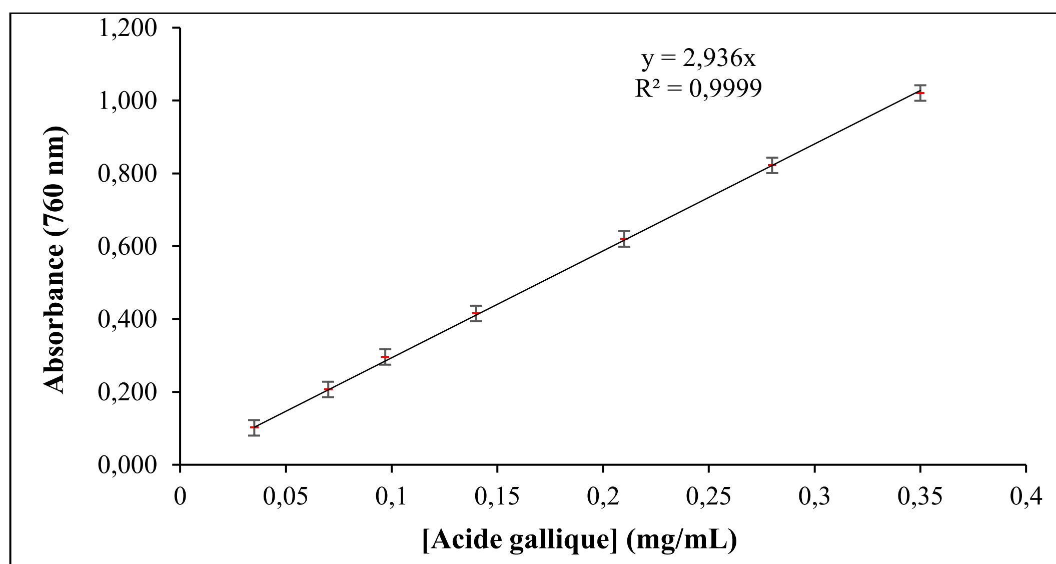

Figure 3: Calibration curve of gallic acid

The results of total phenolic contents expressed as milligrams of gallic acid equivalent per gram of extracted material are summarized in the **Table 03**.

Significant differences among the six honey samples ($p < 0.0001$) revealed substantial variation in TPC depending on botanical origin. M_1 had the highest phenolic content at 2.203 ± 0.068 mg GAE/g, followed by M_5 at 1.340 ± 0.046 mg GAE/g, M_3 at 1.275 ± 0.013 mg GAE/g, M_2 at 1.173 ± 0.019 mg GAE/g, M_6 at 1.099 ± 0.022 mg GAE/g, and the lowest M_4 at 0.813 ± 0.025 mg GAE/g.

A several authors have explained these differences by the variability in floral sources, environmental conditions, and the chemical composition of the nectars collected by the bees **[22] [23] [24] [25]**. Telgouda and Retama-based honeys showed superior phenolic richness, which could be linked to their botanical specificity and possible abundance of secondary metabolites with phenolic structures.

Table 3: Total phenolic contents in the studied extracts.

Extract	Total phenolic mg GAE/g Extract
M_1	2.203±0.068[A]
M_2	1.173±0.019[CD]
M_3	1.275±0.013[BC]
M_4	0.813±0.025[E]
M_5	1.34±0.046[B]
M_6	1.099±0.022[D]

The results of the tests performed are expressed as mean ± standard deviation, n = 3. Mean values followed by a different uppercase letter are significantly different, in same column ($p < 0.0001$).

On the other hand, honeys such as Eruca vesicaria may possess a less complex phenolic profile, resulting in a lower TPC value. **[26]**

These findings are consistent with several studies indicating that floral origin plays a determining role in the phenolic composition of honey. Factors such as soil type, altitude, climate, and floral diversity directly influence the synthesis and transfer of phenolic compounds into the honey matrix.**[27]**

A recent study conducted by Tananaki et al. (2024) on nine monofloral honeys from Greece **(Figure 4)** reported TPC values ranging from approximately 6 to 203.8 mg GAE/100 g honey. Oak honey exhibited the highest TPC (~203.75 mg GAE/100 g), while citrus honey showed the lowest (~11 mg GAE/100 g). When converted to comparable units, the TPC of M_1 in the present study (~220 mg GAE/100 g) was found to be slightly higher than that of oak honey, suggesting a particularly rich phenolic profile. In contrast, M_4 corresponded to ~81 mg GAE/100 g, which is moderate compared to the Greek dataset, ranking higher than citrus but lower than oak or chestnut honeys**.[28]**

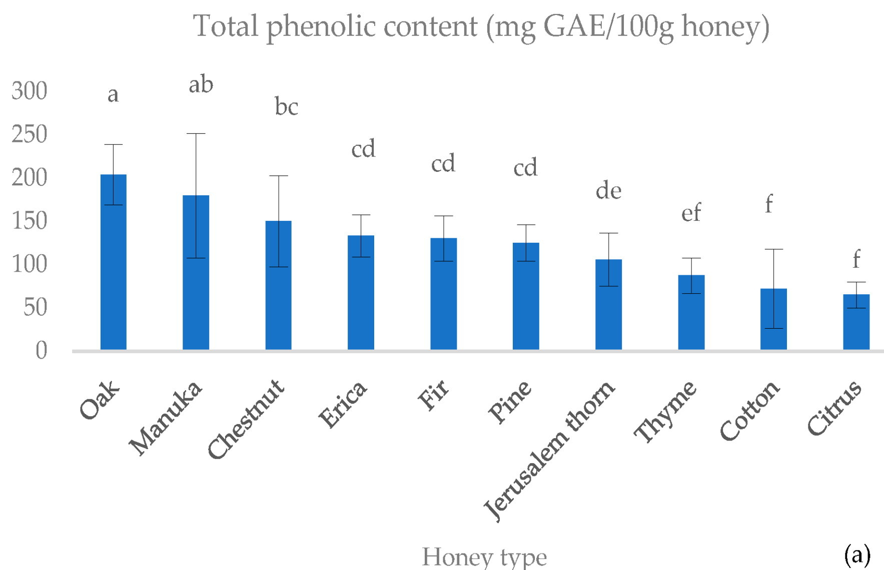

Figure 4: Total phenolic content (mg GAE/100g honey) **[28]**

These results illustrate the strong influence of botanical origin on the phenolic content of honey and highlight the potential of certain Algerian honeys, such as Telgouda, as valuable sources of bioactive phenolic compounds.

