DOI: 10.7251/VETJ1401002D **UDK 616-022.5:579.842.11**

D. Dinkov,[1] D. Stratev,[1] R. Balkanska[2]

Original paper

IN VITRO ANTIBACTERIAL ACTIVITY OF ROYAL GELLY AGAINST PATHOGEN ESCHERICHIA COLI

Abstract

In the study was used a pathogen strain of *E. coli*, caused septicemia for ducks, resistant for different antibacterial agents: Amoxicillin, Lincospectin, Chloramphenicol, Doxycyclin, Enrofloxacin, Sulfonamides and Trimetoprim. Bacterial suspension of *E. coli* icontaminated each from test solutions in TSB of royal jelly (n=6), mixes of royal jelly and rape honey, and independent used rape honey (10–45% v/v). Have in mind exactly counts of colonies before and after incubation from each of test substances was calculated the percent of reduction up to 30 min, and after incubation (24 h and 48 h). In almost all concentrations of royal jelly (10–45 v/v), were found total inhibition effect to *E. coli*. Mixes from royal jelly and rape honey (1:100) possessed a higher antibacterial effect, compared with independent use of rape honey. Up to 45% (v/v), rape honey does not cause total antibacterial reduction. Royal jelly and mixes from royal jelly and rape honey have potential as alternative therapeutics agents against resistant for antibiotics pathogen strains of *E. coli*.

Key words: royal jelly, rape honey, antibacterial, *E. coli*.

[1] Trakia University, Faculty of Veterinary Medicine, 6000 Stara Zagora, Bulgaria.
Универзитет у Тракији, Факултет ветеринарске медицине, Стара Загора, Бугарска.

[2] Department of Special Branches – Bees, Institute of Animal Science, Kostinbrod, Bulgaria.
Департман за специјалистичке студије – пчеле, Институт за науку о животињама, Костинброд, Бугарска.
Е-пошта кореспондентног аутора/ E-mail of the corresponding author: dinkodinkov@abv.bg

Д. Динков,[1] Д. Стратев,[1] Р. Балканска[2]

Оригинални рад

IN VITRO АНТИБАКТЕРИЈСКА АКТИВНОСТ МАТИЧНОГ МЛЕЧА ПРОТИВ ПАТОГЕНА ESCHERICHIA COLI

Кратак садржај

У студији је коришћен патогени сој *E. coli*, који је изазвао септикемију патака, и који је отпоран на различите антибактеријске агенсе: амоксицилин, линкоспектин, хлорамфеникол, доксициклин, енрофлоксацин, сулфонамиде и триметоприм. Бактеријским суспензијама *E. coli* контаминиране су све тест солуције ТСБ (триптон соја бујона) матичног млеча (н = 6), мешавине млеча и репиног меда, као и репиног меда (10%–45% в/в). Имајући на уму тачан број колонија пре и после инкубације од сваке тест супстанције је израчунат проценат редукције до 30 мин., и после инкубације (24 часа и 48 часова). У скоро свим концентрацијама млеча (10%–45% в/в) пронађени су тотални инхибициони ефекти на раст *E. coli*. Мешавина млеча и репиног меда (1: 100) поседовала је веће антибактеријско дејство у поређењу са независном употребом репиног меда. До 45% (в/в), репин мед не изазива смањење укупне антибактеријске редукције. Млеч и мешавина млеча и репиног меда имају потенцијал као алтернативни терапеутски агенси против *E. coli,* која је резистентна на антибиотике.

Кључне речи: матични млеч, репица мед, антибактеријски, *E. coli*.

INTRODUCTION/ УВОД

The antibacterial action of honey was reported for the first time in 1892 (Van Ketel, 1892). The antibacterial effect of honey, mostly against gram-positive bacteria, is very well documented (Molan, 1992a; 1992b; Bogdanov, 1997; Molan, 1997).

