لجمهورية الجزائرية الديمقراطية الشعبية
People's Democratic Republic of Algeria
وزارة التعليم العالي والبحث العلمي
Ministry of Higher Education and Scientific Research
جـامـعـة عمـــار ثـلـيـــجـــي بالأغـــواط
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Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of Master in Chemistry
Specialty: Organic Chemistry

TITLE

Combinatorial effect of plant-honeys on preventing the gout disease: an *in vitro* study

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Academic year 2024/2025

Dedication

To my dearest loved ones,

Grateful appreciation of this work is to those persons who have been my pillar of strength, source of inspiration, and reason for motivation at every step in my career as well as academic pursuit.

To my father, whose wisdom, patience, and unflinching belief in me have been my source of inspiration,

To my mother, whose limitless love, prayer, and sacrifices have made me walk that extra step with confidence

To my brothers and my only sister, whose inspiration and encouragement have never stopped motivating me,

To all my relatives,

I dedicate my purest admiration and gratitude for a lifetime accomplishment. Your encouragement has made it feasible at the moral and emotional levels, your trust in me, and your ongoing guidance in my life.

To this work of love, I want to dedicate a small gesture of my love and appreciation, respect, and gratitude for everything you have done for me.

Acknowledgments

This thesis was completed at the Fundamental Sciences Research Laboratory **of Amar Telidji University** in Laghouat. I would like to thank its director, Mr. **Mohamed Yousfi**, for the warm welcome and the material and scientific conditions he offered me.

I would like to express my sincere gratitude to my thesis director, Mr. **Amar DJERIDANE**, for accepting the responsibility of supervising this thesis. I thank him for his availability, his valuable advice, his guidance and his efforts throughout the writing of this thesis, as well as for his moral and academic support which enabled me to successfully complete this work. I also thank Ms. **Chahrazed HAMIA**, co-director, I extend my sincere thanks for her help and support to the laboratory, as well as for her valuable advice and guidance.

I also thank Mr. **Khaled DJOKHDEM** for welcoming me to the scientific research laboratory of the Laghouat Higher Normal School. I also thank him for his support and guidance.

My thanks go to Mr. **Mohamed BENALIA** for having agreed to chair the jury for this defense and to Ms. **Yasmine AMI** for having agreed to examine this work;

I would also like to express my sincere gratitude to Ms. **Nacira SIYAD**, Ms. **Fatima GUIBADJ, Ms Amira SIGA and Ms Souad LARABI**, doctoral students in the Department of Materials Science at the University of Laghouat, I would like to thank them for their valuable assistance, wise contributions, and kindness in both good and bad times;

Finally, I would like to thank the entire administrative and teaching team of the Department of Material Sciences at **Amar Telidji** University and all those who contributed directly or indirectly to the completion of this work.

List of abbreviations

LFS : Laboratory of fundamental sciences

HCl : Hydrochloric acid

UV/Visible : Ultraviolet-Visible

A_0 : Absorbance in the absence of inhibitor

A_1 : Absorbance in the presence of inhibitor (extracts or standard)

AEAXOC : Allopurinol Equivalent Anti-Xanthine Oxidase Capacity

ANOVA : One-Way Analysis of Variance

BSA : Bovine Serum Albumin

FEAXOC : Febuxostat Equivalent Anti-Xanthine Oxidase Capacity

GAE : Gallic Acid Equivalent

HRBCs : Human Red Blood Cells

LN *: Laurus nobilis*

PI_{Xo} : Percentage of inhibition of XO

PBS : Phosphate-Buffered Saline

PCA : Principal Component Analysis

PH (%) : Percentage of Hemolysis

PI_{BSA} : Percentage Inhibition of BSA denaturation

RO *: Rosmarinus officinalis*

ROS : Reactive Oxygen Species

SD : Standard Deviation

TPC : Total Phenolic Content

UA : Uric Acid

XO : Xanthine Oxidase

XOIs : Xanthine Oxidase Inhibitors

Na : Sodium

K : Potassium

Ca : Calcium

pH : Potential of hydrogen

AEC : Allopurinol equivalent capacity

FEC : Febuxostat equivalent capacity

NLRP3 : NOD-like receptor family pyrin domain containing 3

IL-1 β : Interleukin-1 beta

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Introduction

I. Introduction

Gout is a metabolic disorder of purine characterized by monosodium urate crystals deposition in the joints (articular and non-articular structures), sustained by increase in the levels of blood uric acid (Hyperuricaemia), causing tissue damage and leading to severe inflammation and pain, such as inflammatory arthritis in adults, especially in men (Smith et al., 2011; Yuan et al., 2014; Vargas-Santos and Neogi, 2017; Dalbeth et al., 2021). Gout is most often related to the consumption of foods rich in purines, such as red meat and sea foods (Aihemaitijiang et al., 2020; Zhang et al., 2022), and existing medical conditions such as hypertension and renal disease (Vargas-Santos and Neogi, 2017).

Being an underestimated and extremely painful disease (Smith et al., 2011), many researchers agree that gout is a treatable disease (Doherty et al., 2012; Dalbeth et al., 2021). Gout management recommend lowering serum uric acid concentration, which leads to the dissolution of monosodium urate crystals and long-term prevention of gout flares and tophi and in improved quality of life (Dalbeth et al., 2021); as well as reducing recurrent attacks, destructive arthropathy, renal disease, and comorbidity (Rai et al., 2015). The drugs used to achieve this effect include uricosuric drugs and xanthine oxidase (XO) inhibitors (Yuan et al., 2014). Allopurinol and Febuxostat (XO inhibitors) have been commonly used medications to decrease the circulating urate levels (Hu and Tomlinso, (2008), colchicine and nonsteroidal antiinflammatory drugs by reducing the inflammation, and probenecid and sulfinpyrazone by favouring its excretion (Silvestre et al., 2020). However, the use of these drugs is associated with undesired side effects (El-Tantawy, 2019); they also cause some obvious adverse reactions and the cost of these drugs is very high (Yuan et al., 2014; Rai et al. 2015). Thus, returning to nature is an alternative to overcome these disadvantages.

Plants have been relied upon by humans for disease treatment and health enhancement since ancient times, taking advantage of their biologically active compounds such as flavonoids, terpenoids, and polyphenols. Recent studies confirm that such compounds are enriched with strong antioxidant and anti-inflammatory activity and thus are effective against the prevention and treatment of other chronic diseases (Atanasov et al., 2021). From then on, many studies are purporting traditional herbal medicine as anti-gout products source (Silvestre et al., 2020), and many of them have been patented (Yuan et al., 2014). According to Ling and Bochu (2014) and Silvestre et al. (2020), numerous agents derived from plants have anti-gout potential, by their xanthine oxidase (XO) inhibitory action or their anti-inflammatory effects, in vitro studies; while, in animal studies, they show dual effects including reduction of uric acid production and

increasing uric acid excretion (uricosuric action) or a combination of the two, thus reducing the concentration of uric acid in blood plasma. Besides, the plants with potential antioxidant are much potent in the management of gout (Ling and Bochu, 2014). In the literature review, for example, Abu Bakar et al. (2018) summarize the anti-gout potential of Malaysian medicinal plants which are considered to possess anti-gout activity based on in vitro and in vivo studies.

Among the most valuable medicinal herbs are *Laurus nobilis* (bay laurel), and *Rosmarinus officinalis* (rosemary), which were extensively used in traditional medicine for the treatment of numerous health disorders. Thus, *Laurus nobilis* is reported to possess an antioxidant and anti-inflammatory property (Alejo-Armijo et al., 2017). Also, *Rosmarinus officinalis* had XO inhibitory activity due to the phenolics (flavonoids) content (Hudaib et al., 2011).

In addition, honey, which is a natural product with outstanding nutritional and therapeutic quality, is endowed with antibacterial and antioxidant activity, responsible for improving general health as well as curing many diseases (Pasupuleti et al., 2017). Therefore, Sahin (2016) reported the antioxidant capacities of honey by using three different methods, namely, total phenolic content (TPC), ferric reducing antioxidant power (FRAP), and DPPH radical scavenging activity assay, and the inhibition of the enzymes urease and xanthine oxidase by using the UV spectroscopy technique. Besides, antioxidants play a fundamental role in preventing inflammation and oxidative stress, which is strongly related to cell damage, such as red blood cell hemolysis, and the onset of chronic diseases (Obeagu et al., 2024).

In Algerian traditional pharmacopoeia, many plants were recorded to treat rheumatoid arthritis and/or rheumatism (Cheriti et al., 1995; Chehma et Djebar 2008, Berboucha et al., 2010, Chermat and Gharzouli, 2015; Hadjadj et al., 2015; Benarba, 2016; Boukerker et al., 2016; Lakhdari et al., 2016; Bouasla et Bouasla, 2018; Hamel et al., 2018; Kefifa et al., 2018; Miara et al., 2018; Senouci et al., 2019; Djermane et al., 2022), among them, *Laurus nobilis* L., and *Rosmarinus officinalis*, were used by local population in the treatment of rheumatoid arthritis or listed as an anti-rheumatismal (Cheriti et al., 1995, Hamel et al., 2018, Djermane et al., 2022). Among others, other species, such as, *Helianthemum lippi* (L) (Boukerker et al., 2016), *Zygophyllum album* L. (Lakhdari et al., 2016), *Brassica rapa* L. and *Ononis spinosa* L. (Djermane et al., 2022), *Fraxinus angustifolia* and *Pistacia lentiscus* (Berboucha et al., 2010), and *Crataegus laevigata* L. (Dif et al., 2015), were cited to treat gout disease.

However, all these documented sources described the biological activity of plant species or honey separately, not in mixture or combination. Thereby, and considering this information, the objective of the study is to investigate the potential effect of mixture of three medicinal

plants, namely *Laurus nobilis* L., and *Rosmarinus officinalis*, with honey, in different concentrations, exploring the anti-gout remedies by their XO inhibitory action, their uric acid

Materials and methods

II. Materials and methods

Our study was conducted in the Laboratory of fundamental sciences (LFS) at Amar Telidji University of Laghouat and Applied Chemical Physical Sciences Laboratory at Higher Normal School Taleb Abdel Rahman of Laghouat.

II.1. Chemicals and instruments

This work was supported by a number of material resources listed in Table 1. All chemical products are of purity grade.

Table 1. 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-mete**r** (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), beaker**s** , 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, Filter papers, 96-well microplates, Plate reader (96 and 384 wells MULTISKAN FC) with incubator Thermo.

II.2. Sample collection

Six honey samples from different floral origins were purchased from different beekeepers (Table 2). While, *Rosmarinus officinalis* (ازير او اكليل الجبل) and *Laurus nobilis* (الرند) plants were purchased from commercial market at Laghouat city, as practiced by the local population, in real purchasing conditions.

Table 2. The regions, year of harvest and botanical origin of honey samples

Sample	Botanical origin	Crop Year	Region
M_1	*Euphorbia guyoniana* + *Ratama raetam* honey (عسل اللبين+الرتم)	June 2022	Laghouat
M_2	Mountain honey (عسل جبلي)	June 2024	
M_3	*Eruca vesicaria* (عسل الجرجير)	July 2022	Laghouat
M_4	*Silybum marianum* honey (عسل الشوكي)	June 2022	M'Sila
M_5	*Bunium bulbocastanu* honey (عسل التلغودة)	June 2022	Tiaret
M_6	*Talebi* (عسل طالبى)	June 2022	Laghouat

II.3. Preparation of mixtures

A mixture of honey and grounded plant were performed at proportion of 1g (plant powder) with 10 g (honey).

II.4. Preparation of extracts

II.4.1. Extraction by digestion

0.25 g of the mixture was added to 10 mL of distilled water, shacked using an agitator, and placed in a water bath at 37°C for one hour. Then, the mixture was centrifuged for 10 minutes at 3000r/min until the plant residues that will settle at the bottom of the tube are separated. The prepared extracts were used in the following analyses (anti-OX, uric acid dissolution, TPC, K, Na, Ca).

II.4.2. Extraction by sonication

1 g of the mixture was putted on 10 mL of distilled water and placed in an ultrasonicator at 37°C and frequency with 60Hz for 20 minutes. Then, the mixture was filtered using a filter paper and centrifuged at 3000r/min for 10 minutes until the mixture is completely clear of

impurities. The prepared solutions were used in the following analyses (BSA assay, hemolytic effect, TPC, K, Na, Ca).

II.5. Phytochemical quantification

II.5.1. Determination of total phenolic content

The total phenolic content (TPC) was determined according to the method reported by Singleton and Rossi (1965) involving the Folin-Ciocalteu reagent, and which based on colorimetric change by measuring absorbance at 760nm in UV-Visible spectrophotometer.

The quantification is calculated using the total concentration of hydroxyl groups in the extract. Using a micropipette, 100 µL of each extract was added to test tubes, followed by 500 µL of 10% Folin-Ciocalteu reagent. After 2 minutes of incubation, 2 ml of 2% sodium carbonate Na_2CO_3 was added, and the solutions were shaken quickly before being maintained in the dark for 30 minutes at room temperature. The absorbance of each solution was obtained at 760 nm against a blank using a UV-Visible spectrophotometer (SHIMADZU 1800). The phenolic compound content of each extract was calculated from a gallic acid calibration curve and expressed in milligrams gallic acid equivalent per gram of extracts (mg GAE /g extracts).