According to several recent studies, the determination of phenolic compounds by the Folin-Ciocalteu reagent in honey is influenced by pH and reducing sugars. At alkaline pH, phenols react better but sugars also interfere, resulting in overestimation. Slightly less alkaline conditions reduce these interferences and give a more reliable measurement. **[29,30,31]**

III.1.2 Mineral Content

The mineral composition of honey is an essential quality indicator that reflects both botanical and geographical origin **[32]**. In the current study, the levels of sodium (Na), potassium (K), and calcium (Ca) were evaluated in six honey samples derived from different floral sources. Quantification was based on calibration curves exhibiting excellent linearity for all three elements ($R^2 > 0.998$), confirming the precision and reliability of the analytical method used (**Figure 5**).

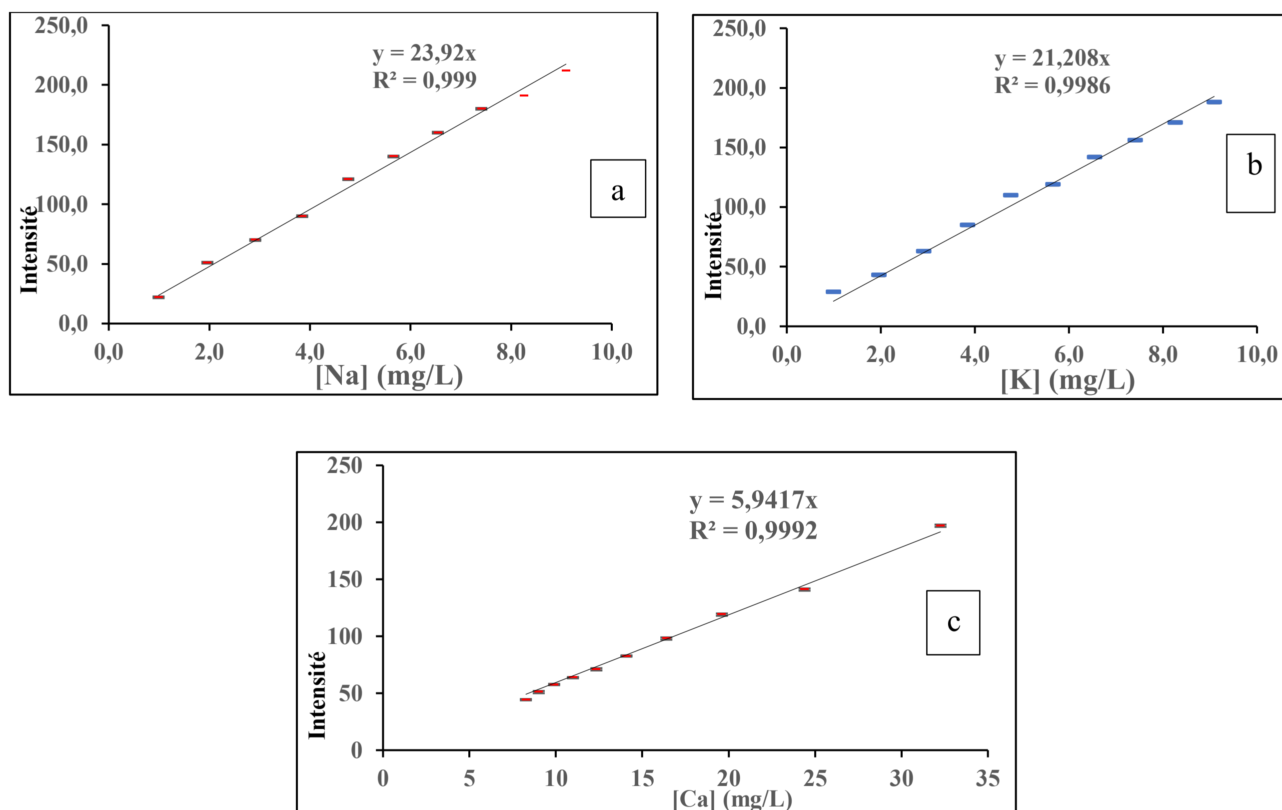

Figure 5: Sodium (a), Potassium (b) and Calcium (c) calibration curves

A significant variation was observed in sodium concentrations across the studied samples ($p<0.0001$). The highest sodium content was recorded in M_5 with a value of 0.469 ± 0.021 mg/g, placing it in a distinct statistical group (A). M_6 followed with 0.307 ± 0.009 mg/g (group B). The remaining samples, including M_1, M_2, M_3, and M_4, presented significantly lower sodium levels ranging between 0.100 and 0.129 mg/g (group C) (**Table 4**). These differences may be attributed to the mineral content of the soil in which the respective floral sources grow, as well as the ion-exchange potential of the nectar collected by bees.**[33]**

Table 4 : Sodium, potassium and calcium contents in the studied mixture extracts

Extract	**Sodium** (mg of Na / g of honey)	**Potassium** (mg of K / g of honey)	**Calcium** (mg of Ca /100g of honey)
M_1	0.116 ± 0.003^{C}	4.227 ± 0.044^{B}	48.134 ± 0.337^{A}
M_2	0.129 ± 0.005^{C}	3.222 ± 0.044^{C}	0.036 ± 0.003^{B}
M_3	0.1 ± 0.007^{C}	1.65 ± 0.023^{E}	0.079 ± 0.02^{B}
M_4	0.127 ± 0.007^{C}	1.664 ± 0.04^{E}	0.03 ± 0.004^{B}
M_5	0.469 ± 0.021^{A}	4.911 ± 0.04^{A}	0.049 ± 0.004^{B}
M_6	0.307 ± 0.009^{B}	4.06 ± 0.04^{D}	0.334 ± 0.004^{B}

The results of the tests performed are expressed as mean ± standard deviation, $n = 3$. Mean values followed by a different uppercase letter are significantly different, in same column ($p < 0.0001$).