The antimicrobial activity of honey is attributed largely to osmolarity, pH, hydrogen peroxide production and the presence of other phytochemical components. In vivo, such activity may occur due to a synergistic relationship between any of these components rather than a single entity (Mavric et al., 2008). It was found, that the honey acids and low pH exert the main antibacterial factors (Bogdanov, 1997).

Pathogen strains of *E. coli* are often case agents for intestinal infections

for animals and humans. According spectrophotometric studies, the MIC_{100} (the lowest concentration of test material which results in 100% inhibition of growth) value for *E. coli* to manuka honey was 12,5% (v/v), (Patton et al., 2006).

Some authors found that growth of *E. coli* was completely inhibited by 30–100% honey concentrations (Noori and Al-Waili, 2004). In other study antibacterial activity of 13 types honeys were tested at four concentrations (10%, 5%, 2,5%, and 1% w/v), against *E. coli*. It was found that several honeys can inhibit *E. coli* and may have potential as therapeutic honeys (Wilkinson and Cavanagh, 2005). As the potential role for honey as a topical agent to manage surgical site or infections is increasingly acknowledged and other types of honeys need to be assessed and evaluated (Gethin and Cowman, 2008).

The hypopharyngeal glands of the honeybee (*Apis mellifera L.*) produces royal jelly (RJ) that is essential to feed and raise broods and queens (Li et al., 2010). RJ may cause allergic reactions in humans, asthma, to even fatal anaphylaxis, thus this product remains unaffordable in most countries (Leung et al., 1997; Lombardi et al., 1998; Takahama and Shimazu, 2006).

From the other hand, it was found more positive effect of RJ: imunostimulating, activating vegetative and central neural systems etc. The main RJ acid, 10-hydroxy-2-decenoic acid (10-HDA), is known to have high antibiotic effect (Blum et al., 1959; Melliou and Chinou, 2005).

Research suggests that the 10-HDA found in RJ may inhibit the vascularization of tumors (Izuta et al., 2007).

Recently, it was found specific antibacterial peptide Royalisin in RJ, displayed certain antibacterial activities against Gram-positive bacteria (Shen et al., 2010).

It was found a few studies about antibacterial effect of RJ to Gram-negative microorganisms (Shirzad et al., 2007). *E. coli* have been used to determine the minimum inhibitory concentration (MIC) of a freshly reaped RJ. The MIC of RJ against *E. coli* was 2% (v/v), (Boukraa et al., 2009).

In available references not found studies for effect of royal jelly for resistant for antibacterial agents strains of *E. coli*, case agents for septicemia for animals.

To avoid acid taste and alergic reactions after consumption of royal jelly many producers recommend mixing of this product with honey, mainly in proportin 1:100. In available references not found studies about exactly degrees of antibacterial activity from this mix for pathogen for animals strains of *E.coli*..

In many studies for detection of antibacterial activities of honey was used agar well diffusion method (Al Jabri et al., 2005).

Usefulness of agar well diffusion method must be interpreted with clear criterions. To the moment some authors used measuring the clear zone around the well, and expressed in phenol concentration possessing equivalent activity (Baltrusaityte et al., 2007).

On the other side standards for methods required for microbiological testing of foods for pathogens used incubation that make objective detection of pathogens.

Only alive cells of microorganisms could survive and made colonies. This is the principle, required for microbiological testing of foods in Europe (Commission regulation (EC) No 1441/2007).

This arguments were used from as to investigate a new principle for antibacterial activity testing for bee products (Stratev et al., 2012).

In our study we use the rape honey because of findings, that the antimicrobial activity of rape honey is higher, similar to that of honeydew honey (Bogdanov, 1997) – a little-known fact, which would be useful of consumers. The potential for production of the additionally processed finely crystallised rape honey, which is especially attractive for many consumers, is also substantial.