II.5.2. Determination of sodium, potassium and calcium in extracts

Sodium, potassium, and calcium concentrations have been measured by flame photometry, based on the method described by Knight et al. (1951)

This technique involves using heat energy, to excite electrons in an atom, causing them to jump to higher energy levels (orbits). When these electrons return to lower energy levels, they release energy in the form of photons (light). Flame photometry compares the emission intensity of a solution containing the element to be determined with that of a standard solution. The apparatus employed was a Sherwood® m410 flame photometer, with air-butane as the flame source. Therefore, the emission intensities of Na, K, and Ca from different extracts diluted in distilled water and centrifuged (3000 r/min for 10 minutes) were measured at the proper wavelengths. The Na, K and Ca content of each extract was calculated from the sodium, potassium and calcium calibration curves using sodium chloride (NaCl), potassium chloride (KCl) and calcium chloride ($CaCl_2$) as standard solutions, respectively. The results are expressed in milligrams equivalent of sodium, potassium and calcium per gram of extracts (mg Na /g extracts), (mg K /g extracts) and (mg Ca /g extracts), respectively.

II.6. Evaluation of anti-gout activity *in vitro*

II.6.1. Evaluation of anti-xanthine oxidase activity (XO)

Total XO (µmole) activity was determined by measuring the rise in uric acid production at 550 nm in the presence of xanthine as an enzyme substrate. The extracts' inhibitory effect on XO activity was investigated spectrophotometrically by measuring the amount of uric acid produced during xanthine oxidation (Fridovich and Handler, 1962).

The extracts' inhibition measurements were as follows: in a 96-well microplate, 80 µL of sodium phosphate buffer (0.2M, pH 7.8) was mixed with 20 µL of extract and 20 µL of XO solution (0.006 units / 2.5 mL of phosphate buffer, pH 7.8). This combination was pre-incubated at 37°C for 5 minutes in a plate reader (Multiskan™ FC Microplate Photometer). Afterward, 20 µL of xanthine solution (0.08 g/L, adjusted with HCl to pH 7.8) was added and the reaction mixture was incubated at 37°C for 30 minutes. The enzymatic reaction was performed using 100 µL of uric acid reagent, and the mixture had been blended and stored for 10 minutes at 37°C. Optical density measurements were performed immediately at 550 nm. The antixanthine oxidase activity of the extracts was expressed firstly as the percentage inhibition of XO activity (PI_{XO}) according to the equation:

$$PI_{XO}(\%) = \frac{(A_0 - A_1)}{A_0} \text{ x } 100$$

Where: A_0: Absorbance in the absence of inhibitor

A_1: Absorbance in the presence of inhibitor (extracts or standard)

The anti-xanthine oxidase activity was determined using the calibration curves of allopurinol and febuxostat. The values were expressed as mg of allopurinol equivalent capacity (AEC) par 1 g of extract, or as µg of febuxostat equivalent capacity (FEC) par 1 g of extract.

II.6.2. Evaluation of the uric acid dissolution effect

The anti-gout activity of extracts produced by the uric acid dissolving method was assessed by determining the dissolved mass of uric acid in the various extract solutions. This model was created and implemented for the first time.

Firstly, 5 mg of uric acid was placed in test tubes with 5 mL of each extract (or distilled water). The solutions are centrifuged at 3000 r/min for 10 minutes. Then, to determine the uric

acid concentration in each extract, 20 µL of each supernatant was mixed with 200 µL of the uric acid reagent. The mixture was mixed and let to stand in the dark at 37°C for 10 minutes. The optical density of each solution was recorded at 550 nm using a Plate reader (Multiskan™ FC Microplate Photometer). The dissolved uric acid content in each extract was calculated from the uric acid calibration curve and expressed as milligrams of soluble uric acid in 1 liter of extract with a concentration equal to 1 mg/mL.

II.7. Anti-inflammatory activity by inhibition of protein denaturation

500 µL of the extracts studied were mixed with 500 µL of bovine serum albumin (BSA; 0.1% w/v prepared in Tris-HCl buffer 0.5 M, pH 6.3). The mixture was incubated at 37°C for 20 minutes, and then the temperature was increased to 72°C for 5 minutes. After cooling in cold water for 2 minutes, the absorbance was measured against a blank using a UV-Vis spectrometer

(SHIMADZU 1800) at 600 nm. The anti-inflammatory effect of the extracts was expressed firstly as the percentage inhibition of BSA denaturation (PI_{BSA}) according to the equation:

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

Where: A_0: Absorbance of BSA without inhibitor

A_1: Absorbance of BSA with inhibitor (extracts or standard)

The anti-BSA denaturation activity was determined using the calibration curve of diclofenac.

The anti-inflammatory values were expressed as mg of diclofenac equivalent capacity (DEC) par 1 g of extract.

II.8. Evaluation of the cytotoxicity by human red blood cell hemolysis test

The cytotoxicity of the extracts has been determined by checking their *in vitro* hemolytic action on human red blood cells (HRBc). The extracts were tested for hemolytic action using Li and Liu method (2008), with little changes. At the start, we produced the red blood cell suspension by collecting fresh blood from a single healthy donor into a heparinized tube and centrifuged at 2000 r/min for 10 minutes. After extracting the plasma, the pellets were washed 5 times with PBS, then diluted with PBS 10, 50, and 100 times. In a hemolysis tube, 500 µL of erythrocyte suspension and 500 µL of extract were incubate at 37°C for 60 minutes. Then, the tubes were centrifuged at 2000 r/min for 10 minutes, and the absorbance of supernatant at 500

nm was measured using a UV-Vis spectrophotometer against a PBS blank. To replicate the experiment, we created a total hemolysis tube with 500 µL of red blood cell suspension and 500 µL of distilled water (there's no extract). The hemolysis rate (PH) of each extract was estimated as a percentage (%) related to the total hemolysis after 60 minutes of incubation, according to the following formula:

$$\mathbf{PH}\ (\%) = \frac{\mathbf{A_{extract\ 60\ min}}}{\mathbf{A_{total\ hemolysis\ 60\ min}}}\ \mathbf{x\ 100}$$

II.9. Statistical analysis

All test results were reported as mean ± standard deviation (SD) with three trials, analyzed using Excel 2018 software. Descriptive analysis was conducted with XLSTAT 2018.5.03 software, applying a tolerance of 00001 and a 95% confidence interval to determine the Pearson correlation coefficient and perform principal component analysis (PCA). One-way analysis of variance (ANOVA) and Tukey's significant difference test were employed to compare group means.

Results and discussion

III. Results and discussion

III.1. Phytoconstituents quantification

III.1.1. Total phenolic content

The first part of researching the biological properties of honey-plants mixture is the extraction of their active compounds. This critical stage should provide a higher yield and enable the extraction of various active compounds. Literature has documented various methods of extraction. In the present study, digestion and ultrasonic extraction methods were employed in the extraction of a broad spectrum of chemicals from various matrices (Sridhar et al., 2021). Following digestion and sonication, the amount of total phenols in the plant-honey mixture was obtained from the calibration curve of gallic acid (Figure 1).

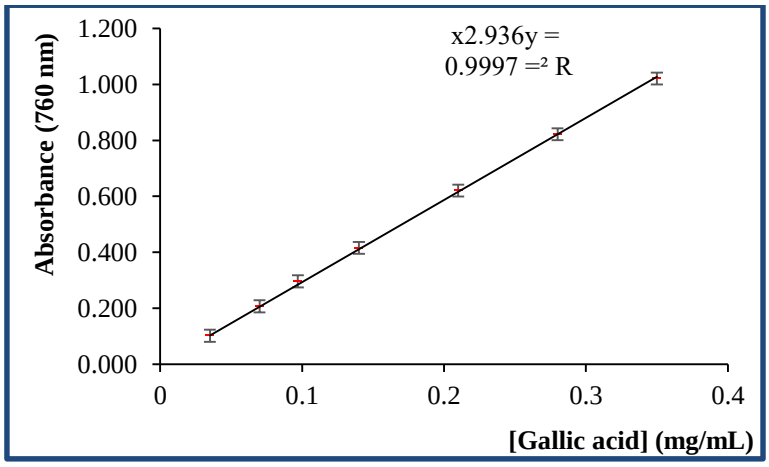

Figure 1. Calibration curve of gallic acid

The results of total phenolic contents expressed as milligrams of gallic acid equivalent per gram of extracted material are summarized in the Table 3.

In general, the estimation of total phenolic content using the Folin-Ciocalteu reagent indicates that the plant-honey mixtures contain phenolics in different levels. However, significative differences were noted between the mixtures and the extraction methods. In contrast, the total phenolic content of the extracts obtained the sonication extraction tends to yield a higher percentage of extraction compared to digestion extraction. The analysis showed that the difference in total phenolic content in all the different extracts was very highly significant at the 0.001% level. On the other hand, the results indicate that there is a very large interspecific variation depending on the plant and honey types. Thus, for the sonication extraction, the highest values were showed for M_5-RO, M_1-RO and M_5-LN with values of 9.429

±0.323 mg GAE/g extract, 8.792 ±0.477 mg GAE/g extract and 8.605 ± 0582 mg GAE/g extract, respectively; and for the digestion extraction, there were three extracts close in values, namely M_3-RO, M_5-RO and M_6-RO with values of 2.297 ±0.038 mg GAE/g extract, 2.288 ±0.053 mg GAE/g extract and 2.315 ±0.053 mg GAE/g extract, respectively. However, for all extract mixtures, M_5-RO showed significantly the higher value of total phenolics. While, the lowest value for sonication procedure was recorded for M_4-LN mixture with values of 6.457 ±0.375 mg GAE/g extract. Nevertheless, M_1-LN mixture extracted by digestion ranked last in terms of total phenolic concentration, recording the lowest value of all extracts and was estimated at 1.342 ±0.047 mg GAE/g extract.

Table 3. Total phenolic contents in the studied extracts.

Extract	mg GAE / g extract	
	Extraction by digestion	**Extraction by sonication**
M_1-LN	1.342 ±0.047 ^{F}	7.431 ±0.576 ^{CDEF}.
M_2-LN	1.408 ±0.037^{EF}	8.155 ±0.385 ^{ABCDE}
M_3-LN	1.473 ±0.077 ^{EF}	7.281 ±0.390 ^{CDEF}
M_4-LN	1.495 ±0.072 ^{E}	6.457 ±0.375 ^{F}
M_5-LN	2.258 ±0.058 ^{AB}	8.605 ±0.582 ^{ABC}
M_6-LN	1.872 ±0.043 ^{D}	7.705 ±0.582^{BCDEF}
M_1-RO	2.029 ±0.009 ^{C}	8.792 ±0.477 ^{AB}
M_2-RO	2.091 ±0.031 ^{C}	6.969 ±0.582 ^{EF}
M_3-RO	2.297 ±0.038 ^{A}	7.094 ±0.319 ^{DEF}
M_4-RO	2.128 ±0.028^{BC}	7.256 ±0.241 ^{DEF}
M_5-RO	2.288 ±0.053 ^{A}	9.429 ±0.323 ^{A}
M_6-RO	2.315 ±0.053 ^{A}	8.430 ±0.478 ^{ABCD}

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 ($p < 0.001$).

Despite this difference in phenolic contents between the mixture extracts, these results remain superior when compared to honey alone, as state by previous studies (Table 4).

Table 4. Phenolic content of some honeys

Honey type / or Origin	mg GAE/100 g	Reference
Chestnut	38.900±0.960 - 65.300±1.306	Sahin (2016)
Oak	36.806±0.972 - 62.260±0.623	
Polyfloral	9.400±0.188 - 29.480±0.737	
linden honey	17.57±0.34 - 31.95 ± 0.49	Zou et al. (2022)
Polish honeys	17.57 - 189.52	Wilczynska, 2010
Western Australian honeys	29.15± 5.46 - 59.4±7.91	Lawag et al. (2023)
United States honeys	81.6 - 105.7	Nyarko et al. (2023)
Yemeni honeys	10.74±0.57 - 86.80±1.99	Wabaidur et al.(2020)
Portugal raw honeys	10.02±0.10 - 30.72±0.07	Paula et al. (2024)
Serbian honeys	30.70 – 127.37	Nedic et al. (2022)
Algerian honeys	50±1 - 200±4	Otmani et al. (2021)
	22.42±1.42 - 96.17±3.50	Amessis-Ouchemoukh et al.(2021)
Ethiopian honeys	17.03±2.49 - 42.04±3.86	Yayinie et al. (2022)
Italian honeys	10.82±6.89 - 14.67±4.64	Perna et al. (2013)
Palestinian honeys	32.49±0.08 - 42.66±2.24	Imtara et al., 2018
Moroccan honeys	12.91±0.85 - 89.53±4.05	

According to many authors, the levels of phenolic content of honey depend on the floral source (Perna et al. 2013), which used as floral markers (Necib et al., 2022), and geographical origins (Nyarko et al., 2023) which explain their large variation.

But when compared to phenol contents of plants used in this study, our results showed lower values, as compared to other studies (Table 5).

