International Journal of Allied Practice, Research and Review
Website: www.ijaprr.com (ISSN 2350-1294)

Antimicrobial Activity of Royal Jelly from Indian Honeybee, *ApisCerana*

AjithaNathKoomankodeGanapathi Ramanathan[#1], Arya Krishna[2]AnanthakrishnanJayakumaran Nair[1] andVethaSundaram Sugunan[3]

[1]Department of Biotechnology, University of Kerala, Karyavattom, Thiruvananthapuram, Kerala, India,
[2]Department of Microbiology, A.J.College of Science and Technology, Thiruvananthapuram, Kerala, India
[3]Department of Zoology, University College, Thiruvananthapuram, Kerala, India

Abstract - Royal Jelly is produced by the honey bee for nurturing the larvae as well as the adult queen. One among the most important properties of Royal Jelly is itsantimicrobial activity. It protects the honeybee against disease and provides immunity.Our study shows that three bacterial strains viz*Bacillussubtilis*, *Salmonellatyphimurim* and *Escherichia coli* were susceptible to RJ. It also exhibited strong antifungal activity against *Aspergillusflavus*.RJ exhibited antimicrobial activity at a concentration of 1000 µg.This is the first report of antimicrobial activity of RJ of *Apiscerana* obtained fromKerala and the effect of RJ against *Aspergillusflavus* is not yet reported.

Keywords - ***Royal Jelly, Apiscerana, Hypopharyngeal gland, Major royal jelly proteins 1, Royalisin, 10 HDA, Jelleines***

I. Introduction

Royal Jelly (RJ) is a nutritious food secreted from the Hypopharyngeal gland (hpg)of 6-12 days old nurse honeybees for feeding larvae and queen bee. Larvae destined to be queen are continuously fed with royal jelly, while other larvae are disconnected from royal jelly after 3 days of hatching after which they are fed with a mixture of honey and pollen[10]. RJ plays an important role in the development of the queen bee and influences caste differentiation. This thick milky white substance has a pH between 3.6-4.2 and is mostly consist of water (60-70%), proteins (12-15%), carbohydrates (10-16%), lipids (3-6%), and traces of salts, vitamins, and free amino acids [5,14,26]. The composition of royal jelly differs with the age and race of nurse bees as well as the regional and seasonal conditions [24, 28]. RJ is one of the unsurpassed neutraceuticalin both traditional and modern medicine because of its antibacterial [7], anti-fungal [12], antiviral [9], antitumor [30], anti-diabetic[23], anti-inflammatory [13], vasodilative [30], hypotensive [22] and anti-hypercholesterolemic [20, 21]activity.

Among these the most remarkable properties of RJ is its antimicrobial activity. Literature show that RJ and its components such as royalisin, Jelleines[15], 10-hydroxy-decenoic acid

(10-HDA) [8, 11]and MRJP1 [4]impart antibacterial activity against both Gram positive bacteriaand Gram negative bacteria. The bactericidal activity of RJ was reported first time by McCleskey and Melampy [16]. However the bioactive principle that provides the activity in RJ was not evident till the identification of fatty acid 10 HDA by Blum et al. [3]. The content of HDA in RJ is considered as an index of its quality [2]. It shows antibacterial activity against both Gram negative and Gram positive bacteria. Melliou and Chinou, [18] identified the antibacterial activity of methanolic and dicholoromethane extracts of RJ. They also found that the fatty acids derivatives 10-acetoxydecanoic acid, trans -10- acetoxydec-2-enoic acid, 11-oxododecanoic acid, (11S)-hydroxydodecnoic acid, (10R,11R)–dihydroxydodecanoic acid, 3,11-dihydroxydodecanoic acid, (11S)-12-dihydroxydodecanoichave antibacterial activity against Gram negative bacteria viz Escherichia coli, Enterobacter cloacae, Klebsiellapneumoniae and Pseudomonas aeruginosa and Gram positive bacteria, Staphylococcus aureus and Staphylcoccus epidermis and antifungal activity three fungus Candida albcans, Candida tropicalis and Candida glabrata.

