

Physicochemical properties, antioxidant, and antibacterial activity of Argania Spinosa honey produced only in Morocco: Application in the care of surgical wounds

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Abstract: The composition of honey is variable according to the current differences in plant types, climate, environmental conditions, and contribution of the beekeeper. Argania Spinosa is an endemic tree from southwestern Morocco, its famous argan oil is well known for its many healing properties including antioxidant and anti-inflammatory effects. This study aims to characterize for the first time the physicochemical, antioxidant, and antibacterial potential of Argania honey. Eight samples of honey were provided by beekeepers of the Tiznit Province. The pollen content was carried out to make the determination of Argania Honey and the characterization of the physicochemical parameters was carried out based on: HMF, pH, total polyphenol content, sugar composition, and diastase activity. The potentiality of Argania honey as an element antioxidant then bactericide agent. The outcomes data showed that the evaluated parameters are according to the guaranteed criteria quality of honey. Interestingly, the high polyphenols content (1295 mg GAE/Kg), presence of only reducing sugars (glucose and fructose), and the antibacterial action of Argania honey demonstrated by the average zone of inhibition between 7.7 mm and 12.07 mm for S. aureus, K. pneumonia, and B. subtilis may be suitable its use for wound healing. The analysis of Argania honey revealed physicochemical, antioxidant, antibacterial, and wound healing properties that were approved according to standard criteria. These characteristics provide an important biological potential that may be reconsidered as a high-value commercial and therapeutic.

Keywords: Argania Honey, physicochemical characteristics, Antioxidant activity, Antibacterial potential, healing surgical, wounds properties, Monofloral

1. Introduction

Honey is a natural substance of great nutritional and prophylactic-medicinal value, produced by bees (*Apis mellifera*) from flower nectar or honeydew (Ulloa *et al.*, 2015). For centuries, honey has been used as a natural remedy for many ailments. It has a wide range of therapeutic properties and has diverse biological potentials, such as accelerated wound healing, anti-inflammatory, antimicrobial

(Jeddar *et al.*, 1985; Subrahmanyam, 1991; Tonks *et al.*, 2003), antifungal, and antioxidant (Aljohar *et al.*, 2018; Bogdanov *et al.*, 2008); effects involved in the treatment of gastrointestinal disorders, skin diseases, cancer, heart disease, and neurological degeneration (Meo *et al.*, 2017). Honey has also been described as having anti-anemic, emollient, preservative, anticarcinogenic, and probiotic properties (Miguel *et al.*, 2017; Nasir *et al.*, 2010; Swellam *et al.*, 2003; Tonks *et al.*, 2003).

Argania Spinosa, an endemic tree with tropical affinity from Morocco, is considered the most notable species of North Africa (H. Faouzi, 2017; K. Faouzi *et al.*, 2015). This tree is considered mythical and unique, and is thermophilic and xerophilic, from arid and temperate bioclimate (Adlouni, 2010; Ecologie.ma, 2012; El Alaoui, 1999; Msanda *et al.*, 2005). Several ancient scripts on *Argania Spinosa* revealed the interest of several scientists from the 10th and 16th centuries such as Ibn al-Baitar, El Bekri, Al Idrissi, and Leon the African. In the 18th century, a great interest was shown in this tree, especially among the researchers of the time (El Fasskaoui, 2009), because of its specific chemical composition and its remarkable potential in culinary preparations, cosmetics, and medicine (Msanda *et al.*, 2005; Belarbi-Benmahdi *et al.*, 2009; Kadda & Belabed (2021; Laaroussi *et al.*, 2021).

This tree has multiple uses, but its oil is the best-known product used in culinary preparations, and the pharmaceutical and cosmetic industry (H. Faouzi & Martin, 2014). Indeed, previous phytochemical studies have reported the presence of large amounts of pharmacologically bioactive and protective compounds implicated in the treatment of several pathologies such as arthritis, skin diseases, and cancer (El Monfalouti *et al.*, 2010). These exceptional results have contributed to the exploitation of all botanical parts of the argan tree, including wood, leaves, and fruits, and especially grains from which the origin of argan oil production, is considered one of the rarest and most expensive oils in the world (Khallouki *et al.*, 2017). Among the still unexploited products of the Argan tree, we find its honey which remains one of the products that may have a growing medical interest. This natural and sweet product coming from plants is collected from the nectar of the flower, or the honeydew through the intermediary of bees according to their physicochemical properties such as fluidity, viscosity, and taste (Anthony & Balasuriya, 2016; Manivanan *et al.*, 2018; Sanz *et al.*, 2005; Veloso *et al.*, 2018; Laaroussi *et al.*, 2022).

Since honey inherits these properties from plants (Gianelli Barra *et al.*, 2010), the most well-known honey of medical interest in the world for their virtues are Lucerne from Canada, Aguacate honey from California, Manuka honey from New Zealand while Malaysia is famous for its Gelam, Coconut, and Tualang honey (Nurul *et al.*, 2013). However, the honey denomination is characteristically based on both melissopalynological and physicochemical analysis (Diez *et al.*, 2004; Elamine *et al.*, 2018). The physicochemical quality criteria for honey are well specified by the European Directive CXS 12-1981 and Codex Alimentarius (Codex Alimentarius, 2019; AOAC, 2005). The main criteria of interest are moisture content, electrical conductivity, ash content, reducing and non-reducing sugars, free acidity, diastase activity, and hydroxymethylfurfural (HMF) content (Gomes *et al.*, 2010). The antibacterial, Antioxidant and Anti-inflammatory activities of Honey issued from several trees and flowers was widely studied in literature (Mahardiani *et al.*, 2022; Mehanned *et al.*, 2022; Cucu *et al.*, 2021; Latkal *et al.*, 2020; Salonel *et al.*, 2017). In other words, the therapeutic benefits of honey is not a recent discovery, but rather dates back to when humankind considered as having both nutritional and health benefits (Papadaki *et al.*, 2021; Samarghandian *et al.*, 2017).

