

An evaluation of the potent antimicrobial effects and unsaponifiable matter analysis of the royal jelly

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Abstract: Royal jelly is complex heterogeneous mixtures of flower's nectar sugars, proteins and bee's glandular secretions. Royal jelly is the cephalic glands excretion of the young worker honeybees (*Apis mellifera*). The wonderful effects of royal jelly on the sexual ability and maturity of the queen have been an interesting topic and controversial issue for researchers for many years. The main purpose of this study was to investigate the antimicrobial effects of royal jelly produced by honeybees (*Apis mellifera*) and collected from Egypt and China against four different types of bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*) and four species of fungi (*Aspergillus fumigants*, *Aspergillus niger*, *Candida albicans* and *Syncephalastrum racemosum*). Three concentrations of pure royal jelly were prepared and added to the bacterial strains seed layer cultured individually. The samples in different concentrations showed antimicrobial activity against tested bacteria and fungi. The diameter of the clear zone formed in each concentration was measured and correlated to the ability of the extracts to inhibit the growth of microorganisms. Interestingly, the results showed the variation of inhibitory effects of royal jelly samples on different strains of the bacteria *in-vitro*. The differences observed may be related to components of RJ associated with their geographical provenance or with genetic variability between bee colonies. The hydrocarbons and sterols from the fresh Egyptian and Chinese royal jelly were characterized by GC-FID. A total of twenty-four compounds were identified, the chemical profile reveals the dominance of C24 hydrocarbon (Egyptian camphor royal jelly: 39.66%, Egyptian citrus royal jelly: 41.9%, Chinese royal jelly: 44.05%).

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1. Introduction

Antibiotic-resistant bacteria and resistant fungal strains continue to be a major health concern world-wide. Since the use of antibiotics became widespread over 50 years ago, bacteria have progressively developed resistance (Hsueh *et al.*, 2005). Consequently, scientific efforts have been made to study and develop new compounds to be used beyond conventional antibiotic therapy. These comprise probiotic strains (Reid, 2001; Petti *et al.*, 2008), new organic compounds (Hoellman *et al.*, 2002), plant extracts (Tereschuk *et al.*, 2004; Chomnawang *et al.*, 2009), natural foodstuffs such as kefir (Leite Rodrigues *et al.*, 2005), honey (Molan, 2002; Basualdo *et al.*, 2007), propolis (Sawaya *et al.*, 2004) and royal jelly (Ratanavalachai and Wongchai, 2002; Eshraghi, 2005). Moreover, the use of alternative medicines has increased substantially over the past fifteen years (Romero *et al.*, 2005). It is, therefore of great interest to study the biological properties of natural products likely to be used as new health remedies.

Royal jelly (RJ) is a secretion produced by the hypopharyngeal and the mandibular glands in the

head of the nurse bees. It has a thick, milky appearance, with a slightly acid, pungent odour and a somewhat bitter taste. The water content is fairly uniform at greater than 60%, and with an activity (a_w) above 0.92. The dry matter of RJ is composed of protein (27-41%, including free amino acids), carbohydrates (approximately 30%), lipids (8-19%), trace elements and some vitamins (Sabatini *et al.*, 2009). Royal jelly serves as nourishment for honeybee larvae during their first three days of life and as the sole food for the queen bee during its entire life span. Beyond its excellent nutritious properties, RJ has widely recognized biological actions, including vasodilative and hypotensive activities, antiseptic action, antitumour, antihyper-cholesterolemic, and anti-inflammatory activities (Eshraghi and Seifollahi 2003, Nagai and Inoue, 2005). It was also reported that RJ has antibacterial activity against both Gram positive and Gram negative bacteria mainly due to fatty acids present in RJ, such as trans- 10-hydroxydec-2-enoic acid, 3-hydroxydodecanoic acid, 11-oxododecanoic acid, and 11-S-hydroxydodecanoic acid (Melliou and Chinou, 2005). However, some peptides (Fontana *et al.*, 2004) and proteins (Fujiwara *et al.*, 2004) present

in RJ have also been shown to possess strong antibacterial properties.

The present study was carried out to determine the antimicrobial activity of royal Jelly against important pathogens such as *Candida* which cause candidiasis (mild inflammation of skin and vaginal thrush in female genital tract in immunocompromised individuals (Ryan and Ray, 2004), *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* which implicated in infections of skin wounds (Garcia *et al.*, 2010). Also, this study can be considered as a trial to discover the chemical components other than peptides and fatty acids which are usually under focus (hydrocarbons and sterols).

