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Antibacterial Effects of Royal Jelly on Different Strains of Bacteria

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Abstract

Honey and royal jelly are complex heterogeneous mixtures of flower s' nectar sugars proteins and bee s ' glandular secretions. Royal jelly is the hypo-pharyngeal gland excretion of the young worker honeybees (*Apis mellifera*). The wonderful effects of royal jelly on the sexual ability and maturity of the queen has been an interesting topic and controversial issue for researchers for many years. The main purpose of this study was to investigate the anti-bacterial effects of royal jelly against six different bacteria (*Escherichia coli, Staphylococcus aureus, Streptomyces griseus,* and three unclassified strains of *Streptomyces*). Four concentrations of water soluble extracts of pure royal jelly were prepared and added drop wise to the bacterial strains seed layer cultured individually. Attempt was made to find out the antibacterial properties of royal jelly by means of agar distribution method (drop-plate). The diameter of the clean zone formed in each concentration was measured and correlated to the ability of the extracts to inhibit the growth of microorganisms. Interestingly the results show inhibitory effects of royal jelly on different strains of the bacteria *in-vitro.* Ether-soluble and non-soluble fractions of the royal jelly were also examined using the above method. It was found that the antibacterial effect of ether-soluble fraction is substantially higher than the effect of ether non-soluble fraction. The zones formed by ether-soluble fractions of royal jelly in the rout of distribution in agar belong to the potency of anti-bacterial properties of fatty acids present in this fraction of royal jelly (*10 -HDA).* It was also shown that the non ether-soluble fraction of royal jelly contains a bactericidal substance called royalisin that was found to have potent antibacterial activity. This fraction of royal jelly revealed weaker anti-bacterial effect than the ether-soluble fraction and even of pure royal jelly.

Keywords: *Royal jelly, Streptomyces, Apis mellifera, Trans-10-hydroxy decenoic acid*

Introduction

Royal jelly is produced in the worker bees' stomach by the incomplete digestion of honeydew. Every cell of the honeycomb which is named shakhoon (Fig.1) is a frame made of bee wax and is the place of the queen's larva which is always filled by excess amounts of royal jelly for nutritional requirements of larvae. The queen's larva is floating in a jelly-like dense and milky white-colored substance (1, 2, 3, 4).

Royal jelly compounds. It is a milky white highly viscous secretion from the salivary gland of the worker hive bee *Apis mellifera* (Apidae) which is essential for the development of queen bees (5, 6). All useful effects of royal jelly cannot be attributed to an Individual component. It is noticeable that the combination of the elements together produces the useful effects (5, 7). It is supposed that royal jelly makes a balance in the human body. It might be because of a similar balance between its components which are mixed in a suitable proportion (5, 6, 9, 8, 10).

Medicinal Properties. A lot of studies done before show a wide range of medical activities in royal jelly. Some of these effects are as follows: anti-microbial effects (11, 12), suppression of allergic reactions (2, 6, 13), lowering the amount of blood cholesterol (14, 15), preventing cell damage in cancer and HIV patients (16, 17), as well as wound healing and growth acceleration (18). The potency of anti-bacterial properties of royal jelly could be related to a particular fatty acid present in ether-soluble fraction of royal jelly called *trans-10-hydroxy decenoic acid* (19, 20). The contents of *10-HDA*, a bioactive component of royal jelly, and several vitamins did not change during storage at 40 ^{o}C for 7 days. The substance has the chemical formula of (C_{10} H_8 O_3) and occupies 10% of the total weight of royal jelly (5, 10, 19).

Microbiology. *Streptomyces* species are aerobic gram-positive bacteria. They produce filaments that are long highly branched and non-fragmenting (21, 22). The bacteria are generally harmless saprophytes of the soil though some strain e.g. *Streptomyces griseus* and *Streptomyces somaliensis* have been found in cases of mycetoma. More than 3000 species of *Streptomyces* have been described which are important as producers of antibiotics (23, 27). Regarding to the results obtained, we were interested to investigate antibacterial effects of royal jelly against above mentioned bacteria.