To our knowledge, no identification or determination of the physicochemical or melissopalynology characteristics of *Argania* honey has been reported, both locally and internationally. The present study aims to describe for the first time the main physicochemical parameters that may have antioxidant and antibacterial potential, especially in wound care.

2. Materials and methods

2.1. Study area

Morocco is characterized by its high plant biodiversity and a very prosperous flora estimated at 7000 species in 920 genera and 130 families, including 4500 species and subspecies of vascular plants (Radford *et al.*, 2011). Indeed, the extraordinary melliferous resources allow Moroccan honey to occupy a very interesting position among the Mediterranean countries producing natural honey from medicinal plants (Scherrer *et al.*, 2005). The honey used for the study was provided from Tiznit in the west of the Souss Massa region.

2.2. Honey samples

Eight honey samples from the 2019 harvest that were artisanally produced, were provided by beekeepers settled in Tiznit, the region of Souss Massa. 500g of each sample were stocked in sterilized, hermetically sealed bottles and glass vials with metal Screws. Then, from each sample, we took 100mL kept in a dry and dark place at a temperature of 21°C in sterilized vials until their analysis. Characterization of Argania honey was carried out through melissopalynological and physicochemical analyses. (Figure 1), located to the west of the Souss Massa region, it is bounded to the north by the provinces of Chtouka Ait Baha and Taroudant, to the east by the province of Tata, to the south by the province of Sidi Ifni, and to the west by the Atlantic Ocean (Centre Régional d'investissement, 2019). Its climate is arid and semi-continental and the most widespread tree in the region is the argan tree (*Argania Spinosa* (L) Skeel), an endemic tree with tropical affinity, and the most remarkable species in North Africa (H. Faouzi, 2017; K. Faouzi *et al.*, 2015).



Figure 1. Geographical localization of Argan forest in Tiznit (Ait Hammou *et al.*, 2018).

2.3. Pollen analysis

Microscopic analysis of honey sediment composition was essentially performed according to the method described by Louveaux *et al.* (1978) and Song *et al.* (2012). Using a non-acetolytic technique

to preserve honeydew elements. A light microscope (Olympus BX 43), fitted with a digital camera for morphologic identification of pollen grains, was used for analysis. Identification of pollen grains was carried out using a collection of reference pollens (Trigo *et al.*, 2008).

2.4. Physicochemical analysis

2.4.1. Water content (moisture)

Moisture was determined by measuring the refractive index at 20°C using a digital handheld refractometer (Abbe NAR-2T) and the corresponding percentage of moisture (g/100g honey) was evaluated according to the protocol reported by Bogdanov.(2002).

2.4.2. pH and free acidity

Briefly, 10g of honey was dissolved in 75 mL of distilled water, and pH was measured in a pH meter (Metrohm, 1840010010115, Switzerland). Dissolve 10 g of honey in 75 mL of distilled water. The titration is carried out with 0.1M Sodium hydroxide free of carbonate. 4 to 5 drops of neutralized phenolphthalein are used as an indicator, and the final color change must persist for 10 seconds (Codex Alimentarius, 2019). The results are expressed in milli-equivalents of acid per Kg of honey (milliequivalent/Kg) calculated as below in Eqn. 1:

$$\text{Acidity} = 10 \times V \text{Eqn. 1}$$

2.4.3. Electrical conductivity

The electrical conductivity of the Argania honey was measured at 20°C in a conductivity meter (Hanna instruments, Hi 2550, USA), and the solution was prepared using distilled water following the method described by Vorwohl.(1964).

2.4.4. Ash percentage

Ash content was determined according to the methods of Azonwade *et al.* (2018); Bogdanov.(2004). Briefly, 3 g of Argania honey was placed in a combustion pot and the sample was incinerated at a high temperature (600°C) in a burning muffle for 2 hours. After cooling at room temperature, the obtained ash was weighted as calculated below in Eqn. 2:

$$\text{Ash content}\% = \frac{\text{weight of ash and combustion pot} - \text{weight of combustion pot}}{\text{weight of honey}} \times 100 \text{Eqn. 2}$$

2.4.5. Density and dynamic viscosity

The liquid density of our substance was determined by dividing the weight of a specific gravity bottle (10 mL) filled with 14g of Argania honey by the weight of the same bottle filled with water. The dynamic viscosity of honey was determined at 28°C by dropping a marble ball into the honey and starting the timer when the bottom of the ball reaches the mark at the top of the test tube. Briefly, we measure the density of the marble ball and its velocity in the honey to find its viscosity by applying this Eqn. 3:

$$[2(ps - pl)ga^2]/9v \text{Eqn. 3}$$

where **ps** is the density of the sphere, **pl** is the density of the liquid, **g** is the acceleration caused by gravity, **a** is the radius of the sphere and **v** is its velocity (Tang, 2016).