Thus, the aims of our study were to found by microbiological method the Real Bactericidal Concentration (RBC) or 100% inhibition (0 CFU/ml), of rape honey, mixes from royal jelly with rape honey (10,20,30 and 45%v/v), and independent used royal gelly, to pathogen strain of *E. coli*, caused septicemia for ducks, resistant for different antibacterial agents.

MATERIALS AND METHODS/ МАТЕРИЈАЛИ И МЕТОДЕ

1. Test substances

The tested rape bee honey samples were obtained from beekeepers owning many hives (from 50 to 210), immediately after the flowering of rape (the centrifugation of honey was performed in June) in different regions of the Stara Zagora district, Bulgaria. During the honey collection period, bees were not supplemented with carbohydrate syrups or treated with antimicrobial drugs. Until the analysis, samples were kept at refrigerator conditions (0–4°C).

Water content, pH, free acidity, electrical conductivity, diastase and invertase activity, specific optical activity and hydroxymethylfurfurol (HMF) content were assayed as per the harmonized methods of the European honey commission (Bogdanov et al., 1997). The botanical origin of the samples was established by their melissopalynological, organoleptic, physical and chemical characteristics (von der Ohe et al., 2004; Oddo et al., 2004)

All data referring to physical and chemical parameters of rape honey were statistically processed by the Student's t-test and presented as mean and standard deviation (SD) (table 2).

Used in the study royal jelly (n=6), was pipette directly from queens cells. The following parameters of samples were determined: sugars (fructose, glucose, sucrose by HPLC after Sesta (2006); proteins by Folin-Ciocalteu reagent; water content by refractometer; dry matter of the sample was obtained by subtracting the water content from 100; pH values – potentiometricaly by pH meter model Mi 150 (1% water solution of royal jelly); total acidity by titration with 0.1 n NaOH according to ON 2576693-84 (ON 2576693-84. Fresh and lyophilized royal jelly (Bg); electrical conductivity of 1% water solution of royal jelly by conductimeter (Bogdanov et al., 1997) (table 3).

From some authors only storage of royal jelly in frozen state prevents

decomposition of biologically active proteins and thus royal jelly should be frozen as soon as it is harvested (Li et al., 2007). For our experiments royal jelly was stored prior to analyze in the dark bottle in frozen conditions (-20°C).

Immediately before conducting microbiological assays in order to aid pipetting during preparation of diluted honey solutions, all test substances were adjusted to 40°C in a water bath. Solutions containing 10, 20, 30, 40 and 45% (v/v) from each of test substances were prepared in sterile TSB. To prevent photodegradation of glucose oxidase, conected with antimicrobial activity in honey (Bogdanov, 1997), all honey samples and mix from royal jelly and rape honey, were stored in the dark and dilutions were prepared immediately prior to testing (Sherlock et al., 2010).

2. Microbiological survey

In the study was used a pathogen strain of *E. coli*, caused septicemia for ducks, resistant for different antibacterial agents: Amoxicillin, Lincospectin, Chloramphenicol, Doxycyclin, Enrofloxacin, Sulfonamides and Trimetoprim (NCCLS, 2002).

Bacterial suspension was with density 0,5 McFarland and be prepared from 24 h bacterial culture of *E. coli*, by taking 3–4 colonies and dissolving in 0,85% sterile saline solution. Received bacterial suspension was with approximate concentration $1,5x10^8$ CFU/ml. From suspension were prepared tenfold dilutions with sterile Triptic Soy Broth (TSB), (Merck), at to 10^7. For detection of exactly count of E.coli from each of dilutions (1 ml) was made cultivation with ChromoCult ® TBX Agar (Merck), followed by incubation with 37°C for 24 h.

Used quantity of 0,6 ml for diluted test suspension of *E. coli* in each from test solutions in TSB (11.4 ml), maintain the mean final concentration of log 1,9–2,11 CFU/ml (table 3).