2. Material and Methods

Royal jelly samples

Three royal jelly samples were used, the first and second samples were Egyptian royal jelly. The first one was collected in camphor season (camphor royal jelly) and the second was collected in citrus season (citrus royal jelly). The samples were collected from an apiary in Beekeeping Research Department, Plant Protection Research Institute, Agriculture Research Center, Giza, Egypt. It was freshly collected 3 days after grafting larvae into the artificial queen cells. After collection, it was kept in several small brown bottles at deep-frozen temperature. The third sample was Chinese royal jelly which imported from China and purchased commercially in Egyptian market.

Assessment of antimicrobial activity:

Antimicrobial activities were investigated in Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University by using agar well diffusion method' (Jinga and Sumitra, 2007). The activity of tested samples was studied against *Staphylococcus aureus* (RCMBA 2004), and *Bacillus subtilis* (RCMBA 6005) as Gram-positive bacteria and *Pseudomonas aeruginosa* (RCMBA 1002) and *Escherichia coli* (RCMBA 5003) as Gram-negative bacteria. The tested samples were screened separately *in vitro* for their antifungal activity against *Aspergillus fumigatus* (RCMBA 06002), *Aspergillus niger* (RCMBA 06106), *Candida albicans* (RCMBA 03004) and *Syncephalastrum racemosum* (RCMBA 005003).

Hundred μ l of three concentrations of water soluble royal jelly extracts (5, 10, 15 mg/ml) were loaded into the wells of the agar plates. The plates were kept for incubation at 30°C for 3-4 days for fungi and 37°C for 24hrs. for bacteria and then the plates were examined for the formation of inhibition zone. The test was performed three times for each fungus and bacterium culture and the inhibition zones were measured by caliper to get an average value.

Clotrimazole, penicillin and streptomycin were used as standard drugs against fungi, Gm + ve and Gm -ve bacteria, respectively.

Determination of MIC

The minimum inhibitory concentration (the lowest concentration which completely inhibited microorganisms growth) values of the samples were estimated for each tested organisms in triplicates according to Doughari method (2006) in RCMB. To 0.5 ml of different concentrations of the samples, 2 ml of nutrient broth (for bacteria) and sabourad dextrose broth (for fungi) were added and then loopful of the tested organism previously diluted to 0.5 Mcfarland turbidity standard for each bacterial and fungal isolate was introduced to the tube. Tubes containing the media only were seeded with the test organisms to serve as control. Tubes containing bacteria and fungi cultures were then incubated (at 30°C for 3-4 days for fungi and 37°C for 24 hrs. for bacteria) and examined for microorganisms growth by observation of turbidity.

Analysis of hydrocarbons and sterols by GC-FID

Isolation of Lipids: About 5 g of fresh royal jelly was extracted with petroleum ether (40-60). The pet. ether extract was evaporated and the residue was dissolved in boiling acetone, cooled and the formed amorphous precipitate (3.1g) was separated out. The acetone soluble fraction was saponified (N/2 alc. KOH) and the unsaponifiable matter was separated (Radwan and Abdel-Shafeek, 2006), then the unsaponifiable matter collected and prepared for injection into GC-FID instrument.

Apparatus and techniques: GC-FID. Gas liquid chromatography, Thermo Trace GC Ultra. Flame Ionization Detector, analyses of the hydrocarbons and sterols were carried out according to the following conditions: Column: TR-5MS, 30m length x 0.25 μ m. Temperature program:- Initial Temp. 70 °C, Initial Time 2min., program rate 5°C/min., F. temp. 280°C, Final time 30 min., Injection temp. 270°C, Detector (FID). Flow rate of carrier gas N₂: 30 ml/min., H₂: 50 ml/min., Air : 350 ml/min.

3. Results

Antimicrobial activity and MIC of RJ samples

In the present study royal Jelly samples had been tested in various concentrations (5-15 mg/ml) to determine the antimicrobial activity against different microorganisms (Table 1). The results showed a relative high antimicrobial activity of Egyptian citrus and camphor royal jelly if compared with Chinese one. The fungicidal activity of citrus royal Jelly was concentration dependent, while the increase of camphor royal Jelly concentration didn't increase the inhibition zone. The three tested royal Jelly samples

didn't show antifungal effect against *Syncephalastrum racemosum*.