Potassium was the most abundant mineral in all samples (**Table 4**), in agreement with previous findings in honey mineral profiling [**34,35,36**]. The highest concentration was found in M_5 (4.911 ± 0.040 mg/g, group A), followed by M1 (4.227 ± 0.044 mg/g, group B). M_6 and M_2 occupied intermediate positions (4.060 ± 0.040 mg/g and 3.222 ± 0.044 mg/g, respectively), while M3 and M_4 recorded the lowest potassium levels (1.650 ± 0.023 mg/g and 1.664 ± 0.040 mg/g, group E). Potassium is known to be a predominant macroelement in honey due to its natural abundance in plant tissues and its water solubility, which facilitates its transfer into nectar. The high potassium levels observed in M_5 and M_1 suggest that their floral origins are particularly rich in this element, possibly due to the physiological characteristics of Retama and Telgouda plants. **[37]**

Calcium content also varied significantly between samples ($p < 0.0001$). M_1 exhibited a markedly elevated level of calcium (48.134 ± 0.337 mg/100g), which is far higher than in any other sample (group A). The rest of the samples presented considerably lower values ranging from 0.030 ± 0.004 mg/100g to 0.079 ± 0.020 mg/100g (group B), without statistically significant differences among them. The exceptional calcium level found in M_1 may be explained by the botanical properties of the Telgouda plant or by its environmental conditions, such as calcium-rich soil. This result may also reflect the potential bioaccumulation capacity of this floral source. **[38]**

When compared to honeys from Middle Anatolia, Turkey, where potassium ranges from 161 to 598 mg/kg, calcium from 40 to 190 mg/kg, and sodium from 9 to 46 mg/kg, the values observed in the current samples—particularly for potassium and calcium—are remarkably higher. **[39]**

In Venezuelan samples, average levels were around 1774 mg/kg for K, 353 mg/kg for Na, and 0.76 mg/kg for Cu, confirming the quantitative predominance of K **[40]**. A detailed report covering several regions of the world corroborates this pattern: K largely dominates the mineral profile, followed by sodium and calcium. Copper, however, is still present in trace amounts but remains of interest for its potential role in differentiating the botanical origin of honey **[41,42,43]**.

These differences may be attributed to environmental factors, including soil mineral richness, specific floral sources, and climatic conditions in the regions where the honeys were produced **[44]** . Darker honeys or those derived from resinous plants have been previously associated with higher mineral contents, which could explain the substantial enrichment observed in certain samples from the present study**. [45]**

III.2 In vitro biological activities

III.2.1 Evaluation of anti-gout activity

Previous studies have reported that several types of honey have been reported to contain bioactive compounds with potential anti-gout properties **[46] [47] [48]**, particularly through the inhibition of xanthine oxidase. However, to the best of our knowledge, limited studies have specifically evaluated the anti-gout activity of different floral honeys. Therefore, this study aims to assess and compare the xanthine oxidase inhibitory effect of six honey samples of various botanical origins, without any mixture with plant extracts.

III.2.1.1 Uric acid dissolution activity

This study represents the first application of the uric acid dissolution (AU) test in honey research. The uric acid dissolution activity of the tested samples was determined using a spectrophotometric method based on a calibration curve of uric acid that exhibited excellent linearity ($R^2 = 0.9992$), confirming the reliability and precision of the assay **(Figure 6).** The results, expressed in mg of uric acid dissolved per gram of extract (mg AU/g extract), showed statistically significant differences among the samples ($p < 0.0001$), reflecting the impact of botanical origin on the urate-dissolving capacity. **(Table 5)**

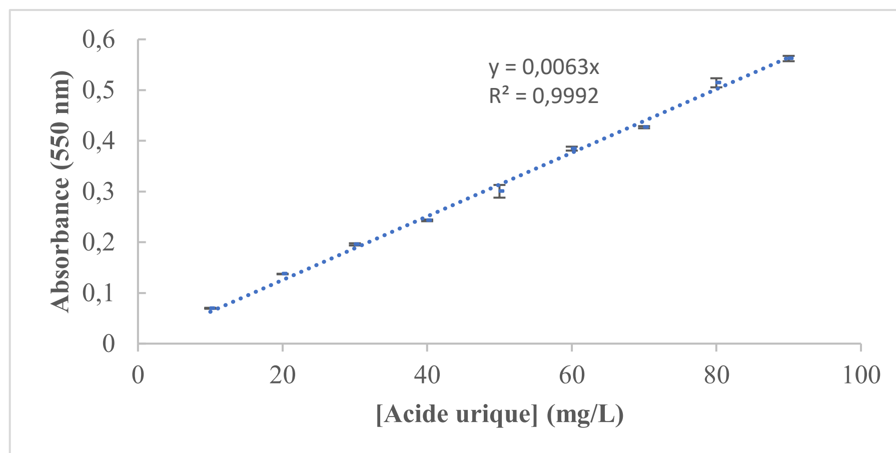

Figure 6 : Calibration curve of uric acid

The sample M_6 exhibited the highest dissolution activity, with a value of 1.51 ± 0.002 mg AU/g extract, and was placed in a distinct statistical group (A) **(Table 5)**. This result suggests the presence of a high concentration of active compounds capable of increasing UA solubility, possibly including polar phenolic compounds, flavonoids, or organic acids. These compounds are known to enhance uric acid dissolution through mechanisms such as hydrogen bonding, pH modulation, or formation of soluble complexes.**[49]**

M_5 recorded a relatively high value as well (1.213 ± 0.028 mg AU/g extract) **(Table 5)**, classified in group B, indicating notable dissolution potential. The observed activity in both M_5 and M_6 may be attributed to their higher content of bioactive molecules, consistent with the results of phenolic content analysis, suggesting a correlation between the richness in secondary metabolites and the capacity to dissolve uric acid.

Intermediate activities were recorded in M_4 and M_1, with values of 0.967 ± 0.017 and 0.912 ± 0.024 mg AU/g extract, respectively **(Table 5)**. These samples fell into statistical groups C to D, suggesting a moderate presence of urate-solubilizing constituents. M_2 and M_3 exhibited the lowest dissolution capacities, with values of 0.879 ± 0.013 and 0.853 ± 0.029 mg AU/g extract, respectively, indicating limited ability to interact with or solubilize UA crystals.