2.4.6. Color analysis

The honey color was determined according to the method reported by Kaškonienė *et al.* (2009). Briefly, 10g of Argania honey is added to 20 mL of distilled water and heated up to 40°C to dissolve sugar crystals. The color was determined by measuring the absorbance at 635 nm in a spectrophotometer (Vis-721, China). The Pfund value was calculated using the following formula Eqn. 4:

$$mmPfund = -38,7 + 371,39 \times Absorbance \text{ Eqn. 4}$$

2.4.7. Refractive Index and Brix Value

The refractive index and Brix value of honey are obtained by the Abbe refractometer (ATAGO, NAR-2T, Japan). For measurement at different temperatures, the refractive index should be increased by a value of 0.00023 for each degree Celsius above or below 20°C, depending on the honey sample temperature (AOAC, 2005; Bogdanov, 1997).

2.5. Chemical properties

2.5.1. Determination of sugar composition

The sugar content (sucrose, D-glucose, and D-fructose, maltose) was determined by liquid chromatography according to the modified method described by Bouhlali *et al.* (2017). 5 grams of honey sample was weighed and dissolved in 50 mL of water and transferred to a volumetric flask. The solution was filtered through a 0.45 µm membrane filter (Millipore) before injection into the HPLC column. The equipment consisted of an LC-10 AT Shimadzu pump, (HP 1047A) refractive index detector, Shimadzu SIL 10ADVP autosampler, and Shimadzu C-R8A integrator. The chromatogram separation was accomplished using the column LiChrospher® 100 RP-18 (250 × 4.6 mm, 5 µm) temperature was at 30°C. The mobile phase was acetonitrile/water (80/20, v/v) and the elution was performed at a flow rate of 1.2 mL/min. The injection volume was 20 µL. Identified sugars were quantified based on peak areas compared with the corresponding standards.

2.5.2. Determination of HMF

The 5-hydroxymethylfurfural (HMF) content of the honey sample was analyzed using the modified method described by Bouhlali *et al.* (2019). Briefly, 5g of the honey sample was dissolved in 25 mL of deionized water and transferred to a 50 mL volumetric flask, diluted to 50 mL with deionized water, and filtered through a 0.45 µm membrane filter before injection into the HPLC column. The high-pressure liquid chromatography system consisted of a Shimadzu LC-20A HPLC system (Shimadzu Co., Kyoto, Japan) equipped with a pump, online degasser autosampler, column oven, and PDA detector. Separation was performed on a reversed-phase using LiChrospher® 100 RP-C18 (250 x 4.6mm; 5µm). The column temperature was set at 30°C. Elution was conducted using water acetonitrile (80:20) as the mobile phase, at a flow rate of 0.7mL/min. The injection volume was 20 µL. The detection was carried out at 285 nm by UV spectra. The HMF content was quantified based on peak areas compared with a standard HMF.

2.5.3. Determination of diastase activity

Diastase activity (DA) was evaluated by using the Phadebas amylase test tablets according to the protocol described by Abselami *et al.* (2018). 1g of honey was dissolved in 15 mL distilled water and 5 mL acetate buffer solution (0.1M, pH 5.2), and transferred to a 100 mL volumetric flask. 5 mL of white solution / Honey is taken: transferred to a test tube and incubated in a water bath at 40°C for a few minutes, the white solution is prepared to take 5 mL of acetate buffer and treated in the same way as

the sampled honey. The Phadebas tablets were added to both test tubes and stirred until the tablets disintegrate. After 35 minutes, 1 mL sodium hydroxide solution (0.5M) was added to each test tube to stop the reaction. The test tubes were stirred again and filtered through filter papers and the absorbance was measured at 620 nm using water as a reference. The diastase activity is expressed in Gothe units (or Schade). The formula is [Eqn. 5](#):

$$DA = -4,37 \times Abs + 31,38 \times Abs + 0,03 \text{ Eqn. 5}$$

(DA: diastase activity; Abs: Absorbance of final solution at 620 nm).

2.5.4. Determination of polyphenols

The Folin–Ciocalteu modified method, described by [Beretta et al.\(2005\)](#), was used to determine total phenolic content. Argania honey sample 0.5 g was diluted to 10 mL with distilled water. 0.5 mL of t solution was then mixed with 0.5 mL of 0.2 N Folin and Ciocalteu's phenol reagent (LOBA, Chemie PVT.LTD, India) for 3 min and 1 mL of 75 g/L sodium carbonate (Na₂CO₃) (13418, Riedel de Haen, Germany) was then added. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 765 nm against a methanol blank (Spectrophotometer Varian Cary 50 BIO, Australia). Gallic acid (Sigma–Aldrich Chemie, Steinheim, Germany) (0- 20 mg/L) was used as a standard to produce the calibration curve. The mean of three readings was used and the total phenolic content was expressed in mg of gallic acid equivalents (GAE)/ Kg of honey.

2.6. Antioxidant activity

2.6.1. Materials

Absolute ethanol (96 %) and 2,2-diphenyl-1-picrylhydrazyl hydrate radical (DPPH) were obtained from Sigma-Aldrich and used as received.