This microbiological survey was done up to 30 min after inoculation (without incubation), and after 24 h and 48 h incubation at 37°C from all dilutions in TSB of contaminated with bacterial suspense test substances. With a view to calculate percent of reduction we adopt as 100% the initial (up to 30 min) and bacterial count after 24 h incubation in positive controls. Have in mind exactly counts of colonies before and after incubation, from each of test substances was calculated the percent of reduction (table 1).

RESULTS/ РЕЗУЛТАТИ

After 30 min before incubation of samples were found log 0,3–0,78 CFU/ml of E.coli. This is below data for positive controls (log 1,9–2,11 CFU/ml) (table 1).

In almost all concentrations of royal jelly (10-45 v/v) were found total inhibition effect to E.coli. Only for sample 2 in 10% (v/v) after 24 h and 48 incubation was found higher content of E.coli. This could be connected with content of sugars in this sample. Five from samples of royal jelly have a higher content of glucose, but only sample 2 a higher content of fructose (table 2). A higher contamination with E.coli in this case also could explain a final multiplication in 10% concentration of royal jelly (log > 8 and 7,4 CFU/ml) (table 1).

Table 1. *Antibacterial activity of royal jelly (J), mix from royal jelly and rape honey (1:100), (JH) and rape honey (H), against resistant pathogen strain of E. coli.*

Test substance	% (v/v)	30 min		24 h		48 h		Test substance	% (v/v)	30 min		24 h		48 h	
		log CFU/ml	reduc-tion,%	log CFU/ml	reduc-tion,%	log CFU/ml	reduc-tion,%			log CFU/ml	reduc-tion,%	log CFU/ml	reduc-tion%	log CFU/ml	reduc-tion,%
J-1	10	0,3	84,2	-	100	-	100	J-4	10	0,48	75,1	-	100	-	100
	20	0,7	63,2	-	100	-	100		20	0,78	59,6	-	100	-	100
	30	0,78	59	-	100	-	100		30	0,78	59,6	-	100	-	100
	40	-	100	-	100	-	100		40	-	100	-	100	-	100
	45	-	100	-	100	-	100		45	-	100	-	100	-	100
JH-1	10	0,9	52,6	> 8	0	> 8	0	JH-4	10	0,95	49,2	> 9	0	> 9	0
	20	0,6	68,4	> 8	0	> 8	0		20	0,7	63,7	> 9	0	> 9	0
	30	0,3	84,2	> 8	0	> 8	0		30	0,48	75,1	> 9	0	> 9	0
	40	0,48	74,7	-	100	0	99,9		40	0,48	75,1	-	100	-	100
	45	0,3	84,2	-	100	-	100		45	0,3	84,5	-	100	-	100
H-1	10	0,7	63,2	> 8	0	> 8	0	H-4	10	0,78	59,6	> 9	0	> 9	0
	20	0,48	74,7	> 8	0	> 8	0		20	0,6	68,9	> 9	0	> 9	0
	30	0,3	84,2	> 8	0	> 8	0		30	0.6	68,9	> 9	0	> 9	0
	40	0,48	74,7	-	100	1	88,9		40	0,48	75,1	-	100	0,95	89,5
	45	0,48	74,7	-	100	0,7	92,2		45	0,48	75,1	-	100	0,7	92,2
Positive control (TSB)		1,9	0	8,98	0	8,98	0	Positive control (TSB)		1,93	0	9	0	9	0
J-2	10	0,3	85,8	> 8	0	7,4	80,3	J-5	10	0,48	75,1	-	100	-	100
	20	0,78	63,3	-	100	-	100		20	0,78	59,6	-	100	-	100
	30	0,7	66,8	-	100	-	100		30	0,78	59,6	-	100	-	100
	40	-	100	-	100	-	100		40	-	100	-	100	-	100
	45	-	100	-	100	-	100		45	-	100	-	100	-	100

