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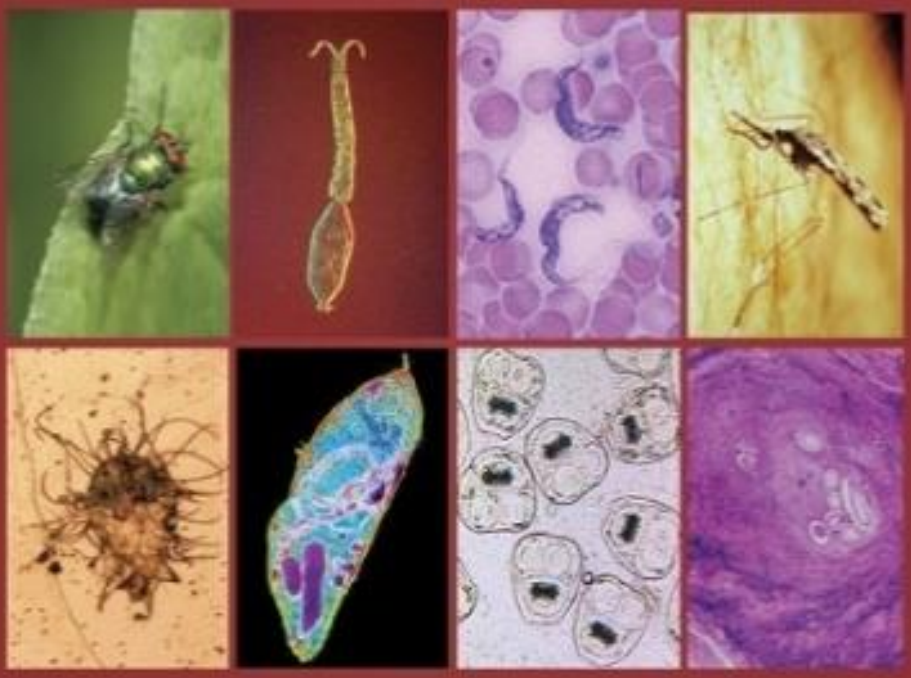

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The Antibacterial Activity of Three Samples of Natural Honey Collected from Algerian Territory on The Growth of *Helicobacter Pylori*.

Zouaouia Chama[1], Esma Zineddine[1], Khedoudja Kanoun[2,] Amina Benabbou[1], Nawel Benchiha[1], Lynda Klouche[1], Chaya Soumia[3] and Sadouni Hadjira[3]

1-Laboratory of Nutrition, Pathology, Agro Biotechnology and Health, Djillali Liabes University, Sidi Bel-Abbès, Algeria

2-Molecular Microbiology and Proteomics and Health, Department of Biology, Faculties of Natural and Life Sciences, Djillali Liabes University, Sidi Bel-Abbès, Algeria.

3- Department of Biology, Faculties of Natural and Life Sciences, Djillali Liabes University, Sidi Bel-Abbès, Algeria.

E-mail : zouaouia_chama@yahoo.fr.

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ABSTRACT

Honey has been used since ancient times due to its nutritional and therapeutic values. It plays an important role as an anti-inflammatory, antioxidant, anti-bacterial agent.

Helicobacter. pylori is an infection that affects half of the world's population, it is responsible for various gastric pathologies (gastritis, ulcer, cancer, lymphoma).

The aim of this present work is to evaluate the antibacterial effect of three samples of natural honey collected from three Algerian regions (wilaya of laghouat, Sidi bal abbès and Mostaganem) on the growth of *H. pylori*. Our work is based on the evaluation of the antimicrobial effect by the agar diffusion technique. We carried out the physicochemical analysis of the three kinds of natural honey which consists in determining the content of water, dry matter (degrees Brix), electrical conductivity, pH and free acidity. And the organoleptic analysis consists in determining the taste, color and appearance of the three types of natural honey and thus the verification of its purity. *H.pylori* is isolated from a gastric biopsy of a patient with gastric cancer within the Gastroenterology Department at the Hospital University Centre of SBA. Our results reveal that chostrole honey has the highest water content (17.6%) followed by the eucalyptus sample (17%) while jujube honey has the lowest water content (16.8%). The dry matter content of Jujubier honey is 74.8%, that of Eucalyptus is 75.7% while that of chostrole is 78.4%. The three honey samples studied (Jujubier, Eucalyptus, Chostrole) have an electrical conductivity of 537; 357 and 363µS/cm respectively. The pH of all types of honey presented is less than 4.9, this confirms that they are of nectar. The acidity values of the honey analyzed are 40 meq/kg. The three types of honey studied (Jujube; Eucalyptus and Chostrole) have good organoleptic characteristics. We found that Chostrole honey exhibits better inhibitory antibacterial activity with an inhibition zone of 15mm perapport to that of jujube and Eucalyptus honey which exhibit inhibition zones of (9.6) mm respectively on the growth of the pathogenic strain *H. pylori*.