Table 5. Phenolic content of *Laurus nobilis* and *Rosmarinus officinalis*

Plant	mg GAE/ g	Reference
Laurus nobilis	24.43 to 36.74	Dobroslavic et al. (2021)
	10.23	Muniz-Marquez et al. (2014)
	46.79	Lu et al.(2011)
	10.63	Muniz-Marquez et al.(2018)
	21.56 - 24.77	Rincon et al. (2019)
	17.32	Muniz-Marquez et al. (2013)
	5.5	Ishtiaque et al., 2015
Rosmarinus officinalis	33 – 185.71	Fadili et al. (2015)
	34.1 - 119	Celiktas et al. (2007)
	195.45±4.16 - 541.82±3.15	Athamena et al. (2015)
	162	Erkan et al. (2008)
	127 ±3	Ho et al. (2008)
	58.1 ± 0.9	Tsai et al. (2007)
	39.1 ± 3.6	Tawaha et al. (2007)

It is well documented that both conventional and advanced extraction techniques influence the yield of chemical extraction as well as phenols (Dobroslavic et al., 2021). Therefore, as a first observation and according to the literature, we therefore note that the plant-honey mixtures have higher phenol content than the honeys and a lower content than the plants, used in this study, taken separately.

III.1.2. Mineral Content

The most important naturally occurring ions in plants and honey are sodium, potassium, and calcium. Therefore, the concentrations of Na, K, and Ca for all extracts obtained by the different extraction methods were determined using a flame photometer. The contents of these elements were calculated from the Na, K, and Ca calibration curves (Figures 2).

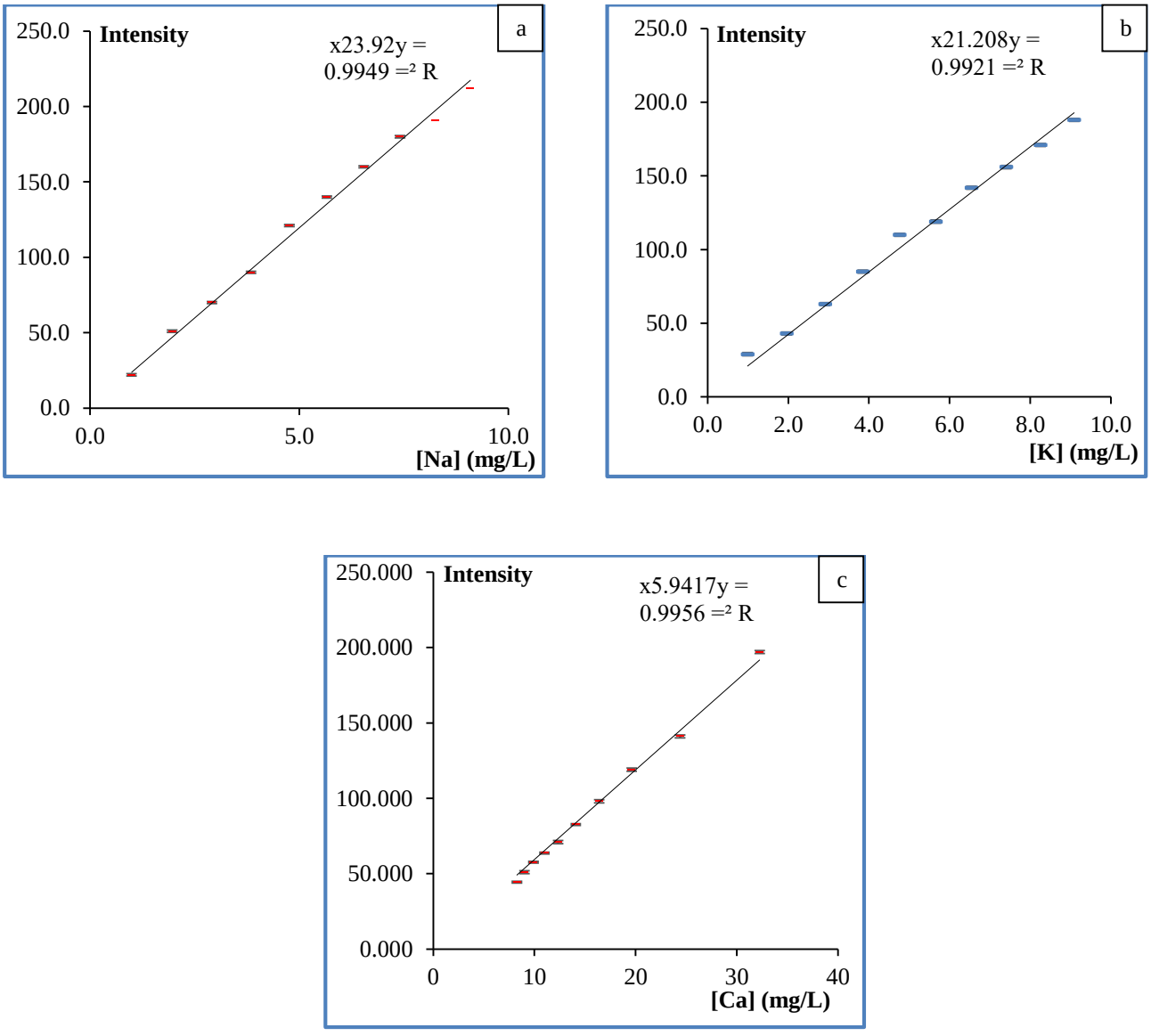

Figure 2. Sodium (a), Potassium (b) and Calcium (c) calibration curves

As shown in Table 6, the type of mixture and the extraction method affect significatly the mineral composition of the extracts; which is represented by the elements Na, K and Ca.

Table 6. Sodium, potassium and calcium contents in the studied mixture extracts

Extract	Extraction by digestion (mg / g extract)			Extraction by sonication (mg / g extract)		
	Sodium	Potassium	Calcium	Sodium	Potassium	Calcium
M_1-LN	0.427 ±0.007 ^{D}	1.791 ±0.012 ^{B}	0.045 ±0.004 ^{D}	0.299 ±0.005 ^{C}	2.210±0.011 ^{A}	0.127 ±0.001 ^{F}
M_2-LN	2.177 ±0.011 ^{A}	1.162 ±0.021 ^{F}	0.236 ±0.000 ^{AB}	0.224 ±0.003 ^{EF}	1.108 ±0.011 ^{GH}	0.073 ±0.001 ^{G}
M_3-LN	0.386 ±0.018 ^{E}	1.051 ±0.012 ^{G}	0.040 ±0.000 ^{D}	0.212 ±0.005 ^{F}	1.153 ±0.011 ^{G}	0.049 ±0.001 ^{H}
M_4-LN	0.534 ±0.007 ^{B}	1.037 ±0.021 ^{G}	0.072 ±0.004 ^{D}	0.366 ±0.003 ^{A}	0.980 ±0.011 ^{J}	0.040 ±0.001 ^{I}
M_5-LN	0.474 ±0.013 ^{C}	1.584 ±0.032 ^{D}	0.081±0.000 ^{D}	0.225 ±0.005 ^{EF}	1.886 ±0.015 ^{B}	0.153 ±0.001 ^{E}
M_6-LN	0.397 ±0.013 ^{DE}	1.411 ±0.021 ^{E}	0.034 ±0.000 ^{D}	0.189 ±0.005 ^{G}	1.680 ±0.006 ^{D}	0.038 ±0.001 ^{I}
M_1-RO	0.560 ±0.007 ^{B}	0.761 ±0.024 ^{H}	0.260 ±0.078 ^{A}	0.360 ±0.009 ^{A}	1.837 ±0.019 ^{C}	0.201 ±0.001 ^{A}
M_2-RO	0.264±0.011 ^{G}	2.199±0.021 ^{A}	0.209±0.000 ^{ABC}	0.211 ±0.000 ^{F}	1.005 ±0.019 ^{J}	0.176 ±0.001 ^{D}
M_3-RO	0.300±0.011 ^{F}	1.093±0.012 ^{G}	0.222 ±0.000 ^{AB}	0.214 ±0.002 ^{F}	1.058 ±0.024 ^{I}	0.194 ±0.000 ^{B}
M_4-RO	0.392 ±0.011 ^{E}	1.162±0.021 ^{F}	0.220 ±0.004 ^{AB}	0.337 ±0.013 ^{B}	1.079 ±0.010 ^{HI}	0.191 ±0.001 ^{C}
M_5-RO	0.380 ±0.011 ^{E}	1.660 ±0.021 ^{C}	0.175 ±0.000 ^{BC}	0.237 ±0.009 ^{E}	1.566 ±0.019 ^{E}	0.199 ±0.002 ^{A}
M_6-RO	0.278 ±0.007 ^{FG}	1.611 ±0.023 ^{CD}	0.153 ±0.004 ^{C}	0.275 ±0.005 ^{D}	1.373 ±0.019 ^{F}	0.192 ±0.001 ^{BC}

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 ($p < 0.001$).

As shown in Table 6, there are significant differences, both between the mineral elements within the same type of extract, and between those of different extracts. In other words, the type of mixture and the extraction method affect the mineral composition of the extracts; which is represented by the elements Na, K and Ca. Sodium concentrations ranged from 0.189 ±0.005 to 2.177 ±0.011 mg /g extract and were significantly different ($p<0.001$) between the extraction methods. Thus, extraction by digestion resulted in a slightly higher estimate of sodium concentrations than sonication. The determined potassium concentrations of the same extracts show the same trend as the sodium content. The determined potassium concentrations ranged from 0.761 ±0.024 to 2.210 ±0.011 mg /g extract. However, extraction by digestion showed higher potassium concentrations than sonication except for M_1-LN, M_5-LN, M_6-LN and M_1-RO mixture extracts. Thus, a significant difference ($p<0.001$) was found between the plant-honey mixtures, that the honey-*Laurus nobilis* mixtures having the highest values compared to *Rosmarinus officinalis*, when extracted by sonication, except for M_4-LN and M_4-RO samples. On the other hand, potassium concentrations were also significantly different ($p<0.001$) between the mixtures, which honey-*Rosmarinus officinalis* extracts were higher than the honey mixtures containing *Laurus nobilis*, when extracted by digestion, except for

M_1-LN and M_1-RO combinations. Regarding calcium contents, the values ranged from 0.034 ±0.000 to 0.260 ±0.078 mg /g of extract. We note also, that the values are linearly consistent between the calcium concentrations in the extracts and the extraction method, we found that M-RO extracts contained more calcium than M-LN extracts. Likewise, significant differences in calcium concentrations between different honey-plant extracts were observed. These differences can arise from various factors, including the plant species, the honey type and the extraction method. Compared to the literature, the concentrations of sodium, potassium and calcium in the plant or honey are significantly higher than in the studied mixtures, since their values are given in mg/kg (Table 7) (Kek et al., 2017; Lomiso and Nigussie, 2019; Atiya et al., 2024; Mrabet et al., 2024).

Table 7. Mineral compositions Na, K and Ca of honey and plants used

	Element mg/kg			**Reference**
Species	**Na**	**K**	**Ca**	
Honey	375.2±10.8 to 944.5±67.8	95.4±0.4 to 1643.9±58.6	37.3 ± 0.4 to 309.3 ± 0.0	Kek et al. (2017)
	205.2±3.04 to 283.7±1.87	47.4±1.5 to 70.17±1.24	420.4±16.2 to 551.9±21.9	Lomiso and Nigussie (2019)
Rosmarinus officinalis L	818.00	1085.24	4462.20	Atiya et al. (2024)
Laurus nobilis	not determined	4645.5	10012.75	Mrabet et al. (2024)

III.2. *In vitro* biological activities

III.2.1. Evaluation of anti-gout activity

According to the literature, numerous phenolic compounds possessing anti-gout activities are available from plants and honey, but there is no information about this activity in a mixture of honey-plant, in our knowledge.

III.2.1.1. Uric acid dissolution activity

The evaluation of the *in vitro* anti-gout power of the extracts thus prepared was determined by measuring their uric acid dissolving powers. Therefore, uric acid dissolved in extract mixtures were expressed in milligrams of uric acid equivalent per gram of extract, according to the linear equation of the calibration curve plotted with standard uric acid solutions at different concentrations (Figure 3).

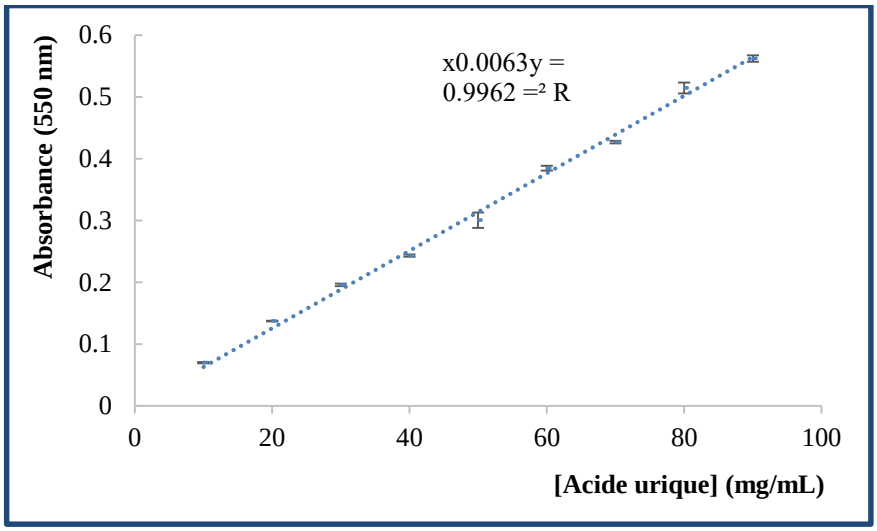

Figure 3. Calibration curve of uric acid

As shown in Table 8, the contents ranged from 0.299 ±0.017 mg UAE/g of extract to 0.960 ±0.090 mg UAE/g of extract in the investigated extracts. Therefore, the statistical analysis did not show any significant difference between the extracts, with LN plant except for M_5-LN, which exhibit the highest value in this group. Nevertheless, a significant difference ($p < 0.001$) is seen between the extracts of the LN-honey and RO-honey mixtures, where it is noted that the highest percentage is the one that contains the LN plant, except for M_2-RO and M_1-RO mixtures that getting closer to the mixture containing LN in terms of efficacy.