JH-2	10	0,85	59,7	> 9	0	> 9	0	JH-5	10	0,95	49,2	> 9	0	> 9	0
	20	0,78	63,3	> 9	0	> 9	0		20	0,7	63,7	> 9	0	> 9	0
	30	0,7	66,8	> 9	0	> 9	0		30	0,48	75,1	> 9	0	> 9	0
	40	0,6	71,6	-	100	-	100		40	0,48	75,1	-	100	-	100
	45	0,48	77,3	-	100	-	100		45	0,3	84,5	-	100	-	100
H-2	10	0,78	63,3	> 9	0	> 9	0	H-5	10	0,78	59,6	> 9	0	> 9	0
	20	0,7	66,8	> 9	0	> 9	0		20	0,6	68,9	> 9	0	> 9	0
	30	0,6	71,6	> 9	0	> 9	0		30	0.6	68,9	> 9	0	> 9	0
	40	0,48	77,3	-	100	1,1	88,1		40	0,48	75,1	-	100	1,1	87,8
	45	0,3	85,8	-	100	0,78	91,5		45	0,48	75,1	-	100	0,8	91,1
Positive control (TSB)		2,11	0	9,22	0	9,22	0	Positive control (TSB)		1,93	0	9	0	9	0
J-3	10	0,48	75,1	-	100	-	100	J-6	10	0,48	75,1	-	100	-	100
	20	0,78	59,6	-	100	-	100		20	0,78	59,6	-	100	-	100
	30	0,78	59,6	-	100	-	100		30	0,78	59,6	-	100	-	100
	40	-	100	-	100	-	100		40	-	100	-	100	-	100
	45	-	100	-	100	-	100		45	-	100	-	100	-	100
JH-3	10	0,95	49,2	> 9	0	> 9	0	JH-6	10	0,95	50,8	> 9	0	> 9	0
	20	0,7	63,7	> 9	0	> 9	0		20	0,7	63,7	> 9	0	> 9	0
	30	0,48	75,1	> 9	0	> 9	0		30	0,48	75,1	> 9	0	> 9	0
	40	0,48	75,1	-	100	-	100		40	0,48	75,1	-	100	-	100
	45	0,3	84,5	-	100	-	100		45	0,3	84,5	-	100	-	100
H-3	10	0,78	59,6	> 9	0	> 9	0	H-6	10	0,78	59,6	> 9	0	> 9	0
	20	0,6	68,9	> 9	0	> 9	0		20	0,6	68,9	> 9	0	> 9	0
	30	0.6	68,9	> 9	0	> 9	0		30	0.6	68,9	> 9	0	> 9	0
	40	0,48	75,1	-	100	1	88,9		40	0,48	75,1	-	100	1,1	87,8
	45	0,48	75,1	-	100	0,7	92,2		45	0,48	75,1	-	100	0,7	92,2
Positive control (TSB)		1,93	0	9	0	9	0	Positive control (TSB)		1,93	0	9	0	9	0

DISCUSSION/ ДИСКУСИЈА

Physico-chemical parameters of Bulgarian's rape honey are comparable to those reported in the literature and in accordance with European quality standards (Bogdanov, 1997). Active acidity values were lower (3,232) than data reported by Devillers et al. (2004) – 4,019, and than European rape honey from 2004 – from 3,9 to 4,1 (Piaza and Oddo, 2004), and could be related to the organoleptically established slightly acid taste (table 2).