INTRODUCTION

H. pylori is classified as a Type I carcinogen by the World Health Organization (WHO) in 1994 and are now considered the main etiological agent of cancers related to bacterial infections (Parkin and Bray, 2005). It is a persistent colonizer of the human stomach and causes gastritis, ulcers, and stomach cancer whose infection poses a real public health problem in Algeria (Amieva and Peek, 2016). *H. pylori* infection is widespread in the world, their prevalence varies considerably from (20-40%) in industrialized countries, (70-90%) in developing countries and it is 78% in Algeria (Joutei et al., 2010; Woodward et al., 2000). Numerous studies worldwide have shown promising activities of natural remedies towards *H. pylori* in vitro and in vivo studies (Drouin, 1999; Keenan *et al*., 2012; Ayala *et al*., 2014; Takeuchi *et al*., 2014; Han *et al*., 2015). Honey is one of the most coveted products. Due to its inhibitory and therapeutic properties, many studies have focused on the therapeutic properties of honey (Baltrusaityte V *et al*., 2007; Badawy O *et al*, 2004). The honey is classified, according to the local origin, as the mode of obtaining (Lamballais C, 1989; Caillas A, 1974). The mode of treatment and the food source. Therefore, all kinds of honey have common properties, but each honey is characterized by its own therapeutic properties (Huchet E *et al*, 1996; Gout J, 1989). The chemical composition of honey varies according to the quality of the collected nectar and honeydew, the nature of the soil and the physiological state of the colony (Gonnet Michel, 1982). Honey, which is primarily used as a source of nutrients, is also an ancient remedy used in particular in the treatment of wounds since antiquity. This practice, which has long been rooted in traditional and folklore, has shown, since the end of the 19th century, a renewed interest by the medical profession, and in particular when current therapeutic agents fail. (Mandal MD, Mandal S, 2011; Bourlioux P, 2013). The World Health Organization has submitted the interest to study the possibility of using natural products. Could honey, as such, represent an effective "natural remedy» ? (Nayak PA *et al*., 2010; Mandal MD, Mandal S, 2011).In Algeria, it is difficult to obtain the antibiotic profile of *H. pylori*. Thus, it is mandatory to adopt an *H. pylori* eradication treatment in Algeria according to the last recommendations of Maastricht V (Malfertheiner, 2017). Our study is an original work and is the first carried out in our region and in Algeria.

MATERIALS AND METHODS

The aim was to evaluate the antibacterial effect of three samples of natural honey, collected in three regions of the Algerian territory; these are Laghouat, sidi Bel Abbes (sidi Daho), Mostaganem (Achkacha) (table 1), against the pathogenic bacterium *H.pylori*.

Table 1: Presentation of honey samples.

Sample number	Harvest date	Harvest location	Floral origin	Extraction mode
01	Jun 2017	Wilaya de Laghouat	Mono-floral	Manual
02	Jully 2017	Ville de sidi Bel Abbes (sidi Daho)	Multi-floral	Manual
03	Septembre 2017	Wilaya de Mostaganem (Achkacha)	Multi-floral	Manual

Physicochemical Analysis:

Analyzes accredited according to the ISO standard (2005)

1. Moisture Content:

The water content is determined by optical measurement of the refractive index (IR) of honey at 20°C. The correction coefficient is 0.00023 per degree Celsius. The correction is additive if the measurement is made above 20 ° C, subtractive in the opposite

2. Dry Matter (Brix degree):

The rate of dried material can be assessed by the refractometry method. The reading is made on the scale that indicates the dry matter content or "Brix Degree" which is in parallel with the scale of the refractive index.

3. Electrical Conductivity:

It is the measurement at 20 ° C of the electrical conductivity taken in an aqueous solution of honey using a conductivity meter. The value is read on the screen. The conductivity of honey is measured in siemens per cm: s.cm -1 conventionally the conductivity is given in 10-4 S.cm-4.

4. pH

It is the measurement of the hydrogen potential of a honey solution using a pH meter

5. Free acidity

Let V be the volume in ml of soda at 0.1M used during titration. The free acidity of honey is expressed in milliequivalent per kilogram of honey and determined by the following formula:

AL= (Volume of 0.1 N NaOH in ml) x 1

Organoleptic Analysis:

Sensory analysis is the examination of a product through the evaluation of attributes perceptible by the five sense organs, such as color, smell, taste, touch, appearance. First, the defects of the product are judged, then its quality is appreciated (Persano Oddo *et al*., 1995). In addition, sensory evaluation makes it possible to distinguish the botanical origin of the honey and to identify and quantify certain defects (fermentation, impurities, bad odors and flavors) (Piana *et al*., 2004).

Before proceeding with the examination, rules must be followed:

- Honey tasters must not smoke, eat or drink anything other than water 30 minutes before tasting. Do not use flavored toothpaste. Cosmetics can influence the neutral character of the tasting room. The number of samples to be analyzed per tasting session must be limited (maximum 7). Take 30-minute breaks between sessions.
- Perform tastings 2 hours after the main meals. The best results are obtained in the middle of the morning and afternoon (Persano Oddo *et al*., 1995).

