

# Protein Analysis of Royal Jelly Bee *Apis Mellifera Carpatica* as Candidates for Immunotherapy in Reproductive Disorders

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## ABSTRACT

**Background:** The honey bee of the *Apis mellifera* species is the main type of bee cultivated in almost all countries in the world, including Indonesia. Honey has benefits in various aspects, including food, health, and beauty. Royal jelly is a daily food menu as a food supplement to maintain and increase health and vitality.

**Purpose:** Analyzing the immunogenic and toxinogenic proteins of Royal jelly *Apis mellifera carpatica* as immunotherapy. **Method:** Conversion of nucleotides into amino acids. Royal jelly *Apis mellifera carpatica*, analysis of the three-dimensional structure of Protein of Royal jelly *Apis mellifera carpatica*, Analysis of Ramachandran Plots of Royal jelly *Apis mellifera carpatica*, Analysis of epitope and allergen proteins, Analysis of antigens and toxins. **Research result:** The research results found that six proteins of Royal jelly *Apis mellifera carpatica* were epitope, antigenic, and non-toxic. Allergens and three Royal jelly proteins, *Apis mellifera carpatica*, are non-allergenic. In this study, the three-dimensional structure has not been found and opens opportunities for proteomic studies of the Royal jelly *Apis mellifera carpatica* protein, including protein isolation. **Conclusion:** The findings of this study can be used as a basis for the use of immunotherapy materials against the protein Royal jelly *Apis mellifera carpatica* against reproductive disorders.

**Key words:** Analysis, Protein, Royal jelly, *Apis mellifera carpatica*, Immunotherapy.

## INTRODUCTION

With its diverse climate, the Indonesian region allows for the growth of various plants, including various commodities from fruits, vegetables, and ornamental plants that produce nectar or pollen, which is a source of food for honey bees. Honey bee cultivation activities have existed and have been known to the Indonesian people since ancient times; only these activities were not properly cultivated at that time but were carried out with makeshift equipment; thus, results were not optimal. The honey bee of the *Apis mellifera* species is the main type of bee cultivated in almost all countries in the world, including Indonesia. These bees are found in many European countries (France, Greece, Italy, Spain, and Yugoslavia).<sup>1-3</sup>

Health is one of the basic human needs besides food, clothing, and shelter. The Law of the Republic of Indonesia Number 36 of 2009 defines health as a physically, psychologically, spiritually, and socially sound condition that enables everyone to lead a socially and economically useful life. Therefore, everyone tries to maintain his health. One way to maintain health is to take preventive measures by consuming honey. Honey is a sweet liquid produced by bees from plant nectar and kept in the cells of a beehive. Honey has benefits in various aspects, including food, health, and beauty. The honey bee business has great potential to be developed in Indonesia. With 193 million hectares of agricultural and plantation land and 143 million hectares of forest area, Indonesia has vast natural resources for developing the honey industry. The benefits of honey are not just to overcome various kinds of diseases. Many bee products are no less

useful, including royal jelly, pollen (bee pollen), and bee propolis.<sup>4,5</sup>

In Indonesia, Royal jelly is better known as the queen bee's milk. Royal jelly is food for the queen bee and the larvae (prospective) bees whose age is 1 to 3 days; in one bee colony, there is one queen bee, hundreds of male bees, and tens of thousands of worker bees (99% of the number of bees). Secretions from the hypopharyngeal glands of worker bees aged 5-15 days. Royal jelly is food from the queen, supplied by larval and adult bees, young worker bees, and male bee larvae. Public knowledge about the Efficacy of Royal jelly has been known since the beginning of human civilization. Historical records of ancient Egypt inform about the efficacy of this queen bee milk, even though it is said that the beauty of Queen Cleopatra's face cannot be separated from the efficacy of the queen bee's milk in the cosmetic ingredients she uses. The nobility of Europe, the Middle East, and Asia put Royal jelly on a daily food menu as a food supplement to maintain and increase the vitality of their health; even in Islamic civilization, the role of bees and the efficacy of honey has a special place in the world of medicine/medicine.<sup>6,7</sup> This study aimed to analyze the immunogenic and toxinogenic proteins of Royal jelly *Apis mellifera carpatica* as immunotherapy.