Table 5: Uric acid concentration in extracts

Extract	mg AU/g extract
M_1	0.912 ± 0.024^{CD}
M_2	0.879 ± 0.013^{C}
M_3	0.853 ± 0.029^{D}
M_4	0.967 ± 0.017^{DE}
M_5	1.213 ± 0.028^{B}
M_6	1.51 ± 0.002^{A}

The results of the tests performed are expressed as mean ± standard deviation, n = 3. Mean values followed by a different uppercase letter are significantly different, in same column ($p < 0.0001$).

The differences observed among the samples may result from variation in the qualitative and quantitative composition of phytochemicals, particularly phenolic acids, sugars, and organic acids, as well as from factors related to nectar source, environmental conditions, and floral physiology. The botanical origin appears to be a determining factor influencing the extract's ability to alter UA solubility, potentially through synergistic interactions between multiple compounds.

These findings highlight the potential role of specific natural extracts in modulating UA solubility, which may be of interest in the context of functional food development or as complementary agents in the management of hyperuricemia (Antigout) . **[50]**

III.2.1.2 Xanthine oxidase inhibitory effect

Gout is a metabolic disorder characterized by the accumulation of uric acid crystals in joints, causing inflammation and intense pain. The primary source of uric acid in the body is the enzymatic oxidation of hypoxanthine and xanthine to uric acid, a reaction catalyzed by xanthine oxidase (XO). Therefore, inhibiting XO activity is a widely accepted therapeutic strategy for managing gout and hyperuricemia.**[51]**

Currently, Allopurinol is clinically prescribed as an XO inhibitor; however, its prolonged use has been associated with adverse effects, including hepatotoxicity and hypersensitivity reactions. This has driven interest in identifying safer natural alternatives, with honey gaining

attention as a potential candidate due to its richness in phenolic compounds and other bioactive constituents known for their antioxidant properties and ability to inhibit key enzymes involved in uric acid production.

In this study, six honey samples from different botanical origins were evaluated for their potential to inhibit xanthine oxidase. The results of the xanthine oxidase (XO) inhibition assay based on AEAXOC values **(Table 6)** revealed clear and significant differences among the six honey samples studied **(Figure 7)**, reflecting the considerable variation in their chemical composition and botanical origins. The highest value was recorded for sample M_6 (1.953±0.090 mg AAE/g), indicating its strong ability to inhibit the enzyme's activity and suggesting that it contains a high concentration of bioactive compounds capable of interacting with the enzyme's active site, thereby slowing down or preventing the oxidation process it catalyzes.

It was followed by sample M_5 (1.760±0.067 mg AAE/g), which also exhibited high inhibitory activity, albeit slightly lower than M_6. Sample M_2 showed moderate value of 1.651±0.029mg AAE/g, respectively, suggesting moderate inhibitory potential that could still be sufficient for certain therapeutic or preventive applications, but less potent compared to M_6 and M_5.

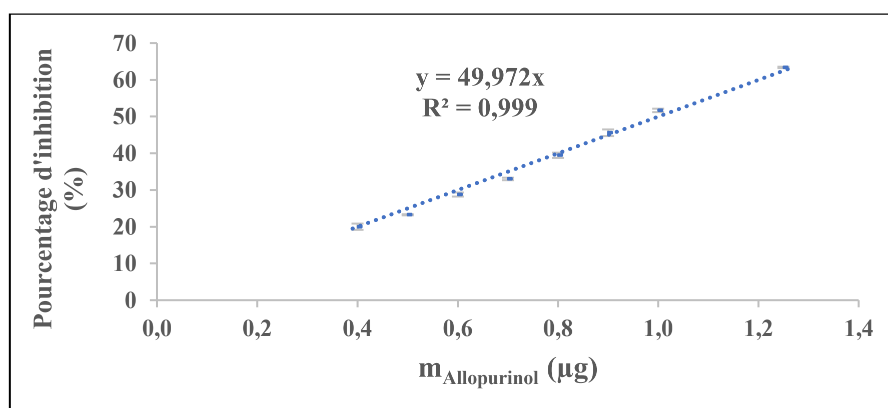

Figure 7: Calibration curve of Allopurinol

In contrast, samples M_1, M_3 and M_4 recorded the lowest values in this assay, with 1.198±0.058 mg AAE/g, 1.311±0.065 mg AAE/g and 1.283±0.065 mg AAE/g, respectively, indicating weak XO inhibition. This could be due to a lower concentration of active compounds responsible for this activity or differences in the nature of these compounds in these samples.

These results show that XO inhibitory activity varies significantly depending on the honey's origin and production conditions. Previous studies have highlighted that the variation in

biological activity among honey types is largely due to differences in chemical composition resulting from floral diversity, as well as environmental factors such as climate, soil type, and the stage of maturity at harvest **[52][53].**

The ability of some samples, such as M_6 and M_5, to achieve high AEAXOC values suggests their potential as effective natural sources for XO inhibition, opening the possibility for their use in reducing uric acid accumulation in the blood and thus helping to prevent the development of gout and other hyperuricemia-related complications **[54][55]**. However, selecting the most suitable type for therapeutic use should not rely solely on efficacy, but also take into account the results of biological safety tests performed on these samples.

Table 6: In vitro anti-gout activity measured by xanthine oxidase inhibition test

Extract	AEAXOC (mg allopurinol / g Extract)
M_1	$1.198{\pm}0.058^{E}$
M_2	$1.651{\pm}0.029^{C}$
M_3	$1.311{\pm}0.065^{D}$
M_4	$1.283{\pm}0.065^{DE}$
M_5	$1.760{\pm}0.067^{B}$
M_6	$1.953{\pm}0.090^{A}$

The results of the tests performed are expressed as mean ± standard deviation, n = 3. Mean values followed by a different uppercase letter are significantly different, in same column ($p < 0.0001$).

When comparing these results with the findings of Zhang et al. (2019) **[53]**, who evaluated the XO inhibitory activity of several honey types, it was found that the values recorded in the present study, especially for M_6 and M_5, exceeded most of those obtained in their study, where values ranged from 1.42 ± 0.05 mg AAE/g to 1.95 ± 0.03 mg AAE/g. This agreement in the ranking of the most active samples, combined with the higher values found here, may be attributed to differences in chemical composition resulting from botanical and climatic diversity, confirming the role of geographical and environmental factors in determining honey's inhibitory activity.