  According to the technique of Gonnet (1991), a little honey (15-20 g) is presented in glasses. It is first appreciated visually, then we examine its smell and at the end its taste
- Visual: color, cleanliness, homogeneity, as well as possible crystallization defects, are recorded.
- Olfactory: these are the odors that are perceived immediately through the direct nasal route.
- Taste: the flavor of honey is first analyzed. A natural or accidental aftertaste (e.g. smoke) can also be recorded.
- Tactile: when honey is crushed in the mouth, we feel the creaminess or firmness of the crystalline texture of the product, we also detect the type of granulation of honey. After the examination of the taste, the mouth must absolutely be neutralized. You can drink water to neutralize it.

Verification of the Purity of Honey:

The purity of honey depends on the total dissolution of honey when there are no artificial additives (especially sugars). Purity depends on the limited sugar content when there is partial oxidation during fire exposure.

There are a few simple tests to check the purity of honey:

-Dissolution Test:

Getting a glass of water and a tablespoon of honey is all necessary for the first test. This is followed by emptying the

honey into the water. If the honey is impure, it will dissolve in water. If pure, honey sticks together and settles as a solid mass at the bottom of the glass.

Isolation and Identification of *H. Pylori*:

1. Sample and Origin of the Material:

The study was carried out on gastric antral biopsies, taken from patients with gastro-duodenal diseases. These patients presented on an empty stomach and had undergone a gastroduodenal endoscopy in the gastroenterology department of the Abdelkader Hassani University Hospital center in the wilaya of Sidi Bel Abbés.

Gastric biopsies are the biological material subject to this study. These are taken in endoscopy and are sent to the biotechnology laboratory of the Department of Biology at the Faculty of Natural and Life Sciences at the University of Djilali Liabes of SBA within a period not exceeding 15 minutes.

2. Sampling:

Biopsies should be taken away from treatment with antibiotics or anti-secretories (Lamouliatte *et al*., 1992; Mégraud, 1994a). The conditions of sterility must be respected at the time of sampling (Mégraud, 1989). Samples were taken endoscopy from the antral region approximately 2 cm from the pylorus using an endoscope and biopsy forceps, previously disinfected and well rinsed due to the inhibitory power of this product on H. pylori (Lamouliatteetal, 1992).

3. Preparation of Biopsies;

Since the gastric biopsies were collected in the endoscopy room and immediately transported to the laboratory, the samples were transported to the Heart brain environment to help the bacteria multiply. Once in the laboratory, the samples are subjected to a direct examination including a rapid urea test, microscopic observations in the fresh state and Gram staining.

4. Urea Rapid Test:

H. pylori has the particularity of having a very active urease. This property is used for the diagnosis of *H. pylori* infection by a rapid urea test (Delchier, 1994). The latter is based on research directly at the level of gastric biopsy.

For this, we carried out this research in the endoscopy room and in the laboratory (Mégraud, 1994).

In the laboratory, we only deposited a biopsic fragment using a sterile handle in a tube containing 0.5 ml of urea-indole medium.

Incubation is done at 37 ° C. The reading of the results (turn of the indicator to red) is done after 20 minutes and 24 hours (Mengraud, 1989; Sobhani *et al*, 1991; Mégraud, 1994)

5. Grinding Biopsies:

With the help of a scalpel blade, the biopsie fragment is deposited on a sterile petri dish and then it is crushed using a scalpel blade.

6. Bacteriological Examination:

H.pylori is a metabolically demanding bacterium (Lamouliatte *et al.*, 1992). Its cultivation requires enriched culture media with the addition of blood or serum (Fauchère and Rosenau, 1991; Sobhani *et al*., 1995).

For its isolation, the gastric biopsy grinded is seeded in a medium of Columbia agar added human blood O +. The boxes are incubated immediately seeded in a jar in a micro-aerophilic atmosphere at 37 ° C for 3 to 7 days. The atmosphere is renewed at least every two days to have optimal growth (Mengroud, 1989; Fauchére et rsenau, 1991; Mégraud., 1994).

6.1 Identification:

Identification is based on the determination of morphological and biochemical characteristics (Mégraud, 1994; Lamouliatte *et al*, 1992).

-Macroscopic examination: the appearance of colonies and their dimensions are studied from bacterial cultures obtained on culture media (Lamouliatte et al, 1992; Fauchère and Rosenau, 1991).

-Microscopic examination: it was carried out on a bacterial smear, prepared from the suspect colonies in pure cultures, then fixed

and stained by the Gram method (Mégraud, 1994).

6.2 Biochemical Study:

The study of biochemical traits is based essentially on the search for oxidase, catalase and urease, because their positivities confirm that it is indeed H.pylori (sobhani *et al*; Fauchére and Rosenau).

-Catalase Test:

Catalase is an enzyme that hydrolyzes hydrogen peroxide into water and oxygen, is a good differentiating element used in bacterial systematics. To highlight this enzyme, part of the suspicious colony is diluted in a drop of hydrogen peroxide on a sterile slide. The release of gas bubbles indicates the presence of catalase.