Table 1: The inhibitory activities of pure royal jelly against fungi and bacteria

Tested strain	Egyptian royal jelly						Chinese royal jelly			St.
	Camphor			Citrus						
	Royal jelly Concentration (mg/ml)									
	5	10	15	5	10	15	5	10	15	
Fungi	Mean of inhibition zone in mm \pm Standard deviation									Clotrimazole
<i>Aspergillus fumigatus</i> (RUMBA 06002)	16.7 \pm 1.02	14.2 \pm 0.32	16.7 \pm 1.02	16.5 \pm 0.2	20.6 \pm 0.5	24.7 \pm 0.2	9.1 \pm 0.18	10.7 \pm 0.3	12.2 \pm 0.09	25.2 \pm 0.5
<i>Aspergillus niger</i> (RCMBA 06106)	15.3 \pm 0.3	12.1 \pm 0.71	15.3 \pm 0.3	14.9 \pm 0.9	18.2 \pm 0.4	20.8 \pm 0.05	10.7 \pm 0.15	13.2 \pm 0.2	14.4 \pm 0.03	21.4 \pm 0.8
<i>Candida albicans</i> (RCMBA 03004)	12.4 \pm 1.24	11.7 \pm 0.24	12.4 \pm 1.24	14.2 \pm 0.7	17.3 \pm 0.2	19.9 \pm 0.3	NA	7.3 \pm 0.22	9.9 \pm 0.08	15.4 \pm 0.1
<i>Syncephalastrum racemosum</i> (RCMB 005003)	NA*	NA	NA	NA	NA	NA	NA	NA	NA	19.8 \pm 0.4
Gram Positive Bacteria:										Penicillin g
<i>Staphylococcus aureus</i> (RCMBA 2004)	8.3 \pm 0.7	12.9 \pm 0.4	16.1 \pm 0.9	18.6 \pm 0.1	21.6 \pm 0.24	23.4 \pm 0.09	8.9 \pm 0.23	10.1 \pm 0.12	13.8 \pm 0.1	24.6 \pm 0.5
<i>Bacillus subtilis</i> (RCMBA 6005)	9.2 \pm 0.5	13.2 \pm 0.3	17.9 \pm 0.1	19.2 \pm 0.5	22.0 \pm 0.18	24.7 \pm 0.1	12.3 \pm 0.4	14.6 \pm 0.1	16.6 \pm 0.03	26.4 \pm 0.4
Gram negative Bacteria:										streptomycin
<i>Pseudomonas aeruginosa</i> (RCMBA 1002)	NA	NA	NA	NA	NA	10.5 \pm 0.1	NA	NA	NA	22.4 \pm 0.3
<i>Escherichia coli</i> (RUMBA 5003)	NA	NA	7.4 \pm 0.5	7.1 \pm 0.27	9.4 \pm 0.19	13.1 \pm 0.2	NA	NA	NA	26.7 \pm 0.9

NA: No Zone of inhibition observed

The elevation in royal Jelly concentration of the samples increased the size of the inhibition zone against the two Gram positive bacteria used (*Staphylococcus aureus* and *Bacillus subtilis*), i.e. the concentrations 15 mg/ml resulted in the largest inhibition zones against the tested Gm + ve bacteria. On the contrary, the concentrations of the used samples didn't affect the growth of the two tested Gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*) except citrus royal Jelly which resulted in inhibition zone 10.5 \pm 0.1 mm against *P.aeruginosa* at concentration 15 mg/ml and 7.1 \pm 0.27, 9.4 \pm 0.19 and 13.1 \pm 0.2mm against *E.coli* at concentrations 5,10 and 15 mg/ml respectively. Camphor royal Jelly also affect *E.coli* growth at 15 mg/ml. It can be observed from table (1) that the citrus royal Jelly was the most effective one against the tested microorganisms.

All these results of the antimicrobial activities of camphor, citrus and Chinese royal Jelly samples were confirmed by the values of MIC (the lowest concentration which completely inhibited microorganisms growth) in table (2), which indicated that citrus royal Jelly had lower MIC values than camphor and Chinese ones, especially against Gm+ve bacteria (15.63 and 7.81 μ g/ml for *S.aureus* and *B.subtilis*, respectively). On other hand MIC values of

Chinese royal Jelly either were high or could not be determined due to its weak effectiveness against the tested microorganisms.