2.6.2. Antioxidants DPPH assay

The antioxidant activity was estimated using the 2,2-diphenyl-1-picrylhydrazyl hydrate radical (DPPH) according to the [Ao et al.\(2008\)](#) method with slight modifications. In a 50 mL flask, the honey samples were diluted in distilled water at different concentrations from 0.05 to 0.25 g/mL, and from each dilution 100 µL was mixed with 900 µL of DPPH (0.1 mM in absolute ethanol (96 %)). The reaction mixture was well vortexed, left at darkroom temperature for 30 min and the absorbance was measured at 517nm. The Radical Scavenging Activity was calculated using the following [Eqn. 6](#):

$$DPPH \text{ radical scavenging \%} = [(OD \text{ control} - OD \text{ sample})/OD \text{ control}] \times 100 \quad \text{Eqn. 6}$$

Where OD control and OD sample are the absorbances of control and sample, respectively.

The IC₅₀ value is the inhibition concentration of the test sample that decreases 50% of the initial radical.

2.7. Antibacterial activity

2.7.1. Preparation of honey solutions

10g of honey was prepared before testing by diluting honey to the required concentrations, undiluted, and 50% w/v.

2.7.2. Test organisms

The microorganisms *Klebsiella pneumonia* ATCC 700603, *Staphylococcus epidermidis* ATCC 12228, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus*

faecalis ATCC29212, Escherichia coli ATCC 8739, and Bacillus subtilis ATCC6633 were provided and tested.

2.7.3. Preparation of inoculums

One single colony of each type was inoculated with a sterile loop and was transferred into 5 mL of sterile physiological water. The cultures were incubated in a shaking incubator at 37°C for 18h.

2.7.4. Kirby-Bauer disc diffusion susceptibility test

The antibacterial activity of honey was evaluated using the Kirby-Bauer disc diffusion susceptibility test protocol according to instructions provided by BioRaD (Marne de la coquette, France). A total of 3 mL of the fresh culture suspension was spread on the respective media Mueller Hinton agar plate. The excess of the solution was removed and kept dry for 15 minutes at 37°C (plate slightly open).

For screening, a sterile 6 mm diameter filter paper disc was impregnated with 20 µl of honey in triplicate after being placed on the surface of inoculated media agar plates. The plates were stood at 4°C for 2h before being incubated under optimum conditions for 24h. Clear inhibitions zones around the discs indicated the presence of antimicrobial activity. The diameters of the inhibition zones were measured in mm and presented as mean \pm standard deviation (SD), including the diameter of the discs. The positive controls were set up with conventional sensitive antibiotics whereas the negative controls were empty discs.

2.8. Complete Healing of surgical wounds using Argania Honey Dressing: Case Report

We present a case of a surgical wound in a 26-year-old man who underwent surgery on the forehead for Lipoma removal. We treated the wound aseptically with a dressing based on Argania honey for 2 weeks.

Before the dressing session, the patient gave us his oral and written consent to receive addressing with Argania honey. The dressing was changed every 3 days until healing, according to the following protocol: Cleaning with normal saline serum; Drying with a sterile compress; Application of gauze impregnated with pure honey. Cover with a sterile compress; Maintain everything with hypoallergenic plaster. The removal of the stitches was performed on the 8th day.

3. Results and Discussion

3.1. Pollen analysis

The melissopalynological analysis is currently the only technique that allows the direct evaluation of the botanical and geographical origin of honey(El Sohaimy *et al.*, 2015).A pollen species is considered dominant when its representation is over 45% of the pollen spectrum, secondary with a percentage between 16 and 45%, important minor pollen from 3 to 15%, and minor pollen when it is less than 3% as described by Elamine *et al.*(2020).The Argania honey characterization is based on the confirmation of its authenticity as a monofloral honey. We proceeded to analyze the eight samples of honey provided by beekeepers by assessing and identifying of total pollen content, and by looking for argan honey to determine its botanical origin. After analysis, we discovered a single sample containing pollen grains of the Sapotaceae with predominance ranging from 65%. Then, this unique sample was retained to complete and to determine the physicochemical parameters of Argania Honey are shown in [Figure 2](#); [Table 1](#).

Table 1. Estimation of pollen grain frequencies.

Family pollen grain	Frequencies
Sapotaceae(<i>Argania spinosa</i>)	65%
Brassicaceae	29.8%
Oleaceae	1.92%
Apiapicia	1.92%
Lamiaceae	0.64%
Compositae	0.64%
Poaceae	Trace

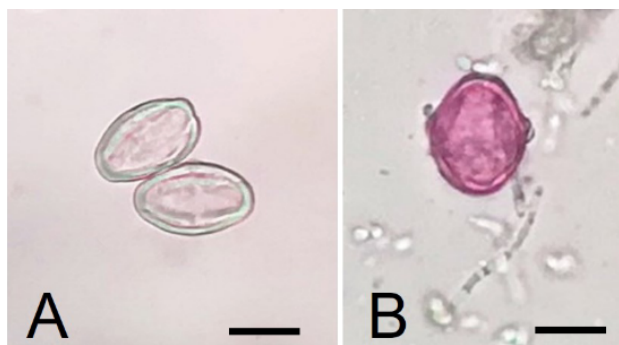


Figure 2. A pollen grain of *Argania Spinosa* (scale bar=10 μ m).
A: nonviable pollen grain, B: viable pollen grain.