-Cytochrome Oxidase Research:

The oxidase test is a means of identifying Gram- bacteria, which allows the presence of cytochrome oxidase enzyme to be demonstrated. The search for this enzyme consists in depositing in a hemolysis tube, an "Ox" disc and soaking it with a drop of distilled water. Then take part of the colony to be studied and spread it on the disk. After about 10 minutes a dark purple coloration appears on the disc and then turns black: oxidase test +.

Evaluation of the Antibacterial Activity of The Three Types of Honey:

The method of evaluating the antibacterial power of honey focused on a standard, simple, fast, economical technique.

1. Preparation of Pure Honey Discs:

-Antibiogram discs 5 mm in diameter were cut out of filter paper sterilized with dry heat and then immersed in pure honey for a few hours.

-The discs were then dried in the oven at 37 ° C for 24 hours

2. Preparation of The Bacterial Suspension of The Pathogenic Strain *H.pylori*:

-From isolated colonies of *H.pylori* scrape with platinum handle some colonies.

-Well unload the handle in a tube containing nutritious broth.

-Homogenize the bacterial suspension then switch to incubation 18h / 37 ° C

-Centrifugation 4000 revolutions for 15min to recover over swimming

3. Technical:

-Soak a sterile swab in the overnatant of the incubated bacterial suspension. This is followed by rubbing the swab over the entire agarized surface (Muller-Hinton agar+ human blood of group O+). Let dry at room temperature for 15min. Squeeze each sterile disc of honey with a well-sterile clamp on the medium and do not move the discs after application. Finally switch to incubation was at 37 ° C for 24 or 48 hours.

RESULTS

Physicochemical Analyses:

The results of the physicochemical study relating to honey samples (Jujube, Eucalyptus, Chostrole) are given in Table 2.

Table 2: Physico-chemical results of the different honey samples.

	Jujubier	Eucalyptus	Chostrole
Moisture content	16 ,8	17	17,6
Dry matter	74,8	75,7	78,4
Electrical conductivity (µS/cm)	537	357	363
pH	4,9	3,48	3,19
Free acidity (Meq/kg)	40	40	40
Refractive index	1,4944	1,4940	1,4925

1. Moisture Content:

The jujube sample has the lowest water content (16.8%). Then the eucalyptus sample (17%) Unlike the chostrole sample it has the highest water content (17.6%) and therefore contains the driest matter (Fig. 1). These values are well below the maximum limit recommended by Codex Alimentarius (2001) which is 20% maximum.

2. Dry Matter:

The dry matter content of Jujube honey is 74.8%, Eucalyptus is 75.7% while chostrole honey is 78.4% (Fig. 2); this variability depends on the water content of the honey.

According to Makhloufi (2001), the variation in parameters (density, refractive index, dry matter rate) is directly related to water content.

3. Electrical Conductivity:

Electrical conductivity is due to the presence of mineral substances; this parameter is an indirect measure of honey mineralization. The three honey samples studied (Jujube; Eucalyptus; Chostrole) have an electrical conductivity of 537; 357 and 363µS/cm respectively (Fig. 3); it meets the standards preconsed by Gonnet (1982). Which indicates that the variation is between 0.1 and 1.5 mS /cm.

4. pH:

The pH of all types of honey presented is less than 4.9 (Fig. 4), this confirms that they are of nectar. We can say a priori that all the honey studied are acidic and are in compliance with the standards of the Codex Alimentarius (2001).

The pH represented the proton concentration in H+ ions of a solution. Nectar honey has a low pH (3.3 to 4.5) while honeydew honey has a slightly higher pH (Pesenti *et al*; 2008).

5. Free Acidity:

The acidity values (Fig. 5) of the honeys analyzed are 40 meq /kg. It is found that the total acidity values have been within the normal range set by the Codex Alimentarius (2001) of 50 meq/kg. This indicates the absence of undesirable fermentation.

Free acidity is an important criterion during extraction and storage, due to its influence on the texture and stability of honey. This acidity comes from organic acids in some are free and others are combined in the form of lactones. Some of these acids come from nectar or honeydew but their main origin comes from the salivary secretions of the bee; the main acid derives from glucose in the form of gluconic acid its formation is accompanied by the release of hydrogen peroxide (Gomes *et al*; 2010; Bogdanov *et al*; 2004; Louveaux, 1985).

The fermentation of honey causes an increase in acidity in honey, although there is considerable natural fluctuation. The old standard prescribes a maximum value of 40 milliequivalents/kg. In the Codex Alimentarius project, it was increased to 50 milliequivalents/kg. Since there are a few kinds of honey that have a higher natural acid content (Cavia *et al*; 2007).