Table 2. *Quality parameters of rape honey*

Quality parameters	Results	References	
		Devillers et al., 2004	Piaza and Oddo, 2004
Water content (%)	X 16,8 SD ± 0,2108 Min 16,6 Max 17	18,46 ± 0.655 17,00 19.80	- - 14,7 21,3
	p<0,05		
Free acidity (meq.kg^{-1})	X 36,3 SD ± 1,1595 Min 35 Max 38	10,66 ± 1,318 6,510 12,30	9,4 - 22 - - -
	p<0.05		-
pH	X 3,232 SD ± 0,01032 Min 3,22 Max 3,25	4,019 ± 0,119 3,700 4,260	3,9 - 4,1 - - -
	p<0.05		
Conductivity (mS.cm^{-1})	X 0,128 SD ± 0,00105 Min 0,127 Max 0,13	0,2031 ± 0,4435 0,110 0,269	0,14 - 0,34 - - -
	p=0.4979		
Diastase activity (Ghote), (DN)	X 12,9 SD ± 0,1051 Min 12,8 Max 13,1	26,85 ± 5,911 11,20 36,80	10 - 46,6 - - -
	p<0.05		

Hydroxymethilfurfurol (HMF), (mg.kg $^{-1}$)	X 14,89 SD ± 0,3528 Min 14,4 Max 15,36	3,196 ± 1,665 0,210 5,950	- - - -
	p<0.05		
Invertase activity (IN)	X 10,643 IN SD ± 0,0241 Min 10,62 Max 10,69	132,9 U/kg ±33,8 [von der Ohe W., von der Ohe K. (1996)]	77,1 U/kg - 39,7 50,7 [Krauze and Zalewski (1991)]
Specific optical rotation, $[\alpha]_D^{20}$	X (–) 12 SD ± 0,8164 Min (–) 11 Max (–) 13	-	-

The minimal and maximal values of the main components of royal jelly samples are in the limits proposed from Sabatiny et al. (2009). They are comparable with those of royal jelly samples produced in other countries (Balkanska et al., 2012).

Table 3. *Quality parameters of royal jelly samples (Balkanska et al., 2012)*

Sample No	Water content, %	pH	Total acidity, ml 0,1n NaOH/g	Electrical conductivity µS/cm	Proteins %	Fructose %	Glucose %	Sucrose %
1	65,10	3,20	4,23	223	14,92	4,14	3,69	2,82
2	64,80	3,50	3,96	211	12,46	5,00	4,54	1,94
3	63,10	3,60	3,68	194	12,92	4,12	3,75	5,08
4	65,80	3,70	2,48	173	12,62	3,64	4,04	3,44
5	63,30	3,80	4,23	180	13,08	4,19	2,69	2,28
6	65,30	3,70	3,86	181	12,23	4,27	5,87	2,74

Mixes from royal jelly and rape honey (1:100) possessed a higher antibacterial effect compared with independent use of rape honey. In 40 and 45 % (v/v) were found 100% inhibition after 24 and 48 h, but in rape honey after 24 h – 100% inhibition, but after 48 h were found different log reduction of

E.coli (88,8–92,22%). This indicate that independent use of rape honey not have total antibacterial effect on pathogen strain of *E.coli*.

CONCLUSIONS/ ЗАКЉУЧЦИ

Royal jelly and mixes from royal jelly and rape honey have potential as alternative therapeutics agents against pathogen strains of E.coli, resistant for antibiotics. Before this have to be done testing for allergy reactions (Leung et al., 1997; Lombardi et al., 1998; Takahama and Shimazu, 2006).