## METHODS

### Conversion of nucleotides to amino acids Royal jelly *Apis mellifera carpatica*

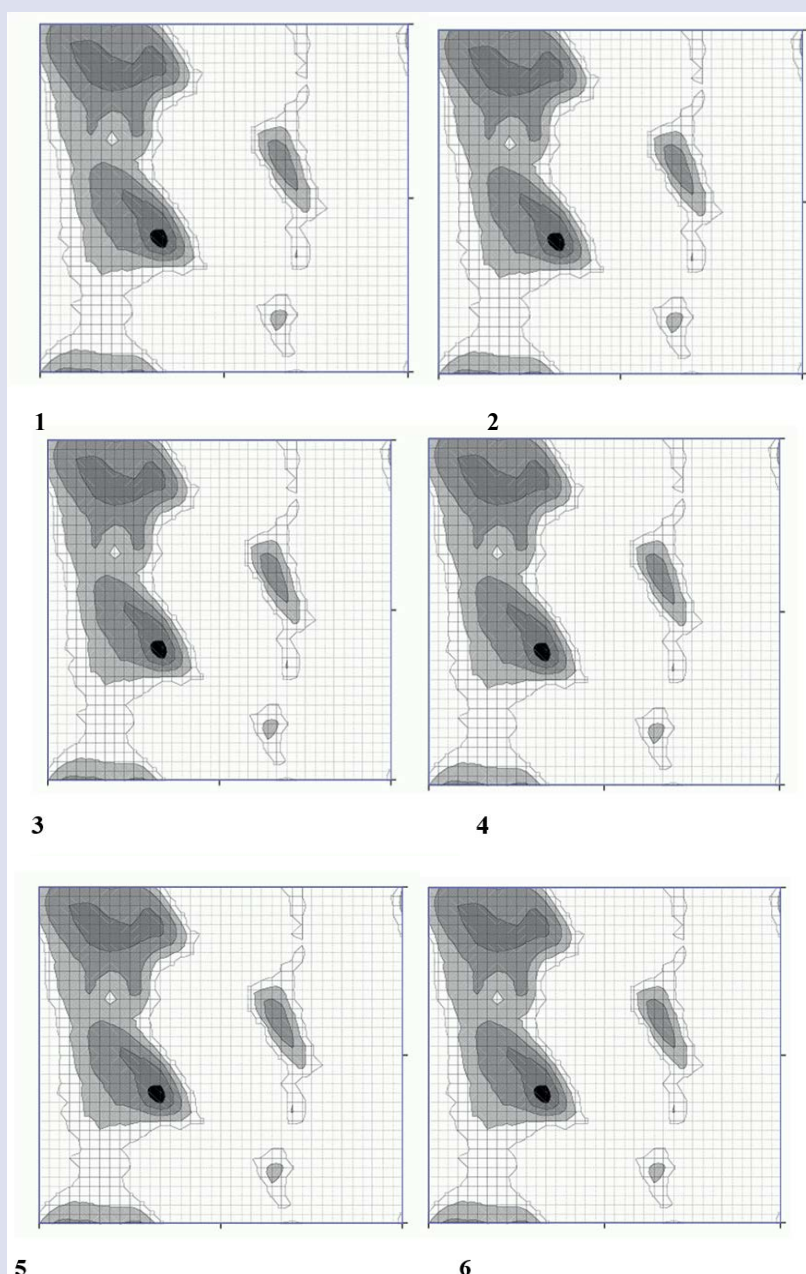
Royal jelly *Apis mellifera carpatica* nucleotides were taken from the NCBI gene bank and converted into amino acids using the ExPASy Translate Tool software.

