


variability from one sample to another and one strain to another.

These results could find a possible application in the treatment of some illnesses caused by pathogenic microbes. The medicinal value of honey as a natural antibiotic is demonstrated more and more scientifically and constitutes the importance of its use in medicine and in the sector of pharmaceutical and cosmetic industry.

references


Baltrusaityte, V., P. Venskutonis, and V. Ceksteryte. 2007. Antibacterial Activity of honey and beebread of different origin against S. aureus and D. epidermidis. Food technology, Lithuania. 45 (2) :201-208.


Donadieu, Y. 1981. Les thérapeutiques naturelles, la gelée royale. Paris. 5ème édition, p. 75


Siess, Mh., Am. Le Bon, Mc. Canivenc-lavier, Mj. Amiot,
* The age of the bee (the honey of the young bee is especially clear and less concentrated compared to the one of the older bee).

* The nature of the flowers and their origins (BirI, 1999; Verdan, 2002).

* The climate of the environment, the season of the breeding of the bee and the production of honey.

* The method of honey extraction.

* The length and the conditions of preservation, as the temperature and the light influence the activity of honey enzymes and their efficiency (Caillas, 1974).

Aging can modify the inhibitory characteristics of honey (Chauvin, 1986). It was found that the oldest sample 2 is the most active sample and this can be interpreted by the fact that the other factors especially the method of extraction and the conditions of preservation intervene strongly. The influence of the genetic variation of the bee strain on the honey characteristics can not be ignored.

* The inhibitory activity of the natural honey on E. coli and Pseudomonas aeruginosa is similar to the one of the most active antibiotics (GM, IPM, YEAR, AMC and SXT), but its effect on Serratia marcescens is relatively weak. Concerning Staphylococcus aureus, an original polyresistant to antibiotics, the inhibitory activity of natural honey on this microbe is excellent with the two samples 1 and 2 (two times more than the antibiotics). The sample 3 doesn’t have an effect on this microbe.

It was observed that the yeast Candida albicans has a sensitivity only to sample 2 of the honey and that its sensitivity is relatively weak in comparison to that of the bacteria.

* The imported honey has no antimicrobial effects on all tested strains and the causes are not known.

**Conclusion:**

According to Kerkvliet (1996), the antimicrobial effect of the honey can partially be explained by its important content of the enzyme glucose oxidase that activates the transformation of glucose to gluconic acid and hydrogen peroxide (Kerkvliet, 1996).

The honey presents a strong bioactivity and an antimicrobial effect ascribed to its enzymes, its content of gluconic acid and to its low pH which is between 3.0 and 4.5 (Brudzynsky, 2006). A lot of the bacteria are inhibited at this pH. (Creemers et al., 2005). Besides, the flavonoides that it contains have a strong antioxidant effect and therefore they arrest the ominous oxygenated radicals (Siess et al., 1996).

These results show clearly that honey is endowed with a large inhibitory activity on four strains of Gram positive and Gram negative bacteria as well as one strain of yeast. This inhibitory effect has been noted for most samples tested with a certain
on Gram positive bacteria and no effect neither on Gram negative bacteria, nor on the tested fungus (samples 1 and 3), and on the other hand of the honey itself.

- The composition of honey depends on many factors, such as: the nature of soil, the race of the bees and the physiological state of the colony (Prost, 1979). In fact, Donadieu (1978) showed that all sorts of honey have common properties, but every sort of mono floral honey is characterized by therapeutic properties ascribed to it (Donadieu 1981 and 2006). Other factors also influence the composition and the nature of the honey and its particularities such as:

Table 5. Effect of the three samples of the natural honey on the five tested microbes (+ an positive effect antimicrobial, - negative effect antimicrobial).

<table>
<thead>
<tr>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bactericidal</strong></td>
<td><strong>Bacteriostatic</strong></td>
<td><strong>Bactericidal</strong></td>
</tr>
<tr>
<td><strong>effect</strong></td>
<td><strong>effect</strong></td>
<td><strong>effect</strong></td>
</tr>
<tr>
<td><strong>E. coli</strong></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Serratia. marcescens</strong></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><strong>Pseudo. aeroginosa</strong></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Staphylo. aureus</strong></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><strong>Candida albicans</strong></td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Graph 1. Antimicrobial activity of the sample 1 of the honey.