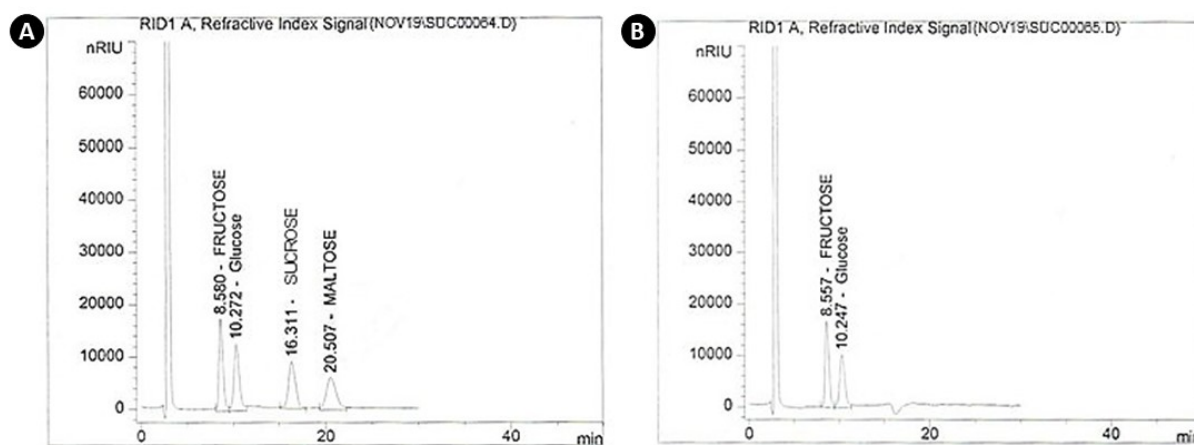


Figure 3. Chromatograms of reducing sugars in *Argania Honey*.
A: Standards used for HPLC, B: estimation of fructose and glucose.

The pollen analysis has several limitations because each country establishes national technical criteria regarding the characteristics of monofloral honey (Persano Oddo & Bogdanov, 2004; Thrasyvoulou *et al.*, 2018). However, these limitations can be overcome by supplementary physicochemical characteristics that give reliable results.

3.2. Physicochemical parameters

Our physicochemical characterization demonstrated that *Argania honey* exhibits parameters in conformity with European Directive 2001/110/EC and the revised Codex standard for honey (Codex Alimentarius, 2019; Directive Council 2001/110/CE, 2002) (Table 2).

Table 2. Physicochemical characteristics of Argania Honey collected in Tiznit in Morocco.

Physicochemical parameters	Values*s.d.	CA**	UE***	
Moisture (%)	18.8±0.2	≤20	≤20	
Determination of diastase activity (Gotheunit)	11.3 ± 0.05	≥ 8	≥ 8	
pH	3.5±0.06	3.5–4.5	3.5–4.5	
Freeacidity (meq/kg)	40 ± 0.3	<50	<50	
Electrical conductivity (mS/cm)	0.264±0.07	≤ 0.8	≤ 0.8	
Ash (%)	0.21±0.03	<0.6	≤ 0.6	
Determination of HMF by HPLC (mg/Kg)	40.9±0.06	≤ 40	≤ 40	
Determination of sugar composition (%)	Fructose	36.8±0.06	>65	>60
	Glucose	31.2±0.04		
	Sucrose	--		
	Maltose	--		
	Total=68%			
Density(g/cm³)	1.414 ± 0.003	1.39 – 1.44 at 20 °C	1.39 – 1.44 at 20 °C	
Viscosity(Pa s)	2,34±0,2	Not determined	Not determined	
Refraction index	1.4895±0.0005	Not determined	Not determined	
Brix value (%)	79,59±0.07	Between 70 and 88	Between 70 and 88	
Color analysis	101.47±0.34	--	--	
Total of phenolic content (mg GAE/Kg)	1295±0.02	Not determined	Not determined	

*Mean of three replications (Average)

**CA : Codex Alimentarius (Codex Alimentarius, 2019)

***EU: European Union(Directive Council 2001/110/CE, 2002)

Results are reported as means ± standard deviation (s.d.).

3.2.1. Chemical properties

The chemical composition of honey makes it possible to evaluate its quality according to its water content, sugars, acidity, ash, enzymes, nitrogen, hydroxymethylfurfural substances (HMF) also called 5-(hydroxymethyl), and others(Ahmed *et al.*, 2014; Persano Oddo & Bogdanov, 2004; Tahir *et al.*, 2019). The moisture content of honey is a very important factor, in controlling its storage stability. The high content can lead to the fermentation of honey during storage (Küçük *et al.*, 2007). The water content of our sample is 18.8%. This result showed that the honey tested recorded a moisture content, slightly below the maximum limit for moisture content according to the Codex standard for the quality of the honey (≤ 20%) (Codex Alimentarius, 2019). The moisture level of the sample analyzed was similar to those reported by other workers on different types of honey such as Indian honey for which the corresponding values ranged from 17.2% to 21.6% (Saxena *et al.*, 2010), Malaysian honey from 11.59% to 19.06% (Moniruzzaman *et al.*, 2013) and Moroccan honey from 14.3% to 20.2%. Overall, the moisture content in our investigated honey sample suggests that the storage and maturity of the studied honey are at the limit. The harvest was in a month from September. The moisture level depends on the harvest season, climatic conditions, the degree of maturity in the hive, and the humidity of the original plant (Fallico *et al.*, 2004; Finola *et al.*, 2007).

