JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

ANTIMICROBIAL POTENTIAL OF ZIZIPHUS AND EUPHORBIA HONEYS HARVESTED IN SEMI-ARID REGION OF ALGERIA AND THEIR POSSIBLE USE IN SOFT MEDICINE

Haderbache Latifa[1*], *Annou Saada*[1], *Mohammedi Arezki*[2]

Address(es):
[1] Research laboratory in food technology (LRTA), M'hamed Bougara University, Avenue de l'indépendance, Boumerdes 35000, Algeria.
[2] Research laboratory in conservation and valorization of biological resources (VALCORE), M'hamed Bougara University, Avenue de l'indépendance, Boumerdes 35000, Algeria.

*Corresponding author: l.haderbache@univ-boumerdes.dz

doi: 10.15414/jmbfs.2020.9.6.1114-1118

ARTICLE INFO

Received 24. 4. 2019
Revised 21. 1. 2020
Accepted 4. 2. 2020
Published 1. 6. 2020

Regular article

OPEN ACCESS

ABSTRACT

Two different botanical origin honey types (*Ziziphus lotus* and *Euphorbia bupleuroides*) from semi-arid regions in Algeria consisting of twelve samples were tested for their antimicrobial efficiency. Global assessment of antimicrobial activity was made by wells method on integer samples and by turbidity test to locate fraction responsible of this activity. Honeys have been tested against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*. Fungal strain was resistant to all honeys at all concentrations, whereas *E. coli* and *S. aureus* were sensitive presenting minimum inhibition concentrations (MIC) between 10 and 50%. Euphorbia honeys appeared to be more active. The fractionation shows that volatile fraction can have great antimicrobial effect, followed by the acidic one. Correlations reveal good relation between inhibitory effect, free acidity and polyphenols. These facts show large possibilities for honey use in soft medicine against some bacterial infections.

Keywords: Honey; *Ziziphus lotus*; *Euphorbia bupleuroides*; Antimicrobial activity; soft medicine; Algeria

INTRODUCTION

The honey efficiency is well known, since long centuries, against the infectious illnesses provoked by several strains such as: *Staphylococcus aureus*, *Salmonella typhi*, *Campylobacter*, *Escherichia coli*, *Listeria monocytogenese* **(Brasson and Gobler, 2008, Lin *et al.*, 2009, Zahoor *et al.*, 2014)**. It has been signaled to be active on more than 60 bacteria species (aerobes and anaerobes, Gram positive and Gram negative), a fungicidal effect has also been signaled on some yeast and *Aspergillus* and *Penicillium* species **(Molan, 1992a)**, as well as on all common dermatophytes **(Brady *et al.*, 1997)**.
Several intrinsic factors participate to this anti-microbial activity; as osmolarity, acidity, viscosity, H_2O_2, non-peroxides inhibines as lyzozymes, flavonoides, acidic phenols, and aromatic acids **(Bogdanov and Blumer, 2001)**. Bogdanov **(Bogdanov, 1997b)** suggests that non-peroxide inhibines depend on the botanical origin of honey, but can come from the bee herself, these molecules have been classified in four groups: neutral, acidic, basic and volatile **(Bogdanov, 1997b)**; however, it is the acidic fraction that had the most marked activity for studied blossom and honeydew honeys **(Bogdanov *et al.*, 2008)**. This activity depends as well on conditioning methods and storage conditions **(Salomon, 2010)**.
Several studies made relation between this AMA (anti-microbial activity) and the floral origin of some honeys (Conifers honeydew, *Castanea sativa* and Manuka honeys, etc.). The relation of this activity with the dark color has always been signaled **(Dustman, 1979)**, but the variations are so big that it is very difficult to make some associations, unless a large number of honey samples are investigated with established floral origin, on several harvests, of several years **(Molan, 1992b, Moniruzzaman *et al.*, 2013)**. Other authors correlated AMA with phenolic compounds and especially flavonoids**(Je-Ruei *et al.*, 2013, Escriche *et al.*, 2014)** or with proteins present in the digestive tube of bees **(Mundo *et al.*, 2004)**.
Researches about antimicrobial activity of honeys, currently have a very big interest because they are becoming used more frequently in the medical domain where medicines do not manage to achieve hoped results, as in the case of the pathogenic strains resistance to various medicinal treatments, we give for example the case of *S. aureus* and *Helicobacter pylori* isolated from gastric ulcers or infected burns **(Al Somal *et al.*, 1994, Cooper, 2001, Cooper *et al.*, 2002b, Willix *et al.*, 1992, Majtan *et al.*, 2013)**, or *Streptococcus mutans* and *Lactobacillus acidophilus* incriminated in the occurrence of tooth decays **(Patel *et al.*, 2011, Jaganathan, 2011)**.
The new interest of Algerian consumer in honeys from semi-arid regions and for soft medicines is pushing scientist to make more investigations on this scope.
Ziziphus lotus is Rhamnaceae shrub called "Sedra, N'beg, or Azar Djerdjer", it is widespread in our arid and semi-arid regions, akin to "sidr" in Middle East countries **(Baba Aissa, 1999, Al Khalifa and Al Arify, 1999, Mekious *et al.*, 2015, Zerrouk *et al.*, 2017)**. Honey from this species was found to be different of common blossom honeys, with a very large shelf life and with particular richness in enzymes, polyphenols and antiradicalar activity **(Haderbache *et al.*, 2013)**. Whereas *Euphorbia bupleuroides L.* is an euphorbiaceae species called "Lebayna, Helayba, Halib elDiba, or tanahout", it is one of native Algerian plants, the most visited by bees among 51 euphorbia species known as toxic plants **(Quezel and Médail, 2003)**. Its honey contains very high amount of flavonoids **(Haderbache *et al.*, 2013)**. The same interest was showed for these two botanical families in morocco **(Chakir *et al.*, 2011)**.
The aim of this work is to quantify their antimicrobial activity against three bacterial strains (*E. coli* / G -; *S. aureus*/G+ and *P. aeruginosa* /G -) and one fungal strain (*C. albicans*), often incriminated in human pathologies. Honeys fractionation in four groups (volatile, neutral, acidic and basic) will enable to identify the nature of active molecules. Until this day no work has been done on the antimicrobial activity of such kinds of honeys, although they are widely used in Maghreb traditional therapy (Sedative and immune-modulatory virtues; Light laxative effect; Hypotensive and anti-diabetic potential; Anti-inflammatory; Emollient and bechic).