GLC analysis for the unsaponifiable matter components

In this study, 24 compounds were isolated from royal jelly samples that could be enrichment for the antimicrobial activity. These compounds were identified as hydrocarbons and sterols by GC-FID. A typical GC-FID chromatogram of unsaponifiable fractions readily separated hydrocarbons and sterols (Fig 1) and the data in table (3) showed the qualitative and quantitative analysis of these compounds which proved to be a mixture of triterpenes, sterols and a series of hydrocarbons. Identification of the compounds was carried out by comparison of their retention times with the available reference compounds. The total hydrocarbons was 90.47 % (camphor royal jelly sample), 84.50% (citrus royal jelly sample) and 88.48% (Chinese royal jelly sample), while the total sterols was 2.99, 3.71 and 3.94% in camphor, citrus and Chinese royal jelly samples, respectively. The ratios of total hydrocarbons to total sterols (TH/TS) in camphor, citrus and Chinese royal jelly samples were 30.20, 22.72 and 22.40%, respectively.

The major constituent in the royal jelly samples was n-Tetracosane hydrocarbon (camphor: 39.66%, citrus: 41.90%, Chinese: 44.05%). Furthermore, the Stigmsterol were (camphor: 0.509%,

citrus: 0.571%, Chinese: 0.430%) and Bsitosterol (camphor: 2.486%, citrus: 3.148%, Chinese: 3.518%) in the royal jelly samples.

Table 2: The minimum inhibitory concentration (MIC) of pure royal jelly against tested microorganism.

Test strain	Egyptian royal jelly		Chinese royal jelly	St.
	Camphor	Citrus		
Fungi	Royal jelly Concentration (µg/ml)			Clotrimazole
	MIC (µg/ml)			
<i>Aspergillus fumigatus</i> (RUMBA 06002)	31.25	31.25	500	0.06
<i>Aspergillus niger</i> (RCMBA 06106)	62.5	62.5	125	0.98
<i>Candida albicans</i> (RCMBA 03004)	125	62.5	NA	31.25
<i>Syncephalastrum racemosum</i> (RCMB005003)	NA	NA	NA	3.9
Gram Positive Bacteria:				Penicillin g
<i>Staphylococcus aureus</i> (RCMBA 2004)	500	15.63	500	0.06
<i>Bacillus subtilis</i> (RCMBA 6005)	500	7.81	125	0.03
Gram negative Bacteria:				streptomycin
<i>Pseudomonas aeruginosa</i> (RCMBA 1002)	NA	NA	NA	0.49
<i>Escherichia coli</i> (RUMBA 5003)	NA	500	NA	0.015

Table 3. GLC analysis of unsaponifiable fractions (hydrocarbons and sterols) in the royal jelly by GC-FID.

Carbons No.	Constituents**	Camphor royal jelly	Citrus royal jelly	Chinese royal jelly
		Relative Area% *		
C8	n-Octane	1.402	0.408	ND
C9	n-Nonane	1.754	0.364	1.403
C10	n-Decane	10.543	3.016	5.557
C11	n-Hendecane	0.800	0.306	0.275
C12	n-Dodecane	11.09	5.455	9.430
C13	n-Tridecane	0.617	0.263	0.317
C14	n-Tetradecane	1.237	1.053	1.571
C15	n-Pentadecane	7.609	5.516	7.774
C16	n-Hexadecane	0.570	1.110	0.515
C17	n-Heptadecane	1.149	1.391	2.259
C18	n-Octadecane	4.549	4.474	5.297
C19	n-Nonadecane	0.321	0.632	0.221
C20	n-Eicosane	3.322	3.136	2.954
C21	n-Heneicosne	0.364	0.521	0.757
C22	n-Docosane	ND	1.662	0.428
C24	n-Tetracosane	39.666	41.904	44.053
C25	n-Pentacosane	0.376	1.274	0.502
C26	n-Hexacosane	1.888	3.977	1.206
C27	n-Heptacosane	0.491	1.740	1.228
C28	n-Octacosane	0.489	0.672	0.916
C29	n-Nonacosane	ND	0.386	0.696
C30	n-Triacontane	2.237	6.3	1.122
TH		90.474	84.507	88.481
	Stigmsterol	0.509	0.5713	0.4306
	Bsitosterol	2.486	3.148	3.518
TS		2.995	3.7193	3.9486
TH/TS		30.208	22.721	22.408

*Percentages obtained by FID peak-area normalization.

