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SAYAH Amira Faiza & CHEKROUNE Hamza

Thème

La comparaison des propriétés physicochimiques et biologiques de quelques miel florales de l'Algérie.

Document déposé auprès du jury composé de :

Nom et Prénom	***Grade***		
Mme CHERIFI A	*MCB*	*Univ. de Bouira*	*Présidente*
Mr KADRI N	*Prof*	*Univ. de Bouira*	*Examinateur*
Mme DJENADI K	MCB	*Univ. de Bouira*	*Promotrice*
Melle GUENAOUI N	*Doctorante*	*Univ. de Bejaia*	*Co Promotrice*

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Field: Nature and Life Sciences **Branch:** Biological Sciences

Specialty: Microbial Biotechnology

By

SAYAH Amira Faiza & CHEKROUNE Hamza

Comparison of physicochemical and biological properties of some Algerian floral honeys

Ahead of the Jury:

Last and first name	***Grade***		
Ms CHERIFI A	*MCB*	*Bouira university*	*Chair*
Mr KADRI N	*Professor*	*Bouira university*	*Examiner*
Ms DJENADI K	*MCB.*	*Bouira university*	*supervisor*
Miss GUENAOUI N	*Doctoral student*	*Bejaia university*	*Co-supervisor*

Scholar year: 2022/ 2023

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Dedication

To my dear parents, for all their sacrifices, love, tenderness, support and prayers throughout my studies

To my brothers and sisters

To my loved ones

To all my friends

I dedicate the fruit of my 18 years of study.

Amira et Hamza

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MGO= glyoxal, 3-deoxyglucosulose, and methylglyoxal

MH= Minimum Bactericidal Concentration

UMF= Unique Manuka Factor

HMF= hydroxy methyl furfural

Abs=Absorbance

RI= Refractive Index

pH= hydrogen potential

HPLC=high performance liquid chromatography

BSA= Bovine Serum Albumin

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Introduction

Introduction

Nowadays, human health is threatened by numerous metabolic dysfunctions and multidrug resistant germs. Therefore, numerous organisms and laboratories are looking for new molecules capable of treating metabolic disorders and inhibiting the growth of pathogenic germs. Among the active compounds being considered to solve this critical situation, scientists have suggested the use of honey bees. For decades, honey bees have been used to cure several infections and physiological dysfunctions.

Honey bee is one of the sweet beekeeping products, composed mainly of sugar and water. Honey is known for many biological activities including anti- inflammatory, antimicrobial, antioxidant and anti-tumor. More than 180 substances are characterized in honey namely: proteins, amino acids, organic acids, enzymes, phenolic components and minerals. The presence of phenolic compounds, such as flavonoids and polyphenols in honey which are transferred through the nectar to honey. These compounds play a vital role in defining essential features such as color, flavor, and functional properties of honey (Peláez-Acero *et al.,* 2022).

Around the world numerous investigations highlighted on the phenolic compounds content and their biological proprieties. Importantly, they revealed that each type of honey exhibits distinct and individual characteristics based on its particular floral source *(Bobis et al., 2020, Santos-Buelga and Gonzale Paramas, 2017).* Moreover, they reported that treatment with ultrasound and /or high temperature increase in the phenolic compound content, thus reduce the biologicalproperties. (Peláez-Acero *et al.,* 2022). In addition, others scientists revealed that the phenolic compound have an important anti-diabetic activity related to the inhibition of of α-amylase (Peláez-Acero *et al.,* 2022, Devarajan *et al.,* 2012*).* In Algeria numerous investigations described the honey biological proprieties *(*Otmani *et al.,* 2021*,* Ayad *et al.,* 2021*).* However, few papiers reported on the anti-diabetic and anti-inflammatory proprieties of honey treated with ultrasound.

Therefore, the aim of our investigations is to define the quality parameters, chemical composition and health promoting proprieties of four honeybee collected from Algeria including: eucalyptus, rosemary, jujube and Eruca sativa. Once the honey samples are collected and treated we investigate on the different biological activities including: anti-oxidant, anti-bacterial, anti-inflammatory and anti-diabetic effects.

First Part: Bibliographic Summary

First Part: Bibliographic Summary

Bee honey is a natural sweetener that is widely available across the world. It is produced by the bees known as *Apis mellifera* by using the nectar and some secretions from flowers and plants (floral honey) to transform it into honey. After regurgitation and digestion of nectar, several biological compounds from honeybees are added during the process, enriching the sugary substances. Honeybees store honey in honeycombs to be used during the winter. Their wings fan the honey to evaporate water from it to escape the fermentation of honey (Ranneh *et al.*2021; Libonatti *et al*.2014).

Among other natural products, bee honey is considerably used for a lot of different applications. It contains approximately 200 different chemical compounds [5]. Bee honey is a viscous solution containing various molecules, including fructose and glucose (80–85%); water (15–17%); ash (0,2%); proteins and amino acids (0,1-0,4%); and some enzymes, vitamins, and other substances, like phenolic compounds. After all, bee honey composition varies depending on the type of plants and the source of the nectar consumed by the bees, as well as factors such as climate and environmental conditions. However, all bee honey in the world contains similar types of phenolic acids, such as caffeic and ellagic; flavonoids, such as apigenin and galangin; and antioxidants compounds, such as tocopherols and ascorbic acids; and each of those components has distinctive nutritional and medicinal properties. All these components behave harmoniously, which grants the utility of honey in a different application (Vit *et al* 2015; Libonatti *et al*.2014). . That is explain the interest given to this natural sweetener and his producer -honeybees-.

1.General information on bee honey

1.1. Origin of bee honey

Honey comes from plants, and in particular from their sap. It is extracted in two ways from the vessels: by the nectar glands that produce the nectar, or by parasitic insects that reject honeydew.

1.1.1. Nectar

Nectar is the most common sweet source. Its production depends on the age, size, position, relative humidity of the air, duration of flowering, sex of the flowers, action of flowering, sex of the flowers, species, and surrounding environment (bey *et al* 2017).

Sweet exudation, which is more or less viscous, contains about 90% sugars, the most common being sucrose, glucose, and fructose. The proportions of each are relatively stable for the same plant species. Nectar also contains organic acids (fumaric, succinic, malic, oxalic, etc.), proteins, including enzymes, free amino acids (glutamic and aspartic acids, methionine, serine, tyrosine, etc.), and inorganic compounds (such as phosphates). Some nectars may contain oily compounds, alkaloids, or bactericidal substances. Each plant species provides nectar with its own characteristics that give bee honey its flavor and fragrance (Bonté *et al* .2013).

1.1.2. Honeydew

Honeydew is a thick, viscous liquid. It is denser than nectar and richer in nitrogen, organic acids, minerals, and complex sugars (Bonté *et al* .2013). Honeydew is made from the excretions of some sap-sucking insects left on plants. These insects are homopterous hemiptera: they are aphids but also scales, cicadas, and psylles. The host plants of these honeydew producers are most often forest or ornamental trees such as fir, spruce, Scot’s pine, oak, or larch.

1.1.3 Others origins of bee honey

There are other origins of bee honey, such as "sugar honey", produced by bees fed with sugars and sometimes fruit, sugar cane, etc (Schweitzer .2004). .

1.2. Types of bee honeys

1.2.1. Mono-floral honey

"Mono-floral" bee honeys are produced from nectar and/or honeydew from a single plant species, which necessitates the placement of hives close to the desired plant. These honeys are unusual since bees rarely ruin only one kind of honeyingle plant species, which necessitates the placement of hives close to the desired plant .

These honeys are unusual since bees rarely ruin only one kind of honey. Thus, natural monofloral honeys are derived from a particular plant, but not entirely. As a result, they may include nectar from different flowers, but nectar from the major sources predominates (Bonté *et al* .2013).

1.2.2. Poly-floral honey

This kind of bee honey is made from nectar and/or honeydew from several plant species. In order to enhance their specificity and allow the consumer to recognize their dominant character, beekeepers indicate their geographical origin. This indicates either the production area (region, department...) or a type of landscape referring to an identified flora (garrigue, forest...) (Clément H. 2002).

1.3. Manufacturing Process

After the harvest of the nectar and its storage in the crop of the foraging bee, the mechanism of making honey begins with the reaction of the nectar and the enzymes of the digestive tract. The invertase enzyme transforms the complex sugars of the plant into simple sugars, mainly fructose and glucose. (Desmouliere *et al* .2013). Once at the hive, the trophallaxia begins, which is defined as a successive exchange of the crop contents of a worker bee to another, beginning with the foraging with the continuity of the same enzyme reaction. By the end of the succession, the last worker will regurgitate the taste of the nectar transformed in an alveolus. At this moment, a second enzyme (glucose oxidase) comes into play and transforms part of the glucose into gluconic acid and hydrogen peroxide (Balas .2016). Gluconic acid conforms to a certain honey acidity that protects it from bacteria, fungi, and molds. When hydrogen peroxide is used, it allows the honey to be stored during maturation in its alveoli or during dilution to feed the larvae. The last stage of its manufacture is the evaporation of water, which takes place in two phases: The initial phase is attributable to the warmth generated by the hive and the workers at the same time as its transfer between them (Balas .2016).

The second time, once placed in the alveoli, the workers create an area current with the movement of wing lures, allowing it to evaporate until reaching a humidity level of less than 18% (to prevent the growth of microorganisms). However, the honey has

become walled, and the honeycomb will be sealed by a wax operculum that will protect it from moisture, and the area is then ready to consume (Balas .2016).

2.Composition and physicochemical characteristics of honey

2.1. Chemical composition of honey

Honey is composed of both macro and micronutrients. This composition depends on factors such as bee type, floral availability, environment, and processing conditions. Honey contains around 200 components, including sugar, protein, enzymes, minerals, vitamins, amino acids, and a variety of polyphenols. Because of the varied ratios of these components, each honey has a different color, flavor, viscosity, and medicinal properties. In this respect, the combination of all of these chemicals works synergistically in many applications. The majority of honeys throughout the world share 80% of their physical features and chemical structure. Thus various methods for distinguishing the entomological sources of honey have been developed (Ranneh *et al* 2021).

2.1.1. Water

Water is the second-most important ingredient in honey, and its content may vary from 15 to 23%. Water content influences on some characteristics of honey (viscosity, specific weight, maturity, flavor and crystallization, specific gravity). The moisty proportion depends on numerous factors including: the climatic conditions, the bee variety, the bee colony strength, the humidity and air temperature in the hive, the processing and storage conditions, as well as the botanical origin of honey. The quantity of water in the honey is not constant due to its hygroscopicity, and it changes during storage depending on the humidity of the air. The water content of honey is an important characteristic in assessing its quality and longevity since it impacts its stability and resistance to microbial deterioration during storage. The higher the water content, the greater the possibility of fermentation (pavlova *et al*.2019).

2.1.2. Carbohydrates

There is a remarkable supply of carbohydrates that is the fundamental core of honey and helps to maintain its antiseptic qualities. The carbohydrate ratio ranges from 60 to 95% of its dry weight and includes mono-, di-, and tri-saccharides, with floral type

playing an important role in modifying this ratio. More than 20 distinct forms of carbohydrates have been detected in honey samples from throughout the world. Fructose is the most abundant carbohydrate, followed by glucose at around 28–40% and 20–35%, respectively. Whereas disaccharide and trisaccharide concentrations are approximately 5 and 1%, respectively. Maltose, maltulose, turanose, sucrose, and nigerose are the most often recognized disaccharides, whereas erlose, centose, isomaltotrios, panose, psopanose, and ketose are discovered in trace amounts (Schievano *et al*.2012; Popek.2002; Küçük *et al* .2007).

2.1.3. Acidity and pH

Honey has a modest acid level; however, it is vital for the honey flavor. The honeybees add the majority of the acids. The major acid is gluconic acid, which is produced by the oxidation of glucose-by-glucose oxidase. It is present as an internal ester, a lactone, and does not contribute to the active acidity of honey. Titration is used to determine honey acidity, which is represented in milliliter equivalents per kilogram. Minor levels of the following acids have been discovered: formic, acetic, citric, lactic, maleic, malic, oxalic, pyroglutamic, and succinic. The pH of most honeys is less than 7, indicating that they are acidic. The pH of honey ranges from 3.3 to 4.6. Chestnut honey is an exception, having a pH of 5 to 6. Honeydew honeys have a higher pH value, ranging between 4.5 and 6.5, due to their increased mineral content. Honey is defined as a buffer, which means that its pH does not alter when small amounts of acids and bases are added. The buffer capacity is attributable to the presence of phosphates, carbonates, and other mineral salts

2.1.4. Amino acids, proteins and enzymes

Honey has a protein composition that ranges between 0.2% and 0.5% in the form of enzymes and free amino acids. In general, the total quantity of free amino acid in honey ranges between 10 and 200 mg/100 g honey, with proline accounting for 50% of total amino acid. In addition to β-alanine and α-alanine, honey samples have been found to include g-aminobutyric acid and ornithine. While pollen is the primary source of protein and amino acids, honeybees help to change this composition through regurgitation (Ranneh *et al* 2021).

2.1.5. Vitamins and Mineral salts

The mineral and vitamin profile of honey varies according to flower type and geographic origin, accounting for 0.2-0.5% of the dry weight of the honey. Several studies have been undertaken across the world to measure the mineral content of honey samples in order to indicate the presence of contaminating minerals. Potassium and sodium account for over 80% of total minerals. While iron, copper, and manganese are few. Furthermore, trace elements have recently been recruited for the identification of several unifloral honeys.
In terms of vitamins, one study discovered thiamin, riboflavin, pyridoxine, niacin, and ascorbic acid in different honey samples; nevertheless, the amounts detected did not match the required daily requirement of humans (Solayman *et al* 2016; Pisani *et al*.2008).

2.1.6. Hydroxymethylfurfural (HMF)

Hydroxymethylfurfural (HMF) is a chemical molecule that is created slowly and naturally during the storage of honey and much more quickly when honey is cooked. It is a breakdown product of simple sugars (such as fructose). Several factors impact HMF levels, including temperature and heating time, storage conditions, pH, and honey type. Therefore, it offers a signal of overheating and storage in bad settings. Many studies have shown that when honey is exposed to high temperatures, poor storage conditions, or the addition of invert sugar, the HMF level rises. It is important to highlight that the HMF is one of the most prevalent degradation products in honey, indicating its aging (pavlova *et al*. 2019).

2.1.7. Phenolic compounds

Honey also has polyphenols in his composition. Many studies have shown that the two major groups of polyphenol compounds contained in honey including phenolic acids (Benzoic and cinnamic acids) and flavonoids (flavones and flavonones) in varying quantities. These two compounds influence on organoleptic features such as the color, taste, and flavor of honey. They also express an antioxidant effects, along with other honey compounds, and some intriguing biological activities: germicides, bacteriostatics, and anti-inflammatory (Ouchemoukh .2012).

2.1.8. Other components of honey

Trace elements, pollens, spores, unicellular algae, osmotolerant yeasts (in charge of fermentation) and microscopic fungi can also be found in honey (Bonté *et al* .2013).

3. Biological properties of bee honey

3.1. Antibacterial properties

Honey is used to treat many bacterial infections since the origin of the mankind and it was one of the few options available to cure those infections. honey is a special food product that contains bioactive molecules originally from bees and plants. These bioactive compounds could have an efficient antimicrobial capacity which has the power of the destruction and inhibiting the growth of some pathogenic microorganisms (Libonatti *et al*.2014).

The high osmotic nature and low pH (3.2-4.5) improve the production of the hydrogen peroxide, and that's plays as the main actor in the antibacterial activity of honey by degrading or interfering with the ability to damage the bacterium cells (Libonatti *et al*.2014).

studies have shown that bee honey has an effective antibacterial capacity over many bacteria including, aerobes and anaerobes, Gram-negative and Gram-positive, and most of the human pathogenic bacteria were found to be sensitive to honey such as *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli and Streptococcus pyogenes*. However, the origin and the concentration as well as the methods of honey processing can be as factors in antibacterial activity of honey and this variation in the antibacterial capacity of the deferent types of honey gave it a well-preserved place in modern medicine (Wasihun et kasa.2016).

Furthermore, the non-peroxide honey exhibits antibacterial properties. Thus, the antibacterial propriety is related to related to the presence of glyoxal, 3-deoxyglucosulose, and methylglyoxal (MGO). The MGO concentration depends on the origin of the honey, and its present in all types of honey. The antibacterial capacity of honey depend on the MGO content. In a study by Girma et al. (Girma et al .2019). MH(Minimum Bactericidal Concentration) of lower UMF (Unique Manuka Factor) grade has showed raised antibacterial effect contrasted to higher UMF grade honey in

opposition to *S. aureus and E. coli*. And a 10+ UMF values were enough to come up with antibacterial power (Tashkandi .2021).

3.2. Antioxidant Properties

Antioxidants are compounds that prevent any damage caused by oxidants such O_2, OH, superoxide, and/or lipid peroxyl radicals. Oxidative stress can cause cancer, the development of mutagens, aging, atherosclerosis, as well as numerous chronic and lasting diseases, Free radicals and other protective oxidative substances make up this defensive mechanism. It has been shown that honey from separate territories and floral sources possesses significant antioxidant properties. Although the precise mechanism of antioxidant activity is uncertain. Some studies have been put forth, including free radical sequestration, hydrogen donation, metallic ion chelation, flavonoid action as a hydroxyl substrate, and superoxide radical effects (Rao *et al.*2016).

The antioxidant activity of the deferent types of the Algerian bee honey is mainly related to the presence of natural components, like phenols, flavonoids and anti-oxidants compounds such as ascorbic acid and tocopherols. They are the most found natural components in the Algerian bee honey, and they are known for their ability to wipe out free radicals and stop oxidative stress (Rao *et al*.2016).

3.3. Anti-inflammatory properties

The immune system's intrinsic, natural reaction to infections is inflammation, which leads to the development of diverse cellular and humoral immune systems. When the equilibrium continues to favor the overproduction of free radicals over the antioxidant components, oxidative stress is present. Numerous signaling pathways link oxidative stress and inflammation together, to put it another way, the development of an uncontrolled inflammatory process and the presence of oxidative stress are crucial in the pathophysiology of chronic illnesses such mental, cardiovascular, traumatic, metabolic, and autoimmune diseases. Honey may have an inhibitory effect on chronic inflammation, oxidative stress, and their respective gene expression, according to recent studies. Honey has been proposed as an immune-modulating substance with two potential functions: first, anti-inflammatory actions by suppression of the generation of pro-inflammatory cytokines and/or downregulation of inflammatory

transcription factors (NF-B and MAPK). And second process: promote the synthesis of inflammatory mediators such cyclooxygenase-2 (COX-2) and prostaglandin E2 (PGE2). However, the molecular mechanisms of polyphenols rich in honey are not fully illustrated, to fully understand the effects of honey on patterns of extracellular signaling pathways, global gene expression, protein expression, and metabolite production in response to specific compounds, additional research in the trigonometric analysis is still required (Ranneh *et al* .2021).

3.4. Anti-diabetic properties

The non-antioxidant components present in honey are responsible for the mechanisms that contribute to its anti-diabetic effects. Related to that honey has been found to include at least 181 compounds, the precise mechanism of its antidiabetic activity is complicated and will require further exploration. Although the mechanisms by which honey improves glycemic control and other diabetes characteristics are unknown. , Existing research shows that fructose in honey may alter honey's hypoglycemic or antidiabetic action. Certain types of honey have been shown to boost fructose plasma concentrations in healthy people. Chromium, one of the mineral components found in honey, is known for helping to lower high blood sugar levels, maintain normal glucose tolerance, and stimulate the release of insulin from pancreatic beta cells. Other research has found that copper and zinc can increase insulin sensitivity, lowering blood glucose levels (Rao *et al*.2016; Erejuwa *et al*.2012).

Additionally, there is a potential mode of action in which honey acts as an antioxidant and scavenges free radicals. There is plenty of compelling evidence that oxidative stress has a role in the development of and/or damage to glycemic control in diabetes mellitus. Increased glucose absorption in both skeletal muscle and adipose tissue increases ROS production and oxidative stress, impairing glucose uptake and glycogen synthesis. Insulin resistance is caused by oxidative stress via altered insulin signaling pathways such as interference with the insulin receptor, insulin receptor substrate 1, and protein kinase B/Akt. Honey may boost insulin sensitivity in the liver and muscle, increasing glucose absorption and resulting in lower hyperglycemia. Several investigations have shown that ROS or oxidative products impede insulin secretion in pancreatic cells, block glucose-induced insulin secretion and biosynthesis, deplete pancreatic cell insulin content, and promote cell death. As a result, honey

supplementation may reduce oxidative stress in the pancreas, protect the pancreas from oxidative damage, and therefore increase insulin production, resulting in better glycemic control. Furthermore, evidence suggests that antioxidants may decrease protein glycosylation in diabetics irrespective of changes in plasma glucose. Antioxidants have been proven to reduce oxidative stress, boost C-peptide and insulin levels, and improve insulin resistance in type 2 diabetes. Antioxidants may reduce intestine oxidative stress and increase BBM fluidity, promoting GIT health and maybe improving glucose management in diabetes. Given the liver's function in glucose regulation, honey's antioxidant action may help reduce hepatic oxidative stress and damage. These hepatic effects might improve liver function and lead to better glucose management (Rao *et al*.2016; Erejuwa *et al.*2012).

Many studies have demonstrated that oxidative stress is the principal mechanism by which glycemic or metabolic memory promotes tissue damage, and honey might be a useful aid in reducing diabetes problems due to its antioxidant impact. This is where honey's antioxidant properties may complement its anti-diabetic properties. For diabetic individuals, this might have a synergistic and positive impact. Honey's antioxidant action may protect against the depletion of intracellular NADPH and the inhibition of intracellular GAPDH. These two actions may boost antioxidant defenses in tissues or organs prone to oxidative stress-induced diabetes problems. These include the kidney (diabetic nephropathy), retina (diabetic retinopathy), nerve (diabetic neuropathy), and heart (diabetic cardiomyopathy). Through up-regulation of Nrf2 activity and expression, honey supplementation may also boost the expression of cytoprotective genes. Patients with diabetes are also likely to benefit more if honey is administered in conjunction with traditional anti-diabetic medication or as a complimentary treatment (Rao *et al.*2016; Erejuwa *et al.*2012).

Second Part: Experimental study

Materials and methods

Second Part: Experimental study

I. Materials and methods

In order to highlight on the biological proprieties of bee honey obtained from different area in Algeria.

1. Bees honey Sampling:

Our research is based on four local honeys from the 2022's (figure n°1), the samples are kept at room temperature and protected from light and humidity. The table … illustrates the coding used for these different samples: harvest region, consistency and color.

Table N°1: table of seeds of the honeys used

Sampel	Origin of honey	Code	Harvest Area	GPS data
Honey 1	Rosemary	R	Hammam Fraksa (BOUIRA)	5XP7+C26 Hammame FRAKSA, El Hachimia 10000
Honey 2	Jujube	S	Ain Oussara (DJELFA)	FW43+964, Aïn Oussera
Honey 3	Eruca sativa	D	Ain Lahjel (MSILA)	PWF7+8H8 ، Unnamed Road, Aïn El Hadje
Honey 4	Eucalyptus	E	Oued Dhouss (BOUIRA)	9W64+QGX Bouira

Table N°2: Characteristics of the honey samples analysed

Sample	Code	Consistency	Color
Honey 1	R	Semi-crystallized	Light yellow
Honey 2	S	Slightly crystallized	Deep yellow
Honey 3	D	Very crystallized	Very light yellow
Honey 4	E	Liquid	Dark brown

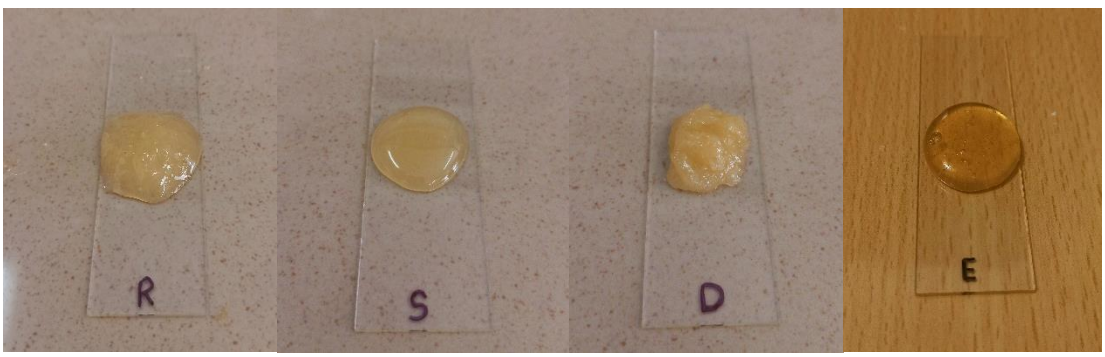

Figure n°1: aspects of different honeys

2. Honey treatment

2.1. Distilled water wash

One gram of honey is resuspended in one milliliters of distilled water and mix well (Tsavea *et al*.2022). The mixture is stored at 4°C until the experimental investigation.