Graph 2. Antimicrobial activity of the sample 2 of the honey.

Graph 3. Antimicrobial activity of the sample 3 of the honey.
The action of the natural honey on the five tested microbes varies according to the sample of the honey and to the microbial strain.

- The strains of *E. coli* and *Pseudomonas aeruginosa* are the most sensitive to the effect of the three samples of honey. *Staphylococcus aureus* is fairly sensitive. Whereas *Serratia marcescens* can be considered relatively resistant to the two samples 1 and 2, completely resistant to sample 3. *Candida albicans* is weakly sensitive.

- The imported honey has no effect on the five studied micro-organisms.

**Table 4.** Effect of honey samples on the tested strains. The numbers represent the diameters of the zones of inhibition in mm, the places and the dates of harvest or manufacture are mentioned.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Dilutions</td>
<td>A  B  C  D</td>
<td>A  B  C  D</td>
<td>A  B  C  D</td>
<td>A  B  C  D</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>24 00 00 00</td>
<td>23 19 16 07</td>
<td>23 00 00 00</td>
<td>00 00 00 00</td>
</tr>
<tr>
<td><em>Serr. marcescens</em></td>
<td>08 06 04 00</td>
<td>15 14 10 00</td>
<td>12 10 00 00</td>
<td>00 00 00 00</td>
</tr>
<tr>
<td><em>Pseud. aeruginosa</em></td>
<td>27 26 14 11</td>
<td>30 21 21 21</td>
<td>25 24 00 00</td>
<td>00 00 00 00</td>
</tr>
<tr>
<td><em>Staphyl. aureus</em></td>
<td>19 14 10 05</td>
<td>21 17 00 00</td>
<td>00 00 00 00</td>
<td>00 00 00 00</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>00 00 00 00</td>
<td>12 10 10 09</td>
<td>00 00 00 00</td>
<td>00 00 00 00</td>
</tr>
</tbody>
</table>
Photo 4. Effect of honey on *Pseudomonas aeruginosa*.

Photo 5. Effect of honey on *Staphylococcus aureus*. 
Photo 2. Effect of honey on *E. Coli*.

Photo 3. Effect of honey on *Serratia marcescens*. 
Results and discussion

1 - Results:

The photo of the antibiogram of *E. coli* Petri dish (Photo 1) is presented for comparison with the tests of honey. The results of the antibiograms are summarized in tables 2 and 3.

The antimicrobial activity based on measuring diameters in (mm) of the zones of inhibition of different dilutions of honey samples is shown in (Photos 2, 3, 4, 5 and 6).

The results are summarized in table 4 and illustrated by the graphs 1, 2 and 3.

2 - Discussion:

According to the results of the assessment of the antimicrobial activity, one can notice the following:
- All tested strains are sensitive to the inhibitory action of the three samples of natural honey, with differences from one type to another and one strain to another, which indicates its large antibacterial action specter and antifungal.
- The antibacterial effect of the honey is more important with the undiluted samples, it decreases with successive dilution.
- Sample 2 is the most efficient sample that possesses an antibacterial effect and antifungal on the five tested microbes. Whereas sample 3 is the weakest sample, and it acts only on Gram negative bacteria.
- The natural honey has two types of effects on Gram negative bacteria (*E. coli*, *Serratia marcescens* and *Pseudoonas aeroginosa*): a bactericidal effect on the nearest zones of the impregnated disks of honey and a bacteriostatic effect on the zones relatively far from the disks. In the first case, the microbial growth is inhibited definitely since the microbes are killed, whereas in the second case, a microbial carpet reappears after the inhibition since the microbes are not killed. The effect of the three samples of honey on the five tested microbes is illustrated in the table 5.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>AMC</th>
<th>AN</th>
<th>CN</th>
<th>CZ</th>
<th>CS</th>
<th>IPM</th>
<th>GM</th>
<th>P</th>
<th>SXT</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>20</td>
<td>22</td>
<td>4.0</td>
<td>14</td>
<td>20</td>
<td>32</td>
<td>24</td>
<td>00</td>
<td>24</td>
</tr>
<tr>
<td><em>Serr. marcescens</em></td>
<td>20</td>
<td>24</td>
<td>00</td>
<td>6</td>
<td>00</td>
<td>28</td>
<td>30</td>
<td>00</td>
<td>20</td>
</tr>
<tr>
<td><em>Peudo. aeroginosa</em></td>
<td>20</td>
<td>20</td>
<td>00</td>
<td>18</td>
<td>15</td>
<td>22</td>
<td>20</td>
<td>00</td>
<td>26</td>
</tr>
</tbody>
</table>