Table 8. Uric acid concentration in extracts

Extract	mg UAE /g extract
M_1-LN	0.887 ±0.035^b
M_2-LN	0.878 ±0.054^b
M_3-LN	0.812 ±0.030^b
M_4-LN	0.868 ±0.051^b
M_5-LN	0.960 ±0.090^a
M_6-LN	0.810 ±0.066^b
M_1-RO	0.787 ±0.093^b
M_2-RO	0.851 ±0.064^b
M_3-RO	0.299 ±0.017^d
M_4-RO	0.457 ±0.036^c
M_5-RO	0.378 ±0.022^d
M_6-RO	0.557 ±0.052^c

The results of the tests performed are expressed as mean ± standard deviation, n = 3.
Mean values followed by a different lowercase letter are significantly different ($p < 0.001$).

It is well settled that raised uric acid levels are significantly influenced by the consumption of high purine-containing foods such as meat, beans, mushrooms, and certain seafood (Jakse et al., 2019). Among other things, there is growing interest in fruits, vegetables and herbs containing phytochemicals and which are used as alternative medicines or supplements in the treatment of gout. Purines are naturally present in all plant-derived foods; these later can have 100 to 500 mg of uric acid/100 g of food (Hafez et al., 2017). Therefore, some foods like those high in fructose can increase uric acid levels, certain plants and honey, particularly those rich in flavonoids and antioxidants, can help lower uric acid (Zhou et al., 2022).

III.2.1.2. Xanthine oxidase inhibitory effect

Xanthine oxidase inhibitors (XOIs) are very promising medications for the treatment of hyperuricemia, a metabolic disorder responsible for gout, kidney stones, myocardial ischemia, and disease because of reactive oxygen species (ROS). Active xanthine oxidase inhibitor

extracts have been identified to decrease the level of serum uric acid significantly. They have been found in other plants used in traditional medicine, and their beneficial influence on rheumatism and gout therapy has already been widely acknowledged (Ceylan et al., 2016; Li et al., 2007; Roleira et al., 2018; Alam et al., 2021; Olugbodi et al., 2019). Apart from plant compounds, natural products such as honey have also been reported to exhibit potent xanthine oxidase inhibitory activity (Di Petrillo et al., 2018). Honey contains high levels of phenolic acids and flavonoids, which are accountable for its antioxidant, anti-inflammatory, and enzyme-inhibiting effects. There are several studies that have confirmed the therapeutic effectiveness of honey as an adjunct in the treatment of gout disease and other reactive oxygen species-induced diseases (Habib et al., 2021; Ahmed et al., 2018). Allopurinol has been recognized pharmacologically as a purine oxidase inhibitor having a synergistic effect on reducing uric acid and oxidative stress (Sahin, 2016). However, its clinical efficacy is sometimes weakened by the formation of oxypurinol, a metabolite whose side effect profile encompasses hypersensitivity reactions (Hussan et al., 2019). Alternatively, another medication, Febuxostat, a non-purine oxidase inhibitor, has been found to be more effective in reducing serum uric acid levels among patients with intolerance or resistance to Allopurinol (Gao et al., 2021). It has been demonstrated in clinical trials its potent uric acid-lowering effect with few side effects (Becker et al., 2005; Schumacher et al., 2008). To determine the *in vitro* anti-gout activity of the plant-honey mixture, the xanthine-xanthine oxidase system was used together with a therapeutic model, the uric acid dissolution test. The inhibitory activity of the extracts against xanthine oxidase was determined using Allopurinol and Febuxostat calibration curves (Figures 4 and 5).

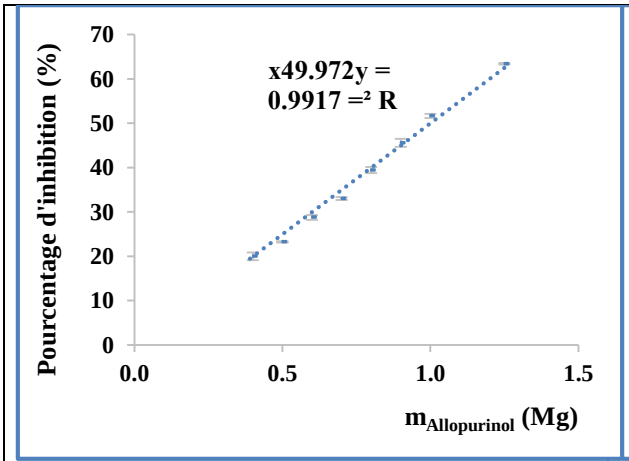

Figure 4: Calibration curve of Allopurinol

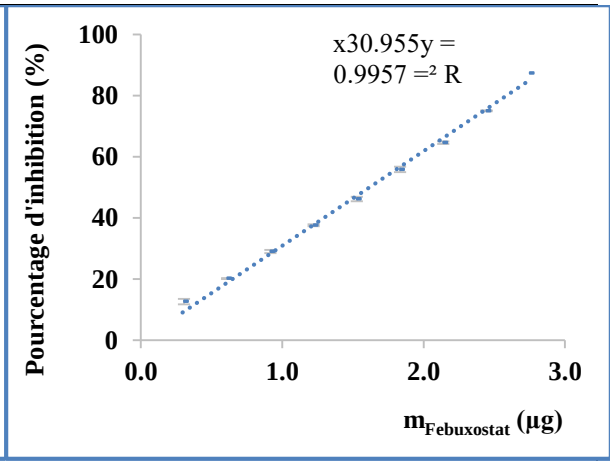

Figure 5: Calibration curve of Febuxostat

The extracts' anti-xanthine oxidase efficacy was then expressed in AEAXOC (Allopurinol Equivalent Anti-Xanthine Oxidase Capacity) and FEAXOC (Febuxostat Equivalent Anti-Xanthine Oxidase Capacity), which are defined as the number of micrograms of Febuxostat and milligrams of Allopurinol that give one gram of the extracts under study the same inhibitory power (µg Febuxostat /g extract) (mg Allopurinol /g extract). Results are presented in Table 9.

Table 9. *In vitro* anti-gout activity measured by xanthine oxidase inhibition test

Extracts	**AEAXOC** **mg Allopurinol /g extract**	**FEAXOC** **µg Febuxostat/ g extract**
M_1-LN	0.990 ± 0.194^{E}	1.595 ± 0.313^{E}
M_2-LN	2.166 ± 0.206^{BC}	3.489 ± 0.332^{AB}
M_3-LN	2.234 ± 0.193^{AB}	3.598 ± 0.311^{BC}
M_4-LN	2.574 ± 0.166^{A}	4.146 ± 0.267^{AB}
M_5-LN	1.708 ± 0.157^{CD}	2.751 ± 0.254^{CD}
M_6-LN	2.533 ± 0.054^{A}	4.079 ± 0.087^{AB}
M_1-RO	2.513 ± 0.152^{A}	4.047 ± 0.244^{A}
M_2-RO	0.963 ± 0.281^{E}	1.551 ± 0.453^{E}
M_3-RO	0.904 ± 0.098^{E}	1.456 ± 0.157^{E}
M_4-RO	0.999 ± 0.037^{E}	1.609 ± 0.059^{E}
M_5-RO	1.581 ± 0.101^{D}	2.547 ± 0.163^{D}
M_6-RO	1.547 ± 0.194^{D}	2.492 ± 0.313^{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 ($p < 0.001$).

The results showed that all extracts exerted a highly significant ($p < 0.001$) concentration-dependent inhibition of XO activity. The AEAXOC values of the different extracts tested showed a wide variation from 0.904 ±0.098 to 2.574 ±0.166 mg Allopurinol /g extract, and the FEAXOC values of the different extracts varied from 1.456 ±0.157 to 4.146 ±0.267 µg Febuxostat/g extract (Table 9). However, compared to Allopurinol and Febuxostat, all extracts appeared to have greater activity. Based on the AEAXOC and FEAXOC values of the different extracts, it appears that the extracts containing *Laurus nobilis* in the mixture had a greater effect than those containing *Rosmarinus officinalis*, except M_1-RO who exhibit higher value.

Therefore, these extracts could be explored as effective xanthine oxidase inhibitors by preventing substrate binding to the enzyme's active sites. Therefore, the inhibition of XO activity by the extracts is reflected in the presence of one or more compounds acting on the enzyme's active sites. However, several studies have shown that plants and honey have an effect on OX, and this inhibitory activity can be attributed to the presence of different bioactive compounds such as phenolics (Ouyang et al., 2018; Chen et al., 2015). Therefore, the higher inhibitory effect of *Laurus nobilis* extracts compared to *Rosmarinus officinalis* extracts is probably due to the presence of different phenolics. Although Allopurinol and Febuxostat are the most widely used OX inhibitors, it is estimated that approximately 5% of patients cannot tolerate their adverse effects, which include gastrointestinal irritation, bone marrow suppression, fever, deterioration of renal function, and hypersensitivity syndromes ranging from mild skin rashes to life-threatening conditions where patients develop toxic epidermal necrolysis. (Lai et al., 2023). Therefore, given the limitations of available XO inhibitor drugs, the development of new drugs with increased therapeutic activity and fewer side effects is a very active area of research. Then, plant-honey extracts, which are XO inhibitors, could be used as a therapeutic approach for diseases associated with this enzyme.

III.2.2. *In vitro* evaluation of cytotoxicity by red blood cell hemolytic assay

Evaluating the hemolytic effect of natural products is essential, even if they have significant anti-gout activity, in order to determine the dose at which the plant-honey mixture becomes toxic. Thus, hemolysis is an indicator of cytotoxicity. However, an *in vitro* cytotoxicity test, which consists of measuring the hemolysis rate of human red blood cells (HRBCs), was performed using human red blood cells from a healthy donor. Concentrations of 1g/10 mL were tested for each extract. 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 Table 10.

Table 10. *In vitro* cytotoxicity activity measured by red blood hemolysis assay

Extracts	Percentage of hemolysis (PH) (%)		
	HRBCs (10%)	**HRBCs (50%)**	**HRBCs (100%)**
M_1-LN	9.578 ±0.321^{B}	100.000 ±1.783^{A}	100.000±4.769^{A}
M_2-LN	9.561 ±0.663^{B}	100.000±2.122^{A}	100.000±5.495^{A}
M_3-LN	9.263 ±0.684^{B}	76.149±0.288^{D}	100.000±3.306^{A}
M_4-LN	5.273 ±0.544^{C}	100.000±1.989^{A}	100.000±2.183^{A}
M_5-LN	0.000 ±0.000^{D}	3.084±0.607^{H}	35.662±4.587^{E}
M_6-LN	12.956 ±0.169^{A}	20.432±1.994^{EF}	100.000±1.099^{A}
M_1-RO	0.000 ±0.000^{D}	2.320±0.589^{H}	100.311±3.782^{C}
M_2-RO	0.000 ±0.000^{D}	9.079±0.865^{G}	12.940±0.351^{FG}
M_3-RO	0.000 ±0.000^{D}	0.000±0.000^{H}	0.000±0.000^{G}
M_4-RO	0.000 ±0.000^{D}	17.521±1.247^{F}	31.784±0.953^{EF}
M_5-RO	0.000 ±0.000^{D}	22.344±0.733^{E}	93.668±1.785^{D}
M_6-RO	0.000 ±0.000^{D}	8.496±0.189^{G}	17.612±0.198^{EF}
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

It is clear, that hemolysis rates vary greatly within the same species, between 0.000 ±0.000% and 100.000 ±2.183%. Thus, for HRBCs (10%), extracts containing *Rosmarinus officinalis* had showed nontoxic effect, but low toxicity exposed by mixtures of *Laurus nobilis*. Whereas, higher toxicity of mixture extracts was found in HRBCs (100%) than in HRBCs (50%). It is also suitable to note that M_3-RO mixture not producing any toxicity in all cases. Compared to the drugs (Diclofenac, Allopurinol, Febuxostat), it is notable that some extracts had lower toxicity than the drug. In the same manner, previous studies have also shown that both plants and honey have some degree of toxicity (Al-Zeidaneen and Atrooz, 2025; Atrooz et al., 2024; Chansiw et al., 2018). The toxicity of the extracts depends on the concentration and also on the

components of the extracts. At high concentrations, phenolic compounds participate in the oxidation of hemoglobin, disrupting the membrane structure, increasing electrical conductivity, and thus causing hemolysis of red blood cells, due to the oxidative effects they can exert (Bukowska and Kowalska, 2004). However, it is clear from this study that the hemolytic effect of the extracts is probably due to the type of HRBCs. It is well known that red blood cells differ from one person to another in terms of surface structure (antigens), shape and size, chemical composition and count, these differences depend on genetics, nutrition, environment and diseases (Daniels, 2008; Hoffbrand, 2024; Keohane et al., 2015).