2.2. Ethanol wash

Each 1g of honey is mixed with 1 ml of 50% ethanol; after homogenization, the solution is centrifuged at a speed of 15000 rpn per minute for 10 minutes, and the supernatant is recovered (Pelaez *et al*.2022). The mixture is stored at 4°C until the experimental investigation.

2.3. Honey Sonication

Three grams of each of the honey samples were placed in a tube with 20 ml of water distilled at 20 °C. After they were treated by sonication for 5 and 20 minutes in an ice bath at 42 kHz (name of the sonizer), the probe was immersed in the prepared solution. (Pelaez *et al*.2021).

On another hand, three grams of each of the honey samples were placed in a tube with 20 ml of 50% ethanol at 20°C. After being treated by sonication for 5 and 20 minutes in an ice bath at 42 kHz, the probe was immersed in the prepared solution (Pelaez *et al*.2021).

2.4. Water bath sonication

Three grams of each of the honey samples were placed in a tube with 20 ml of distilled water at 20°C. Afterwards, they were treated by sonication for 5 and 20 minutes in a water bath sonizer (J.P. Selecta,s.a. 50/60 Hz). (Pelaez *et al*.2021) (Amend).

Table N°3: table of abbreviations used for different honey treatments

abbreviation	Mining
Brute	Distilled water wash
Extrait	Ethanol wash
Son/S/ED/5	Water Sonication using probe for 5 minutes
Son/S/ED/20	Water Sonication using probe for 20 minutes
Son/S/E/5	Ethanol Sonication using probe for 5 minutes
Son/S/E/20	Ethanol Sonication using probe for 20 minutes
Son/BM/ED/5	Water bath sonication for 5 minutes
Son/BM/ED/20	Water bath sonication for 20 minutes

3. Physico-chemical analyses

3.1. Refractive Index, Water Content, and BRIX percentage

Moisture is a conservation index that determines the crystallinity and stability of honey. It is determined by optical measurement of the refractive index (RI) of honey at 20°C, easily achieved using a refractometer following the Bogdanov *et al*. (1997) method.

RI measurements are performed as follows: A drop of liquid honey is applied in a thin layer on the prismatic plate of an Abbe refractometer, previously calibrated with distilled water.

The reading is taken through the refractometer eyepiece after the horizontal line divides the light and dark areas. This line intersects two vertical scales, directly scaled in percent Brix and refractive index, respectively. This index is used to determine the moisture content of honey at 20°C using the CHATAWAY correspondence table (Appendix A table) (Bogdanov *et al*., 1997).

3.2. Calculate the dry mass

The dry mass varies according to the humidity of our sample and is calculated as follows (Bogdanov *et al.*, 1997):

$$Ms = \frac{5 \times 100}{100 - humidity}$$

3.3. The pH measurement

In general, honey from flower nectar has a pH between 3.5 and 4.5, and honeydew honey has a pH between 4.5 and 5.5. The pH meter measures the voltage between two electrodes: a reference electrode ("saturated calomel") and a glass electrode ("pH indicator"). This is directly related to the pH of the solution in which the probe appears (Bogdanov *et al*., 1997).

Firstly, an aqueous solution of honey (10%, m/v) is prepared. After rinsing the electrode with distilled water, we read the pH with a pH meter (pH 213, Hana Instruments). The pH value is determined directly on the device screen. Each sample was analyzed in triple (Bogdanov *et al*., 1997).

3.4. The Electrical conductivity measurement

According to Bogdanov *et al*. (1997) , the electrical conductivity (EC) of honey was measured with a conductivity meter (EC214, Hana Instruments). The aim of this method, is to immerse the probe in an aqueous solution of dry matter. It differentiates honeydew honey (>0.8 mS/cm) from nectar (0.8 mS/cm) and indicates that they are rich in minerals.

Take X g of honey and dissolve it in 25 ml of distilled water. X = (5 .100) / MS (MS is the dry matter of the honey sample). Insert the conductivity meter cell into the prepared solution containing 20% dry matter at 20°C and read the value in mS/cm on the device (Bogdanov *et al*., 1997). Conductivity is calculated according to the following equation:

$$\mathbf{CE\ (mS/cm)\ = Measured\ value - A}$$

A: (measured value × 0,032) × (T° = 20 °C).

0,032: correction factor.

T°: ambient temperature (in our case between 16 and 18°C).

The Color essay:

The color of honey is related to its rich pigments, including flavonoids and carotenoids. The lighter the honey, the less pigment, and conversely (Blanc, 2010). The determination of the coloring power of honey was carried out using the method described by Al *et al*. (2009). Dissolve 1 g of honey in 4 ml of distilled water and homogenize with a magnetic homogenizer. The optical density was measured by a spectrophotometer at 450 nm.

3.5. The determination of hydroxymethylfurfural (HMF)

Hydroxymethylfurfural is a natural compound for the breakdown of sugars in an acidic medium. Its rate increases with temperature. It is an indicator of the freshness and heat of honey.

Mix the honey solution (20%, m/v) with 1 ml of Carrez I solution (15% potassium hexacyanoferrate, m/v) and 1 ml of Carrez II solution (30% zinc acetate, m/v). Adjust the mixture to 50 ml with distilled water. The solution has been filtered through Whatman filter paper, and the first tens of milliliters of filtrate must be discarded. Add 5 ml of filtrate to a volume of 5 ml of distilled water (sample tube); take the same volume of filtrate plus 5 ml of sodium bisulfite (0.2%) as a blank sample. Read the absorbance at 286 nm and 336 nm by UV spectrophotometer (BIOTECH ENGINEERING, MANAGEMENT CO. LTD. (UK)). Dilution is recommended if the absorbance exceeds 0.6. HMF is expressed in mg/kg and given by Bogdanov *et al*. (1997) (Bogdanov *et al*., 1997) :

$$\mathbf{HMF}\left(\frac{\mathbf{mg}}{\mathbf{kg}}\right) = (\mathbf{A284} - \mathbf{A336}) \times \mathbf{149,7} \times \mathbf{5/W}$$

A_{284} et **A_{336}** : absorbances at 284 nm et 336 nm. **149,7 :** constant

W: masse (g) of honey sample. **5:** nominal weight of the sample.

3.6. The proline content determination

The determination of the proline amino acid is based on the fact that ninhydrin acts on the proline in acid medium and turns it pink. The shade of color is related to the proline

content and indicates the maturity and consistency of the honey. When honey is adulterated by adding sugar, its incidence is significantly reduced (Lokossou *et al*., 2017).

The proline content of honey was determined by the method of Bogdanov *et al*. (1997). The colorimetric method consists of preparing three test tubes containing 500 µl of honey in water (5%, m/v), three 500 µl reference tubes of proline solution, and one 500 µl white test tube of distilled water. In each test tube, 1 ml of formic acid and 1 ml of ninhydrin ethanol (3 %, m/v) were added.
After stirring and heating at 100°C for 15 minutes in a water bath, transfer the solution prepared at 70°C for 10 minutes. Add a 5 mL volume of 2-propanol (50%, v/v) to all tubes. After 45 minutes of incubation, read the absorbance at 510 nm. The proline concentration was obtained by the following equation:

$$\textbf{Proline (mg / kg)} = (A_E \times M_P \times 80) / (A_P \times M_E)$$

AE: Absorbance of honey sample solution.**; MP:** mg proline for standard solution.

AP: Absorbance of the standard solution of proline.**; ME:** Quantity of honey taken in kg.

80 : Dilution factor.

3.7. The Protein content measurement

Bradford's method is a colorimetric test to measure the concentration in solution at 595 nm. The assay uses the absorption principle of Coomassie blue G 250, which binds to the NH3+ groups of amino acids in proteins (arginine, tyrosine, tryptophan, histidine, and phenylalanine) and makes the medium blue reactionary.
Protein content was determined by Azeredo *et al*. (2003). Mix 0.1 ml of honey solution (50%, v/v) with 5 ml of Bradford reagent. In the white, replace 100 µl of honey solution with 100 µl of distilled water. After 2 minutes of incubation, the absorbance was measured at 595 nm. The results are expressed in equivalent milligrams of bovine serum albumin (BSA) per 100 g of honey, as referenced in the BSA calibration curve (Appendix Calibration Curve, Figure Photo Color) (y = 0.904 x - 0.0936; R2 = 0.9932).

3.8. The determination of phenolic components composition

3.8.1. The Polyphenols content

Folin-Ciocalteu reagent is an amalgam of phosphotungstic acid (H3 PW12 O40) and phosphomolybdic acid (H3 PMo12 O40). This reagent is reduced when phenols are oxidized into a mixture of blue tungsten oxide and molybdenum. The resulting color is directly proportional to the polyphenol content of honey (Boizot et Charpentier, 2006).

The amount of polyphenols in the four honeys studied was determined using the method identified by Naithani *et al*. (2006) [35]. The reaction medium contains a mixture of 100 µl of honey solution (0.1 g/ml), 100 µl of Folin-Ciocalteu reagent (50%, v/v), and 2 ml of sodium carbonate solution (Na2CO3, 2%). After incubation for 30 minutes in the dark, we read the absorbance at 750 nm. The results were expressed in milligrams of gallic acid equivalents per 100 g of honey samples (mg EAG/100 g) using a standard gallic acid curve (calibration curve appendix) (y = 1.131x + 0.0504; R2 = 0.986).

3.8.2. The flavonoids compounds content

The determination of flavonoids in honey depends on the formation of flavonoid-aluminium complexes that give a yellow color with an absorbance maximum at 510 nm (Djeridane *et al*., 2006).

The flavonoid content of honey samples was determined by the colorimetric method described by Al *et al*. (2009) [31]. Mix 1 mL of honey solution (50%, w/v) with 300 µl of sodium nitrite (5%, w/v) and 4 mL of distilled water. After 5 minutes, add aluminum chloride (10% w/v) in a volume of 300 µl. After 6 minutes of incubation, 2 mL of sodium hydroxide (1 M) was added to the mixture. Read the absorbance at a length of 510 nm. Using a quercetin standard curve (Appendix calibration curve) (y = 0.2317 x + 0.0041; R2 = 0.976), the results are expressed as milligrams of quercetin equivalent per 100 g of honey sample (mg QE/100 g).

4. In vitro evaluation of the biological activity of tested honey.

4.1. The estimation of the antioxidant activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) essay

The compound 2,2-diphenyl-1-picrylhydrazyl is a stable free radical with a purple color. In the presence of antioxidants, proton transfer, resulting in a decrease in color strength (Chaabi, 2008), reduces it.
The method of Meda *et al*. (2005) [38] evaluated the ability of antioxidants in honey to reduce DPPA free radicals by adding 500 µl of aqueous honey solution (2.5%, w/v) to 1 ml of DPPH ethanol solution (6x10-5 M). Prepare a blank tube containing 1 ml of ethanol and a positive control tube containing 1 ml of DPPH solution. After 15 minutes of incubation, take absorbance readings at 517 nm.
The percentage reduction is given by the following formula:

$$\text{Anti-free radical activity (\%)} = [(A_C - A_E / A_C)] \times 100$$

A_C: Control absorbance (1 ml of DPPH). **A_E:** Sample absorbance.

4.2. The evalutaion of the anti-inflammatory propriety

In vitro anti-inflammatory activity of the honey samples is performed according to the BSA denaturation inhibition method (Williams *et al*., 2008) (with some modifications). A volume of 0.45 ml (0.2% w/v) of the aqueous BSA solution prepared with tampon phosphate adjusted to pH 6.3 is mixed with 50 µl of each honey dilution (1 mg/ml, 1 g/1 ml, 0.15 g/ml) or distilled water for the blank. After incubation for 15 min at 37°C and 5 min in a water bath at 71°C, 1.5 ml (100 mM; pH 6.3) of phosphate buffer is added. The absorbance is measured at 660 nm.

$$\text{Percentage inhibition of BSA (\%)} = ((AC-AE)/AC) \text{ X } 100$$

Where :

Ac: Absorbance of BSA soluti mon without sample.; **AE**: Absorbance with sample.

The results of the inhibitory activity of BSA are expressed as ICso, which is calculated by regression analysis.

4.3. The estimation of the antidiabétic activity

Inhibition of α-amylase

To determine the inhibition of α-amylase in honeys, a methodology was employed based on the work of Armando Pelaez *et al*. (2022), with some modifications. Initially, a mixture of 100 µL of the sample (1/16, 1/32, and 1/64 dilutions of the stock solution) and 100 µL of 0.02 mol/L sodium phosphate buffer (pH 6.9) was prepared, as well as 100 µL of α-amylase buffer solution (1 U/mL). This mixture was then pre-incubated at 37°C for 10 minutes. After 10 minutes, 100 µL of 0.1% aqueous starch solution was added to the mixture, which was then incubated for 60 minutes at 37°C. To stop the reaction, 1 mL of dinitrosalicylic acid reagent was added. The samples were then placed in a water bath set at 90°C for 5 minutes before being rapidly cooled in an ice bath. Acarbose was used as a positive control in this particular evaluation. A negative control was also established by introducing distilled water without inhibiting compounds. The percentage inhibition is calculated as follows:

$$\text{Percentage inhibition of α-amylase (\%)} = ((AC-AE)/AC) \times 100$$

Where :

Ac: Absorbance of the positive control (acarbose).

AE: Absorbance with sample.

4.4. The assessment of antibacterial properties

In our investigation, we carried out the microbiological essays on serial bacterial strains; including: *Enterococcus faecalis (ATCC 29212), Pseudomonas aeruginosa (ATCC 6633), Acenitobacter baumannii (610) Escherichia coli (ATCC 25922).*

4.4.1. Preparation of the inoculum and adjustment of the bacterial load

From a pure culture of bacteria on isolation medium (nutrient gelose), having A few well-isolated and identical colonies are scraped off with a sterile platinum ance. The platinum ring is then discharged into 5 ml of sterile physiological water, and the The bacterial suspension is homogenized; its opacity must be equivalent to 0.5 McFarland. which corresponds to 108 CFU/ml. For bacteria, the inoculum is adjusted to bacteria, the inoculum is adjusted to 10-8 cells/ml (an OD of 0.08 to 0.1) by reading the optical density at a wavelength of 625 nm (BOUSSENA, 2020).

4.4.2. Agar diffusion method (well method)

This is the basic technique used to study the ability of a substance to exert an effect, it is also called the agar dilution technique for the determination of active determination of active extracts, Petri dishes containing Luria bertani agar medium (for bacteria) are aseptically inoculated with a suspension of 108 cells/ml from a young culture of bacteria culture of bacteria, respectively. Inoculation is done by swabbing. After drying the plates, the agar is perforated with a Pasteur pipette. The cavities thus formed are filled with the aqueous solution of the extract (approximately 50 µL per well).

The dishes are incubated in an oven at 37°C for 24 hours.
The inhibitory action is manifested by the formation of a halo around the wells. The reading of the results are read by measuring the diameters of the inhibition zones. A product is considered active if the diameter of the inhibition zone is greater than 8 mm (Balouiri *et al*, 2016).

5. The HPLC characterization of the active compound

In order to characterize the actives compounds in the test honey we carry out the HPLC analysis following Zhu and co-worker. Five grams of tested honey were mixed with 10 ml of acidified water (HCl 37% pH = 2.5). After that, the solution was treated with ultrasound at 60khz for 5 minutes. Then the mixture was centrifuged at 5000 rpm for 10 minutes. Once the supernatant is collected, 10ml of acetonitrile acid was added then the mixture was centrifuged at 5000 rpm. After 10 minutes, 2ml of acetonitrile acid was added to the mixture, then the supernatant is discarded after centrifugation at

5000rpm for 10 minutes. Then the precipitate is evaporate under rotavapor , and the residue is recovered in 2.5 milliliters of water-ethanol (30%-70%) (Zhu et al, 2019) (modified)

Results

II. Results

1. The physicochemical properties evaluation of tested honeys:

1.1. Brix prevalence

Various factors could be responsible for the fluctuations in Brix percentage, including the botanical origin of the honey. The sugar levels in various types of flowers can vary, altering the Brix percentage. For example, the sugar profiles of rosemary honey (R) generated from rosemary flowers and eucalyptus honey (E) collected from eucalyptus blossoms may differ. Moreover, variations in Brix percentages may be explained by differences in honey processing procedures and regional variations (Alvarez-Suarez *et al*, 2013; Bogdanov *et al*, 1999).

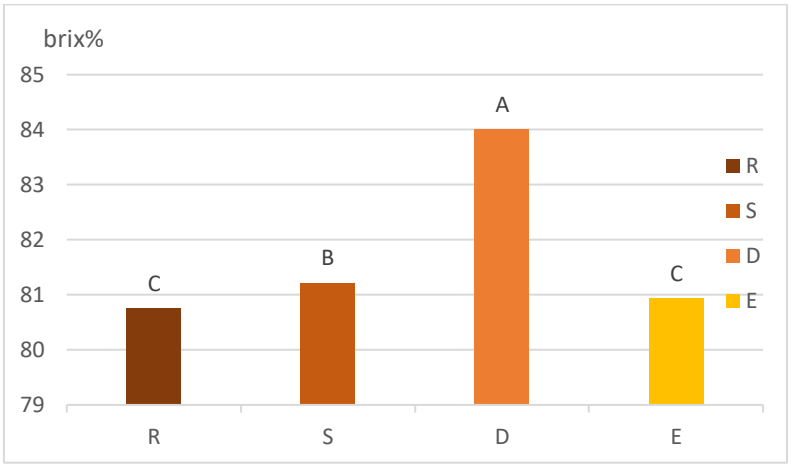

Figure n°2: the brix percentage of the different honeys

The results obtained from the evaluation of the Brix prevalence within the honey samples (R, S, D, and E) highlight on the sugar concentration in the bee's honey samples as well as potential changes in flavor and quality.

Based on the findings presented in Figure n° 02, it was observed that honey sample D (*Eruca sativa* honey) exhibited the highest Brix percentage of 84.003333%. This indicates a significantly higher sugar content and ripeness in comparison to other samples However, honey sample R (rosemary honey) had the lowest Brix percentage (80.75%). The brix percentages for honey samples S (jujube honey) and E (eucalyptus honey) were 81.2083333% and 80.9333333%, respectively.

1.2. Refractive Index (RI)

The refractive Index is often measured as part of the quality control process for honey due to its correlation with several important parameters such as: moisture content, adulteration detection, classification and authentication and consistency and uniformity (Ghosh *et al*, 2017; Bogdanov and Martin ,1997)

Comparing the refractive index values of the honey samples R (rosemary honey), S (jujube honey), D (*Eruca sativa* honey), and E (eucalyptus honey), it is notified that D (1.5121) has the highest refractive index value, indicating a relatively higher optical density compared to the other samples. Otherwise, the refractive index for rosemary, jujube, and eucalyptus honey falls within the range of 1.49(1.4925, 1.4935 and 1.4932, respectively) (Figure n°3).

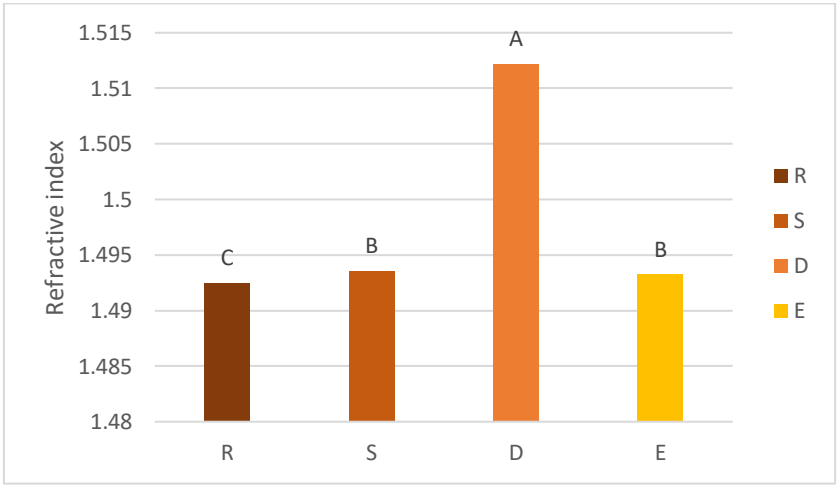

Figure n°3: The refractive index of different honeys

These variances in refractive index values might be attribute to changes in honey content and characteristics. Sugar concentration, water content, and the presence of other substances in honey can all affect its refractive index. The honey samples' individual floral sources and geographical origins may potentially contribute to the observed variances(IR standard is [1.47 -1.51] at 20°C) (ACHOURI *et al*, 2021).

1.3. The moisture contents

The determination of the moisture contents provides valuable information of quality control of the honey. Higher humidity percentages can indicate a higher water

content, which can impair the shelf life and susceptibility of the honey to fermentation. However, the low humidity percentages, indicate a reduced water content, suggesting superior stability and resistance to deterioration. Understanding and managing honey's water content is critical for preserving its quality and preventing fermentation. The humidity percentage of honey can be influenced by a variety of factors, such as ambient conditions, honey processing processes, and storage circumstances (Crane, 1990; White & Riethof, 2001).

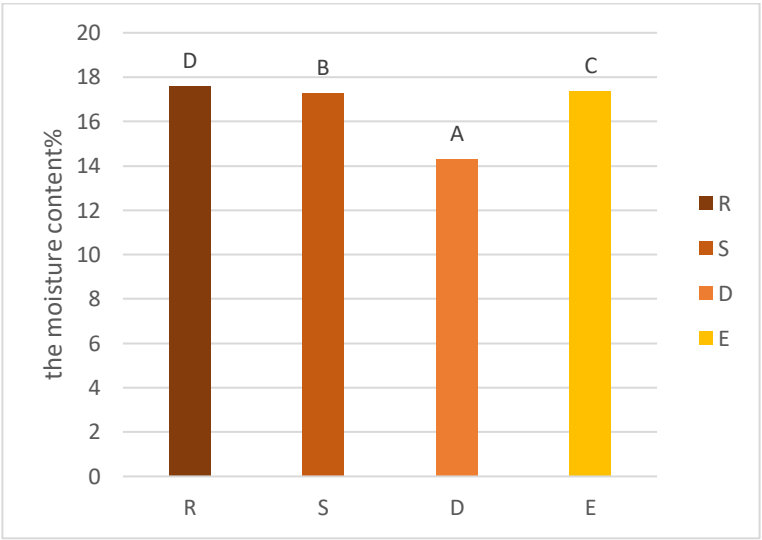

Figure n°4: The Moisture content of different honeys

The finding results illustrated in Figure n°4 indicate that *Eruca sativa* honey (D) had the lowest humidity percentage, indicating a lower water content than the other honey samples. In contrast, the estimation of the moisture value reveals that jujube honey (S), rosemary honey (R) and eucalyptus honey (E) have the same humidity percentages, with an important water content. The provided humidity results for the honey samples R (rosemary honey), S (juube honey), D (eruca sativa honey), and E (eucalyptus honey) were 17.6%, 17.2666667%, 14.3%, and 17.3666667%, respectively. . Higher humidity percentages indicate a higher water content, which can impair the shelf life and susceptibility of the honey to fermentation. However, reducing moisture percentages, suggesting superior stability and resistance to deterioration. Understanding and managing honey's water content is critical for preserving its quality and preventing fermentation. The humidity percentage of honey can be influenced by

a variety of factors, such as ambient conditions, honey processing processes, and storage circumstances.

1.4. Conductivity values (mss)

Conductivity values in honey analysis can provide several insights and information regarding the quality of the honey. The high conductivity levels indicate a high concentration of minerals and dissolved compounds, which can be linked to numerous factors such as the honey's floral source and geographic origin. The conductivity measures can also reveal information about honey's authenticity and adulteration, as well as its nutritional and sensory qualities. It is crucial to remember, that conductivity readings should be interpreted in combination with other quality factors (Meda *et al*, 2005; Puertas-Mejía *et al,* 2003).