The numbers represent the diameters of the inhibition zones in mm.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>AMC</th>
<th>P</th>
<th>CZ</th>
<th>AM</th>
<th>SP</th>
<th>E</th>
<th>GM</th>
<th>CM</th>
<th>AF</th>
<th>SXT</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylo. aureus</em></td>
<td>08</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>24</td>
</tr>
</tbody>
</table>

The numbers represent the diameters of the inhibition zones in mm.
The tested microbial strains have been provided by the laboratory of the hospital “Mohamed Boudiaf of Ouargla”. They were clinical isolates responsible for nosocomial infections and included the following species: Bacteria (*Escherichia coli*, *Serratia marcescens*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*); yeast (*Candida albicans*).

The Gram negative bacteria (*Serratia marcescens*, *E. coli* and *Pseudomonas aeruginosa*) are isolated on the Hektoen medium that inhibits the growth of the Gram positive flora.

The Gram positive bacterium (*Staphylococcus aureus*) is isolated on the Chapman medium, characterized by its high content of NaCl that inhibits Gram negative bacteria.

The Gram negative bacteria are identified and distinguished by the test of the biochemical gallery API 20E (BIO RAD. This system contains twenty wells of biochemical reactions). *Staphylococcus aureus* is identified by test of Coagulase (Bio Rad Pastirextm Strep. 60 Tests. Grouping Streptococci Belonging To Groups A, B, C, D, F, G IVD). These two systems have been provided by the laboratory of microbiology of the hospital “Mohamed Boudiaf of Ouargla”.

The inoculation of the microorganisms is done on the Muller Hinton agar medium in Petri dishes.

The sensitivity to the antibiotics is studied by the antibiogram. The disks of antibiotics have been provided by the laboratory of Microbiology of the hospital “Mohamed Boudiaf of Ouargla” (Bio Rad Susceptibility Disks). The used antibiotics are illustrated in table 1. The disks of antibiotics are deposited in every dish, in contact with the culture. The dishes were incubated for 24 hours at 37°C.

The assessment of the antimicrobial power of the honey is achieved by the technique of diffusion in agar.

The experimentation took place in the laboratory of Bioressources Sahariennes of the University Kasdi Merbah of Ouargla and the laboratory of Microbiology of the hospital “Mohamed Boudiaf of Ouargla /Algeria.”

Sterile disks were impregnated until saturation in different dilutions and deposited in every dish. The Petri dishes are incubated for 24 hours at 37°C. The used dilutions are: A (0%), B (25%), C (50%), D (75%).

The profile of sensitivity of the bacteria to the antibiotics or to the honey was determined by the measure of the diameters of the inhibition zones around the disks on Petri dishes.

Because of the absence of a reading reference that determines threshold of sensitivity, the strain is considered sensitive when the diameter of the inhibition zone is larger than 10 mm, and resistant when the diameter of the inhibition zone is smaller than 10 mm. The sensitivity is intermediate when the diameter is equal to 10 mm.