III.2.3. *In vitro* anti-inflammatory activity measured by protein denaturation assay

Proteins are an important group of large biomolecules found in all living organisms. However, their structure can be altered and modified by the presence of certain substances, including free radicals, which in some cases tend to modify their structure, inhibiting their normal activity (Habib et al., 2021). The anti-inflammatory activity of plant-honey extracts was tested by determining the amount of milligrams of the extract required to inhibit the degradation of BSA protein, and comparing its activity with that of the standard anti-inflammatory compound Diclofenac (Figure 6).

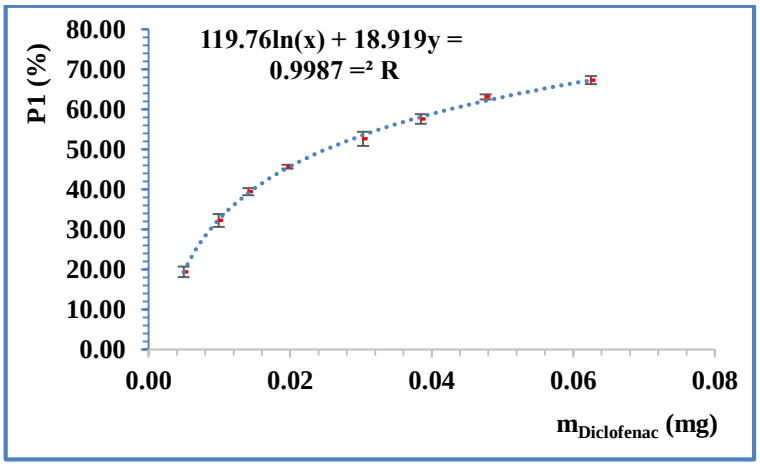

Figure 6: Calibration curve of BSA

The anti-inflammatory potency of the BSA extracts was expressed using Diclofenac, defined as the number of milligrams of Diclofenac that would give one gram of the extracts under study the same inhibitory potency (mg Diclofenac/g Extract). The results are presented in Table 11.

Table 11. Anti-inflammatory activity determined by BSA denaturation assay

Extracts	DEIC mg Diclofenac / g extract
M_1-LN	1.095 ± 0.045^{A}
M_2-LN	0.876 ± 0.052^{B}
M_3-LN	0.143 ± 0.009^{F}
M_4-LN	0.419 ± 0.012^{C}
M_5-LN	0.271 ± 0.018^{DE}
M_6-LN	0.323 ± 0.032^{D}
M_1-RO	0.414 ± 0.037^{C}
M_2-RO	0.194 ± 0.027^{EF}
M_3-RO	0.322 ± 0.023^{D}
M_4-RO	0.000 ± 0.000^{G}
M_5-RO	0.000 ± 0.000^{G}
M_6-RO	0.216 ± 0.007^{EF}

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 ($p < 0.001$).

The values shown in Table 11 range from 0.000 ±0.000 to 1.095 ±0.045 (mg Diclofenac /g of extract). Better results of inhibition rates were observed in the mixture of *Laurus nobilis*. The maximum BSA denaturation inhibition value was found in the M_1-LN mixture. The M_4-RO and M_5-RO mixtures did not exhibit any anti-bovine serum albumin activity. Even though, these findings were under laboratory conditions and they are still promising. 1g of the plant-honey mixture has the potential to produce a more potent anti-inflammatory effect than the synthetic molecule used in our experiments.

It is worth to mention that the anti-inflammatory activity may be partly attributed to the efficacy of the medicinal plants utilized in the combination, even when utilized separately. For example, different researches have set up that *Laurus nobilis* has a specific anti-inflammatory effect. Guedouari and Nabiev (2021) proved that the ethanolic extract of the leaves of *Laurus nobilis* inhibited carrageenan-induced inflammation in mice to a degree higher than Diclofenac.

Lee et al. (2019) also proved that 1,8-cineole (eucalyptol), which is a major constituent of the plant, inhibits NLRP3 inflammasome activation and thereby reduces inflammatory mediator release such as IL-1β. As for *Rosmarinus officinalis*, the anti-inflammatory effect has also been demonstrated when used singly in several studies. Takaki et al. (2008) validated that an ethanolic extract of *Rosmarinus officinalis* exhibited significant anti-inflammatory activity against acute inflammation in a mouse ear oedema model. Rosmarinic acid, the main active component of the herb, was also found to have the potential to inhibit the synthesis of prostaglandins and other inflammatory mediators. Honey, for example, has been shown to have good efficacy, which signifies an action against bovine serum albumin (BSA). As mentioned before and according to the literature, the plant and the honey are both active against bovine serum albumin but with a different inhibition rates. The honey by itself is not as strong as the plant by itself and the plant-honey mixture, and the plant by itself is stronger than the plant-honey mixture. For the two mixtures that were not effective against bovine serum albumin, we suspect one of the following reasons for why they were ineffective:

- A chemical reaction between the two chemicals leads to inactivation: The two chemicals may have chemically interacted when mixed (the formation of a complex, or one decomposing in the presence of the other), leading to the deactivation of the active form.
- A competitive inhibition or antagonism between both compounds: Either of the two components may have an indirect effect that diminishes the action of the other when they are combined. For example, some of the sugars in honey may form a "shell" to inhibit the combination between *Rosmarinus officinalis* compounds and BSA.

III.3. Correlations between all obtained results

Biological testing of many samples using various techniques creates a large data matrix from which it is difficult to gain all the information. For this reason, the use of principal components analysis (PCA) has become a requirement to enable interpretation of the underlying factors accounting for the variation of the sample dosage values and the techniques used. In PCA, variables are spread in scatterplot form, enabling us to identify classes among a group of variables by looking at similar and dissimilar ones, specifically, when the two variables are together, they are positively correlated. When they are orthogonal, they are uncorrelated. When they are symmetrically opposite the center, they are negatively correlated. Therefore, to determine the presence or absence of a correlation between TPC and mineral contents, anti-gout activity, anti-inflammatory effect and cytotoxicity power of the various studied extracts, a PCA has been performed in 12 samples × 15 dosage and activity methods matrix. Firstly, making a correlation matrix allows the assessment of correlations between the explanatory

variables in two dimensions. For that, the Pearson correlation coefficient was calculated between all studied variables. The results are shown in Table 12.

Table 12. 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 (10%)	PH (50%)
TPC	**1,000**								
Na	-0,476	**1,000**							
K	0,204	-0,263	**1,000**						
Ca	0,430	0,295	-0,170	**1,000**					
AUD	**-0,610**	0,309	0,115	**-0,588**	**1,000**				
AEIC	-0,381	0,318	**-0,555**	**-0,636**	0,460	**1,000**			
DEIC	-0,121	0,116	0,391	-0,328	0,501	0,058	**1,000**		
PH (10%)	-0,270	-0,252	0,154	**-0,869**	0,499	0,454	0,546	**1,000**	
PH (50%)	-0,296	0,410	-0,284	-0,449	0,088	0,353	0,068	0,236	**1,000**
PH (100%)	-0,259	0,277	-0,062	**-0,774**	0,385	**0,724**	0,120	0,561	**0,683**

Firstly, the results revealed statistically significant negative correlation between the total phenolic content and uric acid dissolution assay (r = -0.610; p = 0.035). Therefore, no significative correlations between the total phenolic content with other constituents and biological activities were detected. On the other hand, there was significant negative correlation between uric acid dissolution power and calcium level (r = 0.588; p = 0.044). which indicates 58.5 % antagonistic effect to this mineral in the dissolution of uric acid.

Additionally, significative negative correlations between xanthine oxidase inhibition and potassium (r = - 0.555; p = 0.046) and calcium (r =- 0.636; p = 0.026) were observed. This result indicates that calcium and potassium are the most important noncontributors to the xanthine oxidase inhibition activity. This outcome disagrees with previous researches where there noted that calcium and potassium ions can influence XO activity and its effects (Matuz-Mares et al., 2022; Ryan et al., 2011; Xie et al., 1990).

However, calcium content in extracts was strongly negatively correlated with hemolysis effect (r=-0.869; p =0.036). This result indicates that extracts containing high level of calcium could prevent 87 % of the HRBCs hemolysis and are the most important contributors to the antihemolytic activity. Hence, the antihemolytic power of calcium refers to the ability of

calcium or calcium-chelating compounds to inhibit or reduce hemolysis, the breakdown of red blood cells. Additionally, calcium can play a role in regulating the activity of toxins that cause hemolysis (Child and Rafter, 1986; Le et al., 201) .

On the other hand, results of the principal component analysis (PCA) are shown in Figure 7.

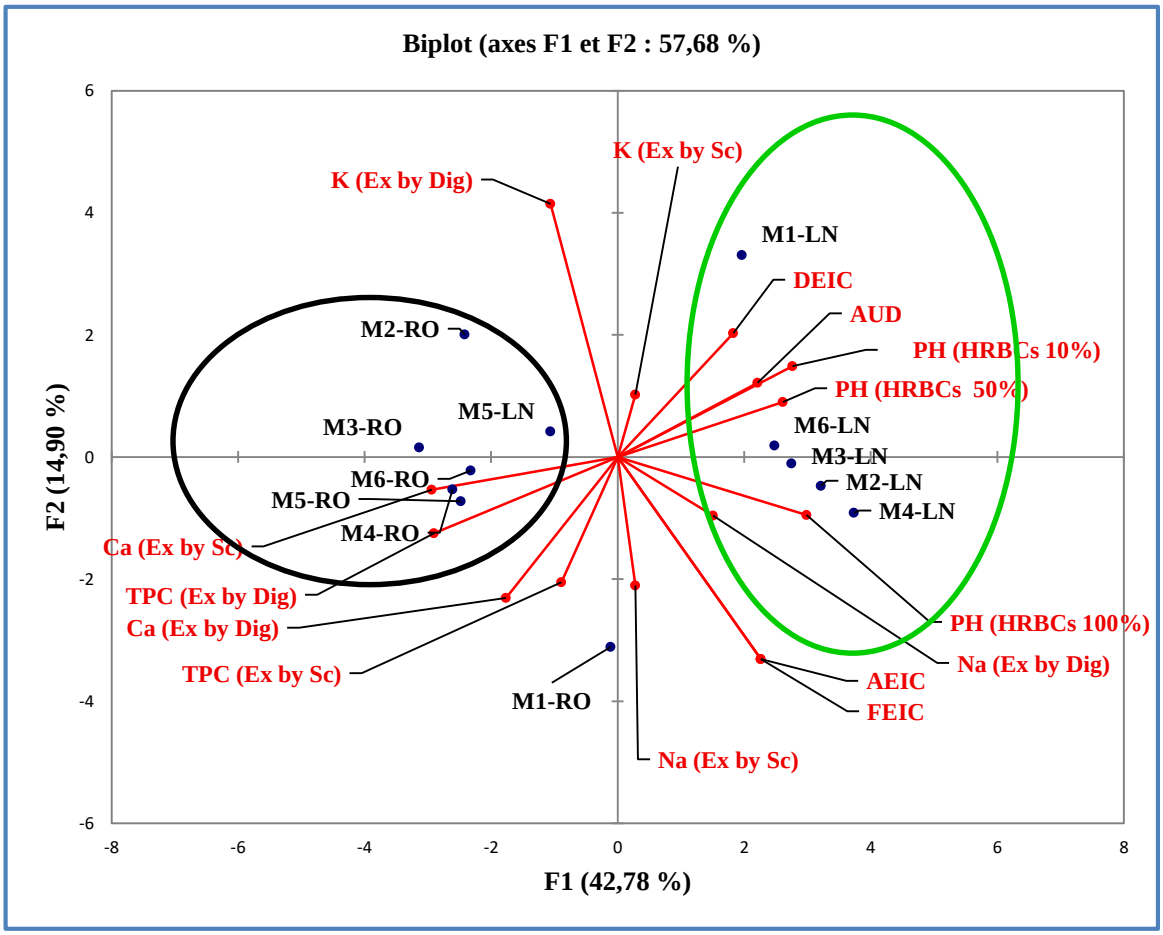

Figure 7: Projections of variables and individuals in the F1-F2 factorial plane (PCA).

According to Figure 7, the two principal components express a total variance of 57.68%. The first principal component (F1) has a dominant influence, with an expression of 42.78% of the total variation. While, the second axis represents a variability of 14.90% of the total variance. The contribution of each variable in the expression of the principal component is given by the value of the correlation ratio between the variable and the factorial axis considered, as much as this ratio is important, as much as the variable contributes to the expression of the axis. The linear combination of the initial variables defines the gradient of each component. Table 13 groups the correlation of the variables to the principal factorial axes.