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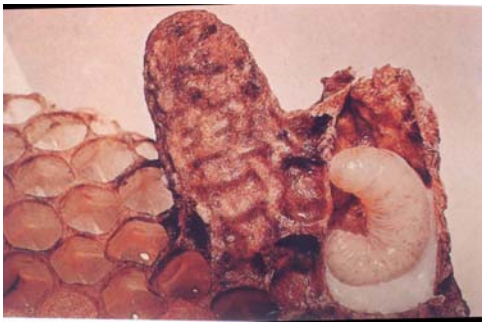

Fig. 1: Cross section of Shakhoon which is filled with royal jelly

Materials and Methods

Streptomyces griseus (ATCC 11746), *Staphylococcus aureus* (ATCC 14776), *Escherichia coli* (ATCC 29532), freeze-dried ampoules obtained from American Type Culture Collection 10801 University Boulevard Manassas USA. Three unclassified strains of *Streptomyces* indicated as ($S._{46}$) ($S._{F8}$) and ($S._{66}$) were provided from School of Public Health, Tehran University of Medical Sciences. The royal jelly was supplied by the faculty of Agriculture, University of Tehran located in Karaj City. Royal jelly was transferred to Tehran in ice. The antimicrobial susceptibility test discs {ceftazidime (31633), neomycin (31313), gentamicin (31299), tobramycin (31569), vancomycin (31353) and streptomycin (31328)} were supplied from Becton Dickinson Microbiology Systems Cockeysville Maryland (BBL products). Different concentrations of water soluble extracts of royal jelly were applied to the surface of Starch Casein Agar plates inoculated with a pure culture of *Streptomyces* strain. The same extracts were applied to the surface of Muller Hinton Agar plates inoculated with a pure culture of *Escherichia coli* and *Staphylococcus aureus* strain. The plates were prepared using seed layer culture procedure. The susceptibility test discs containing appropriate amounts of anti-microbial agents were applied to the surface of Starch Casein Agar plates inoculated with a pure culture of *Streptomyces* strain. Twenty micro liters of each concentration (1000,333, 200 and 143 *mg/ml*) of royal jelly which corresponded with 20, 6.66, 4 and 2.86 *mg* per 20 µl of royal jelly respectively, were added in the middle of every marked area using Gilson samplers. Following incubation, the plates were examined and the clear zones of inhibition surrounding the drop points were measured and compared with established zone size ranges for antimicrobial agent discs. The amount of existing effective matter (X) in each concentration was obtained by the following equation:

$$X = V \times M$$

V = volume of extract, M = dilution of extract.

Ether-soluble (30 *mg/ml*) and non-soluble (30 and 300 *mg/ml*) fractions of the royal jelly were also examined using the above method. At the first stage all fatty acids from the royal jelly were extracted using the solvent diethyl ether. When the solvent was evaporated, 70% ethanol was added to the mixture as an inert solvent. The remaining is ether non-soluble fraction in the royal jelly, which had not been dissolved in di-ethyl ether. Water was also used as an ineffective solvent to the latter fraction.

Results

The royal jelly showed clearly inhibitory effects against a variety of bacteria (Figures 2 and 3) and in similar concentrations demonstrated different inhibitory effects on different strains of *Streptomyces* (Table 1). The antibacterial activity of pure royal jelly is less effective than the ether-soluble fraction of royal jelly (Tables 1 and 2). The inhibitory effect of ether-soluble fraction of royal jelly against the bacteria is clearly stronger than the ether-non-soluble fraction (Tables 2 & 3). The results indicate that non-soluble fraction of royal jelly at a concentration similar to soluble fraction of royal jelly (30 *mg/ml*) does not have any inhibitory activity and even at a higher concentration (300 *mg/ml*) low activity (Tables 2 & 3 and Figure 4). The antimicrobial susceptibility test against *Streptomyces strain* showed that all bacteria are susceptible to most of standard antimicrobial discs (Table 4).