**Identified by authentic samples

TH :Total hydrocarbons

TS :Total sterols

TH/TS :Ratio of total hydrocarbons to total sterols.

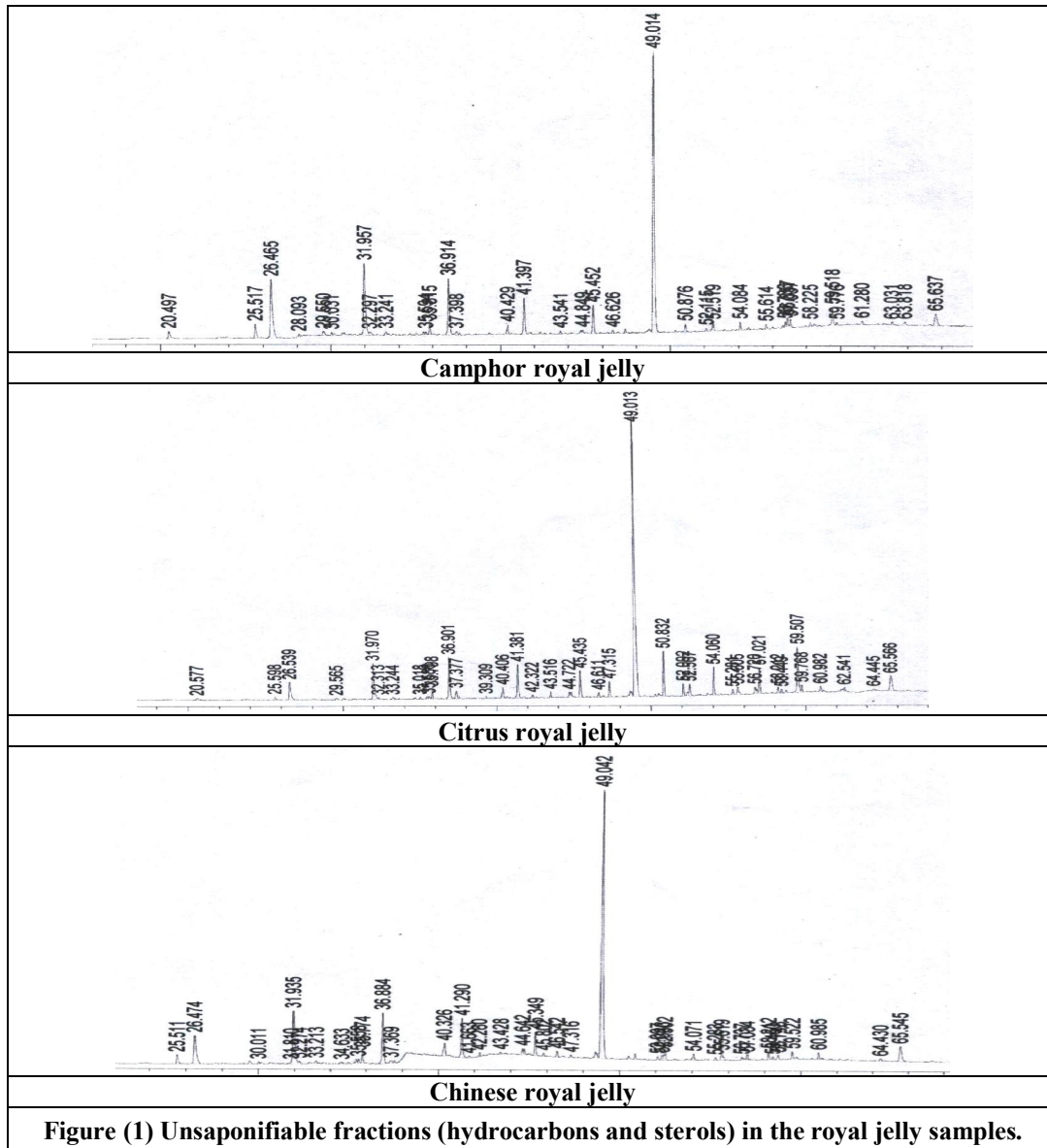


Figure (1) Unsaponifiable fractions (hydrocarbons and sterols) in the royal jelly samples.