Recent research has highlighted the inhibitory capacity of honey against the enzyme xanthine oxidase (XO). This activity appears to be strongly linked to the phenolic content and

botanical origin of the honey. For example, a study on Moroccan Euphorbia honeys (E. officinarum and E. resinifera) showed that extracts, particularly those from E. officinarum, exhibited close to 100% XO inhibition **[56].** Similarly, Petrillo et al. **[57]** observed marked XO inhibition in Sardinian honeys (Arbutus, Eucalyptus, Asphodelus, Thistle, and Sulla), with IC_{50} values comparable to those of allopurinol. Chestnut honey also demonstrated strong inhibitory activity in a biochemical study by Sahin (2016) **[58]**, confirming that some floral sources promote this property. Furthermore, honeys from arid areas showed higher anti-XO capacity than honeys from other regions, suggesting a geoclimatic influence **[59].** Finally, the study by Majid et al. **[60]** on melipona (stingless bee) honeys from different floral origins revealed that some of them outperformed allopurinol in terms of enzyme inhibition, reinforcing the interest of honey as a natural alternative to synthetic XO inhibitors.

III.2.2 In vitro anti-inflammatory activity measured by protein denaturation assay

Inflammation is commonly associated with protein denaturation, during which the structure of native proteins is altered, causing loss of their biological function. In this context, the inhibition of protein denaturation, specifically bovine serum albumin (BSA), was used as a model to evaluate the in vitro anti-inflammatory activity of six honey samples from different botanical origins. The results were expressed as Diclofenac Equivalent Inhibitory Concentration (DEIC), in mg diclofenac per 100 g of extract of honey. The calculation of DEIC values was based on the diclofenac calibration curve illustrated in **Figure 8**.

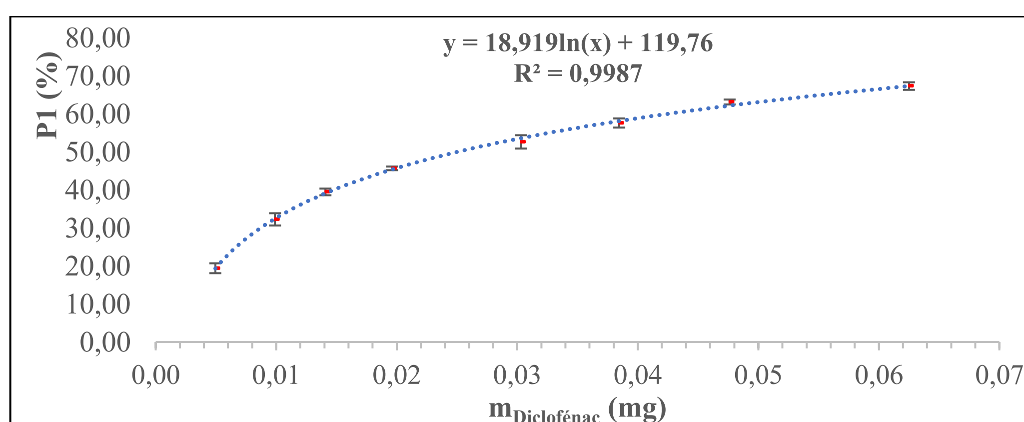

Figure 8: Calibration curve of BSA

The DEIC values of the tested honeys ranged from 2.274 ± 0.164 (M_3) to 14.146 ± 1.052 (M1) mg diclofenac / 100 g (**Table7)**, with a statistically highly significant difference between

samples ($p < 0.0001$). These findings indicate a variable anti-inflammatory potential among honeys, likely influenced by their phytochemical compositions.

The highest DEIC value was recorded for M_1, suggesting a potent inhibitory effect on protein denaturation. This could be attributed to the high content of bioactive compounds such as flavonoids, phenolic acids, and volatile oils known for their anti-inflammatory action**[61]**. In contrast, M_3 displayed the lowest inhibitory capacity, possibly due to the reduced content or weaker bioactivity of its phytochemicals.

Intermediate values were observed for M_4 (8.569 ± 0.802 mg diclofénac / 100g Extract), M_2 (6.836 ± 1.043 mg diclofénac / 100g Extract), M_5 (4.255 ± 0.236 mg diclofénac / 100g Extract), and M_6 (3.914 ± 0.362 mg diclofénac / 100g Extract), reflecting a moderate inhibition capacity. These results suggest a partial correlation between botanical origin and anti-inflammatory efficacy.

Table 7: Anti-inflammatory activity determined by BSA denaturation assay

Extract	DEIC (mg diclofénac / 100g Extract)
M_1	14.146 ± 1.052^{A}
M_2	6.836 ± 1.043^{B}
M_3	2.274 ± 0.164^{D}
M_4	8.569 ± 0.802^{B}
M_5	4.255 ± 0.236^{C}
M_6	3.914 ± 0.362^{CD}

The results of the tests performed are expressed as mean ± standard deviation, n = 3. Mean values followed by a different uppercase letter are significantly different, in same column ($p < 0.0001$).

These findings are in agreement with the results reported by El-Haskoury et al. (2020) **[62]**, who investigated the anti-inflammatory activity of several Moroccan honeys using the same BSA denaturation method. In their study, the DEIC values ranged from 4.75 to 12.94 mg diclofenac/100 g, depending on the floral source and geographic origin. The value recorded in our study for M_1 (14.146 ± 1.052 mg/100 g) exceeds this range, suggesting a particularly high anti-inflammatory potential**.[63]**

On the other hand, samples such as M_3 (2.274 ± 0.164 mg/100 g) fall below the range reported by El-Haskoury et al., indicating a weaker capacity to inhibit protein denaturation. These differences might arise from several factors such as nectar origin, harvest time, environmental conditions, and storage, all of which are known to affect the phytochemical profile of honey**. [64] [65] [66]**

A study conducted on two monofloral honeys from Algeria (arbutus and heather) showed a significant inhibition of protein denaturation, reaching 60.23% and 55.61%, respectively, with IC_{50} values of 0.29 and 0.38 mg/mL. These results, although lower than those obtained with diclofenac sodium (IC_{50} = 0.031 mg/mL), indicate an interesting anti-inflammatory potential attributed in particular to their richness in phenolic compounds **[67]** . Similarly, citrus monofloral honeys also revealed notable anti-inflammatory activity according to the protocol of Fratianni et al., demonstrating a dose-dependent inhibition of BSA denaturation, with variable efficacy depending on the botanical origin **[68].** These results confirm that some honeys can act as natural inhibitors of inflammatory processes, partly due to their richness in flavonoids and phenolic acids.