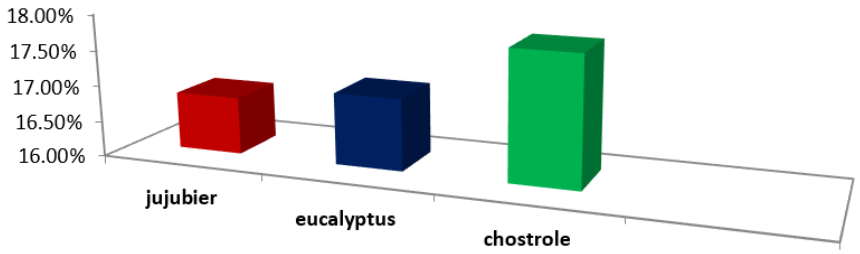

Fig. 1: Water content

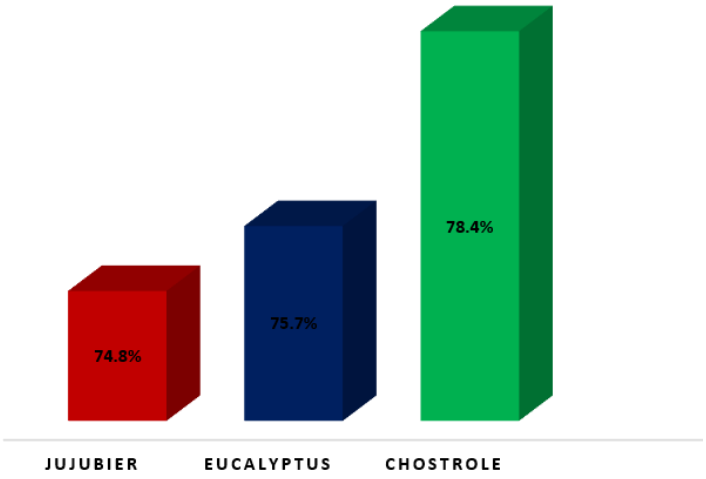

Fig. 2: Dry matter

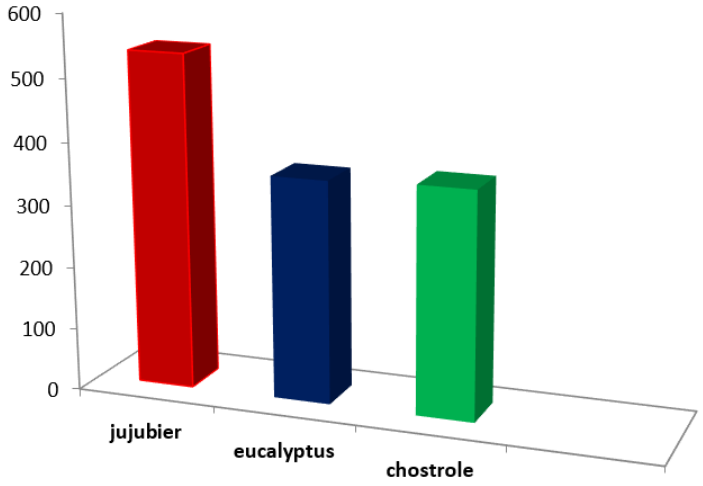

Fig. 3: Electrical Conductivity

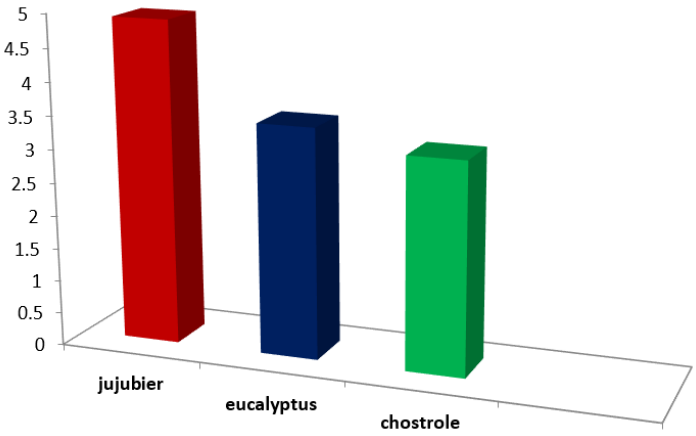

Fig. 4: pH

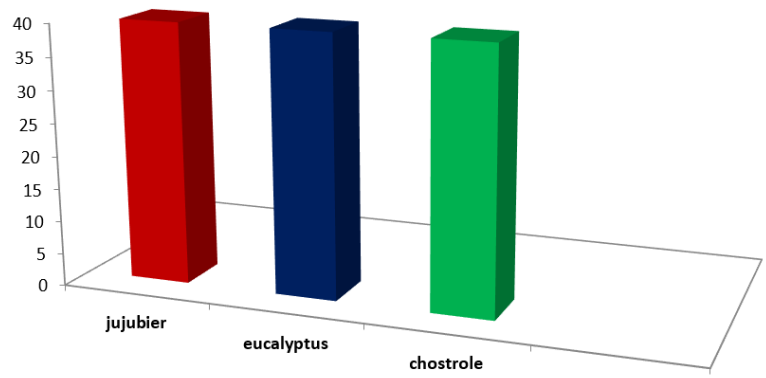

Fig. 5: Free acidity

Organoleptic Analysis:

The three types of honey studied (Jujube; Eucalyptus and Chostrole) have good organoleptic characteristics.