Studies byFujiwara et al. [7] revealed that apeptide in RJ, royalisinhas antibacterial activity against Gram positive bacteria such as *Lactobacillus*, *Bifidobacterium* and *Leuconstoc*at very low concentrations of 1µM, but not against Gram negative bacteria. This protein is also involved in defense mechanisms that protect honeybees against bacterial attack. It is one of the main proteins of RJ accounting for 80% of the total RJ proteins [28].Bilikova et al.[1] reported the antibacterial effects of royalisinagainst Gram positive bacteria, *Bacillus subtilis* and *Paenibacillus larvae larvae.* Recombinant royalisinalso showed higher antibacterialactivity against Gram positive bacteria than Gram negative bacteria [29]. Another peptide isolated from RJ, Jelleines I, II and III inhibit the growth of bacteria but the activity was reduced when itsC and N terminals were modified [25].Bactericidal activity of RJ varies with the geographical origin, the related botanical species and the genetic variability between the colonies[6].

Antifungal activity of RJ was reported by Sauerwald et al. [27]. They investigated water soluble proteins and peptides in RJ for antifungal activity. Bilikova et al. [1] reportedthat royalisinin RJ provides antifungal activity against *Botrytis cinerea*.Koc et al. [12] investigatedthe antibacterial activityof RJ against fungus, *Candida albicans*, *Candida glabrata*, *Candida krusei* and *Trichosporon spp*.

The main objective of this study was to evaluate the antimicrobial activity of RJ of *A.cerana* in Kerala against various bacteria and fungus.

II. Materials and Methods

A. Sample collection

RJ was collected from the hives maintained at Meenachil Bee garden Pala, Kottayam, Kerala and stored at -20° C until use.

B. Antimicrobial activity

Test microorganisms

The Gram positive bacteria: *Bacillus subtilis* MTCC 121 and Gram negative bacteria: *Salmonella typhimurium* MTCC 98and *Escherichia coli*MTCC40 obtained from the Microbial Type Culture Collection (MTCC), The Institute of Microbial Technology, and Chandigarh, India were grown in nutrient agar. The stock cultures were maintained at 4°C on Nutrient agar slants. Overnight grown bacterial cultures on Nutrient broth adjusted to an optical density 0.5 McFarland turbidity standard (approximately $1.5* 10^8$ CFU/mL) [17].The fungal strains used as test organisms were *Aspergillusflavus*, *Asperigillusniger*and*Candida albicans*obtained from the collectionof clinical isolates maintained at Department of Biotechnology, University of Kerala. The stock cultures were

maintained at 4°C onSabourad Dextrose Agar and 48hrs grownfungal cultures were used as inoculum for antifungal activity. The bacteria were selected on the basis of frequency of occurrence in human infections.

c. Agar well diffusion method

Antibacterial activity

The antibacterial efficacy of the RJ was elucidated by agar well diffusion method[32]. The bacterial cultures were spread on Muller-Hinton agar plates (MHA) and dried for 5 min. RJ was diluted with sterile water to get a concentration of 20 mg/mL. The tests were conducted using 500µg and 1000µg concentration of RJ. Wells of 4mm diameter were punctured on the plate using a sterile well cutter and RJ were aseptically loaded onto the wells. Streptomycin and sterilized water were used as positive and negative control respectively. The plates were incubated overnight at 37°C and the diameter of the zone of inhibition was measured. The experiments were repeated as triplicate and the average diameter was calculated.

Antifungal activity

The fungal cultures were swabbed uniformly on Sabourad Dextrose Agar (SDA) plates. Wells of 4 mm were punched on the agarplates andtests were conducted using 500µg and 1000 µg concentration of RJ. After 72 hrs of incubation at 37 °C, the diameter of the inhibition zone was measured. All the assays were carried out in triplicates and the average diameter was calculated[32].

III. Results and Discussion

RJ was tested for antimicrobial activity against *B. subtilis, E. coli* and *S. typhimurium, A. flavus, A. niger* and *C. albicans* which were represented in Fig.1. In the present study RJ exhibited both antibacterial and antifungal activity a concentration of 1000 µg. The maximum antibacterial activity was observed against *B. subtilis* (12 mm) followed by *S. typhimurium*(7 mm) and *E.coli*(5 mm) respectively. RJ had maximum antifungal activity against *A. flavus*(15 mm) and no inhibitory activity was observed on *A. niger* and *C. albicans.*