Interestingly, the total phenol content per UV spectrophotometer was 1295 mg GAE/Kg. this content is a little less than buckwheat honey (1498 mg GAE /kg) but higher than Thyme honey had phenol content (1138.53 mg GAE/kg), and Acacia honey (965.81mg GAE/kg) (Bouhlali *et al.*, 2016)

and Manuka honey (899 mg GAE/kg) that is considered the highest phenolic content among the four floral honeys examined in previous studies (Alzahrani *et al.*, 2012; Deng *et al.*, 2018).

Previously, Tahrouch *et al.* (2011) used chromatographic and spectroscopic methods in argan leaves to quantify phenolic components, the main flavonol glycoside that was detected and well-known for their broad spectrum of biological activity, mycitrin, quercetin, hyperoside, and myricetin-3-O-galactoside. Some studies explain that the increase of phenolic compounds biosynthesis in some plants under UV irradiation and during periods of water stress and nutrient deficiency might be an adaptation phenomenon by enhancing an antioxidant system in fruit (Ackah *et al.*, 2021; Rabelo *et al.*, 2020). The plants that occupy arid areas and infertile habitats such as the Argania Spinosa tree could increase differentially high levels of phenolic constituents. The high phenolic content and their localization in the peripheral tissues might contribute to understanding the relationship between the accumulation of polyphenols and the adaptation of the Argania tree to his area (Tahrouch *et al.*, 2011). These reports have shown a significant correlation between phenol content and the antioxidant activity of honey. Therefore, the high phenolic value of Argania honey may indicate that it has great antioxidant activity.

Concerning fructose and glucose content were estimated at 68% by HPLC (Figures 3 and 4), both Codex and Directive required the summation of fructose and glucose content for blossom honey and honeydew to exceed 60% (Codex Alimentarius, 2019). However, the absence of sucrose, in our sample honey may be explained by the fact that the honey is in an advanced state of the ripening process, which is in favor of the total conversion of sucrose into glucose and fructose (Terrab *et al.*, 2003).

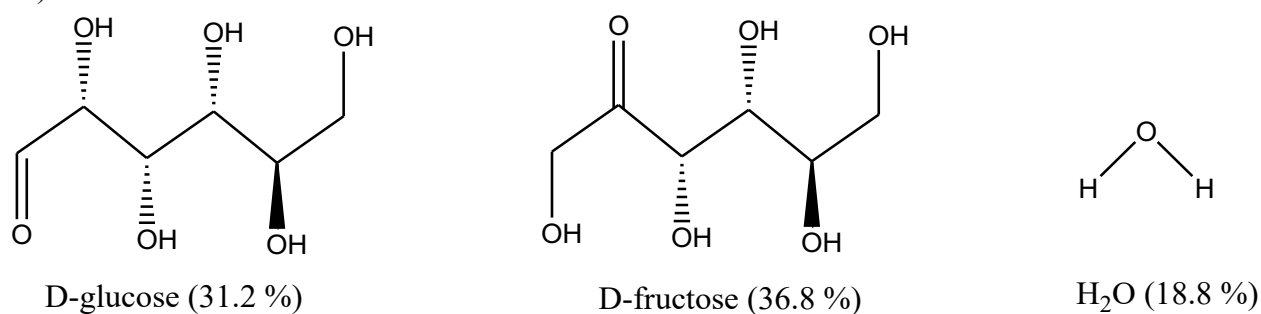


Figure 4. Chemical structures of the main compounds in Argania Honey.

In directive 110/2001, the European Union describes the maximum value of HMF which is a freshness parameter that should not exceed 40 mg/Kg. Our Argania honey registered 40.9 mg/Kg of 5-hydroxymethylfurfural (HMF) is at the limit, this value may be explained by the fact that the climate is semi-arid in the region during the harvesting period. The gap is not resolved at this time. In addition, HMF has been shown to benefit human health by providing anti-oxidative, anti-allergic, anti-inflammatory, anti-hypoxic, and anti-hyperuricemia effects (Shapla *et al.*, 2018).

In general, honey is acidic in nature, regardless of its geographical origins. Our sample is slightly acidic with a pH of 3.5. This acidity is due to the minor acid content of honey, essential amino acids, and organic acids that are responsible for the characteristic taste of honey. This is an acceptable value and comparable to the results obtained in other works (pH4.3 - pH3) (Corbella & Cozzolino, 2006; Feás *et al.*, 2010; Gomes *et al.*, 2010).

The acidity of honey is primarily due to the presence of gluconolactone or gluconic acid (Bouet Kouanou *et al.*, 2020; White, 1975), formed by the action of the enzyme glucose oxidase produced by bees (Alam *et al.*, 2014). In fact, glucose oxidase (GOD) is an enzyme that is secreted by the hypopharyngeal gland of bees into the nectar during the honey maturation process (Kretavičius *et al.*, 2010).