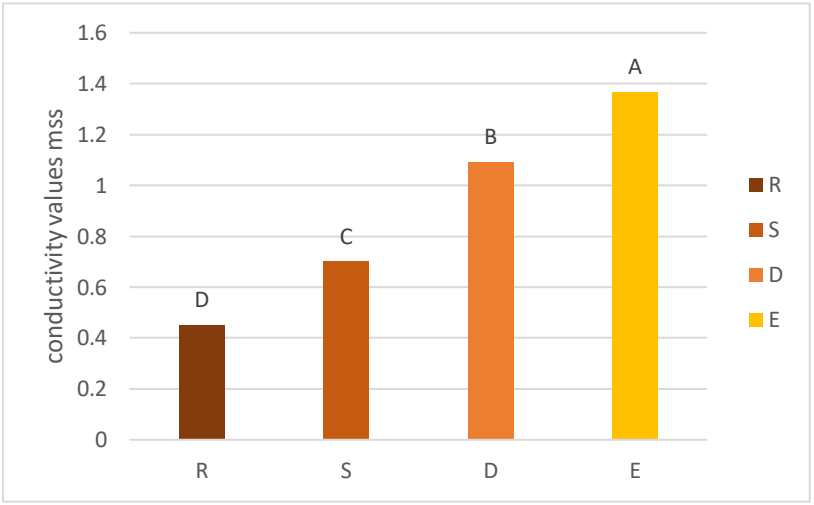

Figure n°5: The Conductivity values of different honeys

The electrical conductivity values reflect the mineral content and dissolved solids in honey. Eucalyptus honey (E=1.3676 mss) has the highest conductivity, indicating a relatively higher mineral content. *Eruca sativa* honey (D=1.0916 mss) has the second-highest conductivity, followed by Jujube honey (S=0.7 mss) and Rosemary honey (R=0.4513 mss), which have lower conductivity values (Figure n°5).

1.5. pH measurement

The pH value of honey can be influenced by various factors, including floral source, geographical origin, and processing methods (Anklam, 1998; White, 1979).

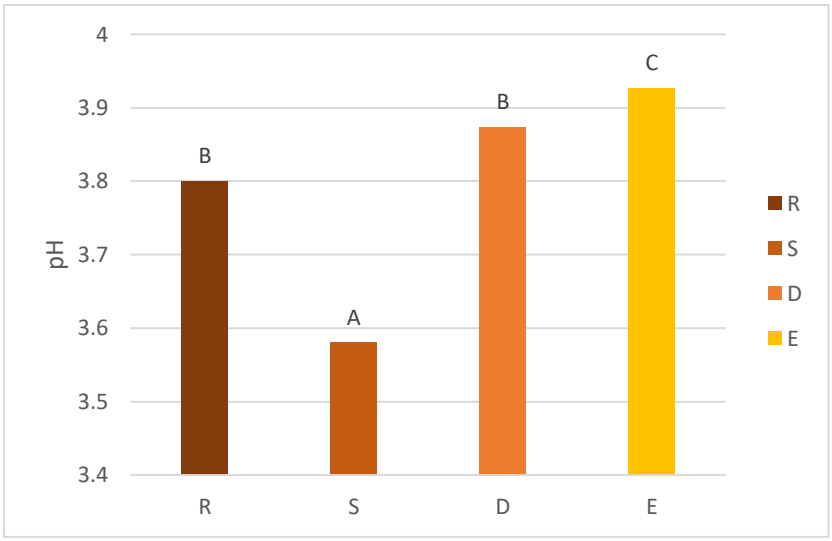

Figure n°6: The pH values of different honeys

The pH values indicate the acidity or alkalinity of honey. Jujube honey (S) has the lowest pH value (3.58), suggesting a more acidic nature compared to the other samples. Eucalyptus honey (E) has the highest pH value (3.92), indicating a relatively more alkaline nature. Rosemary honey (R) and *Eruca sativa* honey (D) have similar pH values (3.80) (3.87) respectively, falling between the extremes of Jujube honey (S) and Eucalyptus honey (E) (Figure n°6).

1.6. Color essays (Abs)

The strength of light absorption by honey samples at distinct wavelengths is shown by the color obtained using the spectrophotometric technique. The changes in color values indicate the presence of pigments such as flavonoids, carotenoids, and maillard reaction products, all of which contribute to the color of honey (White *et al*, 1963; Gheldo & Engeseth, 2002).

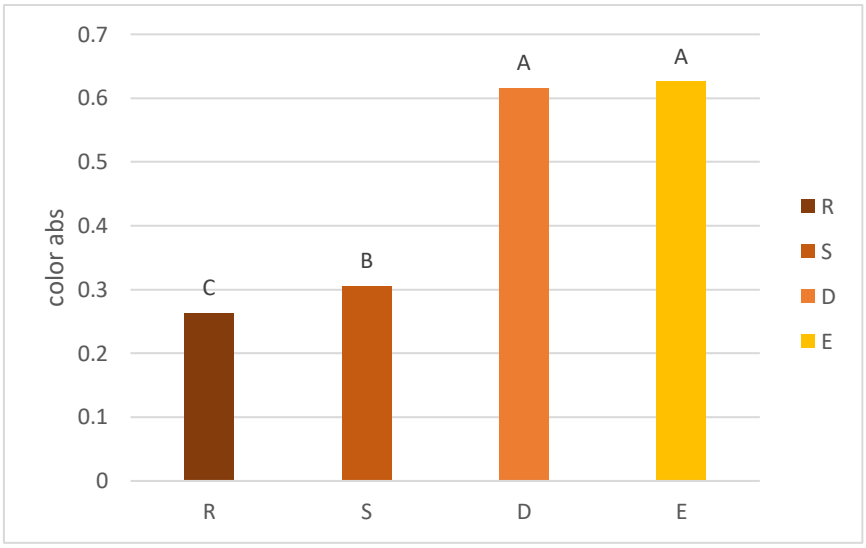

Figure n°7: The color absorbent values of different honeys

Regarding the obtained results on color essays (Figure n°7) reveal that the Eucalyptus honey (E) exhibited the highest chromatic intensity among the tested honey samples, as indicated by its color test score of 0.62566667. Eruca Sativa honey (D) displayed the second highest chromatic intensity with a color test score of 0.61533333. On the other hand, Rosemary honey (R) exhibited the lightest hue among the samples, with a color test value of 0.26233333. Jujube honey (S) demonstrated a slightly higher chromatic intensity compared to Rosemary honey, with a color test result of 0.305.

1.7. protein content

The protein content of honey is generally considered to be low and varies depending on factors such as floral source and honey processing methods (Pasini *et al*, 2017).

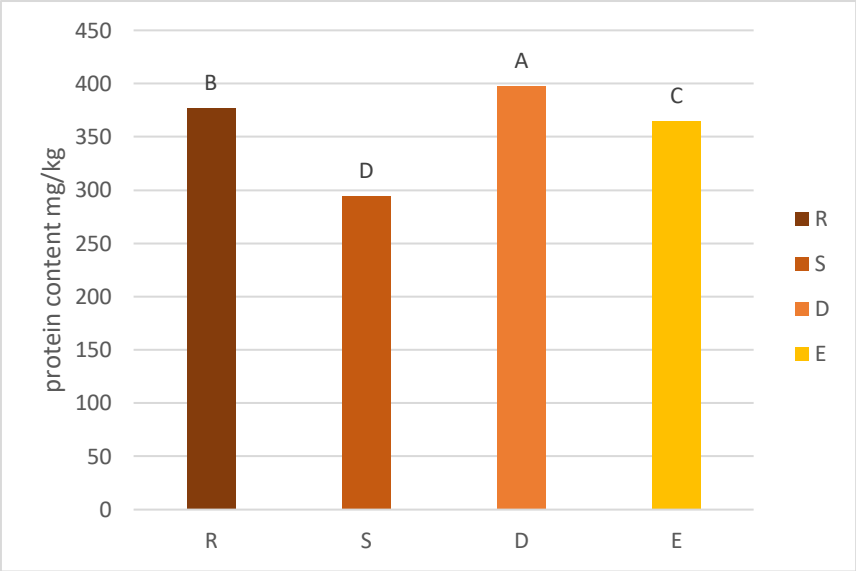

Figure n°8: The protein content of different honeys

Among the samples tested, *Eruca Sativa* Honey (D) showed the highest protein content with a value of 397.196667 mg/kg, followed by Rosemary Honey (R) and Eucalyptus Honey (E) with protein content values of 376.906667 mg/kg and 364.746667 mg/kg, respectively. In contrast, Jujube Honey (S) express the lowest protein content, measuring 294.323333 mg/kg. had It's important to note that while there are slight variations in protein content among these honey samples, overall, the protein content remains relatively low in honey (Figure n°8).

1.8. Proline content

Proline is an amino acid found in honey with variable concentration. The proline value is influenced by numerous factors such as floral source, geographical origin, and processing processes. Moreover, the higher quantities of proline in honey are frequently associated with nectar from certain floral sources, such as eucalyptus (Bogdanov *et al*, 1997; meda *et al*, 2005).

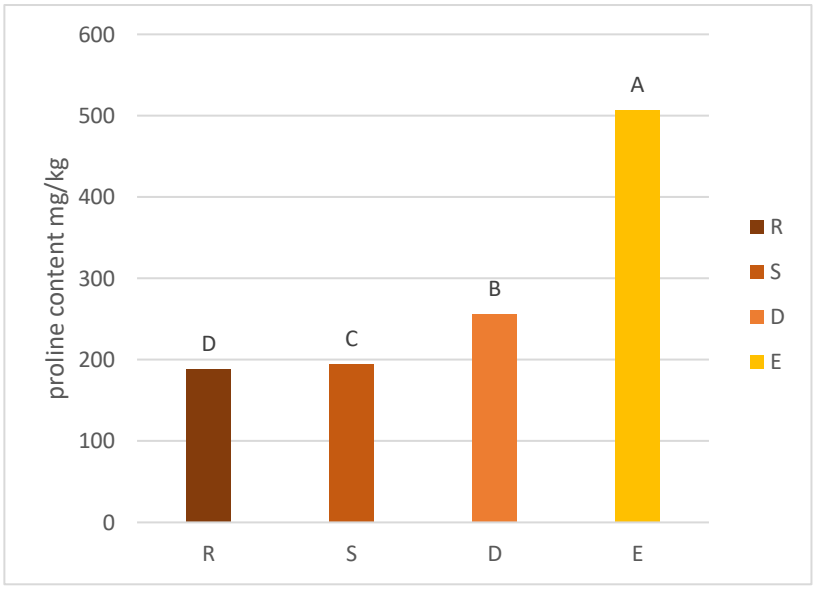

Figure n°9: The Proline content of different honeys

The Figure n°9 shows that Eucalyptus Honey (E) exhibited the highest proline content, measuring 506.443411 mg/kg followed by Eruca *Sativa* Honey (D) with proline content of 255.603101 mg/kg. In contrast, jujube Honey (S) had a moderate proline content of 194.083721 mg/kg. and rosemary Honey (R) showed the lowest proline content among the samples, with a result of 188.924031 mg/kg. These variations in proline content indicate differences in the composition and quality of the honey samples, with Eucalyptus Honey having the highest proline content, suggesting a potentially higher quality or specific floral source.

1.9. Hydroxy methyl furfural (HMF) measurement

HMF is generated during the heating and storage of honey, and its concentration rises with time and exposure to high temperatures. Higher HMF levels may suggest poor storage procedures or low-quality honey. International guidelines indicate that the HMF level of honey should not exceed particular limitations, which generally range from 5 to 40 mg/kg depending on the honey type (Codex Alimentarius Commission, 2001; White, 1979).

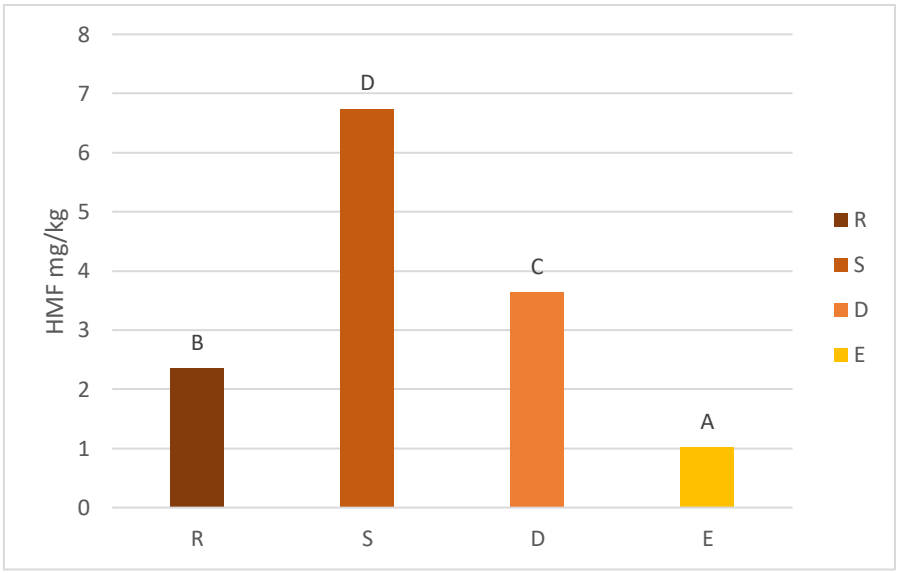

Figure n°10: The (HMF) measurement of different honeys

Among the samples tested, Eucalyptus Honey (E) exhibited the lowest HMF content, measuring 1.02333333 mg/kg followed by rosemary Honey (R) with the HMF content (2.363 mg/kg) (Figure n°10). On another hand, *Eruca Sativa* Honey (D) exhibit a content of 3.643 mg/kg while, jujube Honey (S) express the highest HMF content among the others samples 6.74466667 mg/kg.

These variations in HMF content indicate differences in the freshness and storage conditions of the honey samples. Higher HMF content suggests a longer storage duration or unfavorable storage conditions, while lower HMF content indicates fresher and better-preserved honey. Therefore, Eucalyptus Honey (E) can be considered to have the best freshness and storage conditions, while Jujube honey (S) could potentially have undergone extended storage or suboptimal storage conditions, resulting in an elevated HMF content.

1.10. polyphenolic assay

The polyphenol content in honey plays a significant role in its potential health benefits and antioxidant activity.

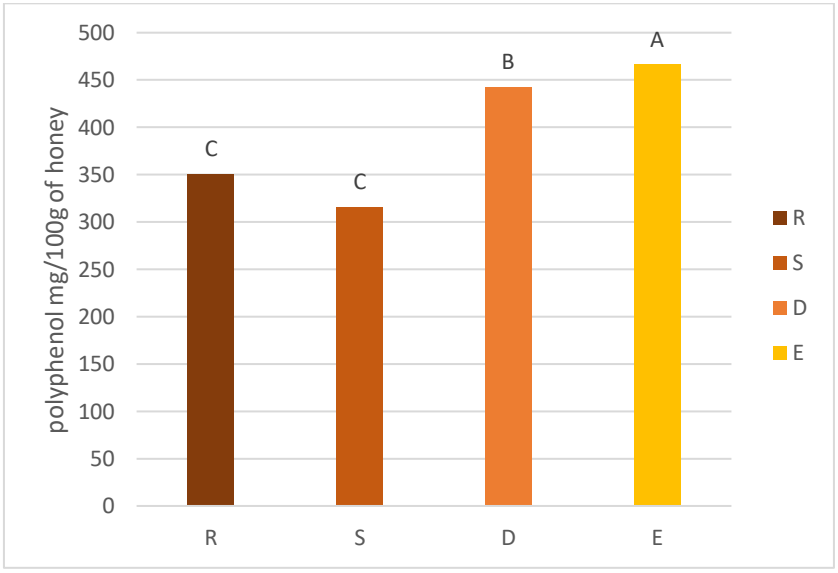

Figure n°11: The polyphenolic assay of different honeys

As illustrate on Figure n°11 the obtained results reveal that eucalyptus honey (E) exhibit the highest polyphenol content with a value of 466.196667 mg GAC /100g , followed closely by Eruca *sativa* honey (D) with a polyphenol content of 442.623333 mg GAC /100g . However, rosemary honey (R) expresses a low polyphenol content with 350.666667 mg GAC /100g, while jujube honey (S) showed the lowest polyphenol content at 315.593333 mg GAC /100g . These findings suggest that eucalyptus honey and Eruca-sativa honey possess higher levels of polyphenols, potentially indicating stronger antioxidant properties, when compare to rosemary honey and jujube honey

1.11. Flavonoid's assay

Flavonoids are natural chemicals present in plants that are transported to honey *via* bee nectar. These bioactive chemicals contribute to honey's antioxidant capacity and have been linked to a variety of health-promoting characteristics. The differences in flavonoid concentration among honey samples might be attribute to various floral sources and geographical considerations. Because of the intrinsic features of the

plants, honey obtained from certain plant sources, such as eucalyptus, may have higher flavonoid levels (Gheldof, 2002; Alvarez-Suarez *et al*, 201).

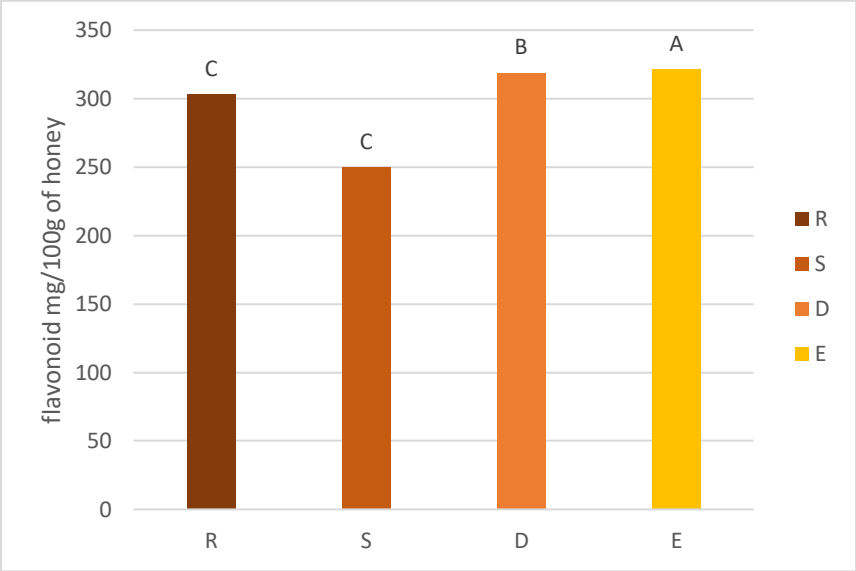

Figure n°12: The Flavonoid's assay of different honeys

The flavonoid content in honey is known for its health-promoting properties and antioxidant activity. The flavonoid essays results of the honey samples (Figure n°12), reveal that eucalyptus honey (E) had the highest flavonoid content: 321.826667 mg Catechin/g, followed closely by rosemary honey (R) and *Eruca sativa* honey (D) with flavonoid contents of 318.946667 mg Catechin/g and 303.123333 mg Catechin/g , respectively. Jujube honey (S) exhibit the lowest flavonoid content at 249.893333 mg Catechin/g . These findings suggest that eucalyptus honey may have the highest potential for health benefits and antioxidant properties among the tested samples, while jujube honey may have a comparatively lower flavonoid content.

1.12. Anti-free radical activity

The DPPH% essays results for the honey samples R (rosemary honey), S (juube honey), D (eruca sativa honey), and E (eucalyptus honey) were 79.06%, 78.25%, 79.77%, and 79.60%, respectively. (Figure n°13) indicate that all the tested honey samples exhibit high antioxidant activity, as reflected by the percentage of DPPH radical scavenging. However, there were only minor variations in the antioxidant activity among the different honey samples, with *Eruca sativa* honey (D) demonstrating slightly higher DPPH% value compared to the others.

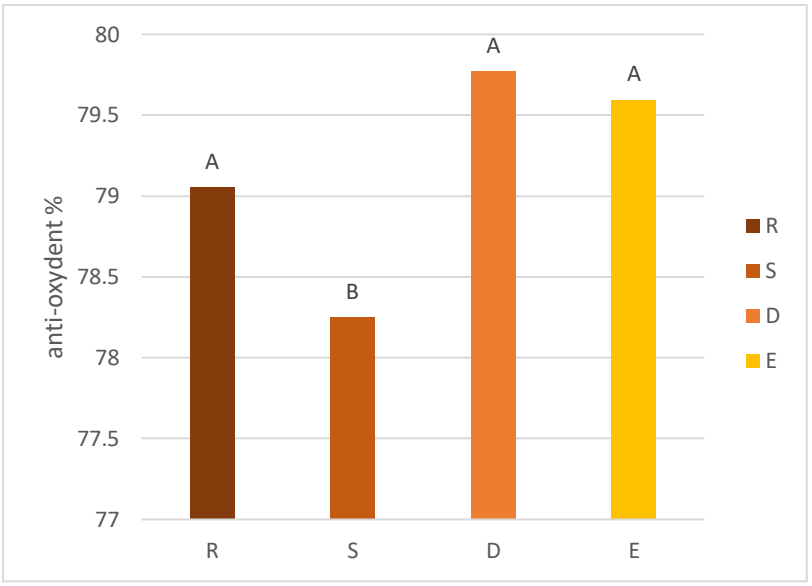

Figure n°13: The Anti-free radical activity of different honeys

These findings suggest that all the honey samples possess notable antioxidant potential, which can contribute to their potential health benefits.

1.13. Anti-inflammatory capacity

The results for the honey samples R (rosemary honey), S (juube honey), D (eruca sativa honey), and E (eucalyptus honey) yielded anti-inflammatory capacity percentages of 83.82%, 83.82%, 85.29%, and 83.33%, respectively. and illustrated in Figure n°14 indicate that all the tested honey samples express considerable anti-inflammatory properties. *Eruca sativa* honey (D) exhibited the highest anti-inflammatory capacity, followed closely by rosemary honey (R) and jujube honey (S), while eucalyptus honey (E) showed a slightly lower percentage.

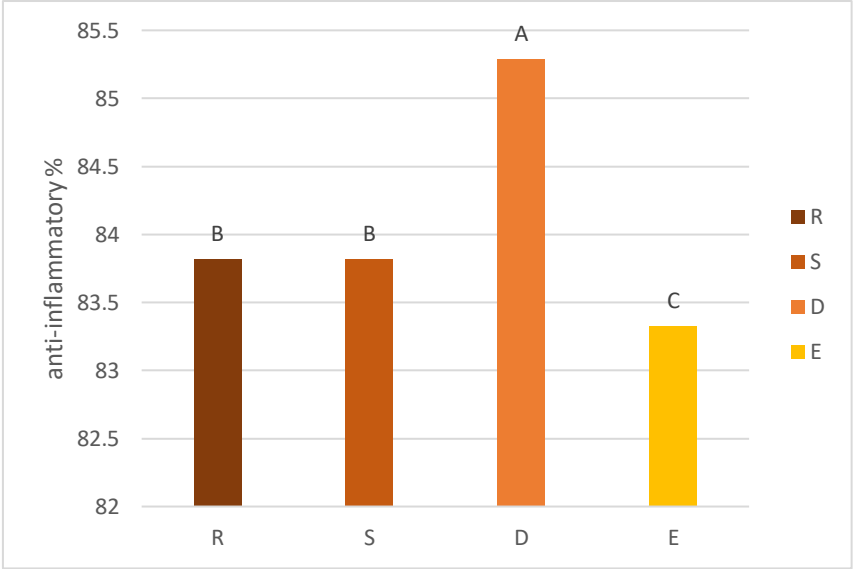

Figure n°14: The Anti-inflammatory capacity of different honeys

1.14. Anti-diabetic activity

anti-diabetic activity refers to their capacity to regulate and control blood glucose levels, which is critical in diabetes management. The anti-diabetic effect of honey is established by examining its influence on several diabetes-related parameters such as glucose absorption, insulin secretion, and glucose metabolism. This study is critical for identifying honey kinds with possible therapeutic advantages for diabetics or those at risk. Researchers want to learn more about honey's bioactive constituents, such as polyphenols and flavonoids, and their mechanisms of action in modifying glucose homeostasis by testing its anti-diabetic ability (Erejuwa *et al*, 2014; Abdulrhman *et al*. 2012).

a) Anti-diabetic capacity at (C1 – dilution 1/16):

These results illustrated in figure N°15 indicate variations in the ability of the different honey samples R (rosemary honey), S (juube honey), D (eruca sativa honey), and E (eucalyptus honey) yielded anti-diabetic capacity percentages of 69.43%, 83.08%, 72.45%, and 84.16%, respectively. to potentially aid in managing diabetes. Eucalyptus honey (E) exhibited the highest anti-diabetic capacity, followed by jujube honey (S) and *Eruca sativa* honey (D). Rosemary honey (R) showed the lowest percentage among the samples. These findings suggest that eucalyptus honey and

jujube honey may have beneficial effects in terms of their potential to regulate blood sugar levels.