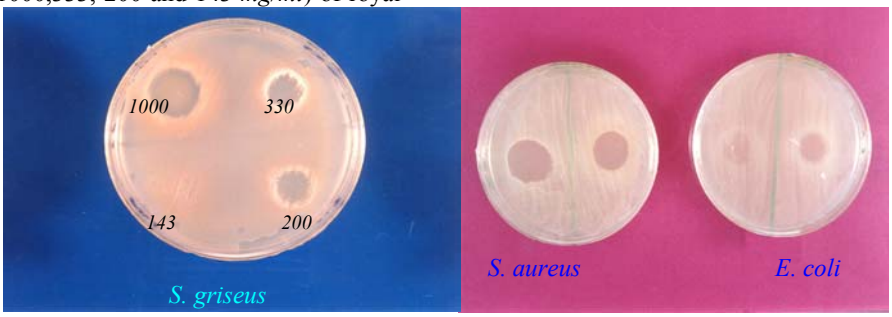

Fig. 2: The inhibitory effects of royal jelly in different concentrations against *Streptomyces griseus*.

Fig. 3: The comparison inhibitory effect of royal jelly against gram positive bacteria (*Staphylococcus aureus*) and gram negative bacteria (*Escherichia coli*)

Table 1: The inhibitory activities of pure royal jelly against Bacteria using drop plate method

	Royal jelly Concentration (mg/ml)			
	143	200	330	1000
Test strain	Inhibitory zone diameter (mm)			
Streptomyces strain (46)	-	7	13	20
Streptomyces strain (F_8)	3	9	13	17
Streptomyces strain (66)	1	8	10	16
Streptomyces griseus (ATCC 11746)	6	13	14	20
Staphylococcus aureus (ATCC 14776)	3	13	15	20
Escherichia coli (ATCC 29532	-	10	12	17

Table 2: The inhibitory activity of ether-soluble fraction of royal jelly against bacteria using drop plate method

Test strain	Royal jelly Concentration 30 (mg/ml)
	Inhibitory zone diameter (mm)
Streptomyces strain (46)	23
Streptomyces strain (F_8)	22
Streptomyces strain (66)	24
Streptomyces griseus (ATCC 11746)	29
Staphylococcus aureus (ATCC 14776)	40
Escherichia coli (ATCC 29532)	22

Table 3: The inhibitory activity of ether-non-soluble fraction of royal jelly against Bacteria using drop plate method

	Royal jelly Concentration (mg/ml)	
	30 (mg/ml)	300 (mg/ml)
Test strain	Inhibitory zone diameter (mm)	
Streptomyces strain (46)	_	9
Streptomyces strain (F_8)	_	10
Streptomyces strain (66)	_	7
Streptomyces griseus (ATCC 11746)	_	10
Staphylococcus aureus (ATCC 14776)	_	12
Escherichia coli (ATCC 29532)	_	4

Table 4: The antimicrobial susceptibility test against S*treptomyces* strain Usi ng disc plate method

	Antimicrobial discs					
Bacteria strain	Ceftazidime	Gentamicin	Neomycin	Streptomycin	Tobramycin	Vancomycin
Streptomyces strain (46)	_	24	25	19	29	28
Streptomyces strain (F_8)	_	23	29	23	24	28
Streptomyces strain (66)	_	18	20	17	26	23
Streptomyces griseus (ATCC 11746)	_	27	23	25	25	27