MATERIAL AND METHODS

Botanical and biological material

The experimentation involved twelve samples of honey from two botanical origins namely *Ziziphus lotus* and *Euphorbia bupleuroides*, provided by professional apiarists. Samples were harvested in different regions (El Bayadh, Aflou, Laghouat, Ain safra) and on different years (2009 to 2012); The chosen acceptance criteria were: Fresh harvested honeys, known harvesting area, *Ziziphus lotus* and *Euphorbia buploroïdes* supposed honey kind, organoleptic properties reminding the mentioned floral source, sufficient quantity for all analysis and the use of virgin honey combs just before harvest to avoid residue transfer if any. Chosen strains are generally used in testing antimicrobial honey effects: *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923),

Pseudomonas aeruginosa (ATCC 9027) and *Candida albicans* (ATCC 10231) were reference strains, procured from research center & development of SAIDAL (Algerian pharmaceutic laboratory).

Pollinic and physic-chemical analyzes

The floral origin has been confirmed by pollinic analysis based on harmonized methods of melissopalynology **(Von der Ohe *et al.*, 2004)**, Since Ziziphus and Euphorbia honeys are normally represented in pollens, the inclusion criteria was a percentage of the main pollen above 45 % based on 1000-1200 counted and identified pollens. Total phenols and flavonoids have been achieved according to method described by Meda, Lamien **(Meda *et al.*, 2005)**. For polyphenols, the quantification is based on the reduction of phosphotungstic and green color phosphomolibdic mixture in molybdenum tungsten oxide of blue color, tests are made on 10% honey solution and coloration intensity is measured at 760 nm. Lectures are reported on Gallic acid standard curve, and results expressed in Gallic acid Equivalent by kg of honey (mg EGA/kg). Whereas, flavonoids measurement is based on their complexation by aluminum trichlorure. The absorbance is read at 510 nm and the quantification made with Quercitine standard curve, results are expressed in Quercitine Equivalent by kg of honey (mg EQ/kg). Free acidity (FA) is measured by titration until pH 8.3 and diastase activity (DN) by following-up the deterioration of the soluble starch through time (Official methods of the IHC)**(Bogdanov, 1997a, Kumar et al., 2018)**.