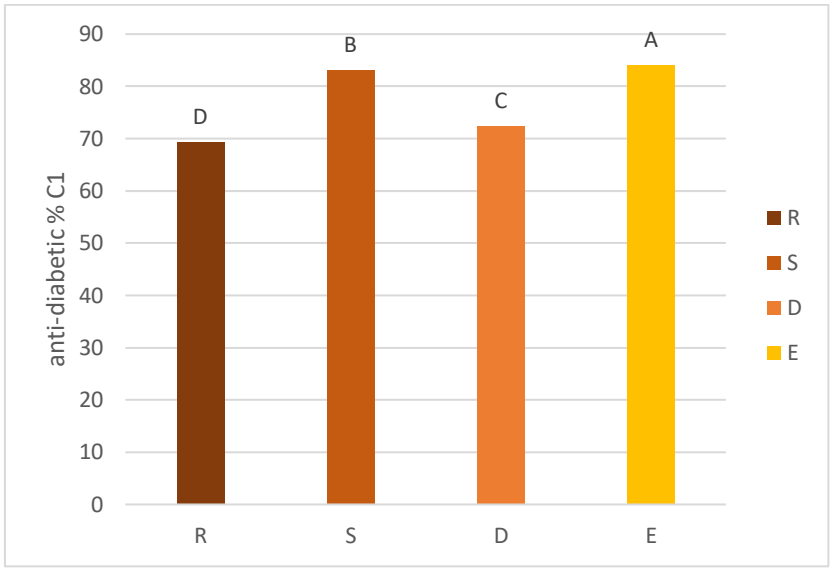

Figure n°15: The Anti-diabetic capacity at (C1 – dilution 1/16) of different honeys

b) Anti-diabetic capacity % (c2 – 1/32)

In the second round of concentration (c2-1/32) of the anti-diabetic capacity essay, the results of the honey samples R (rosemary honey), S (juube honey), D (eruca sativa honey), and E (eucalyptus honey) demonstrated percentages of 77.15%, 82.04%, 78.01%, and 84.44%, respectively, and illustrated in Figure n°16, indicate that all the honey samples showed some level of anti-diabetic capacity, with Eucalyptus honey (E) exhibiting the highest percentage, followed by jujube honey (S), *Eruca sativa* honey (D), and rosemary honey (R).

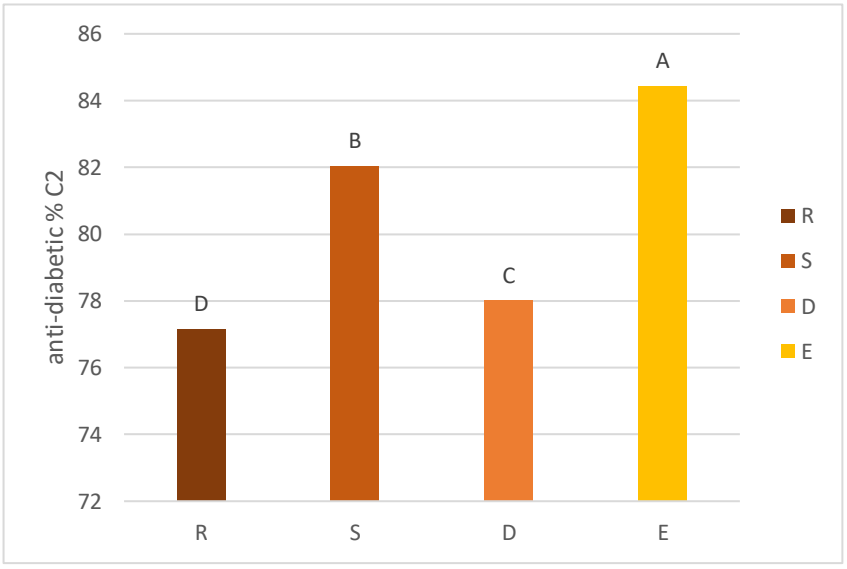

Figure n°16: The Anti-diabetic capacity % (c2 – 1/32) of different honeys

c) Anti-diabetic capacity % (c3-1/64)

In the third round of the anti-diabetic capacity essay (Figure n°17), the honey samples R (rosemary honey), S (jujube honey), D (*Eruca sativa* honey), and E (eucalyptus honey) demonstrated percentages of 87.02%, 86.51%, 86.37%, and 89.25%, respectively. These results indicate that all the honey samples exhibited significant anti-diabetic capacity, with Eucalyptus honey (E) showing the highest percentage, followed closely by rosemary honey (R), jujube honey (S), and eruca sativa honey (D).

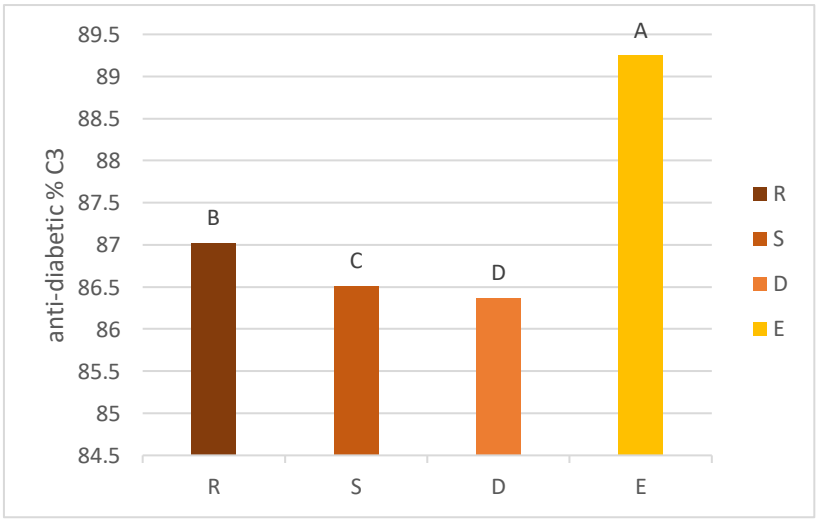

Figure n°17: The Anti-diabetic capacity % (c3-1/64) of different honeys

2. The influence of the treatment process on the composition and biological properties of tested honey.

2.1. Rosemary honey (R)

2.1.1. Polyphenolic assay of rosemary honey

The polyphenol content of the rosemary honey samples exhibited significant variations. The honey diluted in ethanol displayed the highest polyphenol content with a value of 777.424108 mg GAC /100g of honey. In contrast, raw honey had a lower polyphenol content of 350.666667 mg GAC /100g of honey (Figure n°18).

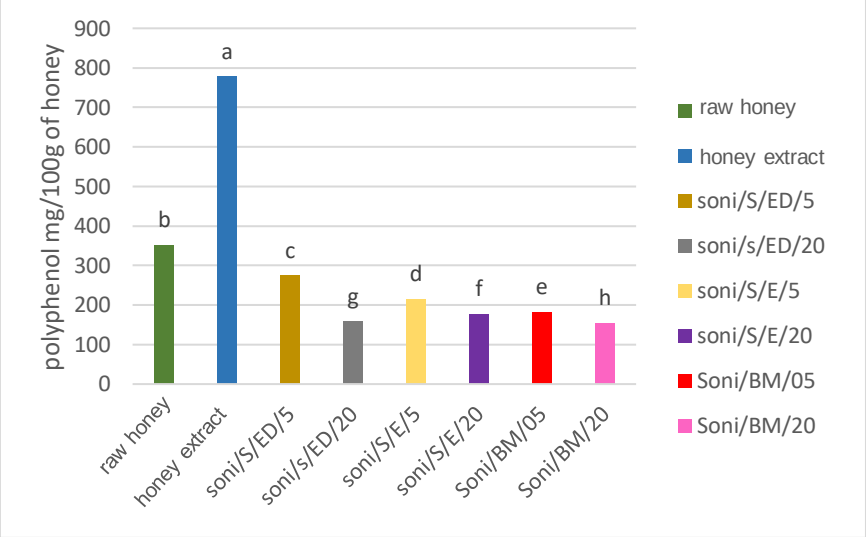

Figure n°18: The polyphenolic assay of different R honey treatments

Among the ultrasound treated honey samples, the treatment with distilled water for 5 minutes (Soni/S/ED/5) yielded with high polyphenol content with 274.034777 mg GAC /100g honey , while the 20-minute treatment (Soni/S/ED/20) showed a lower value of 157.913351 mg GAC /100g honey . The ultrasound treated honey samples with ethanol as the solvent (Soni/S/E/5 and Soni/S/E/20) exhibited polyphenol contents of 214.796667 mg GAC /100g honey and 176.186667 mg GAC /100g honey respectively. The honey treated in bath ultrasound for 5 minutes (Soni/BM/05) resulted in a polyphenol content of 181.78603 mg GAC /100g honey while the 20-minute treatment (Soni/BM/20) showed the lowest value of 153.492485 mg GAC /100g honey. These findings demonstrate the impact of different treatments on the polyphenol composition of rosemary honey (Figure N° 18).

2.1.2. Flavonoid's assay of rosemary honey

The flavonoid content analysis of rosemary honey samples revealed notable variations. The honey diluted in ethanol exhibited the highest flavonoid content with a value of 586.466667 mg Catechin/g . In contrast, raw honey displayed a lower flavonoid content of 303.123333 mg Catechin/g (Figure N° 19).

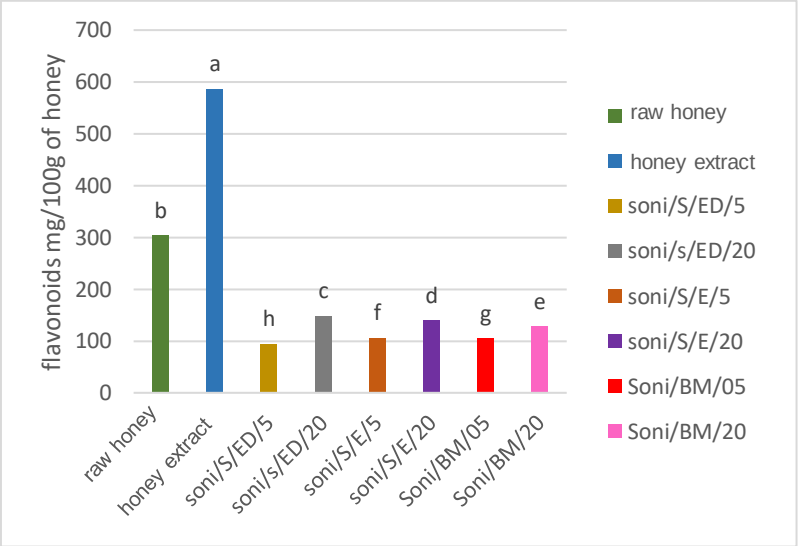

Figure n°19: The flavonoids assay of different R honey treatments

Among the ultrasound treated honey samples, the treatment with distilled water for 5 minutes (Soni/S/ED/5) resulted in a flavonoid content of 94.5187743 mg Catechin/g , while the 20-minute treatment (Soni/S/ED/20) showed a higher value of 147.748525 mg Catechin/g . The sonicated honey samples with ethanol as the solvent (Soni/S/E/5 and Soni/S/E/20) had flavonoid contents of 106.096667 mg Catechin/g and 139.116667 mg Catechin/g, respectively. With ultrasound bath treatment for 5 minutes (Soni/BM/05) yielded with a flavonoid content of 104.589268 mg Catechin/g , while the 20-minute treatment (Soni/BM/20) displayed a slightly higher value of 127.607538 mg Catechin/g . These results emphasize the influence of different treatments on the flavonoid composition of rosemary honey (Figure n° 19).

2.1.3. Anti-free radical activity of rosemary honey

The evaluation of DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity in rosemary honey samples demonstrated varying levels of antioxidant potential. The honey diluted in ethanol displayed a DPPH percentage of 70.0166667, indicating moderate antioxidant activity. Raw honey exhibited a slightly higher DPPH percentage of 79.0566667, suggesting relatively stronger radical scavenging ability (Figure n° 20).

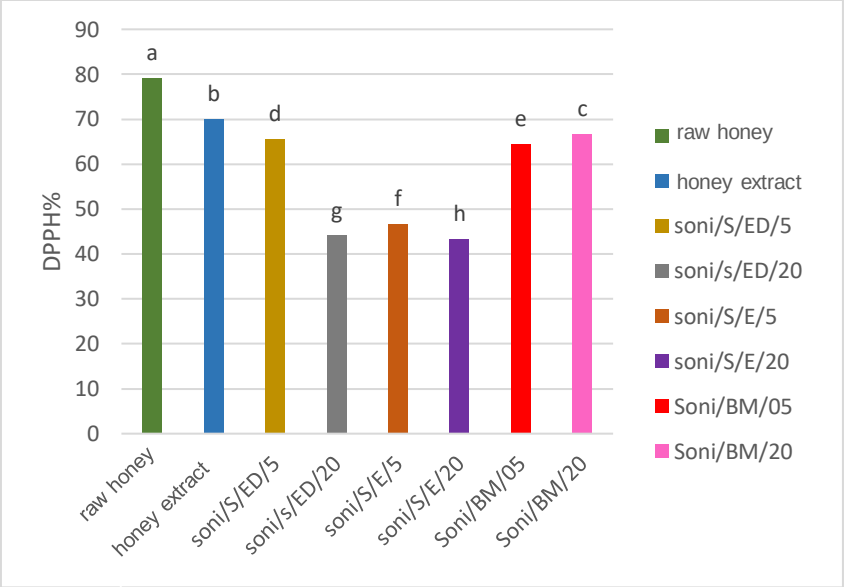

Figure n°20: The Anti-oxidant activity of different R honey treatments

Among the ultrasound treated honey samples, the treatment with distilled water for 5 minutes (Soni/S/ED/5) showed a DPPH percentage of 65.5033333, while the 20-minute treatment (Soni/S/ED/20) displayed a lower value of 44.08. The ultrasound treated honey samples with ethanol as the solvent (Soni/S/E/5 and Soni/S/E/20) exhibited DPPH percentages of 46.7033333 and 43.31, respectively. Bath ultrasound treated honey for 5 minutes (Soni/BM/05) yielded a DPPH percentage of 64.3866667, while the 20-minute treatment (Soni/BM/20) demonstrated a higher value of 66.71. These results highlight the varying antioxidant capacities of rosemary honey samples under different treatment conditions (Figure n° 20).

2.1.4. The anti-inflammatory capacity of rosemary honey

The assessment of anti-inflammatory activity in rosemary honey samples revealed distinct levels of efficacy. The honey diluted in ethanol exhibited a high anti-inflammatory capacity, with a percentage of 97.05%. Raw honey displayed a slightly

lower percentage of 83.82%, suggesting significant anti-inflammatory potential of diluted honey in ethanol. (Figure n°21).

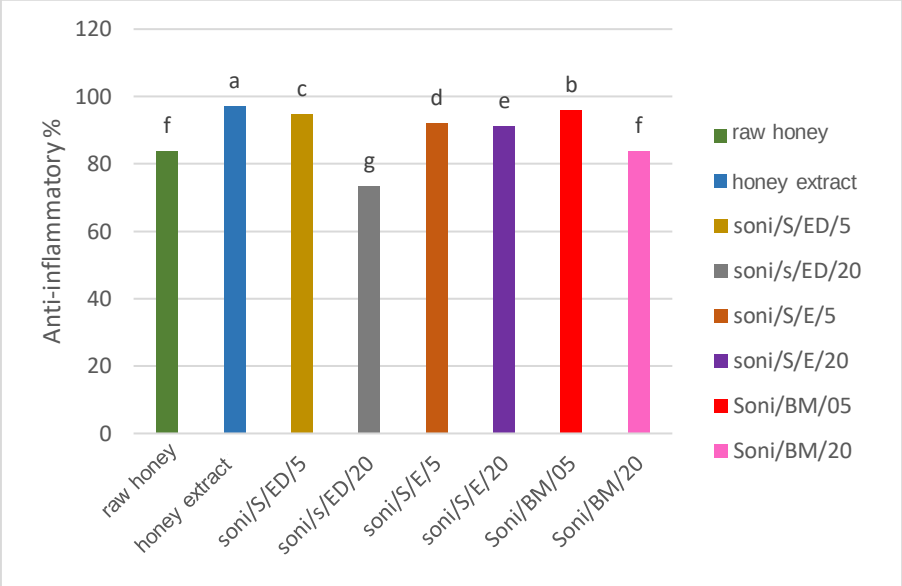

Figure n°21: The Anti-inflammatory capacity of different R honey treatments

Among the ultrasound treated honey samples, the treatment with distilled water for 5 minutes (Soni/S/ED/5) demonstrated a percentage of 94.66 % , while the 20-minute treatment (Soni/S/ED/20) showed a lower value of 73.52%. The ultrasound treated honey samples with ethanol as the solvent (Soni/S/E/5 and Soni/S/E/20) exhibited anti-inflammatory percentages of 92.15% and 91.17%, respectively. Bath ultrasound treatment for 5 minutes (Soni/BM/05) yielded an anti-inflammatory percentage of 96%, while the 20-minute treatment (Soni/BM/20) displayed the same percentage of 83.82%. These findings underscore the variations in anti-inflammatory potential among the different rosemary honey samples. (Figure n°21).

2.1.5. The anti-diabetic propriety of rosemary honey

The evaluation of anti-diabetic activity in rosemary honey samples revealed notable differences in effectiveness. The honey diluted in ethanol exhibited considerable anti-diabetic activity, with an activity of 80.24%. The raw honey displayed a slightly lower activity of 77.15%, suggesting significant anti-diabetic potential of diluted honey. The evaluation of the anti-diabetic activity of rosemary honey samples demonstrated

variations in their effectiveness at different concentrations. Among the tested samples, C3 consistently exhibited the higher anti-diabetic activity in contrast of the others concentration C1, C2 and C4. The specific values for each sample and concentration are as follows:

- For concentration C1, the anti-diabetic activity of the honey diluted in ethanol showed the highest activity (75.43%) than raw honey (69.42%). On another hand the honey treated with ultrasound for 5 minutes in distilled water (soni/S/ED/5) displayed a lowest activity (65.17%), than the honey treat for 20 minutes soni/S/ED/20 (72.95%). Also, the honey treated with ultrasound for 5 minutes in ethanol (soni/S/E/5) showed a lower activity (35.9%) than the honey treated for 20 minutes Soni/S/E/20 (66.6366%). With the honey treated within bath ultrasound for 5 minutes (Soni/BM/05) had a low activity (37.7%), in contrast of the honey treated with bath ultrasound for 20 minutes Soni/BM/20 who had a better activity of 55.9666% (Figure n° 22).

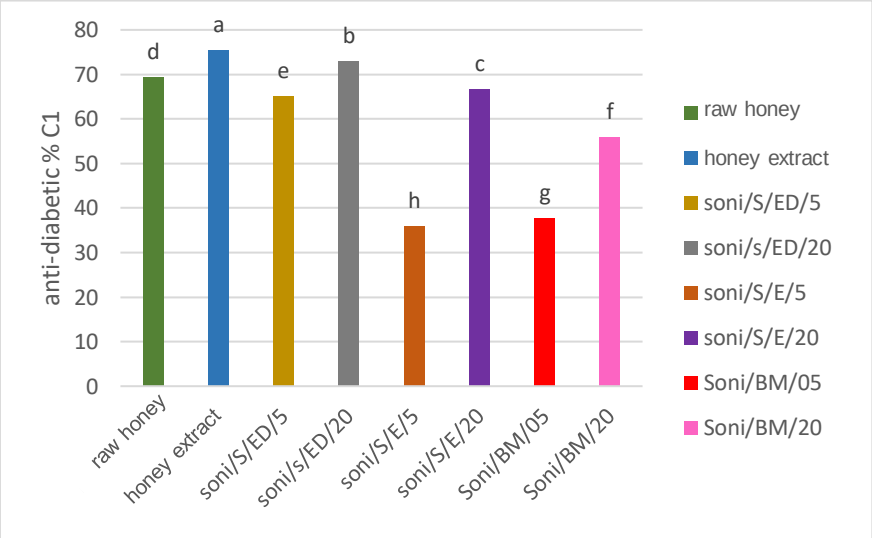

Figure n°22: The Anti-diabetic capacity C1 of different R honey treatments

- ✓ concentration C2, the anti-diabetic activity was as follows: The raw honey displayed a low activity (77.15%), while honey diluted in ethanol showed a slightly higher activity (80.24%). On another hand the honey treated with ultrasound for 5 minutes in distilled water Soni/S/ED/5 exhibited a better activity of 83.1233% than the honey treated for 20 minutes (soni/S/ED/20). The honey treated with ultrasound for 5 minutes in ethanol Soni/S/E/5 showed a highest

activity of 82.33% than the honey treated for 20 minutes. However, the honey treated with bath ultrasound for 20 minutes had a better antidiabetic activity than the honey treated for 5 minutes (Figure n°23).

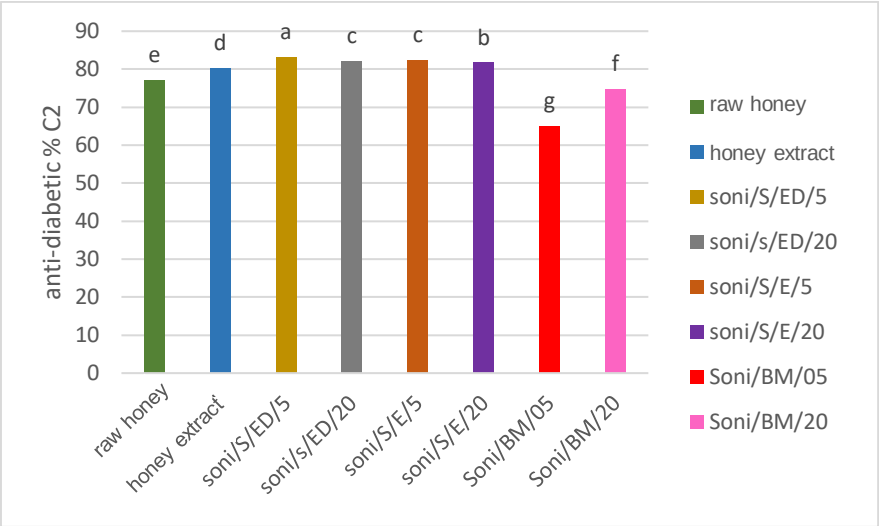

Figure n°23: The Anti-diabetic capacity C2 of different R honey treatments

➢ concentration C3, the anti-diabetic activity of the raw honey (87.02%) is better than the honey diluted in ethanol (85.67%). The honey treated with ultrasound for 5minutes in distilled water Soni/S/ED/5 showed a better activity of 89.3966%, than the treated for 20 minutes soni/S/ED/20 (86.6833%). The honey treated with ultrasound for 5 minutes in ethanol Soni/S/E/5 exhibited a low activity (90.1433%) in comparison to a treated honey for 20 minutes Soni/S/E/20 (91.34%). On another hand the honey treated in bath ultrasound for 5 minutes Soni/BM/05 displayed a low activity (79.2533%) than the honey treated for 20 minutes Soni/BM/20 (83.0033%).(Figure n°24).