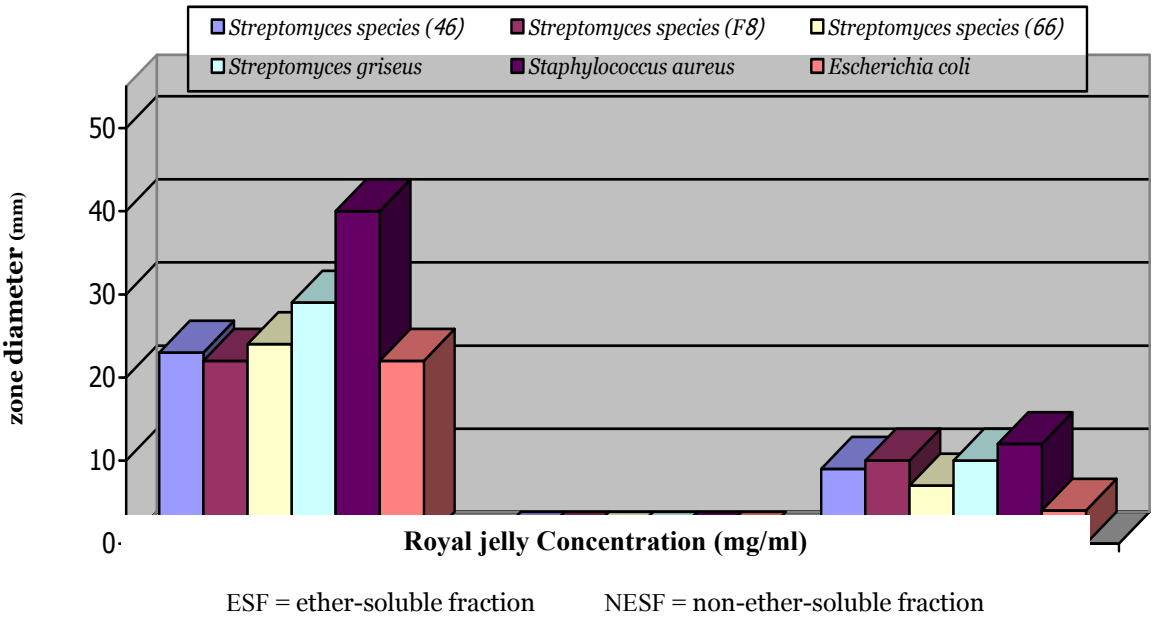

Fig. 4: The comparison of the inhibitory activities of different fractions of royal jelly against Bacteria using drop plate method

Discussion

The results of the experiments on the aqueous solution of the pure royal jelly are indicative of the antibacterial effect on various types of bacteria (Table 1). The studies of royal jelly against a wide variety of microorganisms all over the world, show strong antimicrobial activity (11, 16). However none of the investigation was related to filamentous bacteria (*e.g. Streptomyces* strain) and thus the present investigation is unique. The characterization of major proteins of honeybee and royal jelly has been described by researchers (7, 18). The results of royal jelly fresh specimen analysis that has been presented in the Merck's Index and Martindale books (5,10) are as follows (% wt at pH 5): moisture 65-70, protein 15-20, carbohydrate 10-15, lipid 1.7-6, ash 0.7-2.0; trace elements: Na, K, Fe, Cu, Mg, Mn, Ca. Vitamins (µg/g): thiamine 2, riboflavine 10, pyridoxine 2, nicotinic acid 75, biotine 2, folic acid 0.3, inositol 100, pantothenic 250, ascorbic acid 3-5, vitamin D & E trace. When stored at room temperature it changes to a light yellow gum and after some weeks to brittle amber solid (5, 6, 9, 10). The interesting result in this study was the maximum clear zones of inhibition surrounding the soluble fraction by applying the minimum concentration (30 *mg/ml*) of royal jelly (Table 2 and Figure 4). It seems that the activity is attributed to the 10 HDA content of soluble fraction of royal jelly. Generally the major antibacterial effect of royal jelly is attributed to a particular 10-carbon molecule of fatty acid (10-hydroxy-decenoic acid) (19, 20). Since this acid and the other existing fatty acids are portions of the ether-soluble fraction of royal jelly they create a strong antibacterial activity in this fraction (11, 12) while the non-soluble fraction in ether and the other peptide fractions of royal jelly have weaker antibacterial effect than ether-soluble fraction and even of pure royal jelly (11). The bactericidal activity of decenoic acid which is a long chain fatty acid is stronger than other 10 carbon fatty acids and is not neutralized by addition of alkaline while the short chain fatty acids may be inactivated during the extraction procedures (19, 20). The concentration of 30 mg/ml of ether-soluble fraction of royal jelly (containing *10-HDA*) inhibits all bacteria (Table 2) while ether non-soluble fraction with the same concentration of royal jelly (containing royalisin) was not effective against the same microorganisms (Table 3). Although the inhibitory effect of ether non-soluble fraction of royal jelly increased marginally by application of ten fold higher concentration of the fraction (i.e. 300 mg/ml) however the extent of inhibition was substantially lower than that achieved