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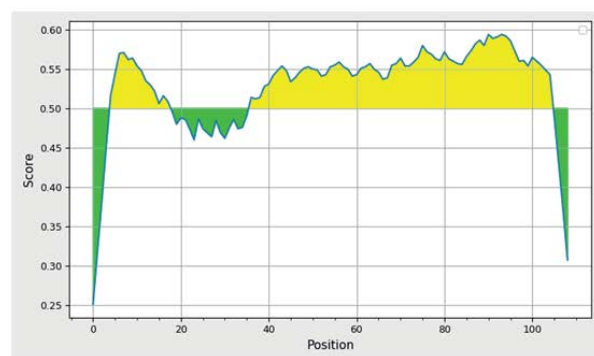
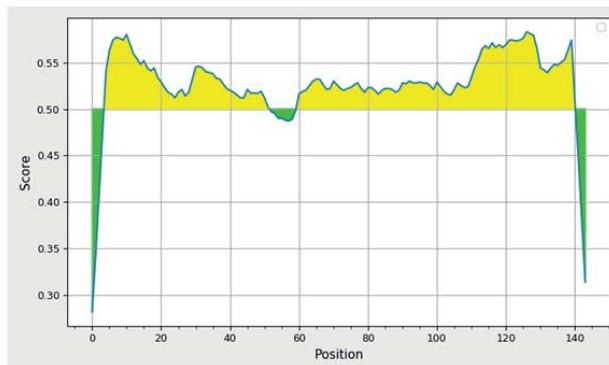
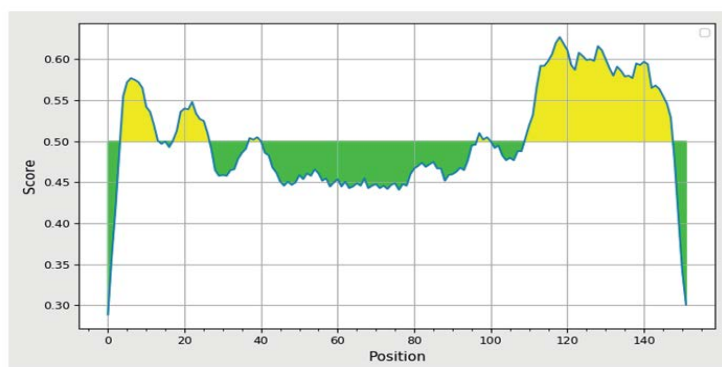


**Table 3: Royal Jelly *Apis mellifera carpatica* protein which is toxic and allergen.**

Protein Royal Jelly <i>Apis mellifera carpatica</i>	Epitope protein	Toxic proteins	Allergenic proteins
1	ISQSECWQSECWQS	Non-toxin	Probable allergen
2	-	-	-
3	-	-	-
4	-	-	-
5	LLSFLFLLSFCLLPFLLLSFCLLPFCLLSF- CLLPFCLLSFCLLPFCLL	Non-toxin	Probable non-allergen
	LLPFCLLSFCLLPFCLLSFCLLPFCLLS- FCLLPFCLLSFCLLSFCLLPFCLLAFL- SAFLSAFLSTFLSAFLPAFLPAFLYL	Non-toxin	Probable non-allergen
	YYHSDYSDCY	Non-toxin	Probable non-allergen
6	CYHSSCYHSV	Non-toxin	Probable allergen
	HFVCHSDYQHSDCQHSDCQHSDC- QHSDCQHSDCQHSDC	Non-toxin	Probable allergen



**Figure 1: Ramachandran Plot Protein Royal Jelly *Apis mellifera carpatica*.**

**Protein number 1****Protein number 5****Protein number 6****Figure 2:** The results of the protein analysis of Royal Jelly *Apis mellifera carpatica*, which are epitope.

number 1, 2 proteins from protein number 5 and 3 pieces of protein from protein number 6. In addition, antigenicity analysis was also carried out on the protein Royal jelly *Apis mellifera carpatica*; from the research results, four proteins were antigenic and two non-antigenic.