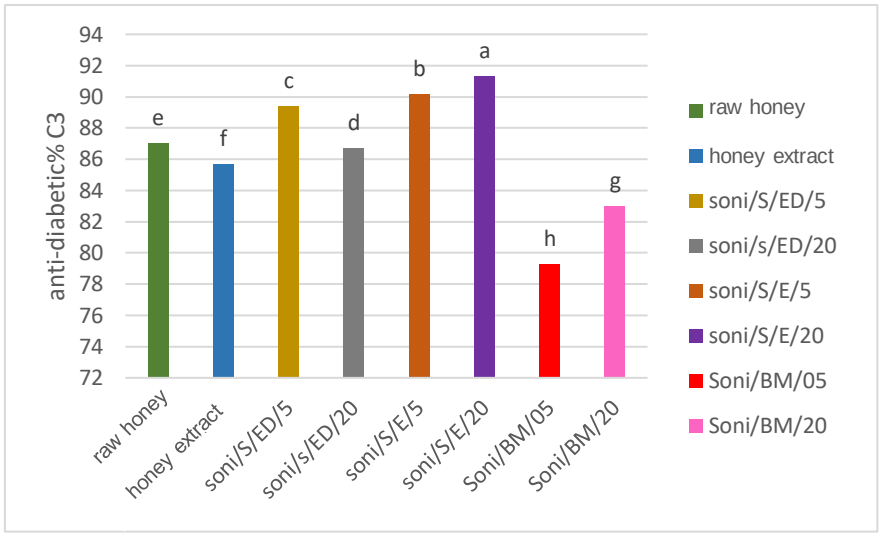

Figure n°24: The Anti-diabetic capacity C3 of different R honey treatments

2.2. Jujube (S) honey

2.2.1. Polyphenols content of Jujube honey

The analysis of polyphenol content in *jujube* honey samples revealed distinct variations. The honey diluted in ethanol extract exhibited the highest polyphenol content (708.16 mg GAC /100g). In contrast, raw honey displayed a lower polyphenol content of 315.59 mg GAC /100g (Figure n°25).

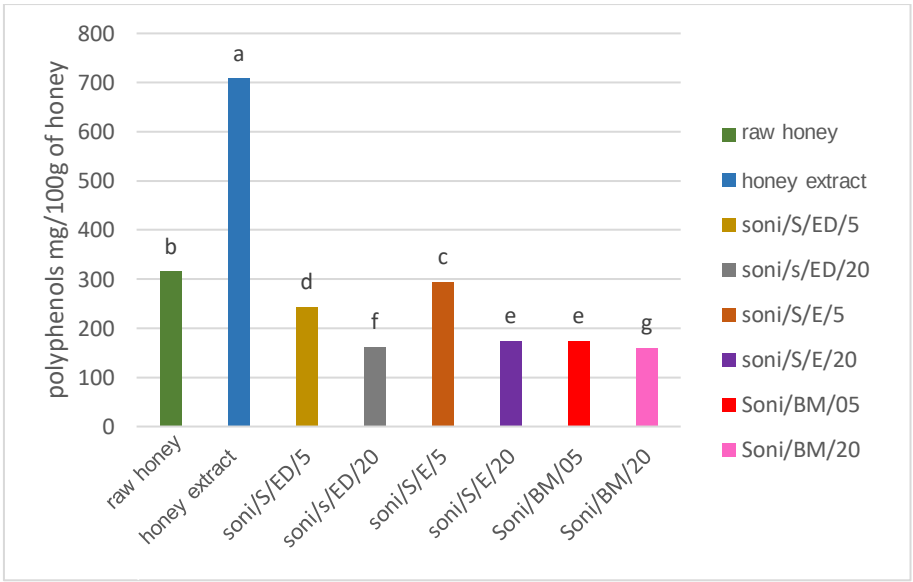

Figure n°25: The Polyphenol's assay of different S honey treatments

Among the ultrasound treated honey samples, the treatment with distilled water for 5 minutes (Soni/S/ED/5) resulted with high polyphenol content of 243.678161 mg GAC/100g while the 20-minute treatment (Soni/S/ED/20) showed a lower value of 161.450044 mg GAC/100g . The honey ultrasound treated in ethanol as the solvent for 5 and 20 minutes (Soni/S/E/5 and Soni/S/E/20) exhibited polyphenol contents of 294.076667 mg GAC/100g and 174.71 mg GAC/100g, respectively. Honey treated with in bath ultrasound for 5 minutes (Soni/BM/05) yielded with a polyphenol content of 173.82847 mg GAC/100g, while for 20-minute treatment (Soni/BM/20) displayed a slightly higher value of 159.386973 mg GAC/100g. These findings underscore the impact of different treatments on the polyphenol composition of jujubie honey. (Figure n°25).

2.2.2. Flavonoids content of Jujube honey

The evaluation of flavonoid content in *jujube* honey samples revealed significant variations. The honey diluted in ethanol displayed the highest flavonoid content with a value of 659.906667 mg Catechin/g. In contrast, raw honey exhibited a lower flavonoid content of 249.893333 mg Catechin/g . Among the ultrasound treated honey samples, the treatment with distilled water for 5 minutes (Soni/S/ED/5) resulted in a flavonoid content of 114.659761 mg Catechin/g , while the 20-minute treatment (Soni/S/ED/20) showed a slightly higher value of 131.923464 mg Catechin/g .

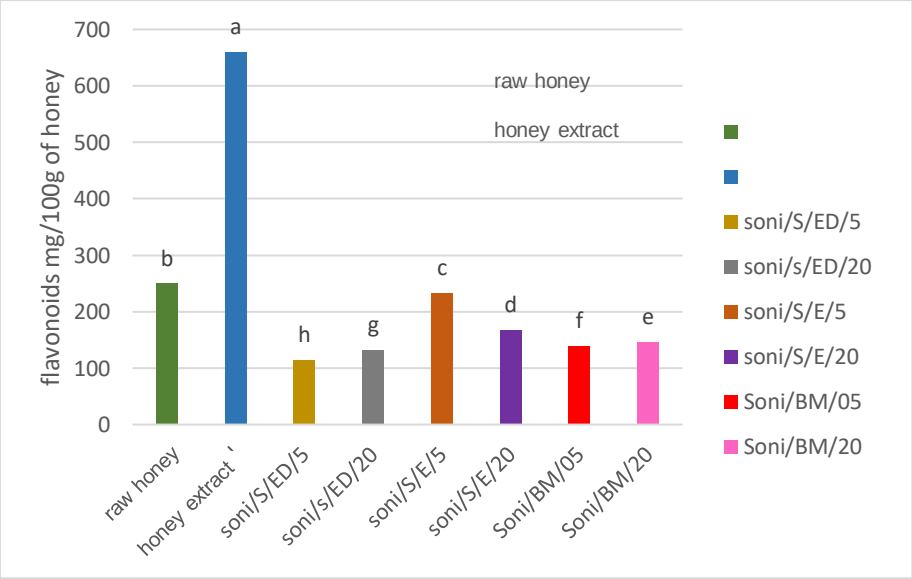

Figure n°26: The flavonoids assay of different S honey treatments

The honey treated with ultrasound using ethanol as the solvent for 5 minutes (Soni/S/E/5) had flavonoid higher contents of 232.628399 mg Catechin/g rather than the treated for 20 minutes (167.89 mg Catechin/g). The honey treated using bath ultrasound for 5 minutes (Soni/BM/05) yielded a flavonoid content of 139.116674 mg Catechin/g, while the 20-minute treatment (Soni/BM/20) displayed a slightly higher value of 146.309922 mg Catechin/g. These results emphasize the influence of different treatments on the flavonoid composition of *jujube* honey. (Figure n° 26).

2.2.3. Anti-free radical activity of Jujube honey

The assessment of DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity in *jujube* honey samples demonstrated varying levels of antioxidant potential. The diluted in ethanol displayed a DPPH percentage of 79.51, indicating moderate antioxidant activity. While, raw honey exhibited a slightly lower DPPH percentage of 78.2533333, suggesting comparable radical scavenging ability (Figure n°27).

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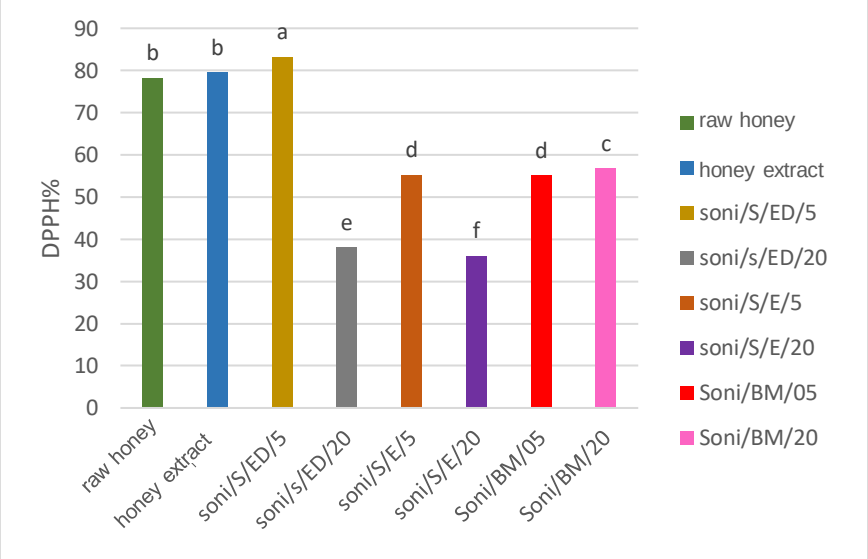


Figure N° 27: The anti-oxidant capacity of different S honey treatments

Among the ultrasound treated honey samples, the treatment with distilled water for 5 minutes (Soni/S/ED/5) showed a DPPH percentage of 83.1766667, while the 20-minute treatment (Soni/S/ED/20) demonstrated a lower value of 38.0733333. The

ultrasound treated honey samples for 5 minutes using ethanol as the solvent (Soni/S/E/5) exhibited DPPH percentages of 55.1666667 higher than those treated for 20 minutes (Soni/S/E/20) (35.9733333). The treated honey in bath ultrasound for 5 minutes (Soni/BM/05) yielded a DPPH percentage of 55.0066667, while for 20-minutes the treatment (Soni/BM/20) displayed a slightly higher value of 56.78. These findings highlight the variations in antioxidant activity among different *jujube* honey samples subjected to various treatments. (Figure N°27).

2.2.4. Anti-inflammatory capacity of Jujube honey

The evaluation of anti-inflammatory activity in jujube honey samples revealed diverse efficacy. The honey diluted in ethanol exhibited a high anti-inflammatory capacity(88.23). However, raw honey displayed a slightly lower capacity (83.82); suggesting significant anti-inflammatory potential. (Figure n°28).

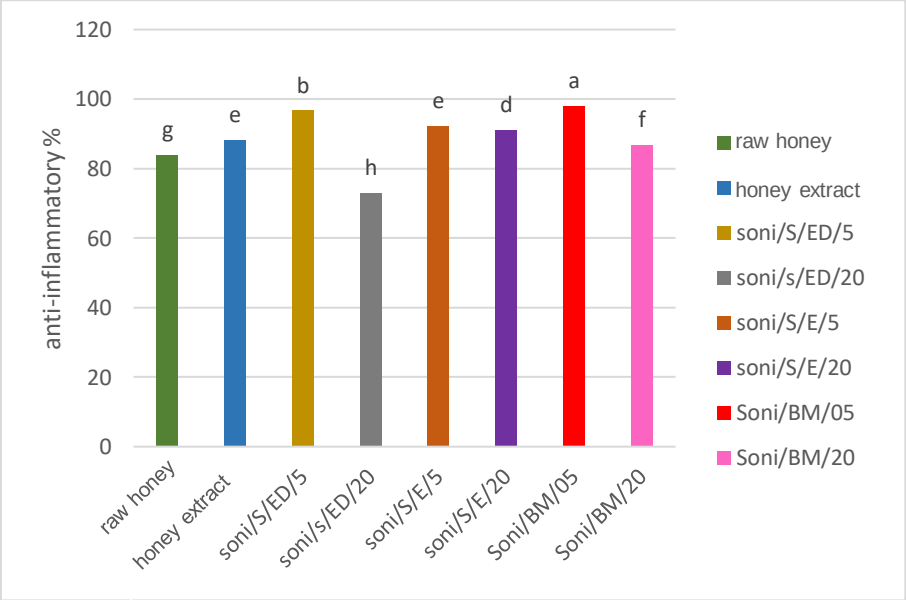


Figure n°28: The Anti-inflammatory capacity of different S honey treatments

Among the ultrasound treated honey samples, the treatment with distilled water for 5 minutes (Soni/S/ED/5) demonstrated a percentage of 96.6666667, while the 20-minute treatment (Soni/S/ED/20) showed a lower value of 73.03. The ultrasound treated honey samples with ethanol as the solvent (Soni/S/E/5 and Soni/S/E/20) exhibited anti-inflammatory percentages of 92.15 and 91.17, respectively. The bath ultrasound

treated for 5 minutes (Soni/BM/05) yielded an anti-inflammatory percentage of 98, while the 20-minute treatment (Soni/BM/20) displayed a slightly lower value of 86.76. These results highlight the variations in anti-inflammatory potential among different jujube honey samples under different treatment conditions. (Figure n°28).

2.2.5. Anti-diabetic capacity of Jujube honey

The evaluation of anti-diabetic activity in jujube honey samples revealed notable differences in effectiveness. The diluted honey in ethanol exhibited considerable anti-diabetic activity. Raw honey displayed a slightly higher activity, suggesting significant anti-diabetic potential. The assessment of the anti-diabetic activity of jujube honey samples revealed variations in their effectiveness at different concentrations. Among the samples, C3 consistently exhibited higher anti-diabetic activity. The specific values for each sample and concentration are as follows:

- For concentration c1, the anti-diabetic activity of the samples is as follows: the raw honey exhibited an activity of 83.0766%. While, the diluted honey in ethanol showed a lower activity of 76.0833%. The ultrasound treated honey using distilled water for 5 minutes (soni/S/ED/5) displayed an activity of 63.3933%, while the honey treated for 20 minutes (soni/S/ED/20) had an activity of 72.02%. On another hand the ultrasound treated honey for 5 minutes with ethanol as solvent (soni/S/E/5) showed a low activity (69.0866%), while the treated for 20 minutes (Soni/S/E/20) exhibited a higher activity (81.2033%). The honey treated in ultrasound bath for 5 minutes (Soni/BM/05) displayed a low activity of 39.3133%. While the treated for 20 minutes (Soni/BM/20) had a higher activity of 53.3%.(Figure n°29).

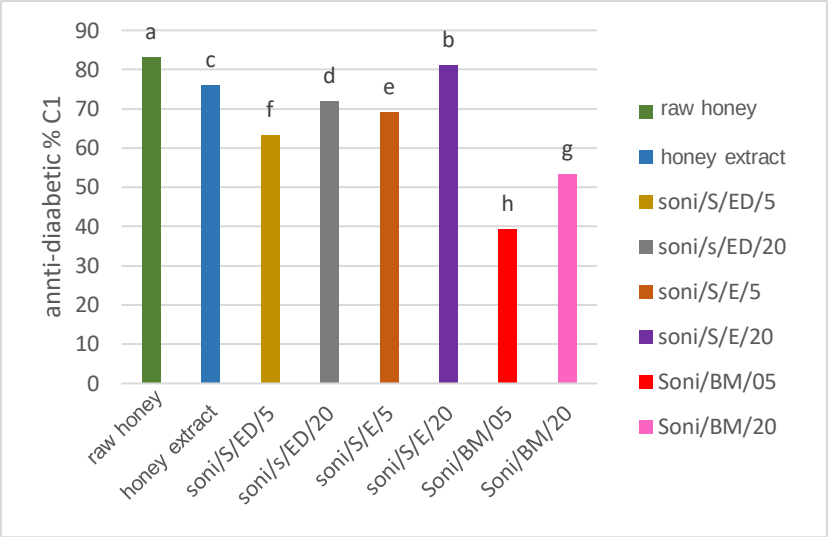


Figure 29: The Anti-diabetic capacity C1 of different S honey treatments

- concentration C2, the anti-diabetic activity was as follows: The raw honey exhibited an activity of 82.0433%, while the honey diluted in ethanol showed a slightly higher activity (78.94%). The extract treated with ultrasound for 5 minutes in distilled water Soni/S/ED/5 exhibited an activity of 79.2533%, while for 20 minutes soni/S/ED/20 had an activity of 83.5833%. However, the treated honey using ethanol as solvent for 20 minutes Soni/S/E/20 had the highest activity at 91.99%, in contrast of the treated for 5 minutes Soni/S/E/5 (81.85%). On another hand, the treated honey in bath ultrasound for 20 minutes had a better activity (72.4033%) than the honey treated for 5 minutes (Soni/BM/05) (Figure n°30).

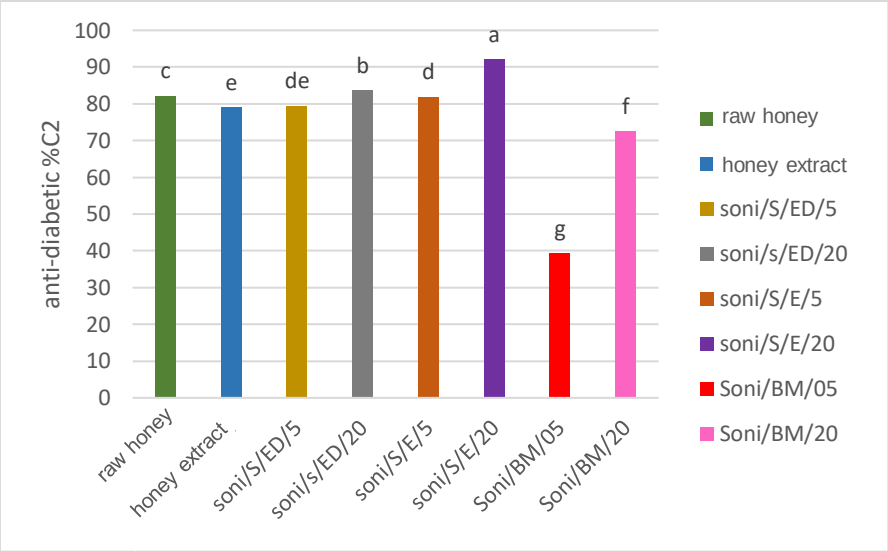


Figure n°30: The Anti-diabetic capacity C2 of different S honey treatments

- concentration c3, the anti-diabetic activity was as follows: The raw honey exhibited an activity of 86.51% and the honey diluted in ethanol displayed a slightly higher activity of 86.73%. The honey ultrasound treated in distilled water for 5 minutes Soni/S/ED/5 showed an activity of 88.17%, while the treated for 20 minutes soni/S/ED/20 had an activity of 86.9466%. In contrast, the honey treated for 5 minutes using ethanol as solvent Soni/S/E/5 exhibited a low activity (89.18%) than the honey treated for 20 minutes Soni/S/E/20, that showed the highest activity at 96.75%. On another hand the honey treated Soni/BM/05 displayed an activity of 77.1633%, and Soni/BM/20 had an activity of 82.8633%.(Figure n°31).

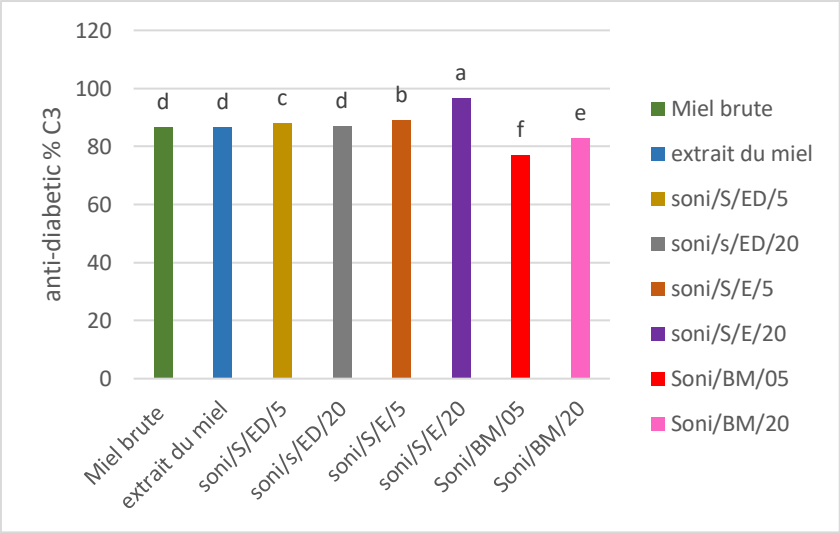


Figure n°31: The Anti-diabetic capacity C3 of different S honey treatments

2.3. Eruca sativa (D) honey

2.3.1. Polyphenols assay of *Eruca sativa* honey

The analysis of polyphenol content in *Eruca sativa* honey samples revealed distinct variations. The honey ethanol diluted exhibit the highest polyphenol content with a value of 791.57 mg GAC /100g . In comparison, the raw honey displayed a lower polyphenol content of 442.623333 mg GAC /100g .(Figure n°32)

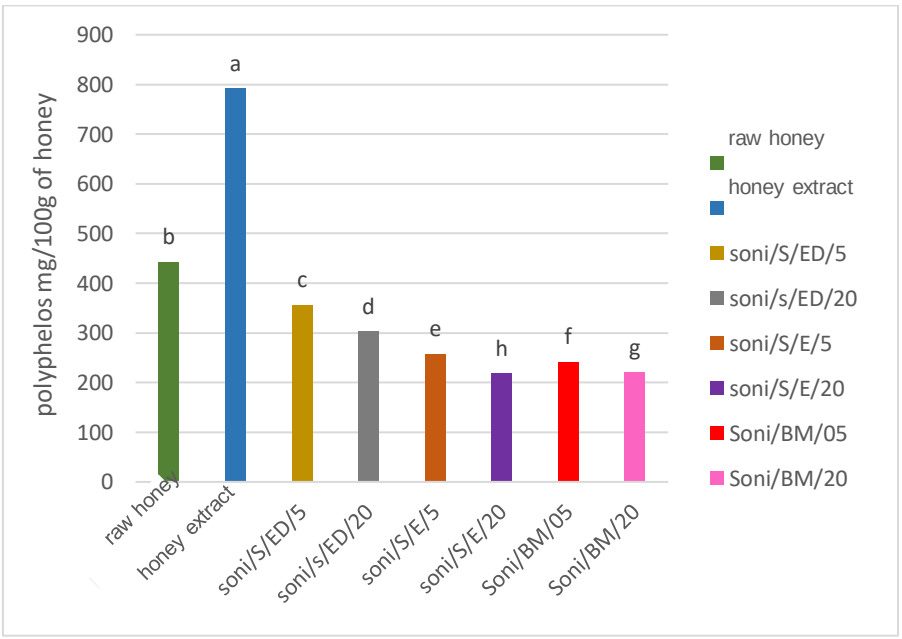


Figure n°32: The polyphenolic assay of different D honey treatments

Among the honey treated with ultrasound, the treatment with distilled water for 5 minutes (Soni/S/ED/5) resulted in a polyphenol content of 356.262894 mg GAC /100g , while the 20-minute treatment (Soni/S/ED/20) showed a lower value of 302.623047 mg GAC /100g . The honey samples treated with ultrasound for 5 minutes and using ethanol as the solvent (Soni/S/E/5) exhibited polyphenol contents of 257.53 mg GAC /100g with the treated for 20 minutes (Soni/S/E/20) express a content of 218.333333 mg GAC /100g , respectively. The treatement in bath ultrasound for 5 minutes (Soni/BM/05) yielded a polyphenol content of 241.025641 mg GAC /100g, while the 20-minute treatment (Soni/BM/20) displayed a slightly lower value of 219.510758 mg GAC /100g. These findings highlight the impact of different treatments on the polyphenol composition of *Eruca sativa* honey. (Figure n°32).

2.3.2. Flavonoids asssay of *Eruca sativa* honey

The evaluation of flavonoid content *in Eruca sativa* honey samples revealed significant variations. The diluted honey in ethanol displayed the highest flavonoid content with a value of 772.12 mg Catechin/g. In contrast, raw honey exhibited a lower flavonoid content of 318.946667 mg Catechin/g. (Figure n°33).