with 30 mg/ml of the ether soluble fraction (Tables 2 & 3 , Figure 4). These results suggest that the inhibitory effect is most likely associated to lipid-containing molecules present in ether soluble fraction of royal jelly. The weaker antibacterial activity in ether-non-soluble fraction of royal jelly may be attributed to royalisin which is a portion of the ether-non-soluble fraction of royal jelly (10) thus the potent antibacterial activity against gram-positive bacteria at high concentrations (300 mg/ml) is probably due to the presence of royalisin. Comparison of the results presented in Tables 3 & 4 revealed approximately similar antibacterial activity in royal jelly and standard antibiotics against the strain of *Streptomyces*. Therefore apart from the wonderful effects of royal jelly in nutritional and medicinal field its bactericidal ability should also be considered and employed.

Reference

1. Gojmerac WL Honey (1993). In: *Honeybees breeding honey producing and pollination*. Translated by Ismaeli M. Golkar Press first press. Tehran, pp. 231-9.
2. Leung R, Ho A, Chan J, Choy D, Lai CK (1997). Royal jelly consumption and hypersensitivity in the community. *Clin Exp Allergy*. 27: 333-6.
3. Loriche N (1968). The biology of the honeybees. In: *The winged pharmacists.* First published in Moscow by Mir Publications Translated in Persian by Haddad-e-Kaveh. Published in Tehran 1988 by S. Saeed-e-now Press, pp. 05-26.
4. Shahrestani N (1982). The biology of the honeybees. In: *Honeybees breeding and maintenance.* Second edition Tehran Univ. Press Tehran, pp. 78-92.
5. Budavari S, O'Neil MJ, Smith A, Heckelman P E, Kinneary JF (1996). *The Merck Index Published* by Merck Research Laboratories division of Merck & Co. INC. Whitehouse Station NJ. USA. P: 1425.
6. Lombardi C, Senna GE, Gatti B, Feligioni M, Riva G, Bonadonna P, Dama AR, Canonica GW, Passalacqua G (1998). Allergic reactions to honey and royal jelly and their relationship with sensitization to compositae. *Allergol. Immunopathol Madr*. 26**:** 288-90.
7. Bonvehi SJ (1991). Contents of minerals and vitamins in royal jelly. *Bull Tech Apicole*, 18**:** 13-20.
8. Iannuzzi J (1990). Royal jelly: Mystery food. 1st part. *Am bee J*, 8**:** 610-12.
9. Iannuzzi J (1990). Royal jelly: Mystery food. 3rd part. *Am bee J*, 10**,** 430-33.
10. Parfitt K (1999). *Martindale*. thirty second edition the complete drug reference published by the pharmaceutical press London SE1 7JN UK. Printed in the USA by Taunton Massachusetts, P. 1626.
11. Lees P, Shojaee Aliabadi F (2002). Rational dosing of antimicrobial drugs: animals versus humans. *International Journal of Antimicrobial Agents*, 19**:** 269-84.
12. Weston RJ, Brocklebank LK, Lu Y (2000). Identication and quantitative levels of antibacterial components of some New Zealand honeys. *Food Chemistry*, 70**:** 427-35.
13. Oka H, Emori Y, Kobayashi N, Hayashi Y, Nomoto K (2001). Suppression of allergic reactions by royal jelly in association with the restoration of macrophage function and the improvement of Th1/Th2 cell responses. *Int-Immunopharmacol.* 1**:** 521-32.
14. Shen X, Lu R, He G (1995). Effects of lyophilized royal jelly on experimental hyperlipidemia and thrombosis. *hung Hua Yu Fang I Hsueh Tsa Chih* ,29:27-9.
15. Vittek J (1995). Effect of royal jelly on serum lipids in experimental animals and humans with atherosclerosis. *Experientia*, 51**:** 927-35.
16. Manfredi R, Chiodo F (2000). The effects of alternative treatments for HIV disease on recommended pharmacological regimens. *International Journal of Antimicrobial Agents.* 13**:** 281–85.
17. Takahashi K, Koshino H, Esumi Y, Tsuda E, Kurosawa, K (2001). SW-163C and E, novel antitumor depsipeptides produced by *Streptomyces* sp. II. Structure elucidation. *J Antibiot*. 54**:** 622-27.
18. Fujii A, Kobayashi S, Kuboyama N, Furukawa Y, Kaneko Y, Ishihama S, Yamamoto H, Tamura T (1990). Augmentation of wound healing by royal jelly in streptozotocin Diabetic Rats. *Japan J Pharmacol*, 53**:** 331-37.
19. Genc M, Aslan A (1999). Determination of trans-10-hydroxy-2-decenoic acid content in pure royal jelly and royal jelly products by column liquid chromatography. *J Chromatogr, A*. 839**:** 265-68.
20. Kitahara T, Sato N, Ohya Y, Shinta H, Hori K (1995). The inhibitory effect of ω-hydroxy acids in royal jelly extract on sebaceous gland lipogenesis.*J of Dermatol Sci*, 10**:** 75-9.
21. Forbes BA, Sahm DF, Weissfeld AS (2002). *Nocardia, Streptomyces, Rhodococcus, Oerskovia,* and similar Organisms In: Bailey & Scott's Diagnostic Microbiology 11th Edition, pp. 351-6.
22. Dong, H, Mahmud T, Tornus I, Lee S, Floss HG (2001). Biosynthesis of the validamycins: identification of intermediates in the biosynthesis of validamycin A by *Streptomyces hygroscopicus* var. limoneus. *J Am Chem Soc*, 123**:** 2733-42.