The organoleptic analysis results were classified in Table 3, below:

Table 3: Results of organoleptic analysis

		Jujubier	Eucalyptus	Chostrole
Visual	Aspect	Dense liquid	Dense liquid	Dense liquid
	Color	Slightly brown	Yellow	Yellow-green
Sensory	Flavor,	Sweet	Sweet	Bitter aftertaste
	Smell	Characteristic of honey	Characteristic of honey	Characteristic of honey

Identification of *H.pylori*

1. Rapid Urea Test:

The use of a urea-indole medium gives a positive reaction which is reflected in a turn of the pH indicator towards fuschia pink (Fig 6) The turn most often occurs at least 20 minutes.

2. Macroscopic Appearances:

The culture of gastric biopsy on the isolation medium revealed the appearance of small colonies, 1 mm in diameter. They are greyish or transparent, shiny, discreetly curved, round and of regular contour (Fig.7). This aspect is encountered in *H. pylori*.

3. Microscopic Appearance:

Gram staining, carried out from the colonies that have appeared, shows the presence of Gram-negative bacteria (Fig.8). They are in the form of bacillus which can be straight, curved in comma, in the shape of C, V, or S, with regular contours.

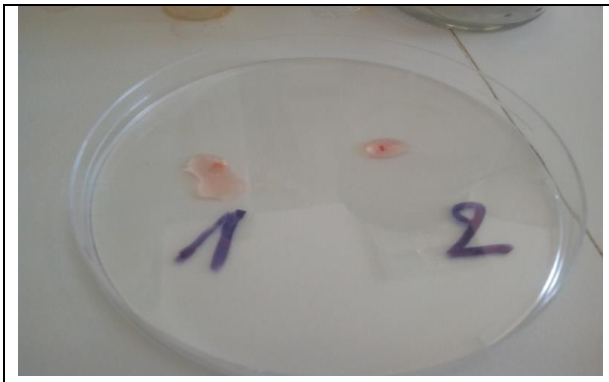

Fig. 6: Urea Rapid Test.

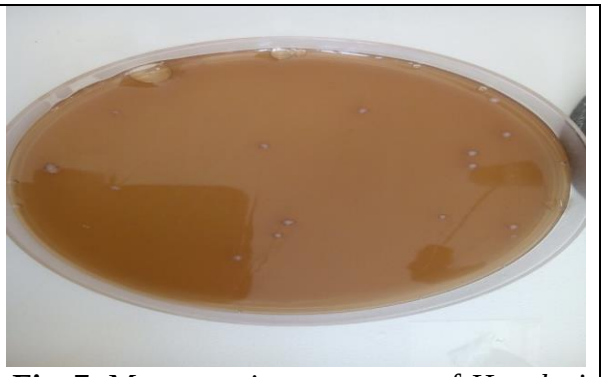

Fig. 7: Macroscopic appearance of *H. pylori* on blood medium.

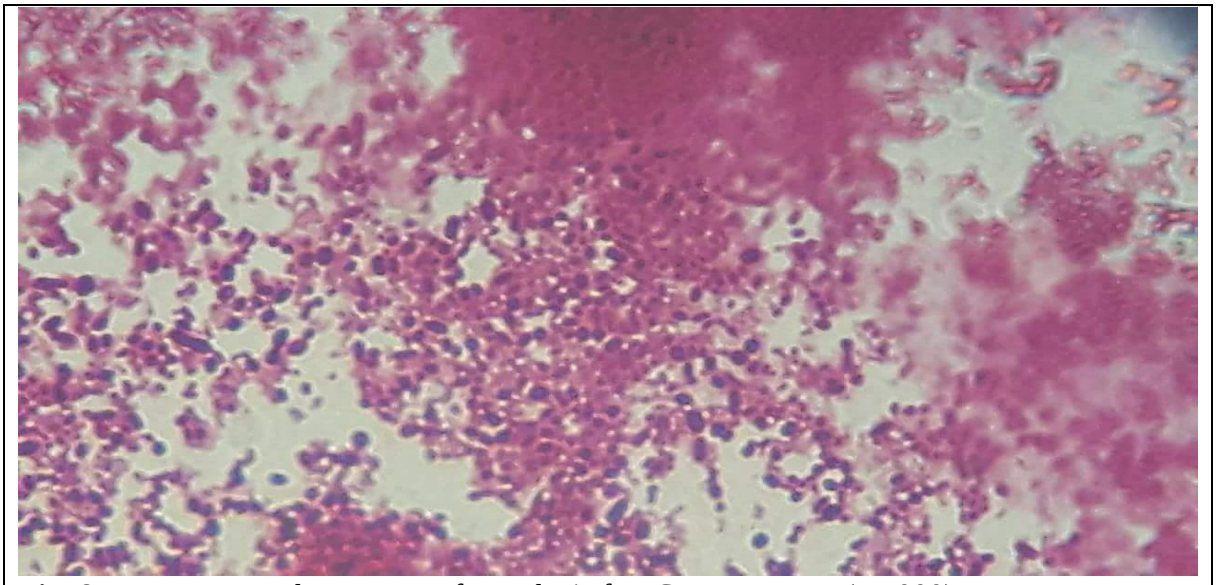

Fig. 8: Microscopic observation of *H.pylori* after Gram staining (× 1000).