Glucose is oxidized by glucose oxidase in the presence of oxygen, producing D-glucono- δ -lactone and hydrogen peroxide (Ball, 2007; M. Wang *et al.*, 2019)(Figure 5). The presence of hydrogen peroxide in honey is considered one of the key antimicrobial constituents of honey (Nolan *et al.*, 2019). The antimicrobial effect of hydrogen peroxide in honey is enhanced upon dilution, allowing glucose oxidase enzyme to more readily bind with glucose, resulting in the continuous production of hydrogen peroxide (Brudzynski, 2006). Additionally, hydrogen peroxide (H_2O_2) promotes wound healing by facilitating leukocyte recruitment, enhancing the release of vascular endothelial growth factor, and improving blood flow (Rieger & Sagasti, 2011; Schreml *et al.*, 2011).

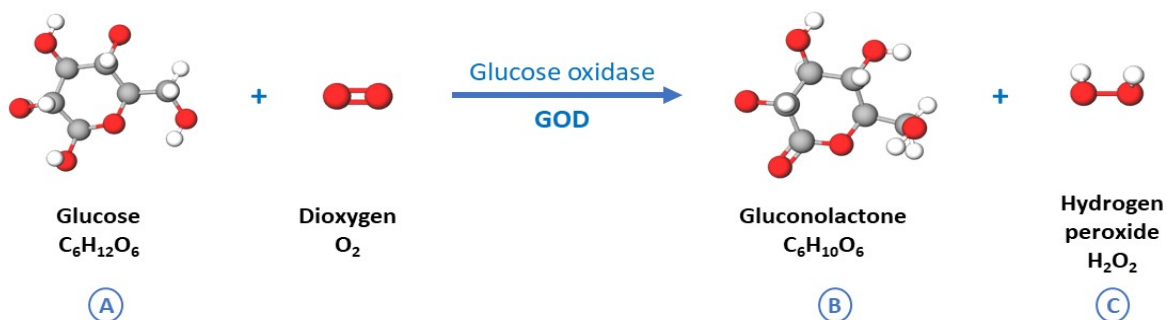


Figure 5. Reaction of gluconolactone (B) and hydrogen peroxide (C) formation from glucose (A) catalyzed by glucose oxidase (GOD)

The pH of honey is correlated with its stability in the storage and growth of microorganisms (Pontis *et al.*, 2014; Smetanska *et al.*, 2021). pH variation among honey samples could be returned to plant floral types. The total acidity of all samples was in accordance with international legislation (Codex Alimentarius, 2019; Directive Council 2001/110/CE, 2002), which required a value of ≤ 50 meq/kg. The sample showed a value of 40 meq/kg (Table 2). Among the factors that influence total acidity are the geographical origin and the harvest season (Alves *et al.*, 2013). Another indicator that confirms the freshness of our Argania honey is the diastase activity which is value is estimated at 11.3 Gothe. It should generally not be less than 8 units of Schade, and in the case of honey with a low natural enzyme content, it should not be less than 3 units of Schade (Codex Alimentarius, 2019; Directive Council 2001/110/CE, 2002). Honey is found with good diastasis activity. A similar result regarding the number of wild carrot honey diastases was 11.3 (Selles *et al.*, 2018). The possible explanation could be attributed to the geographical origin of the honey as well as the time of harvest and floral source of honey.

3.2.2. Physical properties

The chemical composition of honey affects many of its physical characteristics (crystallization, viscosity, hygroscopicity, electrical conductivity, optical properties, surface tension, and color) (Ball, 2007; Batinić & Palinić, 2014). The physical appearance of honey varies depending on the methods of extraction, processing, packaging, and preservation (Ajibola, 2015). The color of honey is an important element used in the identification of the floral source (Moniruzzaman *et al.*, 2013). Differences in the origin and composition of honey have been reported to be significantly reflected in their color intensities (Terrab *et al.*, 2002). As well as the soil the geographical region can influence the color (Alvarez-Suarez *et al.*, 2010). It can also darken with age or when heated (Ajibola, 2016; Moniruzzaman *et al.*, 2013). The value obtained for color according to PFUND Index is 101.47 (Table 2), which means that our sample is dark in color (Amber). Comparing it with other honeys of different origins, the values of Abs_{635} were 113.82 mm for honeys from Saudi Arabia (El Sohaimy *et al.*, 2015), 163,6 mm in Algerian honeys (Rebiai *et al.*, 2015), and 73.88 mm for Egyptian honeys. This property

is related to the mineral content, pollen, and phenolic compounds present in honey (Baltrušaitytė *et al.*, 2007; Bertoneclj *et al.*, 2007). The color intensity test shows that there is a strong correlation between antioxidant activity and phenolic compound content in different types of honey (Dobre *et al.*, 2010). Indeed, the increase in color intensity seems to be related to an increase in the antioxidant properties and polyphenol content of honey (Beretta *et al.*, 2005; Moniruzzaman *et al.*, 2013).

3.3. Antioxidant activity

The botanical origin of honey has the greatest influence on its antioxidant activity, while processing, handling, and storage affect this activity (Beretta *et al.*, 2005; Bertoneclj *et al.*, 2007). Based on extensive research, it has been found that dark-colored honey has a higher total phenolic content, so it has a higher antioxidant capacity (Beretta *et al.*, 2005; Frankel *et al.*, 1998). Molecular oxygen (O₂) is indispensable for a multitude of enzymatic reactions, and therefore, a key element of the metabolism process. However, it can generate some very toxic and reactive derivatives including superoxide (O₂^{•−}), hydroxyl radical (•OH), and hydrogen peroxide (H₂O₂). These reactive molecules can generate serious damage (DNA and fatty acid damage) with age progress. Moreover, the presence of these species in organisms facilitates the generation of pathological and neurodegenerative diseases for example Alzheimer's and diabetes. Thus, the investigation of natural compounds with antioxidant properties became an interesting strategy to study their ability to reduce reactive oxygen derivatives and consequently stop these diseases. The antioxidant activity can be assessed using different assays (DPPH, FRAP, ABTS, and H₂O₂). The DPPH assay is widely performed to estimate the antioxidant activity of an extract, this test is very reactive and sensitive towards some categories of bioactive compounds, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) test gives knowledge on the antioxidant ability to provide hydrogen atoms or electrons.