Table 1. Antibiotics used in the antibiogram.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Symbol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fusidic Acid</td>
<td>FA</td>
</tr>
<tr>
<td>Céfaloxyne</td>
<td>CN</td>
</tr>
<tr>
<td>Imiperem</td>
<td>IPM</td>
</tr>
<tr>
<td>Ampicilin</td>
<td>AM</td>
</tr>
<tr>
<td>Céfalozone</td>
<td>CZ</td>
</tr>
<tr>
<td>Penicillin</td>
<td>P</td>
</tr>
<tr>
<td>Amoxicilin</td>
<td>AMC</td>
</tr>
<tr>
<td>Colistine</td>
<td>CS</td>
</tr>
<tr>
<td>Spiramycine</td>
<td>SP</td>
</tr>
<tr>
<td>Amikacin</td>
<td>AN</td>
</tr>
<tr>
<td>Erythromycine</td>
<td>E</td>
</tr>
<tr>
<td>Sulfamid</td>
<td>SXT</td>
</tr>
<tr>
<td>Clindamycine</td>
<td>CM</td>
</tr>
<tr>
<td>Gentamicine</td>
<td>GM</td>
</tr>
<tr>
<td>Vanomycin</td>
<td>V</td>
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</table>
The obtained results show clearly the impact of natural honey on microbial sensitivity. This inhibitory effect has been noted for the three tested samples, with differences from a sample to another and from a microbial strain to another.

The three samples of natural honey showed two types of antimicrobial effects: a bactericidal effect and a bacteriostatic and fungistatic effect towards the tested strains (bactericidal or bacteriostatic effect or the two together). Whereas the fourth sample (Alshifa) has no antimicrobial effect at all.

Keywords: Algerian honey, Antimicrobial effect, Natural honey, Gram positive and negative bacteria, *Candida albicans*, Alshifa honey.

**Introduction**

The honey has been known and used since the beginning of the history. It was one of the oldest food for human. The Holy Koran present honey as healer from illness (Sourat “El-Nahl” verse 68-69).

The impact of the infective illnesses is always growing in the world. It is generally due to the phenomenon of the antibio-resistance. For this reason, some recent studies are interested in the therapeutic virtues of some natural products, knowing that those products don’t generally cause any side effects. The honey counts among the most coveted of them. Because of its inhibitory and therapeutic properties, numerous studies were interested in the therapeutic properties of honey (Badawy et al. 2004; Baltrusaiyte et al., 2007).

In this perspective, the present work has the evaluation of the therapeutic properties of honey as a main objective, particularly its antimicrobial power towards some pathogenic micro-organisms causing illnesses and serious infections.

The honey can be classified, according to the local origin (Lamballais, 1989; Biri, 1999), the way of obtaining (Caillas, 1974), the method of treatment and the food source. However, all honeys have common properties, but every honey, is characterized by therapeutic properties ascribed to it (Gout, 1989; Huchet et al., 1996).

The composition of honey depends on numerous factors: the species nectar, the nature of soil, the race of the bees and the physiological state of the colony (Prost, 1979). It is constituted of water (16 to 20%); sugars (95 to 99.9% of its dry matter); organic acids (Descottcs, 2004) proteins; mineral salts (0.17%); enzymes and of other constituents (Vitamins, lipids, aromatic substances and colloids and Hydroxy méthyl furfural (H M F) (Whitej and Donner, 1980; Vierling, 2004).

**Materials and Methods**

The present work of the assessment of the antimicrobial effect consists of three samples of multi floral natural honey, harvested in three sites of the Algerian territory namely Jijel, Tizi-Ouzou and Sidi Bel Abbès, and a fourth sample of honey imported from Saudi Arabia (Alshifa).

The three samples of the natural honey are bought from the breeders and are stored under the ambient temperature.

The four honey samples have been diluted directly in the distilled water and tested on five microbial strains (four bacterial strains and a fungus).

For this survey three categories of germs were chosen according to their degree of sensitivity to the antibiotics: very sensitive, fairly sensitive and the resistant strains.
Antimicrobial Effect of The Natural Honey in Algeria

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Abstract

The present work is a contribution to the assessment of the antimicrobial effect of three samples of natural honey obtained from three sites of the Algerian territory (Jijel, Tizi-Ouzou and Sidi Bel Abbès) and a sample of imported honey. The four samples are tested on five microbes of pathogenic nature (and on yeast strains).

Three categories of microbes were chosen for this survey according to their degree of sensitivity to antibiotics, namely: the very sensitive, fairly sensitive and the resistant strains.

This work is based on the assessment of the antimicrobial effect by the technique of diffusion in agar.

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