REFERENCES/ ЛИТЕРАТУРА

1. Al Jabri, A. A.; Al Hosni, S. A.; Nzeako B. C.; Al Mahrooqi, Z. H.; H. Nsanze (2005): *Antibacterial activity of Omani honey alone and in combination with gentamicin*, Saudi Medical Journal 26: 767–771.
2. Baltrusaityte, V.; Venskutonis, P. R.; V. Ceksteryte (2007): *Antibacterial activity of honey and beebread of different origin against S-aureus and S-epidermidis*, Food Technology and Biotechnology 45: 201–208.
3. Blum, M. S.; Novak, A. F.; Taber, S. (1959): 10-Hydroxy-Δ2-decenoic acid, an antibiotic found in royal jelly, Science 130: 452–453.
4. Bogdanov, S. P.; Martin, Lüllman C. (1997): *Harmonized methods of the European Honey Commission*, Apidologie, Extra Issue 1–59, Elsevier/INRA/ DIB/AGIB.
5. Bogdanov, S. (1997): Nature and origin of the antibacterial substances in honey. *Lebensm. Wiss. Technol.* 30: 748–753.
6. Bogdanov, S. (1997): *Nature and origin of the antibacterial substances in honey, Lebensm*. Wiss. Technol. 30: 748–753.
7. Boukraa, L.; Meslem, A.; Benhanifia, M.; Hammoudi, S. M. (2009): *Synergistic Effect of Starch and Royal Jelly Against Staphylococcus aureus and Escherichia coli.* Journal of Alternative and Complementary Medicine 15 (7): 755–757.
8. Commission regulation (EC) No 1441/2007 of 5 December 2007 amending Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs (Text with EEA relevance), Official Journal of the European Union, L 322: 12–29.
9. Devillers, J.; Morlot, M.; Pham-Delegue, M. H.; Dore, J. C. (2004*): Classification of monofloral honeys based on their quality control data*, Food Chemistry, 86: 305–312.
10. Gethin, G.; Cowman, S. (2008*): Bacteriological changes in sloughy venous leg ulcers treated with manuka honey or hydrogel: an RCT*, J Wound Care, 17: 241–244, 246–247.
11. Izuta, H.; Chikaraishi, Y.; Shimazawa, M.; Mishima, S.; Hara, H. (2007): *10-Hydroxy-2-decenoic Acid, a Major Fatty Acid from Royal Jelly, Inhibits VEGF-induced Angiogenesis in Human Umbilical Vein Endothelial Cells*. Oxford Journals, [online] http://ecam.oxfordjournals.org/cgi/content/full/nem152 (Accessed on 02 Oktober 2009).

12. Krauze, A.; Zalewski, R. I. (1991): *Classification of honeys by principal component analysis on the basis of chemical and physical parameters*, Z. Lebensm. Unters. Forsch., 192, 19–23.

13. Leung, R.; Ho, A.; Chan, J.; Choy, D.; Lai, C. K. (1997): *Royal jelly consumption and hypersensitivity in the community*. Clin Exp Allergy, 27(3): 333–336.

14. Li, J. K.; Feng, M.; Begna, D.; Fang, Y.; Zheng, A. J. (2010): *Proteome Comparison of Hypopharyngeal Gland Development between Italian and Royal Jelly-Producing Worker Honeybees (Apis mellifera L)*. Journal of Proteome Research, 9 (12): 6578–6594.

15. Lombardi, C.; Senna, G. E.; Gatti, B.; Feligioni, M.; Riva, G.; Bonadonna, P.; Dama, A. R.; Canonica, G. W.; Passalacqua, G. (1998): *Allergic reactions to honey and royal jelly and their relationship with sensitization to compositae*. Allergol Immunopathol (Madr), 26(6): 288–290.

16. Mavric, E.; Wittmann, S.; Barth, G.; Henle, T. (2008): *Identification and quantification of methylglyoxal as the dominant antibacterial constituent of Manuka (Leptospermum scoparium) honeys from New Zealand*. Mol Nutr Food Res, 52: 483–489.

17. Melliou, E.; Chinou, I. (2005): *Chemistry and bioactivity of royal jelly from Greece*, Journal of Agricultural and Food Chemistry, 53: 8987–8992.

18. Molan, P. C. (1992a): *The antibacterial activity of honey*. 1. The nature of the antibacterial activity, Bee World 73: 5–28.

19. Molan, P. C. (1992b): *The antibacterial activity of honey*. 2. Variation in the potency of the antibacterial activity, Bee World 73: 59–76.

20. Molan, P. C. (1997): Honey *as an antimicrobial agent, Bee Products. Properties, Applications, and Apitherapy*, Symposium Tel Aviv, pp. 27–37.