Global antimicrobial activity

Global assessment of antimicrobial activity was made by wells method: it has been achieved by gelose diffusion technique **(Marghitas *et al.*, 2009)**. Honeys solutions were prepared in normal saline water (0.85% of salt), in different concentrations: 10%, 25%, 50% and 75%. The sensitivity profile is determined by inhibition diameters measurements and MIC (Minimum inhibition concentration) is determined for each honey type. The sensitivity approach is inspired from the works of Meena and sethi **(Meena and sethi, 1994)** and Aboul Ela, El sher **(Aboul Ela *et al.*, 1996)**, who qualify a strain as: Non inhibitory so Ø < 7mm, Slightly inhibitory if 7mm <Ø <13mm, Moderately inhibitory if 13mm <Ø <25mm and Greatly inhibitory so Ø >25mm.
Initially, before the antimicrobial test, strains are revivified by stripes dispersion on gelose surface, previously melted then cooled (nourishing gelose for bacteria and Sabouraud or OGA for fungous strains), followed by an incubation of 37°C/24 h for bacteria and 25°C/48 h to 5 days for yeasts. Strains purity is verified by microscopic test (Gram, shape, gathering and mobility) and standard microbial suspensions are prepared in sterile physiological water to get absorbance (at 520 nm) between 0.22 and 0.32 for bacteria and between 2 and 3.8 for yeasts that correspond to a cell concentration of 10^6 -10^8 CFU/ml **(Leclerc *et al.*, 1993)**.

Evaluation of antimicrobial activity of volatile, neutral, basic and acidic fractions

Honeys are first heated at 70°C during 1h **(Bogdanov and Blumer, 2001)** to destroy glucose-oxydase responsible of peroxide production, this precaution is taken to avoid mistakes in non-peroxide AMA evaluation. Fractionation and turbidity test are achieved according to the method described by **Bogdanov (1997b)**; the volatile fraction is separated by rotavapory on 20% phosphate buffered honey solution, the neutral fraction by passage on a meadow full activated C8 column, the basic fraction by passage on a strong HCl activated cation exchange resin and finally the acidic fraction on a strong NaOH activated exchange resin, to avoid the influence of concentration and acidity, we readjust the fractions to initial Brix by adding or removing water (under vacuum) and pH to the initial value pHi. A SHIMADZU UV-1800 spectrophotometer is used for measuring the absorbance (A) at 520 nm. The sealed glass tubes of 1cm of diameter are used and placed directly on the optic ride of the device after incubation and correct mixing.
The relative inhibition percentage of a fraction is not directly measured but deduced from remaining fractions after each step and calculated by these formulas:

$$\Delta A = A2 - A1 \quad \text{(Eq. 1)}$$
$$G[\%] = 100 * \Delta A / A2 \quad \text{(Eq. 2)}$$
$$I[\%] = 100 - G \quad \text{(Eq. 3)}$$
$$If = If(n+1) - If(n-1) \quad \text{(Eq. 4)}$$

Where: A is the absorbance, A2 the absorbance after incubation, A1 absorbance before incubation, G growth rate, I inhibition rate, If inhibition rate of a fraction, If (n+1) inhibition rate in the step after, If(n-1) inhibition rate in the step before.

Evaluation of osmolarity, pH and H_2O_2 contribution in antimicrobial activity

To evaluate osmolarity effect, artificial honeys are prepared based on global sugar composition and rotatory characteristics of each type of honey by mixing different proportions of fructose, glucose, maltose, KCl and water. There AMA is measured by turbidity test (in liquid medium).
The peroxide effect is estimated by comparing heated (70°C/1h) and non-heated honey treated the same in a solution incubated at 37°C for 1h. Buffered honey solutions (pH7, pH9 and pH3) where compared to water treated honey to show pH influence. For all experiences the solution concentration was the same (50%) to avoid concentration effect.
Bactericidal and bacteriostatic effect
This test is based on a comparison between the absorbance of a yet inoculated and incubated 50% honey solution like used in total AMA and the same solution added by physiological serum and re-incubated for 24h (G+) or 48h (G-) at 37°C. Bactericidal effect is noted when there are no significant changes in A (<2%) and bacteriostatic one when there is an increase of bacterial growth (>30%). The Concentration is preserved.

Statistical analyzes

The comparison between groups averages has been achieved by the t test (coefficient of Pearson to $p < 0.05$) by statistical software SPSS17.0.

RESULTS AND DISCUSSION

Total polyphenols content of studied samples oscillates between 474 and 898 mg EGA/kg, while Flavonoïds ranged between 29 and 3213 mg EQ/kg. Flavonoïds/polyphenols ratio indicates that Ziziphus honeys are poorer in flavonoïds but richer in total phenols than Euphorbia ones.
Ziziphus samples show weak free acidities of 120±21 meq/kg, an important quality that encourage a slow ageing, whereas euphorbia honeys are more acidic with an average of 208±57 meq/kg. Diastase activity, being very important in the assessment of honeys health effects and freshness, reveals that Ziziphus honeys have more homogeneous DN values. There were no statistical differences between Ziziphus and Euphorbia groups for all parameters except for free acidity.