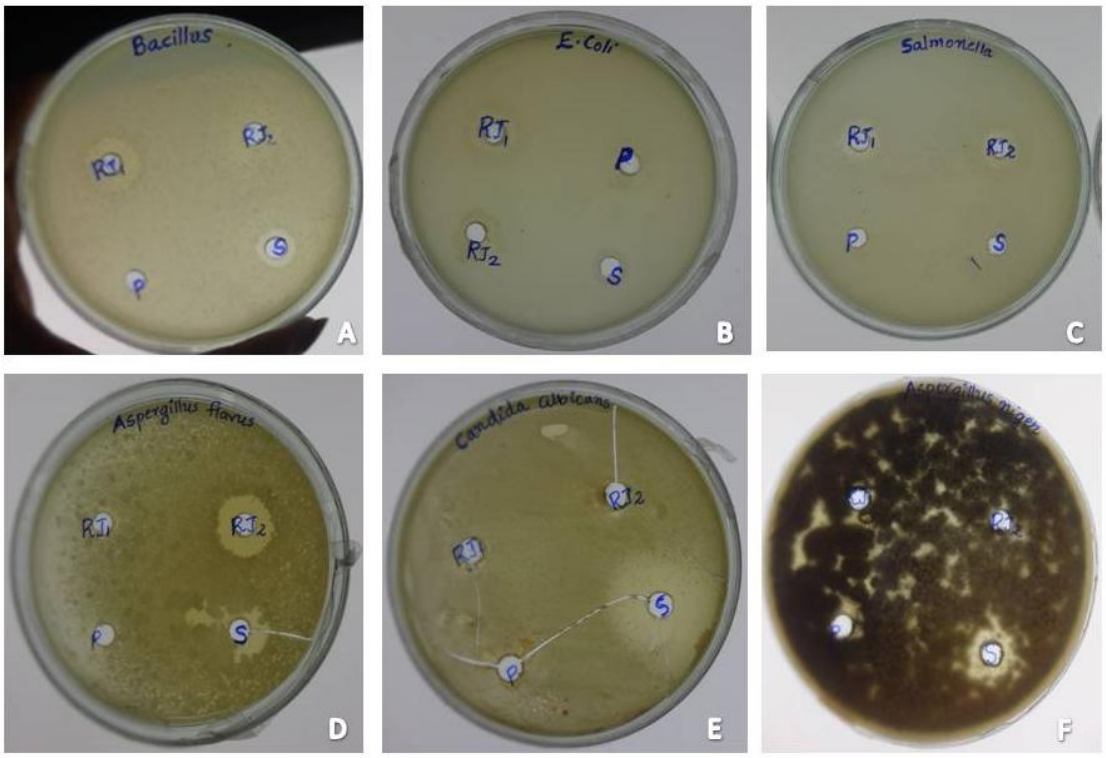

Fig.1:RJ samples showing antimicrobial activity: RJ1(500µg), RJ2(1000µg),S-streptomycin,P-sterile distilled water.

A: Antibacterial activity of RJ against *B. subtilis*, B: Antibacterial activity of RJ against *E.coli*, C: Antibacterial activity of RJ against *S. typhimurium*, D: Antifungal activity of RJ against *A.flavus*, E: Antifungal activity of RJ against *C. albicans*, F: Antifungal activity of RJ against *A. niger*.

The study demonstrates the effect of RJ from *A. cerana* againstbacteria and fungi. The present results indicatethat RJ was more effective against Gram positive bacteria compared to Gram negative bacteria.These results also agree with the studies of Fujiwara et al. [7]. Among the three tested bacterial strainsRJ exhibited strong antibacterial activity against *B. subtilis*at a concentration of 1000µg compared to *E. coli* and *S.typhimurium*. Moselhy et al. [19]and Bilikova et al. [1]also demonstrated the effects of RJ against *B. subtilis*.

RJshowedstrong antifungal activity against *A.flavus*, whichis the most common *Aspergillus*to affect humans after *A. fumigates*, the causative agent of Aspergillosis in immune compromised individuals.Patients with asthma, cystic fibrosis, diabetes mellitus, lung disease and on immunosuppressive drugs are at a high risk of developing problems caused by*Aspergillus*. It also infects beneficial insects such as honeybees and developsinfections to crops such as maize and peanuts and produce potent mycotoxins.

IV. Conclusion

RJ is very important food with nutritional as well as biological and functional properties. The results showed that RJ has both antimicrobial and antifungal activity. It is also confirmed that RJ produced by *A. cerana* from Kerala is a good source of antimicrobial agents that can be used to treat microbial infections.

V. Acknowledgement

This paper was supported by the grant from UGC-RGNF. We thank Mr. Biju and Mrs. RencyBijuofMeenachil Bee Garden, Paala, Kottayamfor providing Royal Jelly for our studies.