In this study, an analysis was also carried out on the protein of Royal jelly *Apis mellifera carpatica*, a non-toxic and toxin protein, and a protein of Royal jelly *Apis mellifera carpatica*, which is allergen and non-allergenic. The research results found that six proteins of Royal jelly *Apis mellifera carpatica* were non-toxic. In addition, based on the analysis of Royal jelly *Apis mellifera carpatica* protein on allergen and non-allergenic properties, it was found that three proteins of Royal jelly *Apis mellifera carpatica* were allergen and three proteins of Royal jelly *Apis mellifera carpatica* were non-allergenic.

## DISCUSSION

Traditional medicine is still used and trusted by the community, one of which is honey. Since ancient times honey has been used for treatment such as wounds, fever, internal heat, and mixed with food ingredients to increase body fitness. Honey is generally adequate for creating energy, enhancing endurance, and enhancing stamina. The magnesium mineral content of honey is identical to the magnesium mineral content of blood serum. In addition, the Fe content in honey can increase the number of erythrocytes in human blood and can increase hemoglobin levels.<sup>10</sup> Royal jelly functions as a tonic to restore energy, get rid of pain, and improve appetite.<sup>11</sup> Royal jelly mostly contains protein, sugar, fat (fatty acid), and minerals. Royal jelly is the most effective for maintaining stamina when combined with honey; royal jelly is useful as an energy

and stamina booster, boosts the immune system, and maintains overall health. Royal jelly relieves various problems such as fatigue, anxiety, mild depression, insomnia, and lack of energy and stamina.<sup>12</sup> Royal jelly also has the ability as a stimulant hormone to stimulate and regulate endocrine function and secretion of other hormones and its involvement in sexual manifestations and endocrine disorders. Royal jelly is associated with therapy to accelerate the restoration of disturbed normal functions through its action on the adrenal cortex.<sup>13</sup> Royal jelly is said to increase appetite, increase memory, treat diabetes, overcome infertility, and in people who are recovering, honey is used to accelerate healing and help form body cells.<sup>14</sup>

Royal jelly has a high protein content obtained from pollen processing, although it is believed that honey is also a secretion. Royal jelly is a thick, milky white liquid with a strong sour taste, rich in nutrients, pungent taste, and slightly bitter taste. Scientists and nutritionists from various countries with sophisticated laboratories have repeatedly analyzed what is contained in this royal jelly. Investigations of the content of natural compounds were started in 1852 by a chemical analyst, LL Langstroth; the content of natural compounds is very complex. The most recent research conducted by scientists and nutritionists demonstrated that royal jelly has a perfect protein composed of 22 types of amino acids that are further split into two groups, necessary amino acids and non-essential amino acids. In detail royal jelly contains: - Protein: 12,50% (22 types of amino acids) - Carbohydrates: 12,50% - Fat (unsaturated fat): 6,00% - Water (H<sub>2</sub>O): 65,00% - Minerals: 0,82% - Bio-Active Agent: 3-4%.<sup>15-17</sup>



This study conducted an immunoinformatics analysis of the protein Royal jelly *Apis mellifera carpatica*. This study found six proteins of Royal jelly *Apis mellifera carpatica*: epitope, antigenic and non-antigenic proteins, allergen and non-allergenic proteins, toxin, and non-toxic proteins. Proteins that are antigenic in minimal portions (8-15 amino acids) can induce the immune system.<sup>18,19</sup> Water-soluble glycoproteins having molecular weights between 10-70 KD are frequently allergenic.<sup>20</sup> Allergies can trigger mild symptoms such as itching, runny nose and eyes, and swelling. Allergies can also cause severe reactions such as anaphylaxis, leading to death.