III.2.3 In vitro evaluation of cytotoxicity by red blood cell hemolytic assay

The red blood cell hemolysis assay is considered a reliable biological method for evaluating the cytotoxicity of natural compounds. This test is based on the ability of certain substances to disrupt the red blood cell membrane, leading to hemoglobin release, and it is widely used to assess the cellular safety of bioactive materials, particularly those with potential therapeutic applications.

The hemolysis rate was assessed by measuring the uptake of hemoglobin released by red blood cells through hemolysis, compared to distilled water. The results are presented in **Table8**.

Table 8 : In vitro cytotoxicity activity measured by red blood hemolysis assay

Extract	HRBCs (10%)	HRBCs (50%)	HRBCs (100%)
M_1	$0.000±0.000^{C}$	$27.969±3.340^{A}$	$106.110±3.075^{A}$
M_2	$0.000±0.000^{C}$	$18.455±2.559^{B}$	$89.226±5.224^{B}$
M_3	$0.000±0.000^{C}$	$0.000±0.000^{D}$	$0.000±0.000^{C}$
M_4	$8.796±0.265^{A}$	$2.109±0.192^{D}$	$20.576±1.346^{B}$
M_5	$0.000±0.000^{C}$	$0.000±0.000^{D}$	$0.000±0.000^{C}$
M_6	$1.332±0.186^{B}$	$54.278±2.876^{C}$	$63.035±2.415^{AB}$
Diclofenac	5.505±0.545	7.387±0.826	40.501±2.694
Allopurinol	5.443±0.247	7.678±0.288	25.738±0.931
Febuxostat	0.000±0.000	0.000±0.000	0.000±0.000

The results of the tests performed are expressed as mean ± standard deviation, n = 3. Mean values followed by a different uppercase letter are significantly different, in same column ($p < 0.0001$).

Recent studies have shown that honey contains biologically active compounds, particularly phenolic acids and flavonoids, which contribute to stabilizing red blood cell membranes and protecting them from oxidative damage, thus reducing cytotoxicity **[69]**.

In the present study, six honey extracts of different botanical origins were evaluated at concentrations of 10%, 50%, and 100%. The results demonstrated clear variation in hemolytic activity across samples **(Table 8).** Extracts M_1, M_2, M_3, and M_5 showed no hemolytic activity at 10% and 50% concentrations, with hemolysis values of 0.000 ± 0.000%, indicating excellent membrane stability and negligible cytotoxic potential. Conversely, extract M_6 showed marked hemolysis at 50% and 100% concentrations (54.278 ± 2.876% and 63.035 ± 2.415%, respectively), suggesting significant cytotoxic effects at high doses. Extract M_4 showed moderate hemolytic activity, with values ranging from 8.796% to 20.576%, increasing with concentration.

When compared to reference drugs, both Diclofenac and Allopurinol exhibited significant hemolysis ranging from 5.44% to 40.5%, indicating that several honey extracts—particularly M_1, M_3, and M_5—demonstrated greater cellular safety than some commonly used

pharmaceutical agents. Febuxostat, on the other hand, showed no hemolytic activity at any concentration, supporting its profile as a low-toxicity drug in this context.

These results suggest that the low cytotoxicity observed in some samples may be attributed to their content of natural antioxidants, which contribute to membrane stabilization and resistance to oxidative stress **[70]**. In contrast, the high hemolytic activity of M_6 may be due to the presence of surface-active components or a higher concentration of bioactive substances capable of disrupting the cell membrane **[71]**.

Several in vitro studies have used the red blood cell hemolysis method as an indicator of cytotoxicity or, conversely, the membrane-stabilizing power conferred by honey. For example, Manukumar & Umesha **[72]** evaluated several varieties (Manoflora, Polyflora, Polyflora forest, Processed) and found an inhibition of hemolysis induced by a hypotonic solution of up to 97.76% at 50 mg/mL, with the Polyfloral forest variety standing out in particular; it also protected red blood cells against thermal hemolysis (21.23%) compared to aspirin (39.38%). Another study by Hilary et al. **[73]** conducted on heterofloral honeys from arid regions measured the protection of erythrocyte membranes against oxidative damage in terms of Trolox equivalent demonstrate that one honey reached 1.300 ± 0.042 mM TE/g, and another 1.122 ± 0.018 mM TE/g, well above the values observed for honeys from temperate regions (0.907 and 0.668 mMTE/g). These results suggest that some honeys, particularly those from arid regions, offer significant membrane protection, likely related to their high antioxidant activity.

III.3 Correlations between all obtained results

The correlation analysis presented in (Table 9) and (Table 10) highlights several statistically significant relationships between phenolic content, mineral composition, and the biological activities of the honey samples.

Table 9: Pearson correlation coefficient matrix (r) values from the 10 different assays (Values in bold are different from 0 at a significance level of α=0.05)

Variables	TPC	Na	K	Ca	AUD	AEIC	DEIC	pH (Sang 10%)	pH (Sang 50%)	pH (Sang 100%)
TPC	**1**									
Na	-0.1151	**1**								
K	0.4938	0.7111	**1**							
Ca	**0.9212**	-0.3012	0.3364	**1**						
AUD	-0.2374	**0.7417**	0.5417	-0.2684	**1**					
AEIC	-0.3641	**0.7537**	0.5545	-0.5352	0.7920	**1**				
DEIC	0.6298	-0.4012	0.1721	**0.8518**	-0.3637	-0.5756	**1**			
pH (Sang10%)	-0.5695	-0.2214	-0.5483	-0.2358	-0.0385	-0.3129	0.1728	**1**		
pH (Sang 50%)	0.1819	0.0732	0.4294	0.2520	0.6207	0.4329	0.1451	-0.2184	**1**	
pH (Sang100%)	0.5244	-0.3562	0.3430	0.6343	-0.0705	-0.0509	0.6806	-0.2533	0.6650	**1**