4. Discussion

The results of this study showed that RJ samples obtained from different ecological regions of Egypt and China, inhibited the growth of fungi, Gram positive and Gram negative bacterial strains used here with various results. The results of inhibition tests against fungi, especially *Candida* agreed with Koc *et al.*, 2011, who found that royal Jelly samples affect the growth of fluconazole resistant fungal strains of *Candida* spp. Similar results have been reported by other authors such as Fujiwara *et al.*, 2004 who proved that the royal jelly has a strong antibacterial *in vitro* action against Gram-positive bacteria, but not against the Gram-negative bacteria, while Garcia *et al.*, 2010 found that the growth of *Pseudomonas aeruginosa* was

inhibited only by high concentrations of RJ samples from different areas and *E.coli* showed variation in sensitivity to different RJ samples. These results agreed with the findings of the present study, in which only the highest concentration of citrus RJ affected *P.aeruginos* and *E.coli* showed higher sensitivity to all concentrations of citrus Egyptian royal jelly. *P.aeruginos* is usually found in skin wounds and causes a variety of systemic infections, particularly in victims of severe burns. Therefore, the antibacterial activity of royal Jelly samples studied against *P.aeruginosa* may be of importance in the development of ointments for the treatment of wounds (Abdelatif *et al.*, 2008).

Bachanova *et al.*, 2002 suggested that, the difference observed in inhibition zones as well as in MIC values of RJ samples obtained from different localities, may be related to components of RJ associated with their geographical provenance or be linked with genetic variability between colonies. Sensitivity of bacteria to bee products varies considerably within the product and the varieties of the same product (Boukraa and Sulaiman, 2009).

Hydrosoluble components of RJ, like proteins and peptides, have a high capacity of inhibition Gram-positive bacteria and fungi (Sauerwald *et al.*, 1998). Parveen, and Rao (2012) suggested that secondary metabolites in RJ such as phenols, flavonoids, flavonoids, glycosides, terpenoids, sterols, lignin and saponins showed antibacterial activity against both Gram positive and Gram negative bacteria, while Andreas *et al.*, 2005 found that biological activities of RJ are variable and have been correlated to their content of trace elements.

Takaisi and Schoncher (1994), Burdock (1998), Cushnie and Lamb (2005) attributed the antimicrobial capacity of the honey bee products to the presence of phenolic compounds and other chemicals (collected by the bees from the plants where they gather nectar) whose action mechanisms are based on the inhibition of bacterial RNA polymerase, or degrading the cytoplasm membrane of the bacteria, which leads to a loss of potassium ions and the damage cause provoking cell autolysis or increase membrane permeability, and dissipates its potential, leading the bacteria to lose their capacity to synthesis ATP, their membrane transport and motility.

Singh *et al.* (2012) found that sterols of some medical plants such as *Terminalia chebula* exhibited good inhibitory activity against most of the pathogens. Among all the bacteria tested, *Enterobacter aerogens* was found to be most sensitive, and inhibition zone produced by extract (sterols of fruits of *Euphorbia hirta*) against this pathogen was the maximum (IZ 21mm) with low MIC values (0.039mg/ml). Sterols of fruits of *Euphorbia hirta* plant exhibited high antibacterial activity against *B. subtilis* (IZ 16.5mm), with modest activity against *E. coli* (IZ 9.833). However *Euphorbia hirta* stem sterols showed maximum activity against *S. aureus* (IZ 19.5mm) followed by stem sterols of *T. chebula* (IZ 18.5mm). In *Withania somnifera* the maximum activity was shown by sterols of stem against *P. aeruginosa* (IZ 20mm). Bactericidal effect of sterols was recorded against *B. subtilis*, *P. mirabilis*, *P. aeruginosa*, *S. aureus*, *E.coli* and *E. aerogens*.

Pavel *et al.* (2011) found that no clear evidence from controlled experiments exists to support claims of internal antibiotic usefulness of RJ and they

suggested that RJ can not exhibited antibiotic and anti-inflammatory effects by ingestion, due to the destruction of the active substances involved through digestion or neutralization through pH changes.

While the antimicrobial capacity of royal jelly is clear, there seems to be no one clear-cut cause, suggesting that there is a combined or synergistic effect at work. All these facts lead us to continue in the near future with studies on the chemical composition of RJ and its relationship with the biological activity. Also, dosage and safety of royal jelly must be tested before its possible *in vivo* application.