Table 13. Correlation ratio between variables and factors (principal components)

	F1	F2	F3
TPC (Ex by Dig)	-0.9029	-0.2289	0.2299
TPC (Ex by Sc)	-0.2767	-0.3761	0.6898
Na (Ex by Dig)	0.4690	-0.1767	-0.2368
Na (Ex by Sc)	0.0866	-0.3858	-0.2489
K (Ex by Dig)	-0.3305	0.7612	0.2587
K (Ex by Sc)	0.0873	0.1876	0.8095
Ca (Ex by Dig)	-0.5490	-0.4239	-0.3887
Ca (Ex by Sc)	-0.9140	-0.0988	0.0550
AUD	0.6883	0.2227	0.2614
AEIC	0.7035	-0.6072	0.2696
FEIC	0.7036	-0.6072	0.2696
DEIC	0.5679	0.3718	-0.0352
PH (HRBCs 10%)	0.8584	0.2733	0.0840
PH (HRBCs 50%)	0.8120	0.1658	-0.3695
PH (HRBCs 100%)	0.9283	-0.1740	0.0769

From Table 13, it is clear that the first axis is positively correlated with HRBCs 100% (92.83%), HRBCs 10% (85.84%), HRBCs 50% (81.20%), AEIC (70.35%), FEIC (70.36%), uric acid dissolution (68.83%). On the other hand, it is moderately or weakly correlated with the other variables (<56.79%), and negatively correlated with variables as TPC, Ca and K (Ex by Dig). The F2 axis is positively correlated with K (ex with dig: 76.12%), DEIC (37.18%), and negatively correlated with AEIC and FEIC (-60.72%) and the anther variables. On the other hand, the F3 axis is positively correlated with total phenols in two different ways of extraction TPC (Ex by Dig: 22.99%), TPC (Ex by Sc: 68. 98%), potassium in two different ways of extraction K (Ex by Dig: 25.87%), K (Ex by Sc: 80.95%), Ca in one way of extraction (Ex by Sc: 05.50%), AUD (26.14%), AEIC and FEIC have the same value (26.96%), HRBCs 10% (08.40%), snag 100% (07.69) , and negatively correlated with the other variables.

Furthermore, the plane representing the projection of the taxa onto the F1 and F2 axes reveals a clear distinction between the different studied extracts. The scatterplots of individuals at factorial levels (1, 2) (Figure 7) do not allow a clear assessment of the effects of variables on the measured quantities. Few individuals truly stand out from the others. In fact, all individuals are generally distributed in an area close to the origin of the axes, which means that the various tests conducted do not present significant differences that reveal distinct families of extracts. A close examination of the projections of individuals reveals that samples M_2-RO, M_3-RO, M_5-LN, M_5-RO, M_6-RO, and M_4-RO (black circle) are characterized by high calcium and phenolic contents, low potassium content, and significant anti-hemolysis activity. The scatterplot (green circle) showing samples M_6-LN, M_3-LN, M_2-LN, and M_4-LN, which exhibit a high hemolysis rate, strong activity against bovine serum albumin, and a high uric acid dissolution. On the other hand, the situation is less clear for the other two samples, confirming the heterogeneity of the analyzed extract components for the observed species. Furthermore, the F1 axis, which is closely related to the variable's uric acid solubilization (68.83%) and xanthine oxidase (70.35%), also shows a significant positive correlation with the individuals in the green circle. In conclusion, the principal components analysis allowed us to graphically visualize the information contained in a large result. Therefore, due to the poor distribution of individuals in the projection space, it is impossible to establish relationships between the various dose and activity parameters and the nature or origin of the studied extracts. This also allowed us to demonstrate that the dosage of the various blended components in the studied extracts depends on several factors, including the source of the extracts, their diverse nature, and their mechanism of action with xanthine oxidase. However, it should be noted that this principal component analysis represents the dosage distribution of only 12 extracts. Therefore, this small number of samples may influence the conclusions of the principal component analysis by highlighting a potential factor not revealed by these extracts. Furthermore, this study demonstrates that the extracts containing the combination of *Laurus nobilis* and honey species, which fall within the same region of the xanthine oxidase and uric acid solubility variables, lead us to conclude that this plant, along with honey, represents the most significant anti-gout potential compared to the other extracts.

Conclusion

IV. Conclusion

Facing to the several mounting limitations of synthetic chemistry in terms of heavy costs, spasmodic problems, and widespread side effects, the pharmaceutical industry is increasingly turning towards natural bioavailability enhancers as green alternatives, particularly medicinal plants, and honey. Therefore, an experiment was conducted to study the *in vitro* curative potential of 12 mixtures of two medicinal plants and six local varieties of honey, by testing their efficacy in the treatment of gout for the first time. Indeed, the results obtained in this work lead us to the following conclusions:

- ✓ Quantitative phytochemical screening revealed the presence of polyphenols, sodium, potassium and calcium, with significant intra- and inter-specific variability depending on the mixture type and extractive method;
- ✓ All the studied extracts showed xanthine oxidase inhibitory and uric acid dissolving properties at varying levels. Thus, the mixture extracts containing *Laurus nobilis* and *Rosmarinus officinalis* with honey (*Euphorbia guyoniana* + *Retama raetam* and Mountain) appeared to have the most potent xanthine oxidase inhibitory effect and the best uric acid dissolving capacity;
- ✓ Extracts containing *Rosmarinus officinalis* and honey showed a weak cytotoxic effect against isolated erythrocytes compared to the mixture containing *Laurus nobilis* and honey, which does not present any significant risk of cytotoxicity;
- ✓ Principal Component Analysis (PCA) analysis showed that extracts containing *Laurus nobilis* and honey exhibited an anti-gout efficacy. Thus, their potent anti-gout effect appears to be moderately correlated with sodium content.

This study remains preliminary and can serve as a baseline for further research, and several perspectives can be considered:

- ✓ Determination of the main compounds of the active extracts for a better understanding of XO inhibition;
- ✓ Study of the anti-gout activities of the active extracts *in vivo* to demonstrate their potential and determine their preventive and therapeutic doses.

References

V. References

- Abu Bakar, F. I., Abu Bakar, M. F., Rahmat, A., Abdullah, N., Sabran, S. F., & Endrini, S. (2018). Anti-gout potential of Malaysian medicinal plants. Frontiers in pharmacology, 9, 261. https://doi.org/10.3389/fphar.2018.00261
- Ahmed, S., Sulaiman, S. A., Baig, A. A., Ibrahim, M., Liaqat, S., Fatima, S., ... & Othman, N. H. (2018). Honey as a potential natural antioxidant medicine: an insight into its molecular mechanisms of action. Oxidative medicine and cellular longevity, 2018(1), 8367846. https://doi.org/10.1155/2018/8367846
- Aihemaitijiang, S., Zhang, Y., Zhang, L., Yang, J., Ye, C., Halimulati, M., Zhang, W., & Zhang, Z. (2020). The Association between Purine-Rich Food Intake and Hyperuricemia: A Cross-Sectional Study in Chinese Adult Residents. Nutrients, 12(12), 3835. https://doi.org/10.3390/nu12123835
- Alam, M., Uddin, G., Rashid, U., Rauf, A., Raza, M., Shah, S. M. M., ... & Khan, A. (2021). In vitro and in silico xanthine oxidase inhibitory potential of Benzofuran isolated from Viburnum grandiflorum Wall. Ex DC. South African Journal of Botany, 143, 359-362. https://doi.org/10.1016/j.sajb.2021.01.010
- Alejo-Armijo, A., Altarejos, J., & Salido, S. (2017). Phytochemicals and biological activities of laurel tree (Laurus nobilis). Natural product communications, 12(5), 1934578X1701200519. https://doi.org/10.1177/1934578X1701200519
- Al-Zeidaneen, E. A., & Atrooz, O. M. (2025). Evaluation of the proteolytic and biological activities of thistle honey. *Journal of microbiology, biotechnology and food sciences*, *14*(5), e10883-e10883. https://doi.org/10.55251/jmbfs.10883
- Amessis-Ouchemoukh, N., Maouche, N., Otmani, A., Terrab, A., Madani, K., & Ouchemoukh, S. (2021). Evaluation of Algerian's honey in terms of quality and authenticity based on the melissopalynology and physicochemical analysis and their antioxidant powers. Mediterranean journal of nutrition and metabolism, 14(3), 305-324. https://doi.org/10.3233/MNM-210561
- Atanasov, A. G., Zotchev, S. B., Dirsch, V. M., & Supuran, C. T. (2021). Natural products in drug discovery: advances and opportunities. Nature reviews Drug discovery, 20(3), 200-216. https://doi.org/10.1038/s41573-020-00114-z
- Athamena, S., Laroui, S., & Athamena, M. (2015). Phenolic composition, antimicrobial activity of *Rosmarinus officinalis*. *Sciences & Technologie*, *41*(3), 21-30.

- Atiya, R. M., Elsherif, K. M., Alkherraz, A. M., & Mohamed, G. A. S. (2024). A Comparative Study on the Chemical Constituents of Four Libyan Herbs: Mentha Piperita, Matricaria Chamomile L, Rosmarinus Officinalis L, and Thymus Vulgaris. Scientific Journal for Faculty of Science-Sirte University, 4(2), 33-40.
- Atrooz, O. M., AL-Naemat, W. H., & Atrooz, M. O. (2024). Insights on the Proteolytic and Biological Properties of Floral Honeys with Various Botanical Sources. Tropical Journal of Natural Product Research, 8(10). https://doi.org/10.26538/tjnpr/v8i10.21
- Becker, M. A., Schumacher Jr, H. R., Wortmann, R. L., MacDonald, P. A., Eustace, D., Palo, W. A., ... & Joseph-Ridge, N. (2005). Febuxostat compared with allopurinol in patients with hyperuricemia and gout. New England Journal of Medicine, 353(23), 2450-2461. https://doi.org/10.1056/NEJMoa050373
- Benarba, B. (2016). Medicinal plants used by traditional healers from South-West Algeria: An ethnobotanical study. Journal of intercultural ethnopharmacology, 5(4), 320-330. https://doi.org/10.5455/jice.20160814115725
- Berboucha, M., Ayouni, K., Atmani, D., Atmani, D., & Benboubetra, M. (2010). Kinetic study on the inhibition of xanthine oxidase by extracts from two selected Algerian plants traditionally used for the treatment of inflammatory diseases. Journal of medicinal food, 13(4), 896–904. https://doi.org/10.1089/jmf.2009.0164
- Bouasla, A. et Bouasla, I. (2018). Ethnobotanical survey of medicinal plants in northeastern of Algeria. Phytomedicine, 36: 68–81. http://dx.doi.org/10.1016/j.phymed.2017.09.007
- Boukerker, H., Salemkour, N., Nouasria, D., Benyakhlef, B., Nacereddine, S., Chalabi, K., Nouidjem, Y., Belhamra, M. (2016). La végétation steppique au profit de la phytothérapie dans la région d'El Bayadh. Journal Algérien des Régions Arides (JARA), 16: 61-73.
- Bukowska, B., & Kowalska, S. (2004). Phenol and catechol induce prehemolytic and hemolytic changes in human erythrocytes. Toxicology letters, 152(1), 73-84. https://doi.org/10.1016/j.toxlet.2004.03.025
- Celiktas, O. Y., Kocabas, E. H., Bedir, E., Sukan, F. V., Ozek, T., & Baser, K. H. C. (2007). Antimicrobial activities of methanol extracts and essential oils of Rosmarinus officinalis, depending on location and seasonal variations. Food Chemistry, 100(2), 553-559. https://doi.org/10.1016/j.foodchem.2005.10.011
- Ceylan, R., Katanić, J., Zengin, G., Matić, S., Aktumsek, A., Boroja, T., ... & Yılmaz, M. A. (2016). Chemical and biological fingerprints of two Fabaceae species (Cytisopsis dorycniifolia and Ebenus hirsuta): Are they novel sources of natural agents for pharmaceutical and food formulations?. Industrial Crops and Products, 84, 254-262. https://doi.org/10.1016/j.indcrop.2016.02.019

- Chansiw, N., Paradee, N., Chotinantakul, K., & Srichairattanakool, S. (2018). Anti-hemolytic, antibacterial and anti-cancer activities of methanolic extracts from leaves and stems of Polygonum odoratum. Asian Pacific Journal of Tropical Biomedicine, 8(12), 580-585. https://doi.org/10.4103/2221-1691.248094
- Chehma, A. et Djebar, M.R. (2008). Les espèces médicinales spontanées du Sahara septentrional algérien distribution spatio-temporelle et étude ethnobotanique. Revue Synthèse, 17 : 36-45.
- Chen, G., Tan, M. L., Li, K. K., Leung, P. C., & Ko, C. H. (2015). Green tea polyphenols decreases uric acid level through xanthine oxidase and renal urate transporters in hyperuricemic mice. Journal of Ethnopharmacology, 175, 14-20. https://doi.org/10.1016/j.jep.2015.08.043
- Cheriti, A., Rouissat, A., Sekkoum, K., & Balansard, G. (1995). Plantes de la pharmacopée traditionelle dans la région d'El-Bayadh (Algérie). Fitoterapia (Milano), 66(6), 525-538.
- Chermat, S. and Gharzouli, R. (2015). Ethnobotanical Study of Medicinal Flora in the North East of Algeria - An Empirical Knowledge in Djebel Zdimm (Setif). Journal of Materials Science and Engineering A 5 (1-2): 50-59. https://doi.org/10.17265/2161-6213/2015.1-2.007
- Child, P., & Rafter, J. (1986). Calcium enhances the hemolytic action of bile salts. Biochimica et Biophysica Acta (BBA)-Biomembranes, 855(3), 357-364. https://doi.org/10.1016/0005-2736(86)90081-7
- Dalbeth, N. Gosling, A.L., Gaffo, A., & Abhishek, A. (2021). Gout. Lancet, 397: 1843–55.
- Daniels, G. (2008). Human blood groups. John Wiley & Sons.
- Dif, M., Benali-Toumi, F., Benyahia, M., Becheikhi, F.A. (2015). Survey phytotherapeutic use of 11 medicinal plants growing in Tessala mountains. Phytothérapie, 13:295-297. https://doi.org/10.1007/s10298-015-0962-y .
- Di Petrillo, A., Santos-Buelga, C., Era, B., González-Paramás, A. M., Tuberoso, C. I. G., Medda, R., ... & Fais, A. (2018). Sardinian honeys as sources of xanthine oxidase and tyrosinase inhibitors. Food science and biotechnology, 27, 139-146. https://doi.org/10.1007/s10068-017-0275-z
- Djermane, N., Rebbas, K., Benhissen, S., & Belkassam, A. (2022). Ethnobotanical study and inventory of medicinal plants in Hammam Dalaa (M'Sila, Algeria). Journal of EcoAgriTourism, 18(1). 44-55.
- Dobroslavić, E., Elez Garofulić, I., Zorić, Z., Pedisić, S., & Dragović-Uzelac, V. (2021). Polyphenolic characterization and antioxidant capacity of Laurus nobilis L. leaf extracts obtained by green and conventional extraction techniques. Processes, 9(10), 1840. https://doi.org/10.3390/pr9101840