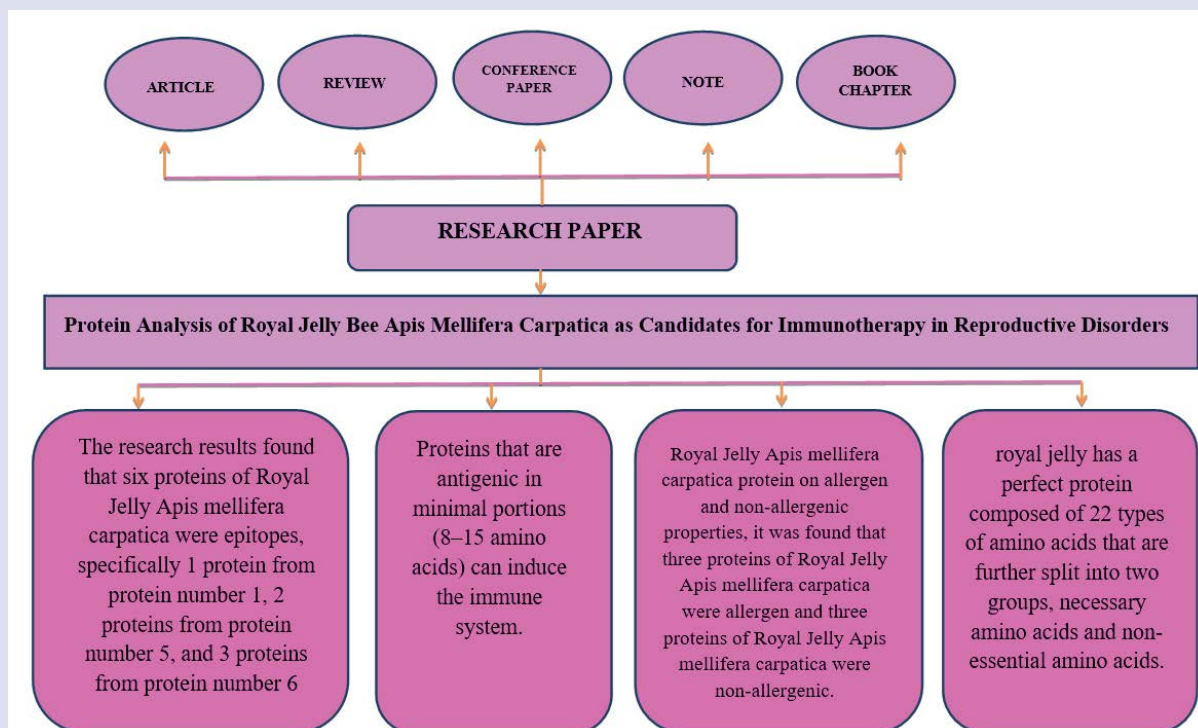
## CONCLUSION

The research results found that six proteins of Royal jelly *Apis mellifera carpatica* were epitopes, specifically 1 protein from protein number 1, 2 proteins from protein number 5, and 3 proteins from protein number 6. In addition, antigenicity analysis was also carried out on Royal protein. Jelly *Apis mellifera carpatica*, from the research results carried out, four proteins were antigenic, and two proteins were non-antigenic. This study also found that six proteins of Royal jelly *Apis mellifera carpatica* were non-toxic. In addition, based on the results of the analysis of the Royal jelly *Apis mellifera carpatica* protein on allergen and non-allergenic properties, it was found that three proteins of Royal jelly *Apis mellifera carpatica* were allergen and three proteins of Royal jelly *Apis mellifera carpatica* were non-allergenic. In this study, the three-dimensional structure has not been found and opens opportunities for proteomic studies of the Royal jelly *Apis mellifera carpatica* protein, including protein isolation.

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## GRAPHICAL ABSTRACT



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