In this work, the antioxidant activity was monitored by a DPPH assay. As presented in Table 3, the sample showed the high scavenging activity of DPPH (IC₅₀ = 6.52 mg/mL). Our result is similar to those of the study by Pontis *et al.* (2014). who report that the value of IC₅₀ ranges from 3.17 mg/mL to 8.79 mg/mL. The antioxidant activity of our sample is high (Table 3).

Table 3. Antioxidant activity of honey.

Samples	Color	G Origin	DPPH assay (mg/ml) IC50 (mg/mL)
Honey	AMBER	Tiznit	6.52 ± 0.09

Values are presented as means ± standard deviation (SD) of three replications.

3.4. Antibacterial activity

Samples of Argania honey have inhibited the growth of some tested bacterial strains. We measured visible clear rings around the disc and the average zone of inhibition (ZOI) was between 7.7 mm and 32.25 mm using undiluted and diluted honey (v/v) 50% (Table 4). No inhibition was observed by Argania honey in the growth of *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis*.

3.5 Surgical wound healing with Argania honey dressing

After the application of a honey dressing, areas of skin showed a healing process with a clean wound bed. The topical application of the pure honey dressings demonstrated their effectiveness in complete wound healing are shown in Figure 6 and Table 5 and the short time of the process was notable, as described in Table. The healing time was 18 days. The wound progressed aseptically; no signs of infection were detected. The scar is almost invisible with an aesthetically remarkable result.

Table 4. Inhibition zone diameters of bacteria in the presence of Argania Honey.

Bacteria	Undiluted honey ZOI (Mean± SD)	50% of honey ZOI (Mean± SD)
K. pneumoniae	9.53 mm ± 0.74	7.77 mm ± 0.81
B. subtilis	7.7 mm ± 0.62	9.7 mm ± 0.61
S. aureus	12.07 mm ± 1.36	No reaction
E. coli	32.25 mm± 1.06	26.5mm ± 2.12

The results of this study showed that topical application of Argania honey allows complete wound healing with a remodeling phase accomplished without scarring. Honey is considered a safe, simple, and effective dressing that offers greater patient satisfaction ([C. Wang et al., 2019](#)). The renewed interest in honey as a modern dressing offers opportunities for patients and clinicians. Honey is being restored as a valuable agent in modern wound care management. The healing effect of argan honey is very promising.

**Figure 6.** Stages of healing of a surgical wound on the forehead treated with argan honey dressing.**Table 5.** Results of the evolution of a surgical wound treated by the dressing based on argan honey.

Surgical wound	Figure of case	Dressing change (Days)	Description
Surgery on the forehead	A	Day 1	Aseptic wound with stitches.
	B	8 days	Ablation of stitches (except 2 points).
	C	10 days	Wound Healing.
	Total	18 days	

The Healing process involves four overlapping phases: 1-hemostasis, 2-inflammation, 3-proliferation, and 4- remodeling; An avascular scar is the last step in the healing process ([Enoch & Leaper, 2007](#)). This post-surgical wound is the result of an incision of the skin, it heals by primary intention when the edges of the wound are joined and fixed by stitches or others ([Dumville et al., 2014](#)). The protection of this type of wound is our main objective in avoiding infections that can occur. In addition, dysregulation of the healing process could lead to excessive collagen deposition and the formation of abnormal scars, such as hypertrophic scars and keloids ([Martin & Nunan, 2015](#)). The clinical use of honey has gained significant importance in regenerative medicine due to its constructive and restorative properties that promote rapid healing of a large number of wounds ([Krishnakumar et al., 2020](#)). Recently, we reported evidence that Argania honey promotes also complete short-term healing by Argania honey dressing in the treatment of a venous leg ulcer in a woman suffering from diabetes ([Khattabi et al., 2021](#)) and a heel bedsore in a man who suffered an ischemic stroke ([Khattabi et al., 2022](#)).

Conclusion

The results obtained in this present study led us to describe the first characterization of Argania Spinosa honey and achieve contribution guidelines. With this value to the characterization of this type of honey from a scientific point of view, the results of the analysis could increase the value of this unique and valuable monofloral honey. All the values obtained for the physicochemical parameters analyzed in the course of this work complied with the standards defined by the Codex Alimentarius and European legislation was also very remarkable. This study shows that Argania honey has performed equally well as other medical honey grades. Its use in wound healing and very promising and it will have an important effect on wound care. These results can be a useful basis on the commercial and therapeutic side. However, the number of samples used leads us to be cautious, further studies are needed to improve specificity and accuracy to provide a robust model to highlight this Moroccan honey.

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Ethical approval: Informed consent was provided by the patient before starting the care according to the World Medical Association Declaration of Helsinki.

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