Table 1 summarize studied parameters generally related to antimicrobial effect (Total polyphenols, poly.; flavonoids, flav. ; free acidity, FA.; and Diastase activity, DN).

Table 1 Polyphenols, flavonoids, free acidity and diastase number results, sorted by botanical origin.

Botanical origin group	Statistic	Poly. [mgEGA·kg^{-1}]	Flav. [mg EQ·kg^{-1}]	Flav/ poly [%]	FA [meq·kg^{-1}]	DN [U shade]
Euphobia	Mean± SD	552 ± 56^a	217 ±113^a	409±229	208 ± 57^b	20.2± 10.8^a
Honeys (n=6)	Min -max	474- 639	59- 313	93- 665	160-320	9.2- 38.6
	α (%)	10	52	56	27	53
Ziziphus	Mean± SD	617 ± 182^a	134±79^a	223±104	120± 21^a	23.2± 7.4^a
Honeys (n=5)	Min - Max	488- 898	29-250	41- 298	100- 150	10.5- 30
	α (%)	30	59	46	18	32

a,b same letters indicate that there is no significant differences at $p<0.05$ (mean comparison test), SD: standard deviation, poly: total polyphenols, Flav: total flavonoids, flav/poly: flavonoids polyphenols ratio, FA: free acidity, DN: diastase number, α (%): homogeneity coefficient.

Global antimicrobial activity

The study of global antimicrobial activities (table 2), show that *C. albicans* is resistant to Ziziphus and Euphorbia honeys at all concentrations for all samples. This result is in accordance with **Zaghloul *et al.* (2001)** who worked on Egyptian honeys. This fact it is not directly related to floral origin but probably due to the greatest tolerance of yeasts and fungal strains to concentrated medium which act only as bacteriostatic **(Molan, 1992b)**, but it does not mean that it is true for all Candida strains and all honeys, many other authors demonstrated the sensitivity of different strains of Candida to Iranian and Indian honeys respectively with MIC about 24 % **(Revathy and Banerji, 1980, Khosvari *et al.*, 2008)**.

Table 2 Inhibition Diameters [mm] of Ziziphus and Euphorbia honeys (based on solution of 50%).

n=10	E. coli	S. aureus	P. aerugi-nosa	C. albicans
Glob Mean.	21.4± 5.6	20.5± 6.0	18.7± 5.9	0.00
Mean. E (n=5)	23.8± 3.3[b]	24.6± 1.7[b]	17.9± 2.9[a]	Total Mean E: 22.1± 3.7
Mean. Z (n=5)	22.9± 3.4[b]	21.6± 1.6[b]	23.1± 0.9[b]	Total Mean Z: 21.9± 1.1

a,b same letters indicate that there is no significant differences at p<0.05; Z: Ziziphus; E: Euphorbia.

In parallel bacterial strains shows different sensitivity levels according to samples, but in general there were important inhibition of *E. coli* and *S. aureus* with average inhibition diameters of 21.4±5.6 and 20.5±6.0 mm respectively.
Euphorbia honeys shows moderate to strong AMA on *S. aureus* followed by *E. coli* and *P. aeruginosa*, whereas, Ziziphus honeys was the strongest against *P. aeruginosa* first, *E. coli*, then *S. aureus*; these results corroborate with investigations made **Halawani and Shohayeb (2011)** on Saudi Ziziphus "Shaoka and Sidr" honeys. It drives us to suppose that Euphorbia honeys are more efficient against Gram positive while Ziziphus against Gram negative strains.
Averages comparison, between both honey groups, by the t test, shows that they have comparable effects on *E. coli* and *S. aureus* but different for *P. aeruginosa* (Table 2). Generally, the AMA was more variable for E. coli than for the other strains.
The MIC for Euphorbia honeys oscillate between 10 and 25 % with an average of 16 % for *E. coli*, between 10 and 25 % for S. aureus with an average of 20 % and between 10 and 50 % for *P. aeruginosa* with an average of 32 %. For Ziziphus the MIC averages are respectively of 16 %, 46 % and 32 % for cited strains in this order. These observations drive us to say that Euphorbia honeys have better performances (dose/effect) against *S. aureus*.