Biochemical Study:

The identification is completed by the search for biochemical characters, and are: - Urease; - Catalase; - Oxidase. The positivity of these three tests confirms that it is indeed *H.pylori* (Table4).Urea test; it gave a positive result, the color change of yellow-orange indole urea medium to pink fuchsia, indicated the presence of the bacterium in the gastric biopsy (Fig.9). Catalase Test; this test gave a positive result, the presence of the enzyme results in the release of gaseous bubbles (Fig.10). Oxidase test; The positive result is a dark purple color, which appears on the disc then turns black after a few minutes (Fig.11).

Table 4: Biochemical characterization of *H.pylori* isolated from gastric biopsies

Strains	Urease	Catalase	Oxidase
H.Pylori	+	+	+

Fig. 9: Urease test

Fig. 10: Catalase test

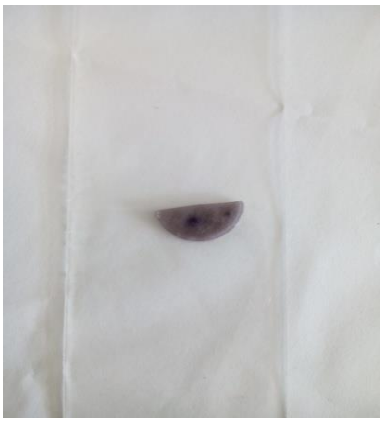

Fig. 11: Oxidase test

4. Evaluation of the antibacterial activity of the three types of honey on the growth of *H.pylori*:

The following Fig.12, Table. 5 show that all three types of honey possess antimicrobial activity that inhibits the growth of *H.pylori*. This shows that the pathogenic strain *H.Pylori* is more sensitive to Chostrole honey this sensitivity translates into a minimum inhibition diameter of 15 mm.

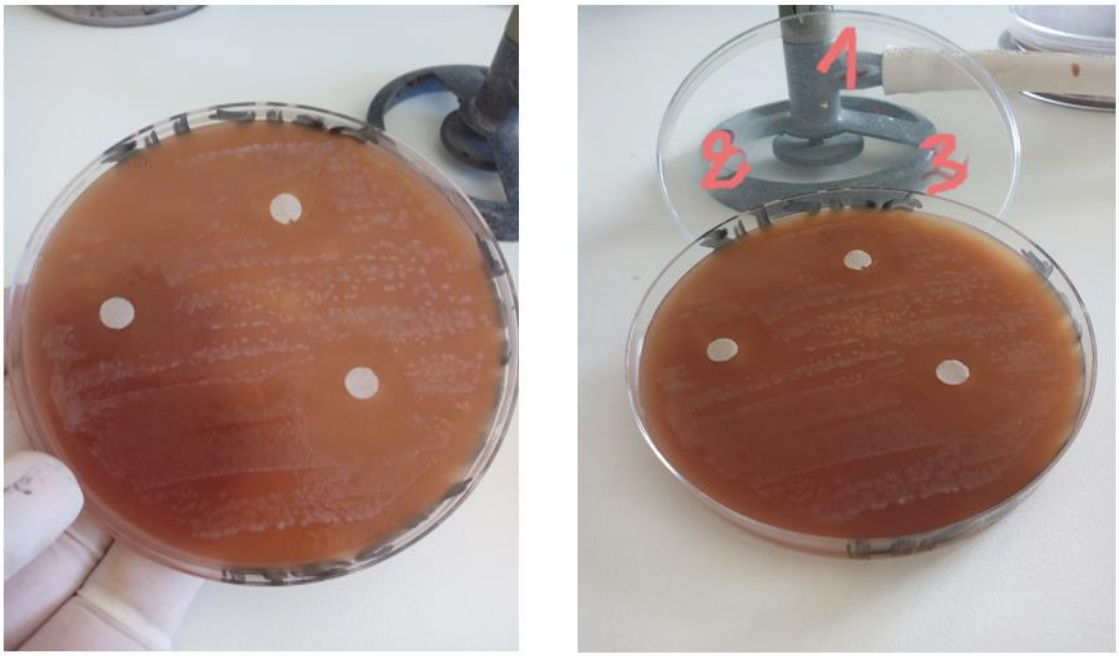

Fig. 12: Result of the antagonism of *H.pylori* with the three types of honey.

Table 5: Result of *H.pylori*'s antagonism towards the three types of honey.

Type of honey	Jujubier	Eucalyptus	Chostrole
H. pylori	+	+	+
MID (mm)	6mm	9mm	15mm

Presence of halo: + / Absence of halo: -.