- Doherty, M. Jansen, T. L., Nuki, G., Pascual, E., Perez-Ruiz, F., Punzi, L., So, A.K., Bardin, T. (2012). Gout: why is this curable disease so seldom cured? Ann. Rheum. Dis., 71:1765–1770. https://doi.org/10.1136/annrheumdis-2012-201687.
- El-Tantawy, W. H. (2021). Natural products for the management of hyperuricaemia and gout: a review. Archives of Physiology and Biochemistry, 127(1), 61-72. https://doi.org/10.1080/13813455.2019.1610779
- Erkan, N., Ayranci, G., & Ayranci, E. (2008). Antioxidant activities of rosemary (Rosmarinus Officinalis L.) extract, blackseed (Nigella sativa L.) essential oil, carnosic acid, rosmarinic acid and sesamol. Food chemistry, 110(1), 76-82. https://doi.org/10.1016/j.foodchem.2008.01.058
- Fadili, K., Amalich, S., N'dedianhoua, S. K., Bouachrine, M., Mahjoubi, M., El Hilali, F., & Zair, T. (2015). Teneurs en polyphénols et évaluation de l'activité antioxydante des extraits de deux espèces du Haut Atlas du Maroc: Rosmarinus officinalis et Thymus satureioides. International Journal of Innovation and Scientific Research, 17(1), 24-33.
- Fridovich, I., & Handler, P. (1962). Xanthine oxidase: V. Differential inhibition of the reduction of various electron acceptors. Journal of Biological Chemistry, 237(3), 916-921. https://doi.org/10.1016/S0021-9258(18)60393-X
- Gao, L., Wang, B., Pan, Y., Lu, Y., & Cheng, R. (2021). Cardiovascular safety of febuxostat compared to allopurinol for the treatment of gout: a systematic and meta-analysis. Clinical cardiology, 44(7), 907-916. https://doi.org/10.1002/clc.23643
- Guedouari, R., & Nabiev, M. (2021). Anti-inflammatory activity of different extracts from Laurus nobilis growing in Algeria. Algerian Journal of Environmental Science and Technology, 7(4). 2115-2120
- Habib, H. M., Kheadr, E., & Ibrahim, W. H. (2021). Inhibitory effects of honey from arid land on some enzymes and protein damage. Food Chemistry, 364, 130415. https://doi.org/10.1016/j.foodchem.2021.130415
- Hadjadj, S., Bayoussef, H., Ould El Hadj-Khelil, A., Beggat, H., Bouhafs, Z., Boukaka, Y., Amina Khaldi, I., Mimouni, S., Sayah, F. and Tey, M. (2015). Ethnobotanical study and phytochemical screening of six medicinal plants used in traditional medicine in the Northeastern Sahara of Algeria (area of Ouargla). J. Med. Plants Res., 9(41): 1049-1059. https://doi.org/10.5897/JMPR2015.5932
- Hafez, R. M., Abdel-Rahman, T. M., & Naguib, R. M. (2017). Uric acid in plants and microorganisms: Biological applications and genetics - A review. Journal of Advanced Research, 8(5), 475–486. https://doi.org/10.1016/j.jare.2017.05.003

- Hamel, T., Sadou, S., Seridi, R., Boukhdir, S., & Boulemtafes, A. (2018). Pratique traditionnelle d'utilisation des plantes médicinales dans la population de la péninsule de l'edough (nord-est algérien). Ethnopharmacologia, 59, 65-71.
- Ho, S. C., Tsai, T. H., Tsai, P. J., & Lin, C. C. (2008). Protective capacities of certain spices against peroxynitrite-mediated biomolecular damage. Food and Chemical Toxicology, 46(3), 920-928. https://doi.org/10.1016/j.fct.2007.10.028
- Hoffbrand, A. V. (2024). Hoffbrand's essential haematology. John Wiley & Sons.
- Hu, M., & Tomlinson, B. (2008). Febuxostat in the management of hyperuricemia and chronic gout: a review. Therapeutics and clinical risk management, 4(6), 1209–1220. https://doi.org/10.2147/tcrm.s3310
- Hudaib, M. M., Tawaha, K. A., Mohammad, M. K., Assaf, A. M., Issa, A. Y., Alali, F. Q., ... & Bustanji, Y. K. (2011). Xanthine oxidase inhibitory activity of the methanolic extracts of selected Jordanian medicinal plants. Pharmacognosy Magazine, 7(28), 320-324. https://doi.org/10.4103/0973-1296.90413
- Hussan, K. S., Thayyil, M. S., Rajan, V. K., & Muraleedharan, K. (2019). DFT studies on global parameters, antioxidant mechanism and molecular docking of amlodipine besylate. Computational Biology and Chemistry, 80, 46-53. https://doi.org/10.1016/j.compbiolchem.2019.03.006
- Imtara, H., Elamine, Y., & Lyoussi, B. (2018). Honey antibacterial effect boosting using Origanum vulgare L. essential oil. Evidence-Based Complementary and Alternative Medicine, 2018(1), 7842583. https://doi.org/10.1155/2018/7842583
- Ishtiaque, S., Naz, S., Soomro, N., Khan, K., & Siddiqui, R. (2015). Antioxidant activity and total phenolics content of extracts from Murraya koenigii (curry leaves), Laurus nobilis (bay leaves), and Camellia sinensis (tea). Quaid-E-Awam University Research Journal of Engineering, Science & Technology, 14(2), 20-25.
- Jakše, B., Jakše, B., Pajek, M., & Pajek, J. (2019). Uric acid and plant-based nutrition. Nutrients, 11(8), 1736. https://doi.org/10.3390/nu11081736
- Kefifa, A., Saidi, A., Hachem, K., & Ouammi, L. (2020). An ethnobotanical survey and quantitative study of indigenous medicinal plants used in the Algerian semi-arid region. Phytothérapie, 18(3-4), 204-219. https://doi.org/10.3166/phyto-2018-0077
- Kek, S. P., Chin, N. L., Tan, S. W., Yusof, Y. A., & Chua, L. S. (2017). Classification of honey from its bee origin via chemical profiles and mineral content. Food Analytical Methods, 10, 19-30. https://doi.org/10.1007/s12161-016-0544-0
- Keohane, E. M., Smith, L., & Walenga, J. M. (2015). Rodak's hematology: clinical principles and applications. Elsevier Health Sciences.

- Knight, S., Mathis, W., & Graham, J. (1951). Mineral analysis with flame photometer. Analytical Chemistry, 23(11), 1704-1706. https://doi.org/10.1021/ac60059a057
- Lai, S. W., Liao, K. F., Kuo, Y. H., Hwang, B. F., & Liu, C. S. (2023). Comparison of benzbromarone and allopurinol on the risk of chronic kidney disease in people with asymptomatic hyperuricemia. European Journal of Internal Medicine, 113, 91-97. https://doi.org/10.1016/j.ejim.2023.04.025
- Lakhdari, W., Dehliz, A., Acheuk, F., Mlik, R., Hammi, H., Doumandji-Mitiche, B., Gheriani, S., Berrekbia, M., Guermit, K., Chergui, S. (2016). Ethnobotanical study of some plants used in traditional medicine in the region of Oued Righ (Algerian Sahara). Journal of Medicinal Plants Studies, 4(2): 204-211.
- Lawag, I.L., Islam, M.K., Sostaric, T., Lim, L.Y., Hammer, K., Locher, C. (2023). Antioxidant Activity and Phenolic Compound Identification and Quantification in Western Australian Honeys. Antioxidants , 12, 189. https://doi.org/10.3390/antiox12010189
- Lee, J. H., Kim, Y. G., Yong Ryu, S., & Lee, J. (2016). Calcium-chelating alizarin and other anthraquinones inhibit biofilm formation and the hemolytic activity of Staphylococcus aureus. Sci Rep 6: 19267. https://doi.org/10.1038/srep19267
- Lee, E. H., Shin, J. H., Kim, S. S., Lee, H., Yang, S. R., & Seo, S. R. (2019). Laurus nobilis leaf extract controls inflammation by suppressing NLRP3 inflammasome activation. Journal of cellular physiology, 234(5), 6854-6864. https://doi.org/10.1002/jcp.27434
- Li, G. X., & Liu, Z. Q. (2008). The protective effects of ginsenosides on human erythrocytes against hemin-induced hemolysis. Food and chemical toxicology, 46(3), 886-892. https://doi.org/10.1016/j.fct.2007.10.020
- Li, M. J., Liu, L., Fu, Y., & Guo, Q. X. (2007). Accurate bond dissociation enthalpies of popular antioxidants predicted by the ONIOM-G3B3 method. Journal of Molecular Structure: THEOCHEM, 815(1-3), 1-9. https://doi.org/10.1016/j.theochem.2007.03.012
- Ling, X., & Bochu, W. (2014). A review of phytotherapy of gout: perspective of new pharmacological treatments. Die Pharmazie-An International Journal of Pharmaceutical Sciences, 69(4), 243-256. https://doi.org/10.1691/ph.2014.3642
- Lomiso, D. F., & Nigussie, G. (2019). Determination of Some Essential Minerals in Honey Samples Collected from Chena District, Ethiopia. Chemistry and Materials Research, 11(7), https://doi.org/10.7176/CMR
- Lu, M., Yuan, B., Zeng, M., & Chen, J. (2011). Antioxidant capacity and major phenolic compounds of spices commonly consumed in China. Food Research International, 44(2), 530-536. https://doi.org/10.1016/j.foodres.2010.10.055

- Matuz-Mares, D., González-Andrade, M., Araiza-Villanueva, M. G., Vilchis-Landeros, M. M., & Vázquez-Meza, H. (2022). Mitochondrial calcium: effects of its imbalance in disease. Antioxidants, 11(5), 801. https://doi.org/10.3390/antiox11050801
- Miara, M. D., Bendif, H., Ait Hammou, M., & Teixidor-Toneu, I. (2018). Ethnobotanical survey of medicinal plants used by nomadic peoples in the Algerian steppe. Journal of ethnopharmacology, 219, 248-256. https://doi.org/10.1016/j.jep.2018.03.0111
- Mrabet, A., Abdelfattah, B., El Mansouri, F., Simou, A., & Khaddor, M. (2024). Bay Laurel of Northern Morocco: A Comprehensive Analysis of Its Phytochemical Profile, Mineralogical Composition, and Antioxidant Potential. Biophysica, 4(2), 238-255. https://doi.org/10.3390/biophysica4020017
- Muñiz-Márquez, D. B., Martínez-Ávila, G. C., Wong-Paz, J. E., Belmares-Cerda, R., Rodríguez-Herrera, R., & Aguilar, C. N. (2013). Ultrasound-assisted extraction of phenolic compounds from Laurus nobilis L. and their antioxidant activity. Ultrasonics sonochemistry, 20(5), 1149-1154. https://doi.org/10.1016/j.ultsonch.2013.02.008
- Muñiz-Márquez, D. B., Rodríguez, R., Balagurusamy, N., Carrillo, M. L., Belmares, R., Contreras, J. C., ... & Aguilar, C. N. (2014). Phenolic content and antioxidant capacity of extracts of Laurus nobilis L., Coriandrum sativum L. and Amaranthus hybridus L. CyTA-Journal of Food, 12(3), 271-276. https://doi.org/10.1080/19476337.2013.847500
- Muñiz-Márquez, D. B., Wong-Paz, J. E., Contreras-Esquivel, J. C., Rodríguez-Herrera, R., & Aguilar, C. N. (2018). Bioactive compounds from bay leaves (Laurus nobilis) extracted by microwave technology. Zeitschrift für Naturforschung C, 73(9-10), 401-407.
- Necib, A., Kefti, L., Draiaia, R., Mohamadi, N., & Rezig, S. (2022). Evaluation of Polyphenols, Vitamin C Contents and Antioxidant Activity of Two Types of Algerian Honey. Turkish Journal of Agriculture-Food Science and Technology, 10(12), 2539-2544. https://doi.org/10.24925/turjaf.v10i12.2539-2544.5475
- Nedić, N., Nešović, M., Radišić, P., Gašić, U., Baošić, R., Joksimović, K., ... & Vovk, I. (2022). Polyphenolic and chemical profiles of honey from the Tara Mountain in Serbia. Front Nutr 9: 941463. https://doi.org/10.3389/fnut.2022.941463
- Nyarko, K., Boozer, K., Greenlief, C.M. (2023). Profiling of the Phenolic Content of Honey from Different Geographical Origins in the United States. Molecules, 28, 5011. https://doi.org/10.3390/molecules28135011
- Obeagu, E. I., Igwe, M. C., & Obeagu, G. U. (2024). Oxidative stress's impact on red blood cells: Unveiling implications for health and disease. Medicine, 103(9), e37360. https://doi.org/10.1097/MD.0000000000037360