Antimicrobial activity of honey fractions

The results of the fractionation test are represented in table 3. The AMA of whole honeys compared to an artificial one show that the osmotic effect can represent 10 to 36% of the global antimicrobial effect, with a range between 50 and 80%, depending on strains sensitivity to medium concentration, a fact already reported by **Bogdanov (1984)**. The antimicrobial effect within the same group changes from one year to another, it is probably due to the contribution of different plants nectars, knowing that every harvest honey is considered to be unique.

Table 3 Relative Inhibition Percentage of the Fractionated Honey Samples.

Honeys	Relative inhibition of different fractions [%]											
	Volatil			Neutral			Basic			Acidic		
	St.	Ps.	Ec.	St.	Ps.	Ec.	St.	Ps.	Ec.	St.	Ps.	Ec.
E2009 (n=2)	2	57	3	90	3	1	1	4	3	7	36	93
E2012 (n=2)	86	84	90	3	2	0	6	2	8	14	17	3
Z2010 (n=2)	5	34	5	2	3	90	90	3	3	3	60	2
Z2012 (n=2)	90	80	74	4	2	2	5	13	2	1	5	22
Mean E (n=4)	44	71	47	47	3	1	4	3	6	11	27	48
Mean Z (n=4)	48	57	40	3	3	46	48	8	3	2	33	12

St. : S. aureus ; Ps. : P. aeruginosa ; Ec. : E. coli ; E : Euphorbia honey ; Z : Ziziphus honey

The global antimicrobial effect survey of the different fractions show that the biggest part of AMA comes from the volatile fraction (> 51 %); followed by the acidic one (>22 %) for euphorbia as well as for Ziziphus honeys. It comes to reinforce the observations of **Bogdanov (1997b)** who signaled important activity of the acidic fraction in some blossom honeys but he also affirms that it is very variable and can also be located in other fractions, for colza honey, it is the neutral fraction, whereas for honeydews it is rather in the basic fraction.
The studied microbial strains show clear differences in their reaction to honeys, *S. aureus* was sensitive to all fractions (volatile, basic and neutral) and slightly sensitive to the acidic one; *P. aeruginosa* was especially sensitive to volatile and acidic fractions, *E. coli* showed a comparable sensitivity for volatile and acidic, followed by neutral fraction but had no reaction to the basic one.

Interrelationship between AMA and the Rates of Polyphenols, Flavonoids, FA and DN

Since we noticed that the two types of honey have comparable effects on *E. coli*, which is a highly sensitive strain to bee products, we made this test to better identify the nature of active molecules. The survey of the existence of a global interrelationship between the AMA of *E. coli* on a honey solution of 75 % (table 4), reveal that no statistically meaningful relation exists between this activity and the studied parameters, but the survey of honey groups apart shows that a strong relation exists between FA and AMA in Ziziphus honeys, proving that the active antimicrobial molecules are free acids but not polyphenols or flavonoids, whereas, for Euphorbia honeys Pearson criterion shows a slight relation with total polyphenols proving the role of phenolic acids but not of the flavonoids in Euphorbia honeys.

Table 4 Interrelationships between the AMA and Composition Parameters.

	AMA	Poly.	Flav.	Flav./ poly.	FA	DN
All samples	Cor.	-.087	-.151	-.157	.215	.089
	P. Sig.	.789	.640	.626	.502	.783
E.	Cor.	.509	-0.189	-0.301	.160	.151
	P. Sig.	.244	.684	.511	.732	.746
Z.	Cor.	-0.261	-0.253	-0.139	* .809	.032
	P. Sig.	.618	.629	.793	.050	.952

*. The correlation is significant at p0.05 level (bilateral).
AMA: antimicrobial activity; Poly: polyphenols; Flav. Flavonoids; FA: free acidity; DN: diastase number. Flav./Poly.: ratio.P. Sig. :Pearson significance; Cor.: Correlation coefficient. E: euphorbia; z: ziziphus.

Contribution of osmolarity, pH, H_2O_2 in antimicrobial activity

In this section, the global activity of the studied honeys is made with a turbidity test, to identify the peroxide, osmolarity and pH contribution in total honey AMA, as well as its nature (bactericidal or bacteriostatic).

According to the results shown in table 5, we can classify used strains from the most sensitive to least sensitive as follows: *E. coli*, *S. aureus* then *P. aeruginosa*. All strains show the greatest sensitivity to non-peroxide fraction, even if we know that *E. coli* and *S. aureus* are "catalase +" and are capable to inhibit the H_2O_2 produced in diluted honey by glucose oxidase enzyme, but according to

Bogdanov and Blumer (2001), mature honeys only contain weak quantities of peroxide, weakly inhibiting bacterial growth and affirm that the mechanism of its production is especially efficient in nectar transformation step into honey.