DISCUSSION

This study has shown that *H. pylori* can be inhibited by the antibacterial activity of honey that the bacterial strain tested is more sensitive to the action of Chostrole honey which is most effective with an inhibition zone of 15 mm, so it is less sensitive to other types of natural honey, which indicates that this type has a wide spectrum of antimicrobial action. Because of their (water content, acidity and eclectic conductivity) which are higher compared to other types. So we find that the action of natural honey on the pathogenic strain tested varies according to the honey sample. Honey is a very bland treatment, and in fact can protect the stomach from the damaging action of other substances (Ali atmm *et al*., 1990). *H. pylori* are known to produce catalase enzyme (Mengraud F, 1989), which would account for its resistance to the honey with the high level of hydrogen peroxide (H_2O_2). However, the results obtained with the agar diffusion assay of honey at higher concentrations clearly show that the H_2O_2 generated in honey and the osmolarity are of no importance. Although there can be problems in comparing different types of antibacterial molecules with this type of assay (Wick WE *et al*.,

1974), others have found that the H_2O_2 activity in honey is detected more readily than the non-peroxide activity by agar well diffusion assay (Bogdanov S., 1984; Willix DJ, 1991). Several studies including those of Ahmadi-Motamayel F *et al*. (2013), Basson NJ *et al*. (1994), Nasser HM *et al.* (2012), Steinberg D *et al* (1996), show a significant antibacterial action or a total inhibition of S. mutans from 20 to 25% honey concentration, On the other hand, Gallardo-Chacon JJ *et al.* (2008). which showed inhibition of S. mutans for honey concentrations of 100% The results of the work on manuka honey also highlighted the effectiveness of this honey on S.mutans. The study by N Al Somall *et al*. (1994) on the antibacterial effect of manuka honey on the sensitivity of H.pylori by, was found that all five isolates tested were sensitive to a 20% (v/v) solution of manuka honey in an agar well diffusion assay, but none showed sensitivity to a 40% solution of honey in which the antibacterial activity was due primarily to its content of H_2O_2.In New Zealand. However, although manuka honey shows potential for use as a low-cost innocuous agent against *H.pylori*, its usefulness clinically is not known. It is believed that the action of natural honey on bacteria depends, on the one hand, on the structure of the wall of the target cell, and on the other hand on the composition of the honey itself. - The composition of the honey itself in turn depends on several factors, such as the nature of the soil, the breed of bees and the physiological state of the colony. Donadieu (1978) showed that all honeys have common properties, but each monofloral honey is characterized by therapeutic properties of its own. Other factors also influence the nature and composition of honey and its peculiarities such as: - the age of the bee (the honey of the young bee is particularly clear and less concentrated by contribution to that of the older bee); - the nature of the bee nutrition flowers and the floral origin of the diet; the climate of the environment, the season of bee breeding and honey production; - the method of honey extraction; - the duration and conditions of storage, such as temperature and light that condition the activity of honey enzymes and their effectiveness. Aging can change the inhibitory characteristics of honey. Several causes are also attributed to the antibacterial power of honey (osmolarity, low pH, H_2O_2, methylgyloxal ...), however, all the antibacterial mechanisms involved still remain to be deepened. According to ASSIE, (2004), the species: *Strptococcus pyogenes* and *Clostridium tetani*, are also sensitive to Jujubier honey, but to a lesser degree. *Clostridium oedematiens* does not seem to be inhibited by this honey. Recently, studies have focused on the antibacterial action of honey on cariogen bacteria, and new aspects of its application, especially in dentistry. Despite their small numbers, these studies have shown that some honeys, such as manuka honey, have antibacterial efficacy. In 2009, the first work on the action of honey on pathogenic oral microorganisms was carried out at the University of Dentistry of Bordeaux by Dr.Badet C. *et al.* (2011) and Muniesa N.(2009) The results obtained showed that honey, and in particular manuka honey, had an interesting antibacterial action on cariogenic bacteria such as (*S.mutans*, *Lactobacillus Rhamnosus*, *Actinomyces viscosus*). The results reported here also raise the possibility that an antibacterial agent useful against *H. pylori* may be found in the pollen or an extract of the chostrole tree. *H.pylori* is susceptible to many antibacterial agents in vitro but only a few are effective in vivo (Goodwin CS *et al.*, 1988). Honey is a very bland treatment, and in fact can protect the stomach from the deleterious action of other substances (Ali atmm *et al*., 1990). Many treatments, and particularly antibiotics have serious side effects, like treatment antibiotic-associated colitis, hypersensitivity and antibiotic resistance. There is also the risk of nephrotoxicity when bismuth treatment is used yet so far bismuth seems to be an

essential element in the therapeutic schemes.

CONCLUSION

These results show that honey is exhibited inhibitory activity on the pathogenic strain *H.pylori*. This inhibitory effect was found for samples tested with some variability from one sample to another with high efficiency in Chostrole honey. These findings could find a possible application in the treatment of deferential gastroduodenal diseases caused by *H.pylori*. The medicinal value of honey as a natural antibiotic is increasingly scientifically demonstrated, which is of great importance for its use in medicine and in the pharmaceutical and cosmetic industry.

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