- Olugbodi, J. O., Tincho, M. B., Oguntibeju, O. O., Olaleye, M. T., & Akinmoladun, A. C. (2019). Glyphaea brevis–in vitro antioxidant and in silico biological activity of major constituents and molecular docking analyses. Toxicology in Vitro, 59, 187-196. https://doi.org/10.1016/j.tiv.2019.04.013
- Otmani, A., Amessis-Ouchemoukh, N., Birinci, C., Yahiaoui, S., Kolayli, S., Rodríguez-Flores, M. S., … Ouchemoukh, S. (2021). Phenolic compounds and antioxidant and antibacterial activities of Algerian honeys. Food Bioscience, 42, 101070. https://doi.org/10.1016/j.fbio.2021.101070
- Ouyang, H., Hou, K., Peng, W., Liu, Z., & Deng, H. (2018). Antioxidant and xanthine oxidase inhibitory activities of total polyphenols from onion. Saudi Journal of Biological Sciences, 25(7), 1509-1513. https://doi.org/10.1016/j.sjbs.2017.08.005
- Pasupuleti, V. R., Sammugam, L., Ramesh, N., & Gan, S. H. (2017)Honey, propolis, and royal jelly: A comprehensive review of their biological actions and health benefits. Oxid Med Cell Longev. 1259510. https://doi.org/10.1155/2017/1259510
- Paula, V. B., Sousa-Dias, M. L., Seixas, N. L., Combarros-Fuertes, P., Estevinho, L. M., & Dias, L. G. (2024). Phenolic Class Analysis in Honey: Comparison of Classical and Single UV Spectrum Methodologies. Processes, 12(10), 2297. https://doi.org/10.3390/pr12102297
- Perna, A. M., Simonetti, A., Intaglietta, I., & Gambacorta, E. (2013). Antioxidant Properties, Polyphenol Content and Colorimetric Characteristics of Different Floral Origin Honeys from Different Areas of Southern Italy. Journal of Life Sciences, 7(4).
- Rai, S. K., Burns, L. C., De Vera, M. A., Haji, A., Giustini, D., & Choi, H. K. (2015). The economic burden of gout: A systematic review. Seminars in Arthritis and Rheumatism, 45(1), 75–80. https://doi.org/10.1016/j.semarthrit.2015.02.
- Rincon, E., Balu, A. M., Luque, R., & Serrano, L. (2019). Mechanochemical extraction of antioxidant phenolic compounds from Mediterranean and medicinal Laurus nobilis: A comparative study with other traditional and green novel techniques. Industrial Crops and Products, 141, 111805. https://doi.org/10.1016/j.indcrop.2019.111805
- Roleira, F. M., Varela, C. L., Costa, S. C., & Tavares-da-Silva, E. J. (2018). Phenolic derivatives from medicinal herbs and plant extracts: anticancer effects and synthetic approaches to modulate biological activity. *Studies in Natural Products Chemistry*, *57*, 115-156. https://doi.org/10.1016/B978-0-444-64057-4.00004-1
- Ryan, M. J., Jackson, J. R., Hao, Y., Leonard, S. S., & Alway, S. E. (2011). Inhibition of xanthine oxidase reduces oxidative stress and improves skeletal muscle function in response to electrically stimulated isometric contractions in aged mice. Free radical biology and medicine, 51(1), 38-52. https://doi.org/10.1016/j.freeradbiomed.2011.04.002

- Sahin H. (2016). Honey as an apitherapic product: its inhibitory effect on urease and xanthine oxidase. Journal of enzyme inhibition and medicinal chemistry, 31(3), 490–494. https://doi.org/10.3109/14756366.2015.1039532
- Schumacher Jr, H. R., Becker, M. A., Wortmann, R. L., MacDonald, P. A., Hunt, B., Streit, J., ... & Joseph-Ridge, N. (2008). Effects of febuxostat versus allopurinol and placebo in reducing serum urate in subjects with hyperuricemia and gout: a 28-week, phase III, randomized, double-blind, parallel-group trial. Arthritis Care & Research, 59(11), 1540-1548. https://doi.org/10.1002/art.24209
- Senouci, F., Ababou, A., & Chouieb, M. (2019). Ethnobotanical survey of the medicinal plants used in the Southern Mediterranean. Case study: the region of Bissa (Northeastern Dahra Mountains, Algeria). Pharmacognosy Journal, 11(4):647-659. http://dx.doi.org/10.5530/pj.2019.11.103
- Silvestre, S. M., Almeida, P. J. S., & El-Shishtawy, R. (2020). Natural Products as a Source for New Leads in Gout Treatment. Evidence-based complementary and alternative medicine : eCAM, 2020, 8274975, 3 pages. https://doi.org/10.1155/2020/8274975
- Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American journal of Enology and Viticulture, 16(3), 144-158. https://doi.org/10.5344/ajev.1965.16.3.144
- Smith, H. S., Bracken, D., & Smith, J. M. (2011). Gout: current insights and future perspectives. The Journal of Pain, 12(11), 1113-1129. https://doi.org/10.1016/j.jpain.2011.06.009
- Sridhar, A., Ponnuchamy, M., Kumar, P. S., Kapoor, A., Vo, D. V. N., & Prabhakar, S. (2021). Techniques and modeling of polyphenol extraction from food: A review. Environmental Chemistry Letters, 19, 3409-3443. https://doi.org/10.1007/s10311-021-01217-8
- Takaki, I., Bersani-Amado, L. E., Vendruscolo, A., Sartoretto, S. M., Diniz, S. P., Bersani-Amado, C. A., & Cuman, R. K. N. (2008). Anti-inflammatory and antinociceptive effects of Rosmarinus officinalis L. essential oil in experimental animal models. Journal of medicinal food, 11(4), 741-746. https://doi.org/10.1089/jmf.2007.0524
- Tawaha, K., Alali, F. Q., Gharaibeh, M., Mohammad, M., & El-Elimat, T. (2007). Antioxidant activity and total phenolic content of selected Jordanian plant species. Food chemistry, 104(4), 1372-1378. https://doi.org/10.1016/j.foodchem.2007.01.064
- Tsai, P. J., Tsai, T. H., & Ho, S. C. (2007). In vitro inhibitory effects of rosemary extracts on growth and glucosyltransferase activity of Streptococcus sobrinus. Food chemistry, 105(1), 311-316. https://doi.org/10.1016/j.foodchem.2006.11.051

- Vargas-Santos, A.B. and Neogi, T. (2017). Management of Gout and Hyperuricemia in CKD. Am J Kidney Dis., 70(3):422-439. http://dx.doi.org/10.1053/j.ajkd.2017.01.055

- Wabaidur, S. M., Obbed, M. S., Alothman, Z. A., Alfaris, N. A., Badjah-Hadj-Ahmed, A. Y., Siddiqui, M. R., ... & Aldayel, T. S. (2020). Total phenolic acids and flavonoid contents determination in Yemeni honey of various floral sources: Folin-Ciocalteu and spectrophotometric approach. Food Science and Technology, 40, 647-652. https://doi.org/10.1590/fst.33119
- Wilczynska, A. (2010). Phenolic content and antioxidant activity of different types of polish honey-a short report. Polish journal of food and nutrition sciences, 60(4), 309-313.
- Xie, Z., Wang, Y., Askari, A., Huang, W. H., Klaunig, J. E., & Askari, A. (1990). Studies on the specificity of the effects of oxygen metabolites on cardiac sodium pump. Journal of molecular and cellular cardiology, 22(8), 911-920. https://doi.org/10.1016/0022-2828(90)90122-I
- Yayinie, M., Atlabachew, M., Tesfaye, A., Hilluf, W., Reta, C., & Alemneh, T. (2022). Polyphenols, flavonoids, and antioxidant content of honey coupled with chemometric method: Geographical origin classification from Amhara region, Ethiopia. International Journal of Food Properties, 25(1), 76-92. https://doi.org/10.1080/10942912.2021.2021940
- Yuan, H. Y., Zhang, X. H., Zhang, X. L., Wei, J. F., & Meng, L. (2014). Analysis of patents on anti-gout therapies issued in China. Expert Opinion on Therapeutic Patents, 24(5), 555-572. https://doi.org/10.1517/13543776.2014.895325
- Zhang, Y., Chen, S., Yuan, M., Xu, Y., & Xu, H. (2022). Gout and Diet: A Comprehensive Review of Mechanisms and Management. Nutrients, 14(17), 3525. https://doi.org/10.3390/nu14173525
- Zhou, M., Huang, X., Li, R., Zhang, Z., Zhang, L., Gao, X., ... & Ma, Y. (2022). Association of dietary patterns with blood uric acid concentration and hyperuricemia in northern Chinese adults. Nutrition journal, 21(1), 42. https://doi.org/10.1186/s12937-022-00789-7
- Zou, S., Tao, H., & Chang, Y. N. (2022). Characterization of antioxidant activity and analysis of phenolic acids and flavonoids in linden honey. Food Science and Technology, 42, e76621. https://doi.org/10.1590/fst.76621

ملخص:

تهدف هذه الدراسة المخبرية إلى تقييم فعالية 12 مستخلصًا ناتجا عن مزيج ثنائي من نباتين طبيين وستة أنواع من العسل المحلي كمثبطات لأوكسيديز الزانثين، وتحلل البروتين، وإذابة حمض اليوريك. كما مكنت من دراسة تأثيرها على خلايا الدم الحمراء، وفحصت بعض المركبات الكيميائية النباتية. أظهر التحليل وجود اختلافات جوهرية في محتوى المركبات الفينولية والأملاح المعدنية وفقًا لنوع النبات والعسل. كشفت التجارب عن تفاوت درجات تأثير جميع المستخلصات في مكافحة النقرس. وكان الأفضل هو مستخلص *Laurus nobilis* ومستخلص Rosmarinus *officinalis* الطبي (نوعان من العسل). وقد أظهرا أقوى تثبيط لأوكسيديز الزانثين وأعلى قدرة على إذابة حمض اليوريك. ومع ذلك، فقد لوحظ أن مستخلص *Laurus nobilis* ضار بخلايا الدم الحمراء، مقارنةً *Rosmarinus officinalis* الطبي، مما يجعله جزيئًا أكثر أمانًا للعلاج العلاجي للأمراض التي يسببها هذا الإنزيم.

الكلمات المفتاحية: لرند(*Laurus nobilis*)، ازير(*Rosmarinus officinalis*)، العسل، المزيج، المستخلصات ، أوكسيداز الزانثين ، حمض البوليك ، السمية ، تحلل البروتين

Résumé :

Cette étude visait à évaluer l'efficacité de 12 extraits issus de combinaison binaire de deux plantes médicinales et six miels locaux comme inhibiteurs de la xanthine oxydase, de l'hydrolyse des protéines et de la dissolution de l'acide urique. Elle a également évalué leur effet sur les globules rouges et examiné certains composés phytochimiques. L'analyse a montré d'importantes différences dans la teneur en composés phénoliques et en sels minéraux selon la plante et l'espèce de miel. Les expériences ont révélé que tous les extraits possédaient différents degrés d'action antigoutteuse. Les meilleurs étaient *Laurus nobilis* et *Rosmarinus officinalis* (deux miels). Ils présentaient la plus forte inhibition de la xanthine oxydase et la plus grande capacité à dissoudre l'acide urique. Cependant, il a été constaté que l'extrait de *Laurus nobilis* est nocif pour les globules rouges par rapport à *Rosmarinus officinalis*, ce qui en fait une molécule plus sûre pour le traitement thérapeutique des maladies causées par cette enzyme.

Mots clés : *Laurus nobilis, Rosmarinus officinalis*, miel, Combinaison, extrait, xanthine oxydase, acide urique, toxicité, dénaturation protéique.

Abstract:

This laboratory study aimed to evaluate the effectiveness of 12 extracts issued of binary combination of two medicinal plants and six local honeys as inhibitors of xanthine oxidase, protein hydrolysis, and uric acid dissolution. It also evaluated their effect on red blood cells, as well as examined some phytochemical compounds. The analysis showed that there were monumental differences in the content of phenolic compounds and mineral salts according to the plant and honey species. The experiments revealed that all the extracts possessed varying degrees of anti-gout action. The best was *Laurus nobilis* and *Rosmarinus officinalis* (two honeys). They presented the strongest inhibition of xanthine oxidase and highest ability to dissolve uric acid. However, it has been noted that *Laurus nobilis* extract is harmful to red blood cells, as compared to *Rosmarinus officinalis*, thus making it a safer molecule for the therapeutic treatment of diseases caused by this enzyme.

Keywords: *Laurus nobilis, Rosmarinus officinalis*, honey, Combination, extract xanthine oxidase, uric acid, toxicity, protein degradation.