In (Table 10), a strong positive correlation was observed between TPC and calcium ($r = 0.9212$, $p < 0.01$), indicating that phenolic compounds and calcium are frequently co-accumulated, which has been previously reported in honeys from different floral origins **[74].** A strong positive correlation was also recorded between sodium and AUD activity ($r = 0.7417$, $p < 0.01$). This relationship may indicate that sodium ions enhance

the solubility of uric acid crystals through ionic interactions that increase dissolution capacity **[75].** Furthermore, a strong association was recorded between xanthine oxidase inhibitory activity (AEIC) and sodium ($r = 0.7537$, $p < 0.01$), underlining the possible synergistic contribution of mineral ions to enzyme inhibition, as discussed in antioxidant-enzyme interaction studies **[76].** Finally, calcium was found to be strongly correlated with DEIC ($r = 0.8518$, $p < 0.01$). This association underlines the potential role of calcium in enhancing the inhibitory activity, possibly by synergizing with phenolic compounds and other bioactive molecules present in honey **[77].**

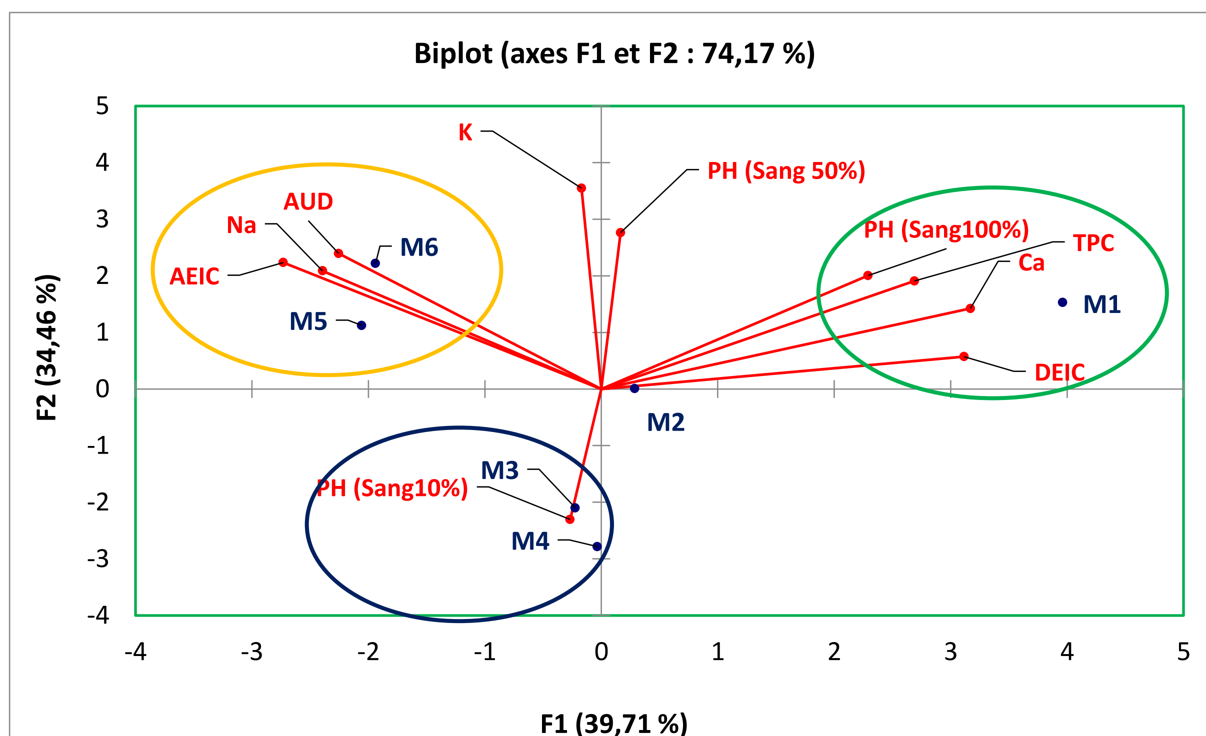

Figure 9: Projections of variables and individuals in the F1-F2 factorial plane (PCA).

To complement the correlation matrix, Principal Component Analysis (PCA) was applied, and the results are displayed in Figure 10. The biplot shows the distribution of variables and honey samples along the first two principal components, accounting for 74.17% of the total variance.

The projection of TPC and Ca in close proximity **(circle green)** reaffirms their strong positive correlation. Likewise, AEIC and DEIC aligned with sodium **(circle orange)**, and AUD was oriented along the potassium vector **(circle orange)**. These spatial patterns support the statistical associations described earlier.

Sample distribution further validated functional grouping: M_6 was placed near AUD and K **(circle orange)**, indicating high solubility activity, M_5 aligned closely with AEIC **(circle orange)**, confirming strong enzyme inhibition, while M_2, M_3, and M_4 were located away from the core functional vectors **(circle blue)**, consistent with their limited bioactivities. (**Figure 9**)

Table 10: Uric acid dissolution by different honey extracts under three experimental conditions (F1, F2, F3)

Extract	F1	F2	F3
M_1	**0.8581**	0.1275	0.0028
M_2	0.0300	0.0000	**0.0345**
M_3	0.0067	**0.5969**	0.1571
M_4	0.0001	**0.7270**	0.1353
M_5	**0.4436**	0.1325	0.3111
M_6	0.3304	**0.4338**	0.2019

The results of the tests performed are expressed as mean ± standard deviation, n = 3. Mean values followed by a different uppercase letter are significantly different, in same column ($p < 0.0001$).

In (Table 11), uric acid dissolution activity showed a strong positive correlation with TPC ($r = 0.8581$, $p < 0.01$), supporting the hypothesis that phenolic compounds contribute to the solubilization of uric acid crystals **[78].** In contrast, the correlation between uric acid dissolution

and sodium was very weak ($r = 0.0345$), suggesting that sodium plays little to no role in this biological process **[79].** Moderate correlations were observed between uric acid dissolution and potassium ($r = 0.5969$, $p < 0.05$) as well as between dissolution activity and calcium ($r = 0.4436$, $p < 0.05$), indicating that these minerals might have a supportive but not predominant effect [**80**]. A stronger correlation was found between dissolution activity and DEIC ($r = 0.7270$, $p < 0.01$), reflecting the partial overlap of mechanisms between crystal solubilization and enzymatic inhibition **[81].** Finally, pro-hemolytic activity was moderately correlated with calcium ($r = 0.4338$, $p < 0.05$), which could suggest that higher calcium concentrations contribute to membrane destabilization in erythrocytes **[82].**

Overall, these findings emphasize the multifactorial interactions between phenolic compounds, mineral content, and the biological effects of honey, demonstrating that both organic and inorganic constituents jointly influence its therapeutic potential.