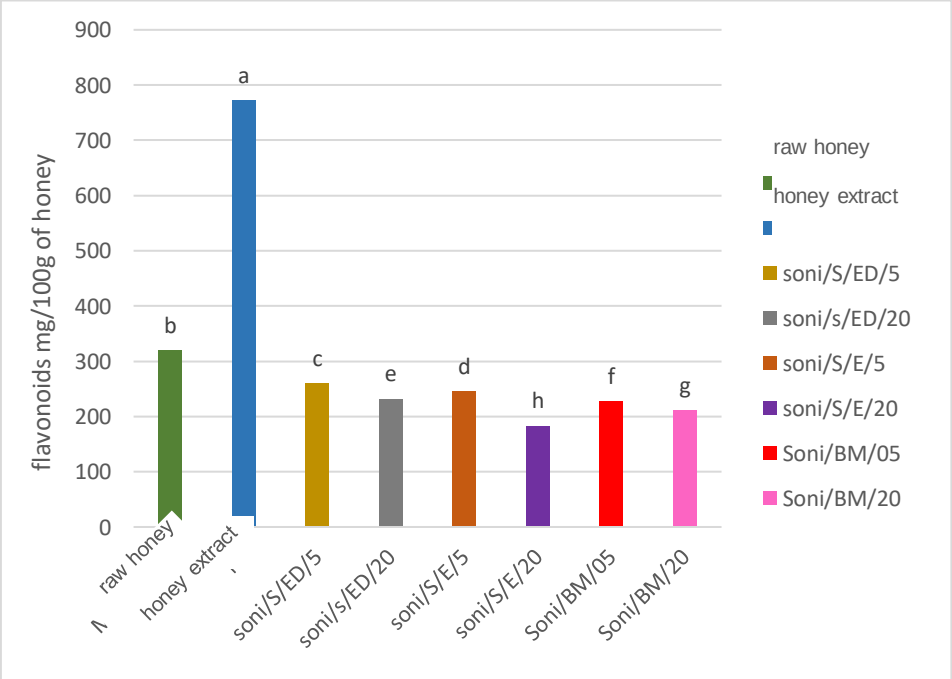


Figure n°33: The flavonoids assay of different D honey treatments

Among the honey samples treated with ultrasound, the treatment with distilled water for 5 minutes (Soni/S/ED/5) resulted with a high flavonoid content of 259.962595 mg Catechin/g, while the 20-minute treatment (Soni/S/ED/20) showed a slightly higher value of 231.189757 mg Catechin/g. The honey samples treated with ultrasound for 5 minutes and using the ethanol as the solvent (Soni/S/E/5) had an important flavonoid contents 245.576667 mg Catechin/g than the honey samples treated for 20 minutes (182.276667 mg Catechin/g). The treatment using the bath ultrasound for 5 minutes (Soni/BM/05) yielded with an important flavonoid content of 226.873831 mg Catechin/g, while the treatment for 20-minute (Soni/BM/20) displayed a slightly lower value of 211.04877 mg Catechin/g. These results highlight the influence of different treatments on the flavonoid composition of *Eruca sativa* honey. (Figure n°33).

2.3.3. Anti-free radical activity of *Eruca sativa* honey

The assessment of DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity in *Eruca sativa* honey samples demonstrated varying levels of antioxidant potential. The diluted honey in ethanol displayed a DPPH percentage of 81.9266667, indicating moderate antioxidant activity. While the raw honey exhibited a slightly lower DPPH percentage of 79.7733333, suggesting comparable radical scavenging ability. (Figure n°34).

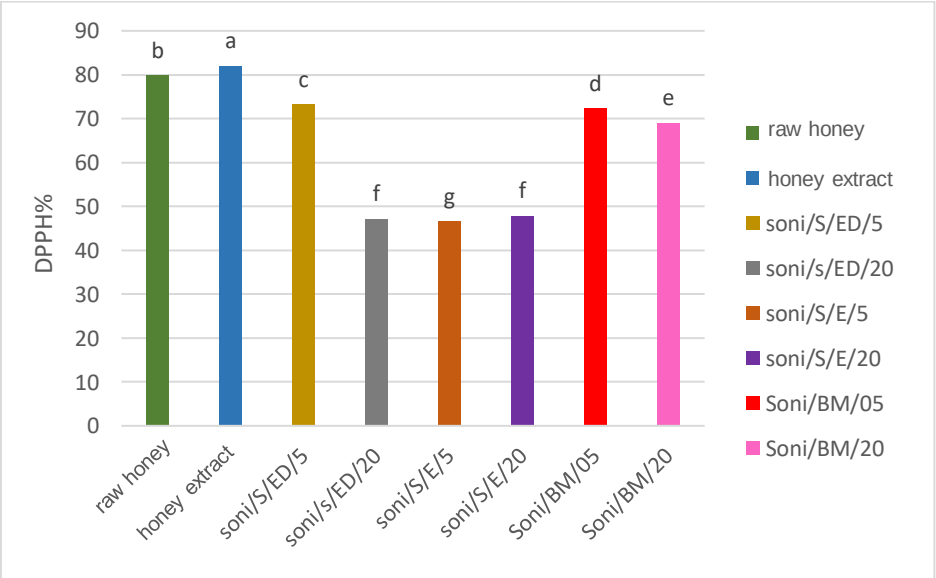


Figure n°34: The anti-oxidant capacity of different D honey treatments

Among the ultrasound treated honey samples, the treatment using distilled water for 5 minutes (Soni/S/ED/5) showed a DPPH percentage of 73.2433333, while the 20-minute treatment (Soni/S/ED/20) demonstrated a lower value of 47.11. The ultrasound treated honey samples using ethanol as the solvent even for 5 minutes (Soni/S/E/5) or for 20 minutes (Soni/S/E/20) exhibited DPPH percentages of 46.58 and 47.74, respectively. The bath ultrasound treatement for 5 minutes (Soni/BM/05) yielded a DPPH percentage of 72.28, while the 20-minute treatment (Soni/BM/20) displayed a slightly higher value of 68.9933333. These findings emphasize the variations in the antioxidant activity of *Eruca sativa* honey under different treatment conditions. (Figure n°34).

2.3.4. Anti-inflammatory capacity of *Eruca sativa* honey

The evaluation of anti-inflammatory activity in *Eruca sativa* honey samples demonstrated diverse efficacy. The diluted honey in ethanol exhibited the highest anti-inflammatory capacity, with a percentage of 98.5233333. While the raw honey displayed a slightly lower percentage of 85.29, suggesting significant anti-inflammatory potential. (Figure n°35).

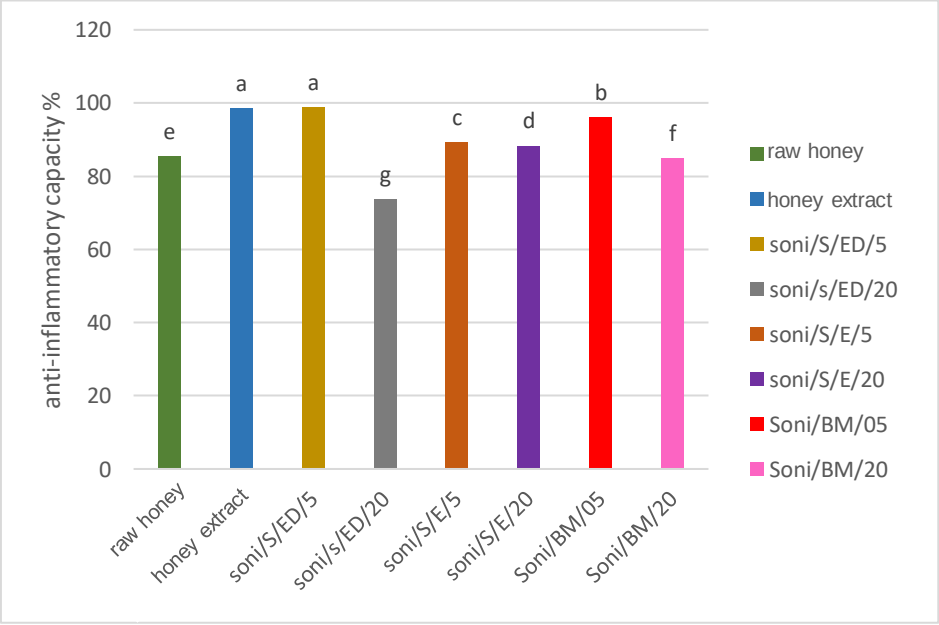


Figure n°35: The Anti-inflammatory capacity of different D honey treatments

Among the ultrasound treated honey samples, the treatment with distilled water for 5 minutes (Soni/S/ED/5) demonstrated an anti-inflammatory percentage of 98.6666667,

while the 20-minute treatment (Soni/S/ED/20) showed a lower value of 73.5233333. The ultrasound treated honey samples using ethanol as the solvent even for 5 minutes (Soni/S/E/5) or for 20 minutes (Soni/S/E/20) exhibited anti-inflammatory percentages of 89.21 and 88.23, respectively. The treatment in bath ultrasound for 5 minutes (Soni/BM/05) yielded an anti-inflammatory percentage of 96, while the 20-minute treatment (Soni/BM/20) displayed a slightly lower value of 84.8. These results highlight the variations in anti-inflammatory potential among different *Eruca sativa* honey samples under different treatment conditions. (Figure n°35).

2.3.5. Anti-diabetic capacity of *Eruca sativa* honey

The evaluation of anti-diabetic activity in *Eruca sativa* honey samples revealed notable differences in effectiveness. The ethanol diluted honey samples exhibited considerable anti-diabetic activity. The raw honey displayed a slightly higher activity, suggesting significant anti-diabetic potential. The assessment of the anti-diabetic activity of *Eruca sativa* honey samples revealed variations in their effectiveness at different concentrations. Among the samples, C3 consistently present higher anti-diabetic activity. The specific values for each sample and concentration are as follows:

- For concentration c1, the anti-diabetic activity of the samples is as follows: The raw honey exhibited an activity of 72.4533%. While the diluted honey in ethanol showed a slightly lower activity of 70.3833%. The honey treated with ultrasound using distilled water for 5 minutes (soni/S/ED/5) displayed an activity of 69.0166%, while the treatment for 20 minutes soni/S/ED/20 had an activity of 71.9733%. The ultrasound treatment using ethanol for 5 minutes (soni/S/E/5) showed a low activity of 64.6166%, while the treatment for 20 minutes Soni/S/E/20 exhibited a higher activity of 67.98%. The bath ultrasound treatment for 5 minutes (Soni/BM/05) displayed an activity of 43.2766%, while the treatment for 20 minutes Soni/BM/20 had an activity of 57.3366%. (Figure n°36).

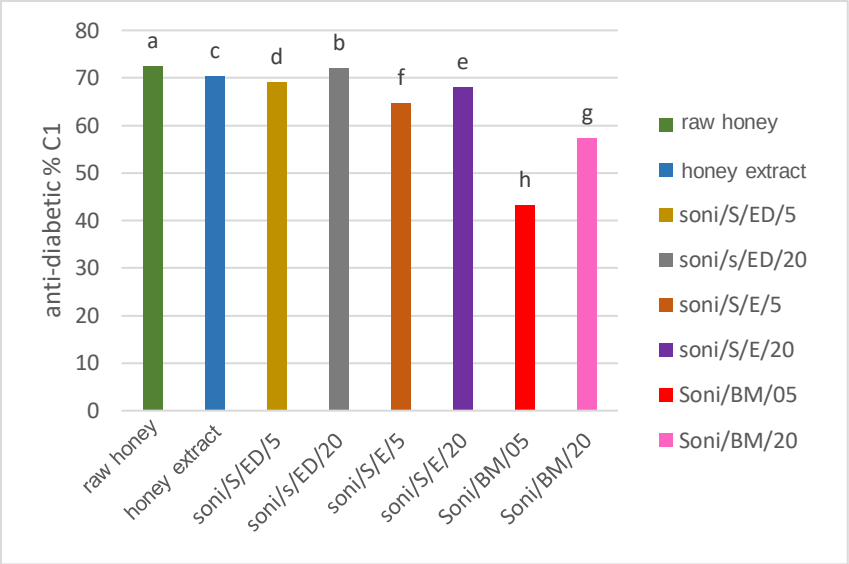


Figure n°36: The Anti-diabetic capacity C1 of different D honey treatments

- concentration c2, the anti-diabetic activity was as follows: The raw honey exhibited an activity of 78.0066%. While the honey diluted in ethanol showed a slightly higher activity of 80.5533%. The treatment with ultrasound for Soni/S/ED/5 exhibited an activity of 79.52%, while the treatment for 20 minutes soni/S/ED/20 had an activity of 82.5033%. On another hand the ultrasound for 5 minutes Soni/S/E/5 showed a lower activity of 81.68% than treated for 20 minutes Soni/S/E/20 that had the highest activity at 88.32%. The treatment for 5 minutes in ultrasound bath Soni/BM/05 displayed a low activity of 70.4333%, while the treatment for 20 minutes Soni/BM/20 had an activity of 74.4% (Figure n°37).

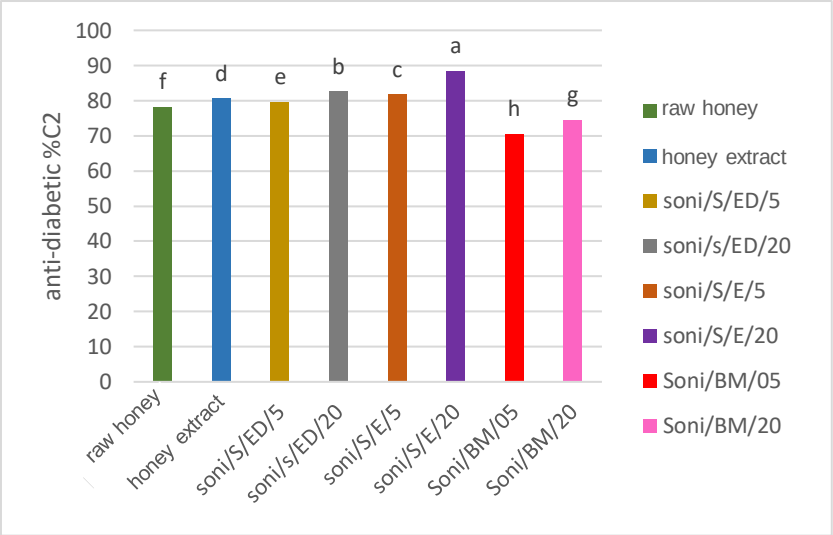


Figure n°37: The Anti-diabetic capacity C2 of different D honey treatments

- concentration c3, the anti-diabetic activity was as follows: The raw honey exhibited an activity of 86.3666% while the diluted honey in ethanol displayed a slightly higher activity of 87.09%. The treatment with sonication for 5 minutes Soni/S/ED/5 using distilled water showed an activity of 84.6633%, while the treatment for 20 minutes soni/S/ED/20 had an activity of 86.8266%. The treatment for 5 minutes Soni/S/E/5 using ethanol as solvent exhibited a low activity of 90.1433% than treated for 20 minutes Soni/S/E/20 that showed the highest activity at 95.09%. The treated honey in bath ultrasound for 5 minutes Soni/BM/05 displayed a low activity of 80.1933%, while the treatment for 20 minutes Soni/BM/20 had an activity of 83.5066%. (Figure n°38).

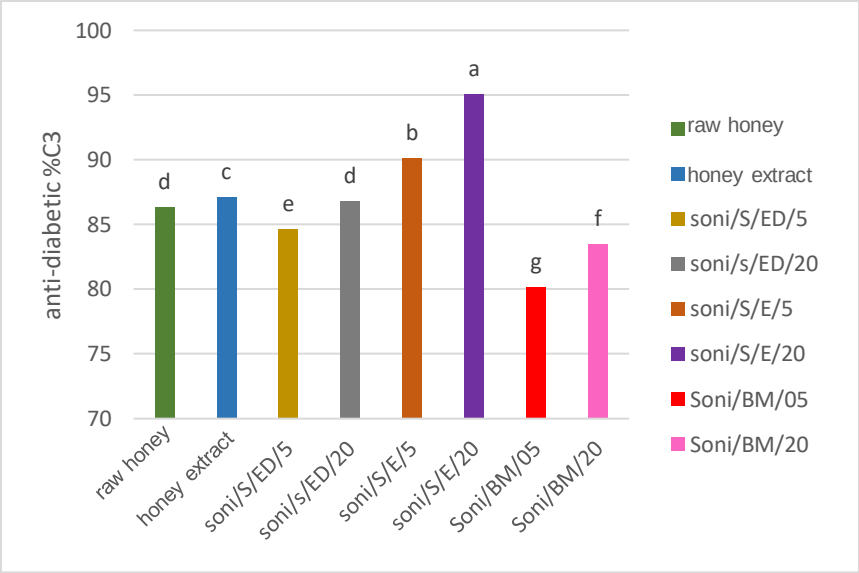


Figure n°38: The Anti-diabetic capacity C3 of different D honey treatments

2.4. Eucalyptus (E) honey

2.4.1. Polyphenols content of Eucalyptus honey

The analysis of polyphenol content in Eucalyptus honey samples revealed significant variations. The ethanol diluted honey exhibited the highest polyphenol content with a value of 800.706667 mg GAC /100g . In comparison, raw honey displayed a lower polyphenol content of 466.196667 mg GAC /100g . (Figure n°39).

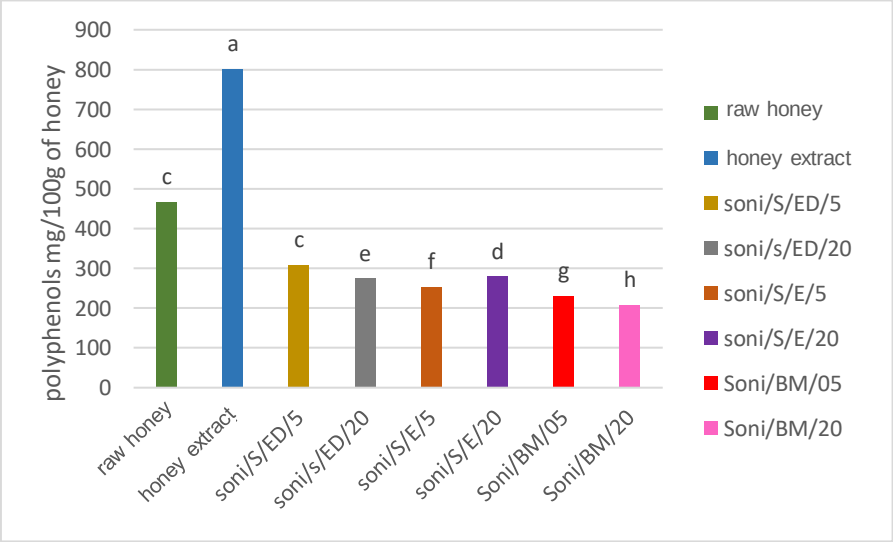


Figure n°39: The Polyphenol's assay of different E honey treatments

Among the sonicated honey samples, the treatment with distilled water for 5 minutes (Soni/S/ED/5) resulted in a polyphenol content of 308.517536 mg GAC /100g , while the 20-minute treatment (Soni/S/ED/20) showed a slightly lower value of 274.918951 mg GAC /100g . The ultrasound treated honey with ethanol as the solvent for 5 minutes (Soni/S/E/5) yielded a polyphenol content of 252.52 mg GAC /100g , while the 20-minute treatment (Soni/S/E/20) exhibited a higher value of 279.343333 mg GAC /100g . The treatment in bath ultrasound 5 minutes (Soni/BM/05) yielded a polyphenol content of 230.120837 mg GAC /100g , and the 20-minute treatment (Soni/BM/20) displayed a slightly lower value of 208.605954 mg GAC /100g . (Figure n°39).

2.4.2. Flavonoids content of Eucalyptus honey

The assessment of flavonoid levels in Eucalyptus honey samples revealed notable variations. Among the samples, diluted honey in ethanol exhibited the highest flavonoid content with a value of 764.19 mg Catechin/g. While, raw honey displayed a slightly lower flavonoid content of 321.826667 mg Catechin/g. (Figure n°40).

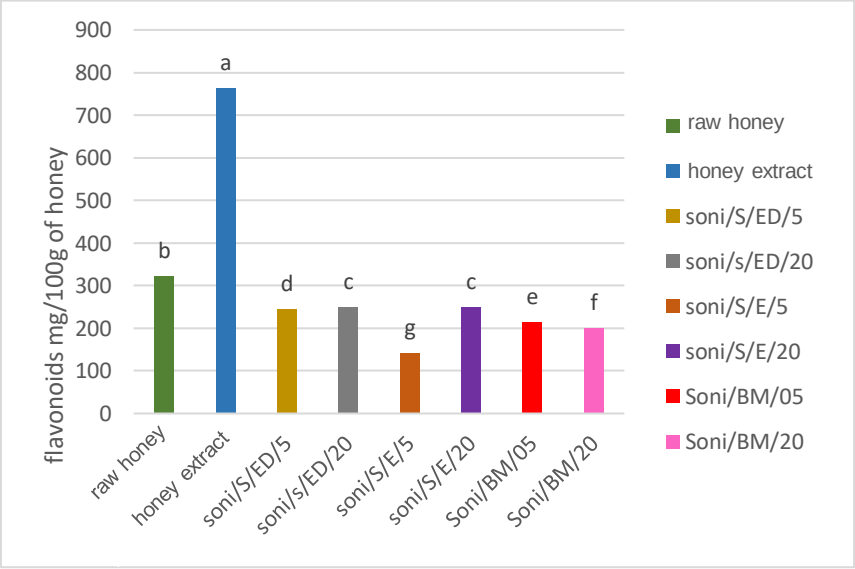


Figure n°40: The Flavonoid's assay of different E honey treatments

The ultrasound treatment of the honey samples showed diverse values in flavonoid contents, with the distilled water treatment for 5 minutes (Soni/S/ED/5) a value of 245.576176 mg Catechin/g is recorded , while for 20 minutes (Soni/s/ED/20) a higher

value of 249.892102 mg Catechin/g is observed. The ultrasound treatment using ethanol as the solvent for 5 minutes (Soni/S/E/5) resulted in a low flavonoid content (141.993958 mg Catechin/g) while the 20-minute treatment (Soni/S/E/20) displayed a comparable value of 249.89 mg Catechin/g. The treatment in bath ultrasound for 5 minutes (Soni/BM/05) yielded with a higer flavonoid content of 215.364696 mg Catechin/g, than with the 20-minute treatment (Soni/BM/20) that exhibited a slightly lower value of 200.978277 mg Catechin/g. (Figure n°40).

2.4.3. Anti-free radical activity of Eucalyptus honey

The evaluation of the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity in Eucalyptus honey samples demonstrated varying degrees of antioxidant potential. The diluted honey in ethanol displayed a DPPH percentage of 74.63, while the raw honey exhibited a slightly higher value of 79.5966667. (Figure n°41).

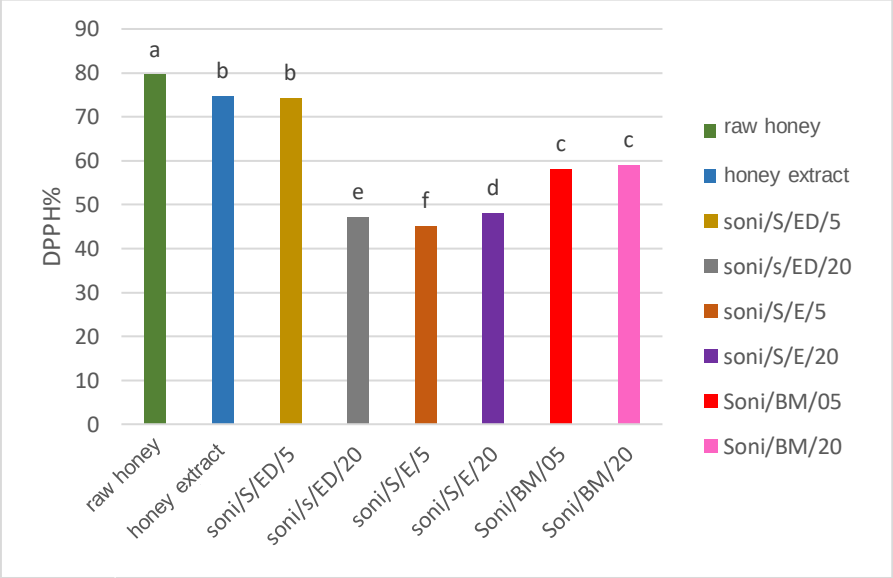


Figure n°41: The anti-oxidant capacity of different E honey treatments

Among the ultrasound treated honey samples, the treatment with distilled water for 5 minutes (Soni/S/ED/5) demonstrated a DPPH percentage of 74.1833333, whereas the 20-minute treatment (Soni/S/ED/20) exhibited a slightly lower value of 47.1333333. The ultrasound treatment using the ethanol as the solvent for 5 minutes (Soni/S/E/5) yielded a DPPH percentage of 45.1, while the 20-minute treatment (Soni/S/E/20) showed a similar value of 48.05. The bath ultrasound treatment for 5 minutes

(Soni/BM/05) resulted in a DPPH percentage of 58.12, but for the 20-minute treatment (Soni/BM/20) exhibited a slightly higher value of 58.9933333. (Figure n°41).