Table 5 pH, peroxide, non peroxyde and osmolarity Contribution in antimicrobial activity of Ziziphus and Euphorbia honeys.

Relative inhibition [%]	*Total AMA*	*Non peroxide effect*	*Peroxide effect*	*Osmolarity*	*pH 3*	*pH 7*	*pH 9*	*Bactericidal or bacteriostatic effect*
				S. aureus				
Z.	100[b]	52.05[a]	47.95[b]	18.55[a]	100[c]	76.29[a]	98.70[b]	**bactericidal**
E.	94.46[a]	71.35[b]	23.11[a]	76.63[b]	100[c]	99.70[b]	100[c]	**bacteriostatic**
P. aeruginosa								
Z.	92.97[a]	81.75[b]	11.22[a]	13.72[a]	100[c]	59.28[a]	97.66[c]	**bactericidal**
E.	100[b]	35.71[a]	64.29[b]	51.69[b]	91.62[b]	100[b]	100[c]	**bactericidal**
E. coli								
Z.	100[a]	66.10[a]	33.90[a]	17.00[a]	100[c]	56.25[a]	100[c]	**bactericidal**
E.	100[a]	60.08[a]	39.92[a]	41.60[b]	100[c]	69.20[b]	100[c]	**bactericidal**

a,b,c Different letters mean statistical significant differences ($p<0.05$) with Duncan test. Z. : ziziphus ; E. : euphorbia (classification by botanical origin)

Although *P. aeruginosa* is the least sensitive strain, but honeys action takes a fundamental importance when we know that more of 50 *P. aeruginosa* strains are incriminated in ear infections, diabetic foot ulcers and infected cutaneous burns, as confirmed by **Mullai and Menon (2005), (Mullai and Menon, 2007)** in their work on 152 Pseudomonas isolates from hospitalized patients; and **Cooper *et al.* (2002a)** in their survey on alternative treatments of the infected burns.

Osmolarity effect proves to be important in most cases, for *E. coli* it represents about 29.3% of the total AMA, this last rises to 32.7% for *P. aeruginosa* and 47.6% for *S. aureus*, which is not negligible.

The antimicrobial effect appears to be maximal in acidic or basic environment. We notice that Ziziphus honeys show a bactericidal action for all studied strains, but this activity is very sensitive to pH changes, it is maximal mainly in an acidic environment (pH 3).

Euphorbia honeys are bactericidal for *E. coli* and *P. aeruginosa* (Gram -) but only bacteriostatic for *S. aureus* (Gram +), its action is divided between peroxides and non-peroxide fractions. The particularity of these honeys is that the osmolarity plays a major role with an average of 56.6% of total AMA, but remains unchanged at different medium pH.

It is necessary to signal that strain behaviors are complex, according to a Bulgarian survey **(Khristov and Mladenov, 1961)** working on 50% honeys solutions and 12 bacterial species, it was Gram+ strains that are first killed (after 1h exhibition) with a complete bactericidal action between 3 and 24h, whereas Gram – species are more resistant and begin to die after 4 to 6h, with a complete extermination in approximately 48h.

CONCLUSION

The study of the antimicrobial potentialities of both honey types (*Z. lotus* and *E. bupleuroides*) revealed good inhibitory effect on the studied bacterial strains (*E. coli*, *S. aureus* and *P. aeruginosa*) but no antifungal effect tested on *C. albicans*; Leading to weak uses in Candida fungal diseases.

Euphorbia honeys presented better performances (dose/effect), but remain especially active thanks to its osmolarity and phenolic acids and show only bacteriostatic effect on *S. aureus* (Gram positive). Ziziphus honeys show total bactericidal effect at pH 3 especially against G- strains. A big heterogeneity was observed within the same honey group, indicating that the antimicrobial effect of a honey is tributary of an important number of factors (visited flora, pedoclimatic conditions, harvest year, etc.). Finally, we can affirm today that these special honeys present interesting possibilities for use in soft medicine against these bacterial infections and can advantageously be used in some human pathologies.

Acknowledgments: The authors thank the staff of CRD-SAIDAL, the fraud repression laboratory (Al Harrach), the pedagogic laboratories of department of food technology (DTA) and the all the staff of food research laboratory (LRTA). This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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