Conclusion

IV. Conclusion

The growing burden of chronic diseases such as hyperuricemia and inflammation has intensified the search for safer and more accessible therapeutic alternatives. In this context, natural products, especially honey, have attracted increasing interest due to their richness in bioactive compounds and their historical use in traditional medicine. Algeria, with its diverse flora, offers a unique opportunity to valorize local honeys as potential natural remedies.

The present work aimed to explore the anti-hyperuricemic and anti-inflammatory potential of six honey samples of different botanical origins. The evaluation was based on in vitro assays targeting xanthine oxidase inhibition and protein denaturation, as well as the quantification of total phenolic content. The results obtained throughout this study led to the following conclusions:

- ✓ A significant variation in biological activity was observed among the studied honey samples, confirming the impact of botanical origin on their therapeutic properties.
- ✓ Honey derived from Ortica dioica (stinging nettle) showed the highest biological efficacy, particularly in:
- ▪ Xanthine oxidase inhibition (1.953 mg allopurinol equivalent/g),
- ▪ Uric acid dissolution (1.51 mg/g),
- ▪ but also exhibited strong cytotoxic effects at high concentrations, which limits its direct therapeutic application without careful dose control.
- ✓ In contrast, honey from Euphobia guyoniana and Retama raetam demonstrated a well-balanced profile, combining:
- ▪ High anti-gout activity (1.760 mg/g XO inhibition),
- ▪ Moderate uric acid dissolution (1.213 mg/g),
- ▪ And complete absence of cytotoxicity, making it a promising natural candidate for safe complementary therapy.
- ✓ Honey from Ziziphus lotus and Euphobia guyoniana showed no cytotoxicity, but its overall biological performance was moderate in the various assays.
- ✓ Correlation analysis confirmed that total phenolic content and mineral composition—especially potassium and calcium—significantly contributed to the observed biological effects.

These findings support the hypothesis that certain Algerian honeys could serve as promising natural agents in managing inflammation and oxidative imbalances. Nevertheless, this work remains preliminary and opens several perspectives:

- ✓ The identification and isolation of the active molecules responsible for the observed effects;
- ✓ The investigation of synergistic effects between bioactive components;
- ✓ The confirmation of efficacy through in vivo models;
- ✓ The assessment of safety, toxicity, and optimal formulation for therapeutic use.

In conclusion, this study contributes to the scientific valorization of Algerian honey and encourages further research aimed at integrating local natural products into evidence-based complementary medicine.

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الملخص:

تهدف هذه الدراسة إلى تقييم الفعالية البيولوجية لبعض أنواع العسل الطبيعي ذات أصول نباتية مختلفة، من خلال سلسلة من الاختبارات شملت قياس المحتوى الفينولي الكلي، وتثبيط إنزيم أوكسيداز الزانثين، وذوبان حمض البول، والسمية الخلوية باستخدام اختبار الانحلال الدموي، بالإضافة إلى دراسة العلاقة الارتباطية بين مختلف النتائج المحصل عليها. أظهرت النتائج تباينًا ملحوظًا بين أنواع العسل المدروسة، حيث سُجلت فروقات في المحتوى الفينولي وانعكاسه على النشاط البيولوجي، كما تم رصد علاقة بين فعالية تثبيط أوكسيداز الزانثين وقدرة ذوبان حمض البول. أبان عسل اللبينة والسدر عن فعالية جيدة في جميع الاختبارات مع انعدام تام للسمية، مما يجعله مرشحًا واعدًا كمكمل طبيعي مساعد في الوقاية أو العلاج المساند لداء النقرس، في حين أظهر عسل الشوكي فعالية عالية في تثبيط الإنزيم لكنه ارتبط بسُمية خلوية مرتفعة عند التركيزات العالية، مما يُقيد استخدامه من الناحية العلاجية دون ضبط الجرعة.

الكلمات المفتاحية: العسل، أوكسيداز الزانثين، داء النقرس، حمض البول، الذوبان، السمية، المحتوى الفينولي

Résumé :

Ce travail vise à évaluer l'activité biologique de différents miels naturels issus de diverses origines botaniques à travers une série de tests comprenant la détermination de la teneur totale en composés phénoliques, l'inhibition de la xanthine oxydase, la dissolution de l'acide urique, la toxicité cellulaire via le test d'hémolyse, ainsi qu'une étude de corrélation entre les différents résultats obtenus. Les résultats ont montré une variation notable entre les types de miel étudiés, notamment au niveau de la teneur phénolique et son impact sur l'activité biologique. Une relation claire a été observée entre l'inhibition de la xanthine oxydase et la capacité de dissolution de l'acide urique. Le miel de *Ziziphus lotus* et *Euphobia guyoniana* s'est distingué par une bonne efficacité dans tous les tests et une absence totale de toxicité, ce qui en fait un candidat prometteur comme complément naturel dans la prévention ou le traitement de la goutte. En revanche, le miel d'*Ortica dioica* a montré une forte activité inhibitrice de l'enzyme, mais accompagnée d'une toxicité cellulaire marquée à fortes concentrations, limitant ainsi son usage thérapeutique sans contrôle de la dose.

Mots-clés : Miel, xanthine oxydase, goutte, acide urique, dissolution, toxicité, composés phénoliques.

Abstract:

This study aims to evaluate the biological activity of various natural honeys from different botanical origins through a series of essays including the determination of total phenolic content, xanthine oxidase inhibition, uric acid dissolution, cellular toxicity via hemolysis assay, and a correlation analysis among the obtained results. The findings revealed notable differences among the studied honey types, particularly in term of phenolic content and its impact on biological activity. A clear relationship was observed between xanthine oxidase inhibition and uric acid dissolution capacity. *Ziziphus lotus* and *Euphobia guyoniana* honey demonstrated strong performance across all assays with no observed toxicity, making it a promising natural candidate for the prevention or complementary treatment of gout. On the other hand, *Ortica dioica* honey exhibited potent inhibitory activity on the enzyme but was associated with significant cytotoxicity at higher concentrations, which limits its therapeutic use without dosage control.

Keywords: Honey, xanthine oxidase, gout, uric acid, dissolution, toxicity, phenolic content.