21. NCCLS (2002): *Performance standards for Antimicrobial Disc-Susceptibility* Tests. Sixth Edition; Development of in vitro susceptibility testing criteria and quality control parameters for veterinary antimicrobial agent Approved guideline, second ed., NCCLS Document M37–A2.

22. Noori, S. Al-Waili (2004): *Investigating the Antimicrobial Activity of Natural Honey and Its Effects on the Pathogenic Bacterial Infections of Surgical Wounds and Conjunctiva*, Journal of Medicinal Food. 210–222.

23. Oddo, L. P.; Piana, L.; Bogdanov, S.; Bentabol, A.; Gotsiou, P.; Kerkvied, J.; Martin, P.; Morlot, M.; Valbuena, A. O.; Ruoff, K.; von der Ohe, K. (2004): *Botanitical species giving unifloral honey in Europe*. Apidologie, 35: 82–93.

24. ON 2576693-84. Fresh and lyophilized royal jelly (Bg)

25. Patton, T.; Barrett, J.; Brennan, J.; Moran, N. (2006): *Use of a spectrophotometric bioassay for determination of microbial sensitivity to manuka honey*. J Microbiol Methods, 64: 84–95.

26. Piaza, M. G., Oddo, L. P. (2004): *Bibliographical review of the main European*

unifloral honeys, Apidologie 35, S94–S111, INRA/DIB-AGIB/ EDP Sciences, DOI: 10.1051/apido: 2004046.

27. Sesta, G. (2006): *Determination of sugars in royal jelly by HPLC*. Apidologie, 37: 84–90.

28. Shen, L. R.; Ding, M. H.; Zhang, L. W.; Jin, F.; Zhang, W. G.; Li, D. (2010): *Expression of Acc-Royalisin Gene from Royal Jelly of Chinese Honeybee in Escherichia coli and Its Antibacterial Activity*. Journal of Agricultural and Food Chemistry, 58 (4): 2266–2273.

29. Sherlock, O.; Dolan, A.; Athman, R.; Power, A.; Gethin, G.; Cowman, S.; Humphreys, H. (2010): *Comparison of the antimicrobial activity of Ulmo honey from Chile and Manuka honey against methicillin-resistant Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa*, BMC. Complementary and Alternative Medicine, 10: 47. [online] http://www.biomedcentral.com/1472–6882/10/47).

30. Shirzad, H.; Shahinfard, N.; Naficy, M. R.; Karami, M. (2007): *Comparison of royal jelly effects with gentamicin and ceftriaxone on the growth of Escherichia coli, Bacillus cereus, Pseudomonas aeruginosa, Staphylococcus aureus, in a laboratory environment*. Tropical Medicine & International Health, 12: 159.

31. Stratev, D.; Dinkov, D.; Vashin, I. (2012): *Antibacterial effect of extracts from Bulgarian's "poplar" propolis and Smoke tree (Cotinus coggigria Scop.) against Aeromonas hydrophila (ATCC 7965)*, Revue Méd. Vét., 163, 10: 443–447.

32. Takahama, H.; Shimazu, T. (2006): *Food-induced anaphylaxis caused by ingestion of royal jelly*. J Dermatol., 33(6): 424–426.

33. Van Ketel, B. A. (1892): *Festnummer der Berichten van den Niederlandsche Maatschappij*, Bevordering der Pharmacie, 67–96.

34. von der Ohe, W.; Piana, M. L.; Morlot, M.; Martin P. (2004): *Harmonized methods of melissopalynology*. Apidologie, 35: 18–25.

35. von der Ohe, W.; von der Ohe, K. (1996): *Charakterisierung einheimischer Rapshonige*, Dtsch. Bienen J. 4: 438–443.

36. Wilkinson, J. M.; Cavanagh, H. M. A. (2005): *Antibacterial Activity of 13 Honeys Against Escherichia coli and Pseudomonas aeruginosa*, Journal of Medicinal Food. 8: 100–103.