5. Conclusion

Royal jelly is a natural bee product with a great potential for use in medicine. Royal jelly has numerous precious therapeutic properties used from ancient times until today. Some of its biological and therapeutic activities have been confirmed, but scientists have only begun to uncover the many health benefits of this amazing superfood and there are just a few solid evidences for those claims. On the other hand, the chemistry and bioactive compounds RJ are not sufficiently known. Further experimentation (in vitro, in animal research and on clinical trials) and validation would be needed to prove any useful benefit and action mechanism of native RJ and of isolated compounds as well.

References

1. Abdelatif, M.; Yakoot, M. and Etmaan, M. (2008). Safety and efficacy of a new honey ointment on diabetic foot ulcers: a prospective pilot study. *Journal of Wound Care*, 17 (3): 108-110.
2. Andreas, S.; Peter, S.; Antonius, K. and Eberhard, B.(2005). Trace and mineral elements in royal jelly and homeostatic effects. *Journal of Trace Elements in Medicine and Biology*, 19(2-3):183-189.
3. Bachanova, K.; Klaudiny, J.; Kopermichy, J. and Šimuth, J., (2002). Identification of honeybee peptide active against *Paenibacillus larvae* through bacterial growth-inhibition assay on polyacrylamide gel, *Apidologie*, 33: 259-269
4. Basualdo, C.; Sgroy, V.; Finola, M. and Marioli, J. M. (2007). Comparison of the antibacterial activity of honey from different provenance against bacteria usually isolated from skin wounds. *Veterinary Microbiology* 124: 375-381.
5. Boukraa, L.; Sulaiman, S. (2009). Rediscovering the antibiotics of the hive. *Recent Patent on Anti-Infective Drug Discovery* 4(3): 206-213.
6. Burdock, G. A. (1998). Review of the biological properties and toxicity of bee propolis *Food Chem Toxicol.* 36 : 347 63
7. Chomnawang, M.; Surassmo, S.; Wongsariya, K. and Bunyapraphatsara, N (2009). Antibacterial Activity of Thai Medicinal Plants against Methicillin-resistant *Staphylococcus aureus*. *Fitoterapia*, 80: 102-104.