VI. References

[1]. Bíliková, K., Wu, G. and Šimúth, J., 2001. Isolation of a peptide fraction from honeybee royal jelly as a potential antifoulbrood factor. *Apidologie*. *32*, 3, 275-283.

[2]. Bloodworth, B.C., Harn, C.S., Hock, C.T. and Boon, Y.O., 1995. Liquid chromatographic determination of trans-10-hydroxy-2-decenoic acid content of commercial products containing royal jelly. *Journal of AOAC International*. *78*, 4, 1019-1023.

[3]. Blum, M.S., Novak, A.F. and Taber, S., 1959. 10-Hydroxy-Δ2-decenoic acid, an antibiotic found in royal jelly. *Science*. *130*, 3373, 452-453.

[4]. Brudzynski, K. and Sjaarda, C., 2015. Honey glycoproteins containing antimicrobial peptides, Jelleins of the Major Royal Jelly Protein 1, are responsible for the cell wall lytic and bactericidal activities of honey. *PloS one*. *10*, 4, e0120238.

[5]. Chen, C. and Chen, S.Y., 1995. Changes in protein components and storage stability of royal jelly under various conditions. *Food chemistry*. *54*, 2, 195-200.

[6]. Fratini, F., Cilia, G., Mancini, S. and Felicioli, A., 2016. Royal Jelly: An ancient remedy with remarkable antibacterial properties. *Microbiological research*. *192*, 130-141.

[7]. Fujiwara, S., Imai, J., Fujiwara, M., Yaeshima, T., Kawashima, T. and Kobayashi, K., 1990. A potent antibacterial protein in royal jelly. Purification and determination of the primary structure of royalisin. *Journal of biological chemistry*. *265*,19, 11333-11337.

[8]. Genç, M. and Aslan, A., 1999. Determination of trans-10-hydroxy-2-decenoic acid content in pure royal jelly and royal jelly products by column liquid chromatography. *Journal of Chromatography A*. *839*,1, 265-268.

[9].Hashemipour, M.A., Tavakolineghad, Z., Arabzadeh, S.A., Iranmanesh, Z. and Nassab, S.A., 2014. Antiviral Activities of Honey, Royal Jelly, and Acyclovir against HSV-1. *Wounds: a compendium of clinical research and practice*. *26*, 2, 47-54.

[10]. Imjongjirak, C., Klinbunga, S. and Sittipraneed, S., 2005. Cloning, expression and genomic organization of genes encoding major royal jelly protein 1 and 2 of the honey bee (Apiscerana). *BMB Reports*. *38*,1, 49-57.

[11]. Kitahara, T., Sato, N., Ohya, Y., Shinta, H. and Hori, K., 1995. 077 The inhibitory effect of ω-Hydroxy acids in Royal Jelly extract on sebaceous gland lipogenesis. *Journal of Dermatological Science*. *10*,1, 75.

[12]. Koç, A.N., Silici, S., Kasap, F., Hörmet-Öz, H.T., Mavus-Buldu, H. and Ercal, B.D., 2011. Antifungal activity of the honeybee products against Candida spp. and Trichosporon spp. *Journal of medicinal food*. *14*, 1-2,128-134.

[13].Kohno, K., Okamoto, I., Sano, O., Arai, N., Iwaki, K., Ikeda, M. and Kurimoto, M., 2004. Royal jelly inhibits the production of proinflammatory cytokines by activated macrophages. *Bioscience, biotechnology, and biochemistry*. *68*, 1, 138-145.

[14].Lensky, Y. and Rakover, Y., 1983. Separate protein body compartments of the worker honeybee (Apismellifera L.). *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*. *75*,4, 607-615.

[15].M Alreshoodi, F. and Sultanbawa, Y., 2015. Antimicrobial Activity of Royal Jelly. *Anti-Infective Agents*. *13*, 1, 50-59.

[16]. McCleskey, C.S. and Melampy, R.M., 1939. Bactericidal properties of royal jelly of the honeybee. *Journal of Economic Entomology*. *32*, 4, 581-587.

[17].McFarland, J., 1907. The nephelometer: an instrument for estimating the number of bacteria in suspensions used for calculating the opsonic index and for vaccines. *Journal of the American Medical Association*. *49*,14,1176-1178.

[18].Melliou, E. and Chinou, I., 2005. Chemistry and bioactivity of royal jelly from Greece. *Journal of agricultural and food chemistry*. *53*, 23, 8987-8992.