2.4.4. Anti-Inflammatory capacity of Eucalyptus honey

The evaluation of the anti-inflammatory activity of Eucalyptus honey samples revealed significant variations in their capacities. The ultrasound treated honey using distilled water in 5 min (soni/S/ED/5) exhibited a high anti-inflammatory activity percentage of 94.6666, otherwise The diluted honey in ethanol exhibited a percentage of 85,29, while raw honey express value of 83,33%. (Figure n°42).

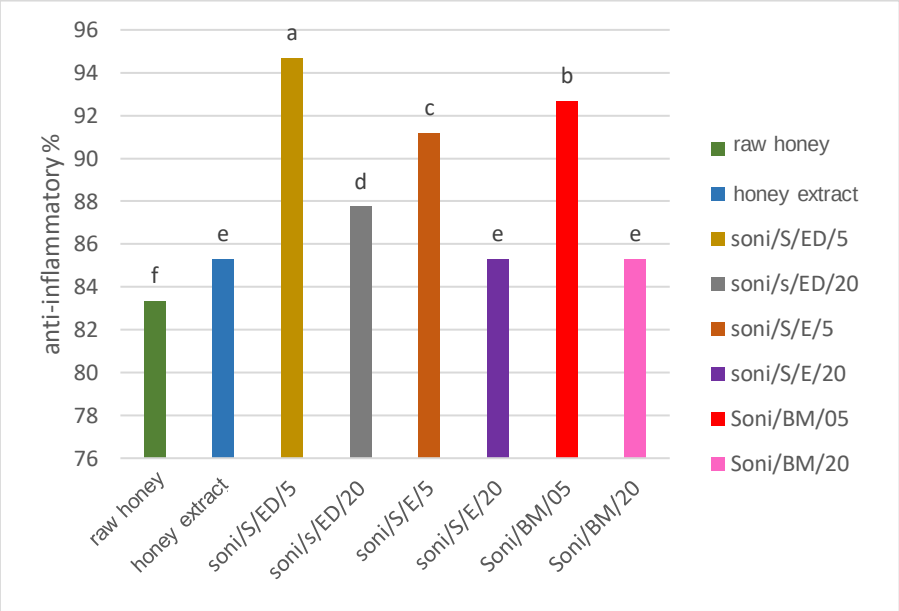


Figure n°42: The Anti-Inflammatory capacity of different E honey treatments

Among the ultrasound treated honey samples, the treatment with distilled water for 5 minutes (Soni/S/ED/5) demonstrated an anti-inflammatory activity percentage of 94.6666. The 20-minute treatment (Soni/S/ED/20) displayed a comparable value of 87.74. The ultrasound treatment using ethanol as the solvent for 5 minutes (Soni/S/E/5) yielded an anti-inflammatory activity percentage of 91.17, while the 20-minute treatment (Soni/S/E/20) showed a value of 85.29. The treatment with bath ultrasound, for 5 minutes (Soni/BM/05) resulted in an anti-inflammatory activity percentage of

92.6666, while the 20-minute treatment (Soni/BM/20) exhibited a slightly lower value of 85.29. (Figure n°42).

2.4.5. Anti-Diabetic capacity of Eucalyptus honey

The evaluation of anti-diabetic activity in *eucalyptus* honey samples revealed notable differences in effectiveness. The ethanol diluted honey exhibit considerable anti-diabetic activity. While the raw honey displayed a slightly higher activity, suggesting significant anti-diabetic potential. The assessment of the anti-diabetic activity of *eucalyptus* honey samples revealed variations in their effectiveness at different concentrations. Among the samples, C3 consistently exhibited higher anti-diabetic activity. The specific values for each sample and concentration are as follows:

- For concentration c1, the anti-diabetic activity of the samples is as follows: the raw honey exhibited an activity of 84.1566%. The honey diluted in ethanol showed a slightly lower activity of 82.0666%. The treated honey with ultrasound for 5 minutes and using distilled water as solvent (soni/S/ED/5) displayed an activity of 63.1733%, while soni/S/ED/20 had an activity of 65.0533%. The treatment with ultrasound for 5 minutes (soni/S/E/5) and using ethanol as solvant showed an activity of 54.43%. the treatment for 20 minutes Soni/S/E/20 exhibited a higher activity of 71.8266%. Treatment in bath ultrasound for 5 minutes (Soni/BM/05) displayed an activity of 68.9433%, and Soni/BM/20 had an activity of 62.36%.(Figure n°43).

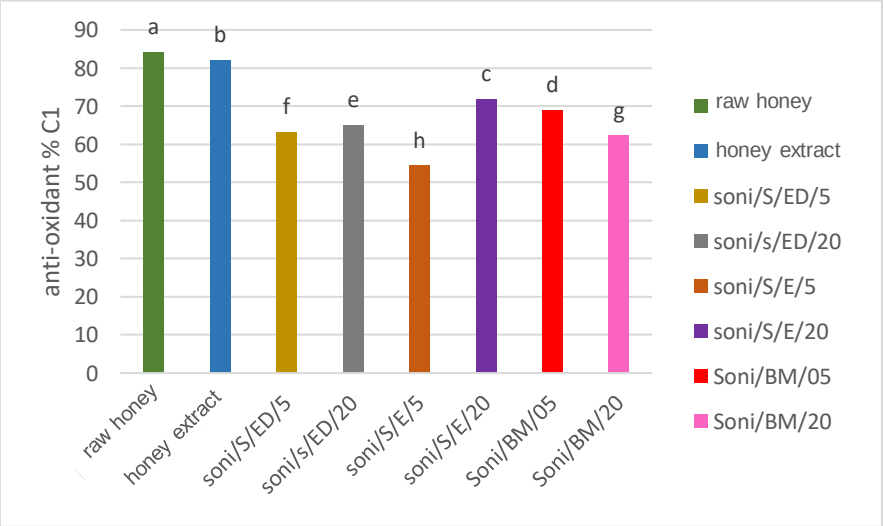


Figure n°43: The Anti-Diabetic capacity C1 of different E honey treatments

- concentration c2, the anti-diabetic activity was as follows: the raw honey exhibited an activity of 84.4433%. While the diluted honey in ethanol showed a slightly higher activity of 83.9433%. The ultrasound treatment for 5 minutes Soni/S/ED/5 exhibited an activity of 83.27%, while the treatement for 20 minutes soni/S/ED/20 had an activity of 77.86%. The treatment with ultrasound for 5 minutes Soni/S/E/5 using ethanol as solvant showed an activity of 74.7833%, and Soni/S/E/20 had the highest activity at 88.3166%. Soni/BM/05 displayed an activity of 82.7133%, and Soni/BM/20 had an activity of 78.0766%. (Figure n°44).

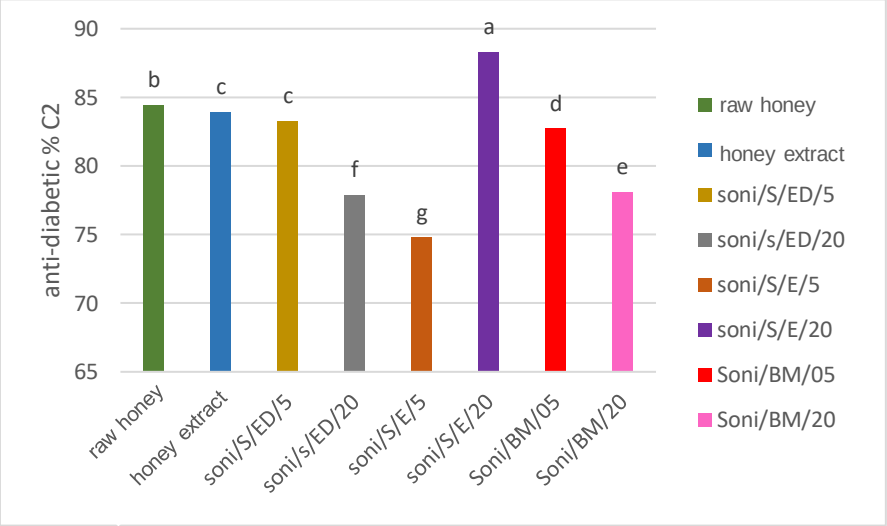


Figure n°44: The Anti-Diabetic capacity C2 of different E honey treatments

- concentration c3, the anti-diabetic activity was as follows: the raw honey exhibited an activity of 89.25%. The diluted honey in ethanol displayed a slightly lower activity of 87.02%. While the treatment with ulyrasound fo 5 minutes and using distilled water as solvent Soni/S/ED/5 showed an activity of 89.7566%, while with treatment for 20 minutes soni/S/ED/20 had an activity of 83.7233%. On another using ethanol as solvent express different result, for 5 minutes treatment Soni/S/E/5 exhibited an activity of 88.1%, while the treatment for 20 minutes Soni/S/E/20 showed the highest activity at 94.1833%. Following bath ultrasound treatment for 5 minutes the honey Soni/BM/05 displayed an activity of 86.8233%, and Soni/BM/20 had an activity of 84.2333%. (Figure n°45).

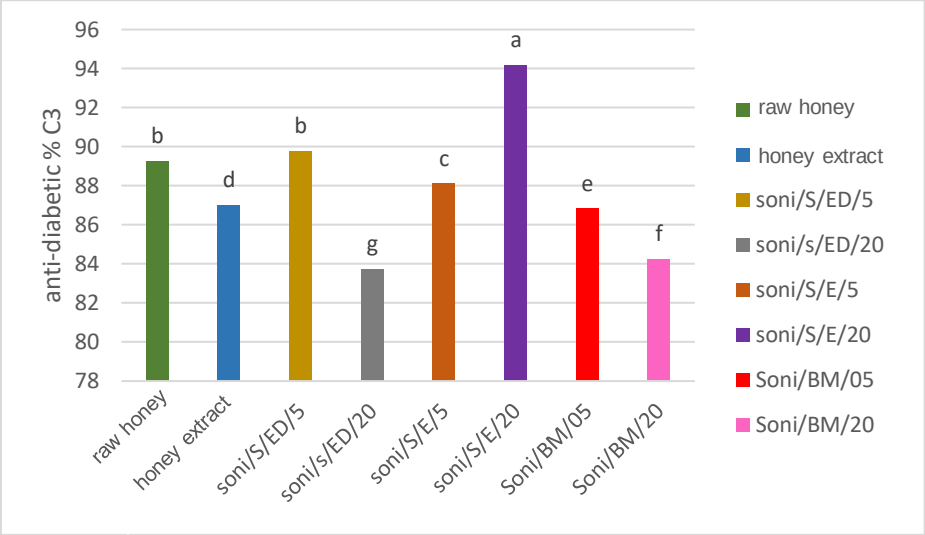


Figure n°45: The Anti-Diabetic capacity C3 of different E honey treatments

3. statistical analysis

3.1. Correlation

3.1.1. Correlation between physicochemical parameters and antioxidants and antioxidant activity:

The failure has a very highly significant correlation with the refractive index (r=0.93/P 0.001) and humidity (r=0.97/P 0.001), and the refractive index has a very highly significant correlation with humidity (r=0.86/P 0.001) and with HMF (0.58/P 0.05). Otherwise, electrical conductivity has a very highly significant correlation with color and proline content (r=0.95, r=0.86/P 0.001) respectively, and highly significant for pH and polyphenols (r=0.78; r=0.75/P 0.01) respectively. The pH showed a very highly significant correlation with polyphenols (r=0.93/P 0.001) and highly significant for colour, proline content, HMF and flavonoid levels (r=0.73, r=0.76, r=0.76, r=0.71/P 0.01) respectively. The colour has a very highly significant correlation with the proline content with a coefficient of r=0.95/P 0.001 and has a highly significant correlation with the polyphenols content (r=0.77/P 0.01), this is similar to the results obtained by Ayad *et al* 2021.

Proteins have a significant correlation with the level of flavonoids (r=0.65/P 0.05), proline has a very highly significant correlation with the level of polyphenols (r=0.86/P 0.01) and a significant correlation with the level of flavonoids (r=0.65/P 0.05). HMF and

proline have a correlation with antioxidants: proline has a very highly significant correlation with polyphenols (r=0.86/P 0.001) and significant with flavonoids (r=0.65/P 0.05), and HMF has a highly significant correlation with polyphenols (r=0.78/P 0.01) and significant with flavonoids (r=0.61/P 0.05), in parallel polyphenols have a highly significant correlation with flavonoids (r=0.80/P 0.01).

3.1.2. Correlation between physicochemical parameters and anti-inflammatory activity and antidiabetic activity:

The correlation matrix shows a significant correlation between proteins and anti-inflammatory activity with a coefficient of (r=0.70/P 0.05) and results is different from those of zaidi *et al* 2019.

And for anti-diabetic activity: Humidity has a significant correlation with the latter C3 (r=0.65/P 0.05) and Electrical conductivity has a highly significant correlation with C1 C2 (r=0.76/P 0.01). Colour has a highly significant correlation with antidiabetic activity C1 (r=0.77/P 0.01) C2 (r=0.76/P 0.01), in fact honey E and D which show a dark color and record high levels phenolic compounds have recorded a better antidiabetic activity. Proline is characterized by a significant correlation with antidiabetic activity C1 (0.64/P 0.05) C2 (r=0.69/P 0.05) different to the results obtained by takezare *et al* 2016, and HMF this manifests with a highly significant correlation with antidiabetic activity C3 (r=0.77/P 0.01).

Moreover, the anti-inflammatory activity has a highly significant correlation with the antidiabetic activity C2 (r=0.78/P 0.01) and its results are different to that of palaez et al 2022.

3.2. ACP

Principal component analysis (PCR), according to **Otmani *et al.* (2021)** is defined as a method of reducing information contained in a large database into many composite variables called "principal components (CPs)". It is used to determine and specify the similarity between the honeys analyzed and the relationship between the variables studied in a two-dimensional space.

The pie chart (Figure n°46) shows the two main components, CP1 and CP2, which represent 80.49% of the total variation used in interpreting the data. CP1, which explained 49.74% of the data, represented by HMF in the positive part and phenolic

constituents (polyphenols and flavonoids), color, pH, conductivity, proline, antidiabetic activity C3 and C2 in the negative part. Otherwise, CP2 represents 34.75% and shows anti-inflammatory activity, Brix, refractive index, protein activity and antioxidant (DPPH) in the positive part; however, in the negative part, we have antidiabetic activity C1 and C2 and water content, because they are grouped in related circles. Close correlations between colour, phenolic compounds and antioxidant activity (DPPH) observed in the circle of correlation (Figure n°46) confirm the correlations obtained by Spearmane. These results are similar to those reported by **Ayad *et al*. (2021)** and **Amessiss-Ouchemoukh *et al*. (2021)** on Algerian honeys.

The CP1 differentiation in Figure n°47 shows three groups of honey samples. The first located to the left of the positive part represents D honey which has the best physico-chemical parameters (Refractive index, Brix, proteins), best anti-inflammatory activity. The second group located in the center of the positive part contains the two honeys R and S0 These were poor and recorded low values in physico-chemical parameters except HMF and biological activities. The last group on the left of the negative part represents honey E, which recorded the best levels of phenolic compounds, proline, electrical conductivity and antioxidant and anti-diabetic activities.

The PCR results show a significant difference between the honeys analysed in terms of physicochemical parameters, antioxidant levels and antioxidant, anti-inflammatory and anti- oxidative activitiesdiabetic, indicating that the correlation circle confirms the correlations obtained in this study.

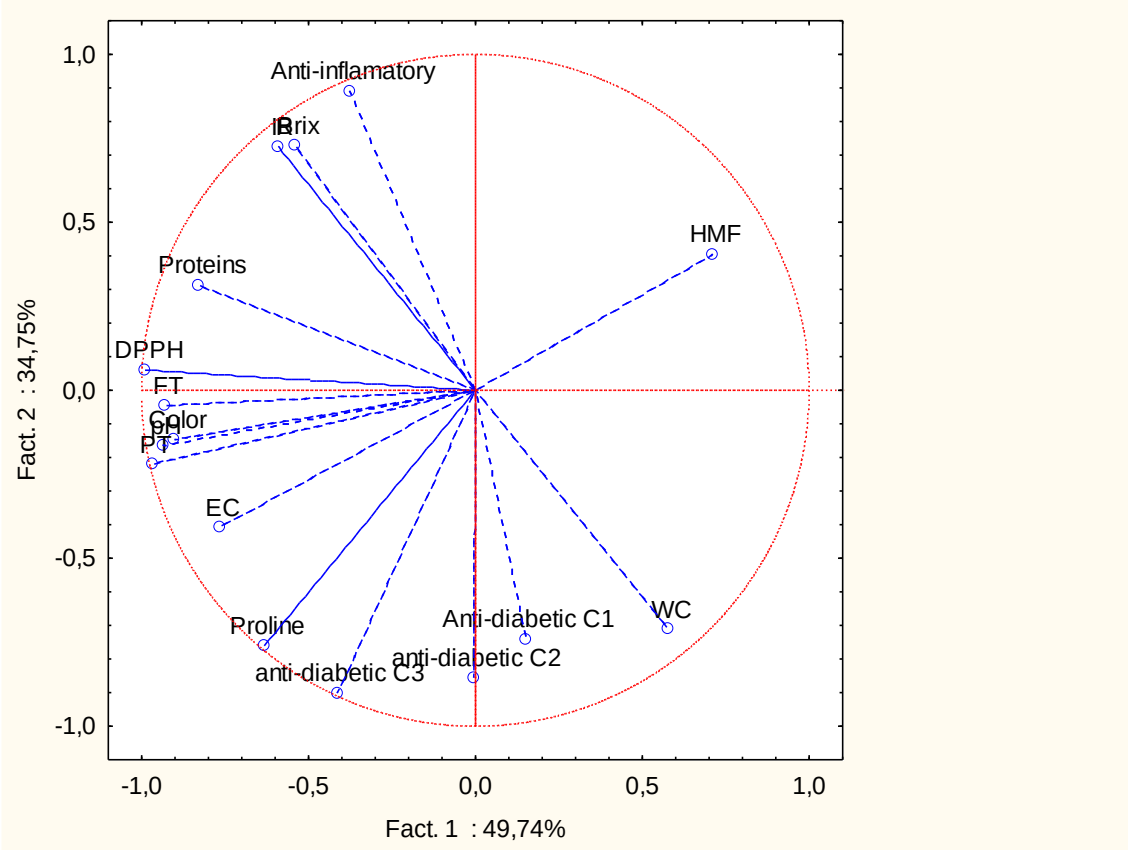


Figure n°46: The Circle of correlation of physicochemical parameters, antioxidants and activities

Biological samples of honey samples analyzed.

Abbreviation: WC: water content, PT: total polyphenols, FT: total flavonoids, EC: electrical conductivity.

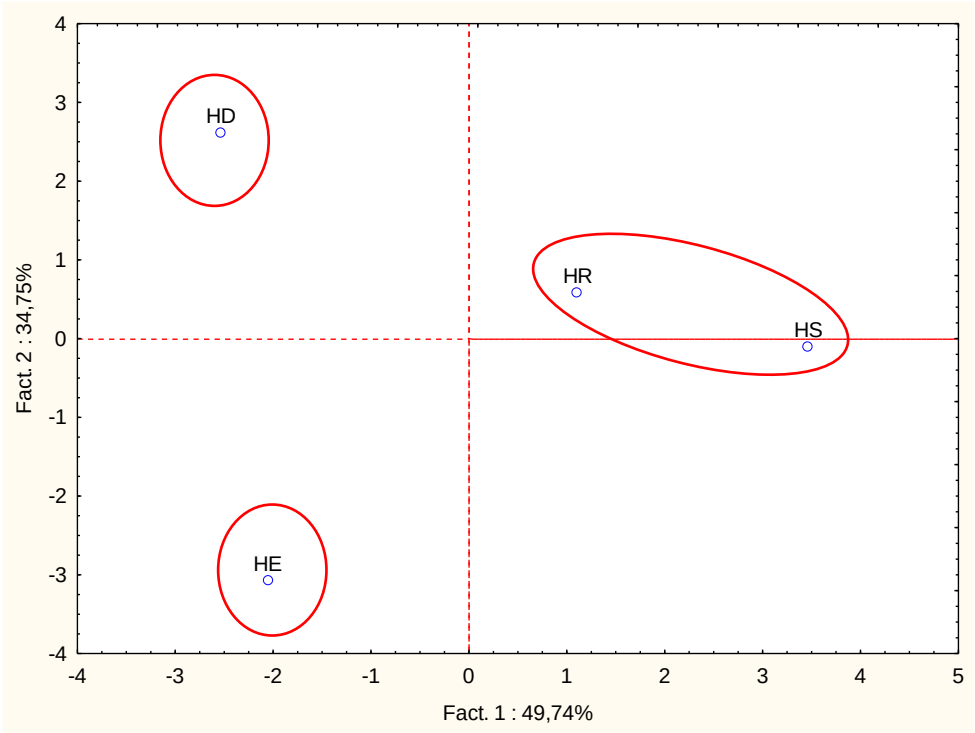


Figure n°47: PCR of physicochemical parameters, antioxidant content and activities of the honeys analysed.

Abbreviation: HE: Honey E; HR: honey R; HS:honey S; HD: honey D

4. Anti-bacterial result

Table N°4: Determination of the diameters of the inhibition zones of the bacterial growth strains by our honeys

		Escherichia coli (ATCC 25922)	*Acenitobacter baumannii (610)*	*Pseudomonas aeruginosa (ATCC 6633)*	*Enterococcus faecalis (ATCC 29212)*
Son/S/E 5 min	R	8	14	8	7
	S	14	15	16	7
	D	11	23	11	7
	E	10	13	18	9
extrait	R	6	6	6	7
	S	6	6	6	11
	D	6	6	6	7
	E	6	6	6	7
senergy water	RS	6	6	9	6
	RD	6	6	11	6
	DS	6	6	9	6
senergy ethanol	RS	6	6	11	6
	RD	6	6	0	6
	DS	6	6	10	6

Table N°4 shows that there is a low antibacterial activity. The highest inhibition zone is noticed in honey D (*Eruca sativa*), treat with Sonication ethanol for 5 minutes against the growth of *Acinetobacter baumanni* with a diameter zone of 23 milometers, and the lowest value was obtained against *enterococcus faecalis* of diameter zone between 7 and 11 millimetres. The synergy between the different honeys also had very weak results and the activity against *Pseudomonas aeruginosa* show a diameter zone between 9 and 11 millimetres

Discussion

III. Discussion

Honey is a natural and sweetie product, produced by Apis mellifera bees. Depending on the raw plant material taken: honey dew or blossom honey, we will result with honey product defined by variable chemical, sensorial and healthy effects. Furthermore, honey composition is defined by its botanical and geographical sources. Moreover, the storage time and condition honey have the potential to undergo significant transformation (Santos-Buelga and Gonzalez-Paramas, 2017).

The aim of our investigations is to define the quality parameters, chemical composition and health promoting proprieties of four honeybee collected from different plants and region from Algeria including: eucalyptus, rosemary, jujube and Eruca sativa honey.

Based on the outcome results, the tested honeys demonstrated good quality as their parameters, including pH, humidity, conductivity, HMF, proline, and protein content, aligned with commonly considered standards for high-quality honey. The pH values fell within the range of 3.4 to 6.1, with the most common range observed between 3.9 to 4.5, indicating acceptable acidity levels. Moisture content remained below 20%, preventing fermentation or spoilage. Electrical conductivity ranged from 0.2 to 1.0 mS/cm, reflecting the mineral content. Furthermore, the HMF content, a marker of honey quality, was below the recommended threshold of 40 mg/kg. Proline, an indicator of floral sources, exhibited higher levels across the honeys. Protein content remained low, typically below 0.5% 5 (Bogdanov et al, 1999). Typically, honey is a highly concentrated solution of sugars, making up approximately 95% of its dry weight. The primary sugars present are fructose (38%) and glucose (31%). In addition to water (around 20%), honey contains various other minor components such as proteins, enzymes, amino acids, minerals, vitamins, organic acids, and phenolic compounds. These constituents play a crucial role in determining the quality and health benefits of honey (Santos-Buelga and Gonzalez-Paramas,2017).