23. Larsen HS (2000). Aerobic Gram-Positive Bacilli. In: Text book of Diagnostic Microbiology, edited by: CR Mahon and G Manuselis. 2nd Edition. WB Saunders Company Philadelphia, pp. 398400.
24. Holtzel A, Jack RW, Nicholson GJ, Jung, G, Gebhardt K, Fiedler HP, Sussmuth RD (2001). Streptocidins A-D, novel cyclic decapeptide antibiotics produced by *Streptomyces* sp. Tu 6071. II. Structure elucidation. *J Antibiot*, 54**:** 434-40.
25. Rodriguez L, Rodriguez D, Olano C, Brana AF, Mendez C, Salas JA (2001). Functional analysis of OleY L-oleandrosyl 3-O-methyltransferase of the oleandomycin biosynthetic pathway in *Streptomyces* antibioticus. *J Bacteriol,* 183**:** 5358-63.
26. Sasaki T, Igarashi Y, Saito N, Furumai T (2001). Cedarmycins A and B, new antimicrobial antibiotics from *Streptomyces* sp. TP-A0456. *J Antibiot,* 54**:** 567-72.
27. White JD, Hanselmann R, Jackson RW, Porter WJ, Ohba Y, Tiller T Wang S (2001). Total synthesis of rutamycin B, a macrolide antibiotic from *Streptomyces aureofaciens*. *J Org Chem*, 66**:** 5217-31.
28. Schmitzova , Klaudiny J, Albert S, Schroder W, Schreckengost W, Hanes J, Judova J,,Simuth J (1998). A family of major royal jelly proteins of the honeybee *Apis mellifera L Cell Mol Life Sci,* 54**:** 1020-30.