[19]. Moselhy, W.A., Fawzy, A.M. and Kamel, A.A., 2013. An evaluation of the potent antimicrobial effects and unsaponifiable matter analysis of the royal jelly. *Life Science Journal*. *2*,10, 290-296.

[20]. Nakajin, S., Okiyama, K., Yamashita, S., Akiyama, Y. and Shinoda, M., 1982. Effect of royal jelly on experimental hypercholesterolemia in rabbits. *YakugakuZasshi*. *36*, 65-69.

[21]. Pasupuleti, V.R., Sammugam, L., Ramesh, N. and Gan, S.H., 2017. Honey, propolis, and royal jelly: a comprehensive review of their biological actions and health benefits. *Oxidative medicine and cellular longevity*. 2017.

****

[22]. Pavel, C.I., Mărghitaş, L.A., Bobiş, O., Dezmirean, D.S., Şapcaliu, A., Radoi, I. and Mădaş, M.N., 2011. Biological activities of royal jelly-review. *Scientific Papers Animal Science and Biotechnologies*, *44*(2), pp.108-118.

[23]. Pourmoradian, S., Mahdavi, R., Mobasseri, M., Faramarzi, E. and Mobasseri, M., 2014. Effects of royal jelly supplementation on glycemic control and oxidative stress factors in type 2 diabetic female: a randomized clinical trial. *Chinese journal of integrative medicine*. *20*, 5, 347-352.

[24]. Qu, N., Jiang, J., Sun, L., Lai, C., Sun, L. and Wu, X., 2008. Proteomic characterization of royal jelly proteins in Chinese (*Apisceranacerana*) and European (*Apismellifera*) honeybees. *Biochemistry (Moscow)*. *73*, 6, 676.

[25]. Romanelli, A., Moggio, L., Montella, R.C., Campiglia, P., Iannaccone, M., Capuano, F., Pedone, C. and Capparelli, R., 2011. Peptides from Royal Jelly: studies on the antimicrobial activity of jelleins, jelleins analogs and synergy with temporins. *Journal of peptide science*. *17*, 5, 348-352.

[26] Santos, K.S., dos Santos, L.D., Mendes, M.A., de Souza, B.M., Malaspina, O. and Palma, M.S., 2005. Profiling the proteome complement of the secretion from hypopharyngeal gland of Africanized nurse-honeybees (Apismellifera L.). *Insect biochemistry and molecular biology*. *35*, 1, 85-91.

[27]. Sauerwald, N., Polster, J., Bengsch, E., Niessen, L. and Vogel, R.F., 1998. Combined antibacterial and antifungal properties of water soluble fractions of royal jelly. *Advances in food sciences*. *20*, 1-2, 46-52.

[28]. Schmitzova, J., Klaudiny, J., Albert, Š., Schröder, W., Schreckengost, W., Hanes, J., Judova, J. and Šimúth, J., 1998. A family of major royal jelly proteins of the honeybee Apismellifera L. *Cellular and Molecular Life Sciences*. *54*,9, 1020-1030.

[29]. Shen, L., Liu, D., Li, M., Jin, F., Din, M., Parnell, L.D. and Lai, C.Q., 2012. Mechanism of action of recombinant acc-royalisin from royal jelly of Asian honeybee against gram-positive bacteria. *PloS one*. *7*,10, e47194.

[30]. Shinoda, M., Nakajin, S., Oikawa, T., Sato, K., Kamogawa, A. and Akiyama, Y., 1978. Biochemical studies on vasodilative factor in royal jelly (author's transl). *Yakugakuzasshi: Journal of the Pharmaceutical Society of Japan*. *98*,2, 139-145.

[31]. Šimúth, J., Bíliková, K., Kováčová, E., Kuzmová, Z. and Schroder, W., 2004. Immunochemical approach to detection of adulteration in honey: physiologically active royal jelly protein stimulating TNF-α release is a regular component of honey. *Journal of agricultural and food chemistry*. *52*,8, 2154-2158.

[32]. Wayne, P.A., 2002. NCCLS (National Committee for Clinical Laboratory Standards) Method for dilution antimicrobial susceptibility tests of bacteria that grow aerobically. *Approved Standard. M100-S12*.

[33]. Zhou, J., Zhao, J., Yuan, H., Meng, Y., Li, Y., Wu, L. and Xue, X., 2007. Comparison of UPLC and HPLC for determination of trans-10-hydroxy-2-decenoic acid content in royal jelly by ultrasound-assisted extraction with internal standard. *Chromatographia*. *66*, 3-4,185-190.