Regarding the honey's chemical composition, the obtained results regarding the prevalence on polyphenol revealed that the highest prevalence is observed with Eucalyptus honey extract (800.70 mg GAC /100g) and the lowest in Rosemary honey

treated with bath sonication (153.50 mg GAC /100g). Other side, the essay on the flavonoid concentration revealed that the highest prevalence is also observed with Eucalyptus honey extract (764.19 mg Catechin/g) and the lowest prevalence is observed with Rosemary honey treated with ultrasonic with distilled water (94.52 mg Catechin/g). Our obtained results express an important prevalence regarding to those obtained from other region all over the word. From Argentina, Brazil, the Euro-Siberian area of the Iberian peninsula, Ecuador, Italy, India and Mexico the content of phenolic compounds in honey samples ranged between 11.29 and 83.86 mg GAC/100 g honey,while total flavonoids were between 4.20 and 13.76 mg quercetin equivalent/100 g honey (Bobis et al., 2020). The difference in the content proportion is mainly related to the botanical and geographical sources (Santos-Buelga and Gonzalez Paramas,2017).

The antioxidant essays demonstrated the highest activity in Jujube honey treated with ultrasonic and distilled water (83.18%) and the anti-inflammatory essay shows that Jujube honey treated with ultrasonic and distilled water express also an interesting anti-inflammatory activity (73.03%). The antioxidant capacity is regarded as a measure of the existence of bioactive substances in honey, thus means that Jujube honey treated with ultrasonic and distilled water has an interesting content in polyphenol (243.678161 mg GAC/100g) correlating with the jujube honey antioxidant and anti-inflammatory activities. Our results are much better than those revealed by Hájek, in his paper he revealed a higher loss of phenolic compounds were observed during ultrasound treatment (48.5%) (Hájek, 2023). However, our results expressing an increase in the phenolic compound content with samples treated with ultrasonic corroborate with those found by Peláez-Acero et al. The phenols reached a maximum concentration of 29.91 ± 1.56 mg EQ/100 g, and the flavonoids of 1.92 ± 0.01 mg EQ/100 g (Peláez-Acero *et al*., 2022).

Regarding the anti-diabetic tests, the highest activity varied across concentrations, with Eucalyptus honey showing the highest at the 1st concentration (84.16%), Eruca sativa honey at the 2nd concentration (88.32%), and Jujube honey at the 3rd concentration (96.75%). The lowest activities were observed in Rosemary honey treated with ultrasonic withethanol (36.9) at the 1st concentration, Jujube honey treated with a bath sonication (39.24%) at the 2nd concentration, and Jujube honey treated with a bath

sonication (72.16%) at the 3rd concentration. Our finding results corroborate with those obtained by Peláez-Acero and collaborator, in addition of the high prevalence in polyphenolic content with ultrasonic treated honey, they notify an inhibition of α-amylase of 37.14 ± 0.09% (Peláez-Acero et al., 2022). This important anti-diabetic activity is attributed to the phenolic compounds present in this honey, such as kaempferol, caffeic acid, and p-coumaric acid (Devarajan *et al*, 2012). Thus, we carried out an HPLC essay to determine the major actives compound present in the most potent honey including: eucalyptus and jujube honey.

These results highlight the variations in activity levels among the different honeys and treatments, suggesting diverse functional properties that warrant further investigation.

Conclusion

Conclusion

Our research focused on the study the biological activity of four types of honey including Eucalyptus, Rosemary, Jujube and *Eruca sativa* honey. Moreover, we explored the underlying mechanisms of honey's biological activity, such as its bioactive compounds (antioxidants, phenolic compounds, etc.). The research highlighted on the variations in the biological activity of honey based on factors such as floral source and treatment methods. The obtained results revealed the importance of the biological activity of honey and its multiple bioactive components. A honey with good biological activity offers many potential applications in various fields.

Related to the obtained results, the tested honeys demonstrated good quality. From the tested honey, the highest prevalence in polyphenol and flavonoid is observed with Eucalyptus honey extract (800.70 mg GAC /100g) (764.19 mg Catechin/g), respectively. However, the antioxidant test demonstrated the highest activity in Jujube honey treated with ultrasonic and distilled water (83.18%). In the anti-inflammatory test, the highest activity was found in *Eruca sativa* honey treated with ultrasonic with ethanol (98.76%). Regarding the anti-diabetic tests, the highest activity varied across concentrations, with Eucalyptus honey showing the highest antidiabetic activities at the 1st concentration (84.16%), Eruca sativa honey at the 2nd concentration (32%), and Jujube honey at the 3rd concentration (96.75%).

Therefor, the antioxidant and anti-inflammatory properties of honey could be exploited in the pharmaceutical and cosmetic industry. Extracted from honey, polyphenols and flavonoids components could be used as active ingredients in the formulation of natural health and beauty products. On another hand, the anti-diabetic activity of honey arouses particular interest in the fight against diabetes and the regulation of blood sugar. The encouraging results obtained in this area open up promising prospects for the development of alternative and complementary therapies for people with diabetes.

In addition, honey with high biological activity could be used as a functional ingredient in the diet, offering additional health benefits. Its antioxidant power and anti-inflammatory properties could help prevent various chronic diseases.

However, further research is essential to better understand the mechanisms of action and complex interactions of honey bioactive compounds. Further clinical and

epidemiological studies are needed to fully assess the potential benefits of using honey in different contexts.

For this purpose, honey with a high biological activity represents a precious resource with many potential applications in the fields of health, cosmetics and food. Its potential remains to be explored and exploited in a responsible manner in order to fully benefit from its beneficial properties for our well-being.

Supplementary data

Honey Suppliers

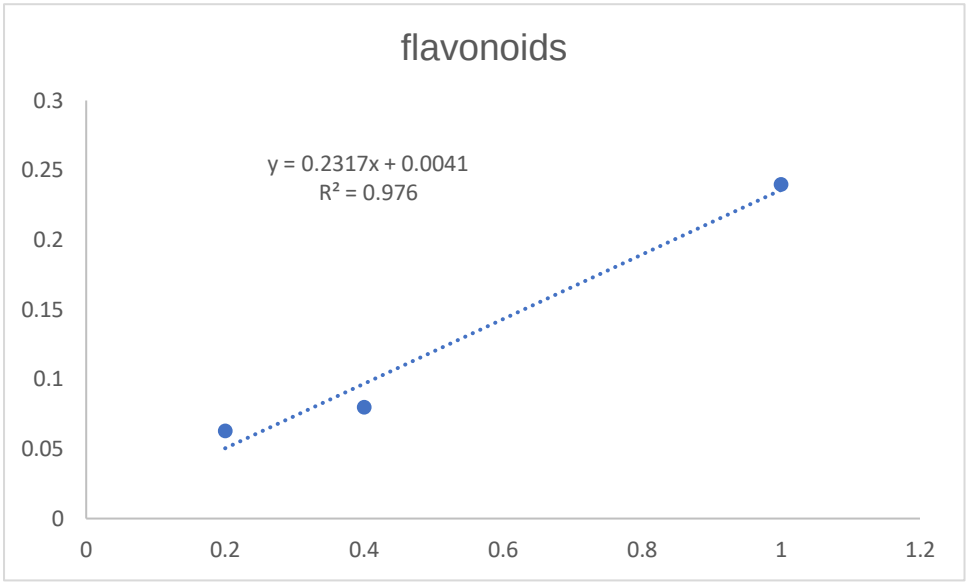
Honey	Supplier
Rosemary	Zaidi rabah Bee Nursery
Jujubier	Beekeeper cassab alaloui
Eruca sativa	Beekeeping nursery Hambli bee
Eucalyptus	Bousbaine fouad beekeeper

CHATWAY table

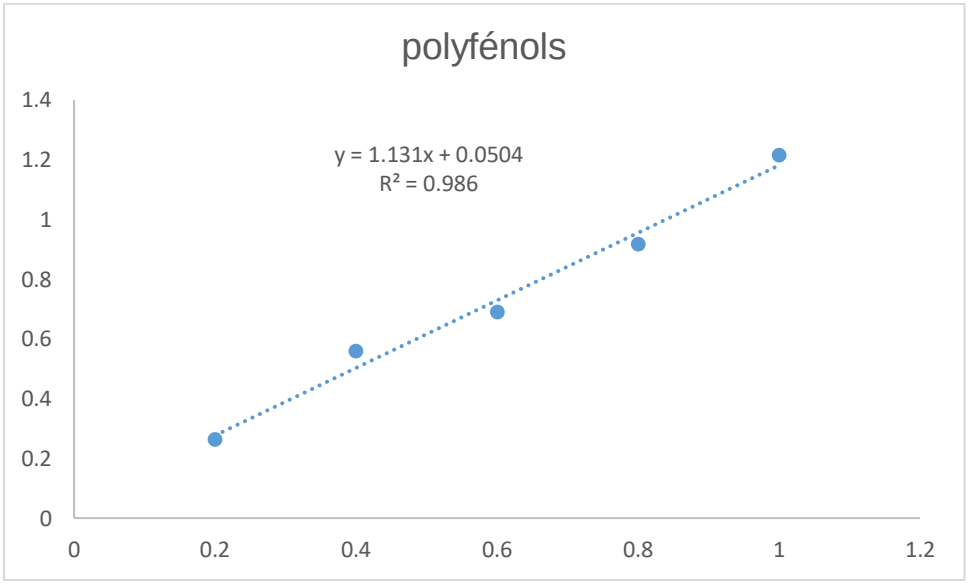
Indice de réfraction à 20 °C	Teneur en eau g/100 g	Indice de réfraction à 20 °C	Teneur en eau g/100 g
1.5044	13.0	1.4880	19.4
1.5038	13.2	1.4875	19.6
1.5033	13.4	1.4870	19.8
1.5028	13.6	1.4865	20.0
1.5023	13.8	1.4860	20.2
1.5018	14.0	1.4855	20.4
1.5012	14.2	1.4850	20.6
1.5007	14.4	1.4845	20.8
1.5002	14.6	1.4840	21.0
1.4997	14.8	1.4835	21.2
1.4992	15.0	1.4830	21.4
1.4987	15.2	1.4825	21.6
1.4982	15.4	1.4820	21.8
1.4976	15.6	1.4815	22.0
1.4971	15.8	1.4810	22.2
1.4966	16.0	1.4805	22.4
1.4961	16.2	1.4800	22.6
1.4956	16.4	1.4795	22.8
1.4951	16.6	1.4790	23.0
1.4946	16.8	1.4785	23.2
1.4940	17.0	1.4780	23.4
1.4935	17.2	1.4775	23.6
1.4930	17.4	1.4770	23.8
1.4925	17.6	1.4765	24.0
1.4920	17.8	1.4760	24.2
1.4915	18.0	1.4755	24.4
1.4910	18.2	1.4750	24.6
1.4905	18.4	1.4745	24.8
1.4900	18.6	1.4740	25.0
1.4895	18.8		
1.4890	19.0		
1.4885	19.2		

Calibration curves:

Of the determination of flavonoids



the determination of polyphenols:



Histogram value tables:

phisicochemical parameters

La Moyenne	BRIX %	INDICE DE REFRACTION	HUMIDITE %	MASSE SECHE g	CONDUCTIVITE	PH	Test De Couleur (Abs)	Dosage des protéines	la proline	HMF mg/kg
R	80,75	1,4925	17,6	6,07	0,45133333	3,8	0,26233333	376,906667	188,924031	2,363
S	81,2083333	1,49355	17,2666667	6,04333333	0,7	3,58	0,305	294,323333	194,083721	6,74466667
D	84,003333	1,51216667	14,3	5,83	1,09166667	3,87333333	0,61533333	397,196667	255,603101	3,643
E	80,9333333	1,49323333	17,3666667	6,05	1,36766667	3,92666667	0,62566667	364,746667	506,443411	1,02333333

biological activities

R	Polyphénols	Flavonoïdes	DPPH %	Capacité anti-inflammatoire %	anti-diabètique % c1	anti-diabètique % c2	anti-diabètique % c3
Brute	350,666667	303,123333	79,0566667	83,82	69,4266667	77,15	87,02
Extrait	777,424108	586,466667	70,0166667	97,05	75,4333333	80,24	85,67
Son/S/ED/5	274,034777	94,5187743	65,5033333	94,6666667	65,17	83,1233333	89,3966667
Son/S/ED/20	157,913351	147,748525	44,08	73,5233333	72,9566667	82,0633333	86,6833333
Son/S/E/5	214,796667	106,096667	46,7033333	92,15	35,9	82,33	90,1433333
Son/S/E/20	176,186667	139,116667	43,31	91,17	66,6366667	81,68	91,34
Son/BM/ED/5	181,78603	104,589268	64,3866667	96	37,7	65,0533333	79,2533333
Son/BM/ED/20	153,492485	127,607538	66,71	83,82	55,9666667	74,7133333	83,0033333

S	Polyphénols	Flavonoïdes	DPPH %	Capacité anti-inflammatoire %	anti-diabètique % c1	anti-diabètique % c2	anti-diabètique % c3
Brute	315,593333	249,893333	78,2533333	83,82	83,0766667	82,0433333	86,51
Extrait	708,166667	659,906667	79,51	88,23	76,0833333	78,94	86,73
Son/S/ED/5	243,678161	114,659761	83,1766667	96,6666667	63,3933333	79,2533333	88,17
Son/S/ED/20	161,450044	131,923464	38,0733333	73,03	72,02	83,5833333	86,9466667
Son/S/E/5	294,076667	232,628399	55,1666667	92,15	69,0866667	81,85	89,18
Son/S/E/20	174,71	167,89	35,9733333	91,17	81,2033333	91,99	96,75
Son/BM/ED/5	173,82847	139,116674	55,0066667	98	39,3133333	39,2433333	77,1633333
Son/BM/ED/20	159,386973	146,309922	56,78	86,76	53,3	72,4033333	82,8633333

D	Polyphénols	Flavonoïdes	DPPH %	Capacité anti-inflammatoire %	anti-diabètique % c2	anti-diabètique % c3	anti-diabètique % c4
Brute	442,623333	318,946667	79,7733333	85,29	72,4533333	78,0066667	86,36666667
Extrait	791,57	772,12	81,9266667	98,5233333	70,3833333	80,5533333	87,09
Son/S/ED/5	356,262894	259,962595	73,2433333	98,6666667	69,0166667	79,52	84,66333333
Son/S/ED/20	302,623047	231,189757	47,11	73,5233333	71,9733333	82,5033333	86,82666667
Son/S/E/5	257,53	245,576667	46,58	89,21	64,6166667	81,68	90,14333333
Son/S/E/20	218,333333	182,276667	47,74	88,23	67,98	88,32	95,09
Son/BM/ED/5	241,025641	226,873831	72,28	96	43,2766667	70,4333333	80,19333333
Son/BM/ED/20	219,510758	211,04877	68,9933333	84,8	57,3366667	74,4	83,50666667

E	Polyphénols	Flavonoïdes	DPPH %	Capacité anti-inflammatoire %	anti-diabètique % c2	anti-diabètique % c3	anti-diabètique % c4
Brute	466,196667	321,826667	79,5966667	83,33	84,1566667	84,4433333	89,25
Extrait	800,706667	764,19	74,63	85,29	82,0666667	83,9433333	87,02
Son/S/ED/5	308,517536	245,576176	74,1833333	94,6666667	63,1733333	83,27	89,7566667
Son/S/ED/20	274,918951	249,892102	47,1333333	87,74	65,0533333	77,86	83,7233333
Son/S/E/5	252,52	141,993958	45,1	91,17	54,43	74,7833333	88,1
Son/S/E/20	279,343333	249,89	48,05	85,29	71,8266667	88,3166667	94,1833333
Son/BM/ED/5	230,120837	215,364696	58,12	92,6666667	68,9433333	82,7133333	86,8233333
Son/BM/ED/20	208,605954	200,978277	58,9933333	85,29	62,36	78,0766667	84,2333333

Table: Correlation matrix between physico-chemical parameters, phenolic compounds and biological activities of Algerian honeys.

Brix	IR	Humidité	CE	pH	Couleur	Protéines	Proline	HMF	TP	TF	DPPH	A,anti-inflammatoire	anti-diabètique (C1)	anti-diabètique (C2)
1,00														
0,93***														
-0,97***	-0,86***	1,00												
0,29	0,19	-0,19	1,00											
0,07	-0,11	0,05	0,78**	1,00										
0,39	0,33	-0,27	0,95***	0,73**	1,00									
0,27	0,11	-0,25	0,06	0,44	0,09	1,00								
0,30	0,23	-0,19	0,86***	0,76**	0,95***	0,24	1,00							
0,48	0,58*	-0,54	-0,41	-0,76**	-0,38	-0,26	-0,47	1,00						
0,04	-0,08	0,06	0,75**	0,93***	0,77**	0,51	0,86***	-0,78**	1,00					
0,13	-0,04	-0,10	0,43	0,71**	0,49	0,65*	0,65*	-0,61*	0,80**	1,00				
0,32	0,30	-0,33	0,36	0,35	0,44	0,39	0,48	-0,28	0,52	0,52	1,00			
0,53	0,43	-0,54	0,08	0,08	0,02	0,70*	0,00	0,34	0,08	0,13	0,22	1,00		
0,13	0,18	-0,04	0,76**	0,35	0,77**	-0,46	0,64*	-0,20	0,36	0,11	0,14	-0,42	1,00	
0,01	0,06	0,08	0,76**	0,39	0,76**	-0,44	0,69*	-0,20	0,42	0,11	0,06	-0,36	0,92***	1,00

Results Statistical Analysis Tables:

		Brix	IR	CE	Color	H	pH	Proline	HMF	Proteine	TP	TF	DPPH	A.infla	A.Diabc1	A.Diabc2	A.Diabc3
Miel R	Brute	C	C	D	C	D	B	D	B	B	Cb	Cb	Aa	Bf	Dd	De	Be
	Extrait										A	a	b	a	A	D	F
	S/ED/5										C	h	d	C	E	A	C
	S/ED/20										G	c	g	g	B	C	D
	S/E/5										D	f	f	D	H	C	B
	S/E/20										F	d	h	E	C	B	A
	S/BM/5										E	g	e	B	G	G	H
	S/BM/20										H	e	c	f	f	f	g
Miel S	Brute	B	B	C	B	B	A	C	D	D	Cb	Cb	Bb	Bg	Ba	Bc	Cd
	Extrait										A	a	b	e	c	e	d
	S/ED/5										D	h	a	b	f	de	c
	S/ED/20										F	g	e	h	d	b	d
	S/E/5										C	c	d	e	e	d	b
	S/E/20										E	d	f	d	b	a	a
	S/BM/5										E	f	d	a	h	g	f
	S/BM/20										G	e	c	f	g	f	e
Miel D	Brute	A	A	B	A	A	B	B	C	A	Bb	Bb	Ab	Ae	Ca	Cf	Dd
	Extrait										A	a	a	a	c	d	c
	S/ED/5										C	c	c	a	d	e	e
	S/ED/20										D	e	f	g	b	b	d
	S/E/5										E	d	g	c	f	c	b
	S/E/20										H	h	f	d	e	a	a
	S/BM/5										F	f	d	b	h	h	g
	S/BM/20										G	g	e	f	g	g	f
Miel E	Brute	C	B	A	A	C	C	A	A	C	Ac	Ab	Aa	Cf	Aa	Ab	Ab
	Extrait										A	a	b	e	b	c	d

	S/ED/5										C	d	b	a	f	c	b
	S/ED/20										E	c	e	d	e	f	g
	S/E/5										F	g	f	c	h	g	c
	S/E/20										D	c	d	e	c	a	a
	S/BM/5										G	e	c	b	d	d	e
	S/BM/20										H	f	c	e	g	e	f

- Upper case letters: a comparison between raw honey samples for each test performed with A> B> C> D

Honeys that have even letter indicate an absence of a significant difference.

- Lowercase letters: comparison between each honey and its different treatments (raw, extracted, sonicated...). With a>b>c>d>e>f>g>h.

Same assigned letter means no significant difference.

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Summary

Honey, which is known for its many health benefits thanks to its biological activity and very minimal toxicity, we wanted to assess the quality and biological activity of our honeys of Algerian origin (rosemary honey, jujube honey, *Eruca sativa* honey, eucalyptus honey). For this we have provided for a working strategy that is based on the analysis of treated honeys with washing water and ethanol 50% and Sonication (different solvents and different times) On different tests: quality(pH, humidity, HMF, proline, conductivity,...), And biological activity (antioxidant, anti-inflammatory, anti diabetic and antibacterial), And to determine the active elements we turned towards HPLC.The result showed significant values in question of quality and biological activity and essentially the honey of the eucalyptus (oregine de bouira) which presented the best results in 50% of the overall of our tests after there is *Eruca sativa* honey with a percentage of 43,75, The jujube 6.25% and in last position the rosemary in with 0%. Compared to the treatment the ethanol washing 50% allowed to accentuate the activity of the active principles and thus to present the best values of results. Without forgetting the ultrasonic, 50% ethanol as solvent, for 20 minutes which comes in seconde position. This results provide a perspective in the agri-food, pharmaceutical and cosmetic fields.

Keys words: honey, treatment, antioxidant, anti-inflammatory, antidiabetic, antibacterial.

Résumé

Le miel qui est connu pour ces nombreux bénéfices pour la santé grâce à son activité biologique et toxicité très minimal, on a voulu évaluer la qualité et l'activité biologique de nos miels d'origine algérienne (romarin, jujubier, *Eruca sativa*,eucalyptus). Pour cela On a apporté pour une stratégie de travail qui se base sur l'analyse des miels traités avec lavage eau et éthanol 50% et Sonication (différents solvants et différents temps) Sur différents tests: de qualité(pH, humidité, HMF, proline, conductivité,...), Et activité biologique(antioxydante, anti-inflammatoire, anti diabétique et antibactérienne), Et pour déterminer les éléments actifs nous nous sommes orientés vers l'HPLC.Le résultat ont montré des valeurs significatives en question de qualité et activité biologique et essentiellement le miel de l'eucalyptus qui a présenté les meilleurs résultats avec une concentration en composants phénoliques de (800.70 mg GAC /100g) et en flavonoid (764.19 mg Catechin/g). Cette proportion importante en composant phénolique reflète la bonne qualité biologique du miel d'eucalyptus. Par rapport au traitement le lavage à l'éthanol 50 % a permis d'accentuer l'activité des principes actifs et donc à présenter les meilleures valeurs de résultats. Sans oublier la Sonication a sonde, l'éthanol 50 % comme solvant, pendant 20 minutes qui vient en deuxième position. Ses résultats permettent d'avoir une perspective dans le domaine agroalimentaire, pharmaceutique et cosmétique.

Mots clés : miel, traitement, antioxidante, antiinflammatoire, antidiabetique, antibacterienne.

موجز

العسل، المعروف بفوائده الصحية العديدة بفضل نشاطه البيولوجي والحد الأدنى من السمية، أردنا تقييم الجودة والنشاط البيولوجي لعسلنا من أصل جزائري)عسل إكليل الجبل، عسل السدرة، عسل الجرجير، عسل الأوكالبتوس). لذلك قدمنا استراتيجية عمل تستند إلى تحليل العسل المعالج بماء الغسيل والإيثانول بنسبة 50٪ والصوت)مذيبات مختلفة وأوقات مختلفة) في اختبارات مختلفة: الجودة ، البرولين، الموصلية،...)، والنشاط البيولوجي (مضاد للأكسدة ومضاد للالتهابات ومضاد HMF(الأس الهيدروجيني، الرطوبة، من ولاية بويرة(الذي قدم أفضل النتائج في 50٪ من) .HPLC للسكري ومضاد للبكتيريا(، ولتحديد العناصر النشطة اتجهنا نحو بنسبة 43.75، و السدرة ب 6.25٪ وفي المركز الأخير إكليل الجبل بنسبة *Eruca sativa* إجمالي اختباراتنا بعد وجود عسل 0٪. بالمقارنة مع العلاج، سمح غسل الإيثانول بنسبة 50٪ بإبراز نشاط المبادئ النشطة وبالتالي تقديم أفضل قيم النتائج. دون نسيان مسبار العلاج بالموجات الصوتية، 50٪ إيثانول كمذيب، لمدة 20 دقيقة والتي تأتي في المركز الثاني. وتوفرهذه النتائج منظورًا في مجالات الأغذية الزراعية والمستحضرات الصيدلانية ومستحضرات التجميل.

كلمات مفتاحية **:**عسل، علاج، مضاد للأكسدة، مضاد للالتهابات، مضاد للسكري، مضاد للبكتيريا