8. Cushnie, T. P. T. and Lamb, A. J. (2005). Detection of galangin-induced cytoplasmic membrane damage in *Staphylococcus aureus* by measuring potassium loss. *J. Ethnopharmacol.*, 101 : 243-248.
9. Doughari, J. H. (2006). Antimicrobial Activity of *Tamarindus indica* Linn. *Trop. J. Pharm. Res.* 5(2):597-603.
10. Eshraghi, S. (2005). An evaluation of the potent inhibitory effects of royal jelly fractions against *Streptomyces bacteria*. *Pakistan Journal of Medical Science*, 21(1): 63-68.
11. Eshraghi, S. and Seifollahi, F.(2003). Antibacterial Effects of Royal Jelly on Different Strains of Bacteria, *Iranian J. Publ. Health*, 32(1): 25-30
12. Fontana, R.; Mendes, M. A.; De Souza, B. M.; Cono, K.; César, L. M. M.; Malaspina, O.; Palma, M. S. (2004). Jelleines: a family of antimicrobial peptides from the Royal Jelly of honeybees (*Apis mellifera*). *Peptides*, 25: 919-928.
13. Fujiwara, S.; Imai, J.; Fujiwara, M.; Yaeshima, T.; Kawashima, T.; Kobayashi, K. (2004). A potent antibacterial protein in royal jelly. Purification and determination of the primary structure of royalisin. *Peptides*, 25: 919-928.
14. García, M. C.; Finola, M. S. and Marioli, J. M.(2010). Antibacterial activity of Royal Jelly against bacteria capable of infecting cutaneous wounds. *Journal of ApiProduct and ApiMedical Science* 2 (3): 93 - 99 (2010)
15. Hoellman, D.; Kelly, L.; Jacobs, M. and Appelbaum, P. (2002). *In vitro* anti-anaerobic activity of the cephalosporin derivative RWJ 54428, compared to seven other compounds. *Clinical Microbiology and Infections* 8: 813-822.
16. Hsueh, P.; Chen, W. and Luh, K. (2005). Relationships between antimicrobial use and antimicrobial resistance in Gram-negative bacteria causing nosocomial infections from 1991-2003 at a university hospital in Taiwan. *International Journal of Antimicrobial Agents*, 26: 463-472.
17. Jigna, P. and Sumitra, V. C. (2007). *In-vitro* antimicrobial activity and phytochemical analysis of some Indian Medicinal Plants. *Turk J. Biol.* 31, 53-58.
18. Koc, A. N.; Silici, S.; Kasap, F.; Hörmet-Oz, H. T.; Mavus-Buldu, H. and Ercal, B. D.(2011). Antifungal activity of the honeybee products against *Candida* spp. and *Trichosporon* spp. *J Med Food.* 14(1-2):128-34.
19. Leite Rodrigues, K.; Gaudino Caputo, L.; Tavares Carvalho, J.; Evangelista, J. and Schneedorf, J. (2005). Antimicrobial and healing activity of kefir and kefir extract. *International Journal of Antimicrobial Agents*, 25: 404-408.
20. Melliou, E. and Chinou, I. (2005). Chemistry and bioactivity of royal jelly from Greece. *Journal of Agricultural Food Chemistry*, 53:8987-8992.
21. Molan, P. (2002). Not all honeys are the same for wound healing. *Bulletin of the European Tissue Repair Society*, 9: 5-6.
22. Nagai, T. and Inoue, R. (2005). Preparation and the functional properties of water extract and alkaline extract of royal jelly. *Food Chemistry* 84:181-186.
23. Parveen, A. and Rao, S. (2012). Antibacterial activity and phytochemical analysis of leaf extracts of *Aristolochia bracteolata*. *Lam. Asian j. Exp. Boil. Asc.* 3(3):577-581.
24. Pavel, C.I.; Marghitas L. Al.; Bobis, O.; Dezmiorean D.S.; Sapcaliu, A. Radoi, I and Madas, M.N. (2011). Biological Activities of Royal Jelly Review. *Animal Science and Biotechnologies*, 44(2): 108-118.
25. Petti, S.; Tarsitani, G. and Simonetti D'arca, A. (2008). Antibacterial activity of yoghurt against viridans streptococci *in vitro*. *Archives of Oral Biology*. 53: 985-990.
26. Radwan, H.M. and Abdel-Shafeek, K. A. (2006). Phytochemical and Bioactivity Investigation of *Chrysanthemum coronarium* (var. discolor) Durv Growing in Egypt. *Egypt. J. Pharma. Sci.*, 47:59-72.
27. Ratanavalachai, T. and Wongchai, V. (2002). Antibacterial activity of intact royal jelly, its lipid extract and its defatted extract. *Thammasat International Journal of Science and Technology* 7: 5-12.
28. Reid, G. (2001). Probiotic agents to protect the urogenital tract against infection. *American Journal of Clinical Nutrition*, 72: 437S-443S.
29. Romero, C.; Chopin, S.; Back, G.; Martinez, E.; Garcia, M. and Bixby, L. (2005). Antibacterial properties of common herbal medicines of the southwest. *Journal of Ethnopharmacology*, 99: 253-257.
30. Ryan K.J. and Ray C.G. (2004). *Sherris Medical Microbiology* (4th ed.) McGraw Hill. ISBN 8385-8529.
31. Sabatini, A. G.; Marcazzan, G. L.; Caboni, M. F.; Bogdanov, S.; Almeida-Muradian, L. B. DE. (2009). Quality and standardisation of Royal Jelly. *Journal of ApiProducts and ApiMedical Sciences* 1(1):16-21.
32. Sauerwald, N.; Polster, J.; Bengsch, E.; Niessen, L. and Vogel, R.F. (1998). Combine antibacterial and antifungal properties of water soluble fraction of royal jelly. *Adv.Food Sci.*, 20(1/2), 52-56
33. Sawaya, A.; Souza, K.; Marcucci, M.; Cunha, I. and Shimizu, M. (2004). Analysis of the composition of Brazilian propolis extracts by chromatography and evaluation of their *in vitro* activity against Gram-positive bacteria. *Brazilian Journal of Microbiology*, 35: 104-109.
34. Singh, G.; Kumar, P. and Jindal, A. (2012). antibacterial potential of sterols of some medicinal plants. *International Journal of Pharmacy and Pharmaceutical Sciences*. 14(3):159-167.
35. Takaisi, N.B. and Schoncher, H. (1994). Electron microscopy and microcalorimetric investigations of the possible mechanism of the antibacterial action of a defined propole provenance *Planta Med*, 60: 222-227.
36. Tereschuk, M.; Baigori, M. and Abdala, L. (2004). Antibacterial activity of *Tagetes terniflor*. *Fitoterapia*, 74: 404-406.