Pharmacogn J. 2023; 15(1): 175-182
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com

Research Article

Analysis of Royal Jelly *Apis Cerana* as Therapeutic Candidate in Cbavd Based on Bioinformatics Studies

Maslichah Mafruchati[1,*], Jonathan Makuwia[2]

Maslichah Mafruchati[1,*], Jonathan Makuwia[2]

[1]*Department of Veterinary Anatomy, Faculty of Veterinary Medicine (60115), Universitas Airlangga, Mulyorejo, C Campus, Surabaya, INDONESIA.*

[2]*Malawi University of Science and Technology 5196 Limbe Malawi*

Correspondence

Maslichah Mafruchati

Department of Veterinary Anatomy, Faculty of Veterinary Medicine (60115), Universitas Airlangga, Mulyorejo, C Campus, Surabaya, INDONESIA.

E-mail: maslichahmafruchati01@gmail.com

History

- Submission Date: 30-09-2022;
- Review completed: 29-10-2022;
- Accepted Date: 10-11-2022.

DOI : 10.5530/pj.2023.15.26

Article Available online

http://www.phcogj.com/v15/i6

ABSTRACT

Background: *Apis cerana* honey bee is a honey bee native to Asia that spreads from Afghanistan, China, and Japan to Indonesia. The productivity of *Apis cerana* honey bees can produce as much as 2-5 kg of honey per colony in a year. Royal jelly is the queen's nourishment, and it is produced by larvae, adult bees, young worker bees, and male bee larvae. CBAVD contributes to 1–2% of the 20–25% of males with subfertility. **Aim**: The objective of this study was to evaluate Royal Jelly *Apis Cerana* as a potential therapeutic candidate for CBAVD using a bioinformatics approach. **Method:** This research method consisted of converting nucleotides into amino acids, analyzing the three-dimensional structure of *Apis Cerana* Royal Jelly Protein, Ramachandran Plot Analysis, Analysis of Epitope and Allergen Proteins, and Analysis of Proteins that were antigens and toxins. **Results:** The research results were conducted on six three-dimensional *Apis Cerana* Royal Jelly bee protein structures and had very good validity based on the Ramachandran plot, GQME value, and QmeanDisCo value. In addition, this study also obtained the results of proteins that are epitope, antigenic, non-allergenic, and non-toxic. **Conclusion:** The findings of this study can be used as a basis for therapy against CBAVD.

Key words: *Apis Cerana*, Bioinformatics, CBAVD, Therapeutic Candidates, Public Health.

INTRODUCTION

Apis cerana honey bee is a honey bee native to Asia that spreads from Afghanistan, China, and Japan to Indonesia.[1] The productivity of *Apis cerana* honey bees can produce as much as 2-5 kg of honey per colony in a year. *Apis cerana* is widely developed by people in Indonesia because this bee is more resistant to disease; besides, it also has higher adaptability to the environment than *Apis mellifera*.[2,3] *Apis cerana* can be developed in the highlands and lowlands. It has been kept for centuries in various parts of Asia, including Indonesia. Moreover, some maintenance methods are still traditional, among others, by placing them in a log or other simple place. Modern maintenance has been carried out in movable boxes.

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. There is one queen bee, hundreds of male bees, and tens of thousands of worker bees in a single bee colony (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, which is 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 have a special place in the world of medicine/medicine.

About 1 in every 6–10 couples have fertility problems. Sub-fertility derives from males in 20–25 % of the cases, females in 30–40 % of the cases, and both in 30 % of the cases. The causes of sub-fertility remain unexplained in 15% of the cases.[4] Between the 20–25 % of males with sub-fertility, CBAVD accounts for 1–2 %.[5] The purpose of this study was the analysis of Royal Jelly Apis Cerana as a therapeutic candidate for CBAVD based on a bioinformatic study.

METHOD

Conversion of nucleotides to amino acids

Apis Cerana Royal Jelly nucleotides were taken from the NCBI gene bank and converted into amino acids using the Expasy Translate Tool software.

Analysis of the three-dimensional structure of *Apis Cerana* royal jelly protein

The research steps were carried out to predict the three-dimensional structure of the *Apis Cerana* Royal Jelly protein by homology according to the Protein Structure Homology Modeling Using SWISS-MODEL Workspace protocol.

Ramachandran plot analysis

The outputs of the three-dimensional protein structure study of *Apis Cerana* Royal Jelly were entered into the Ramachandran Plot Server program in the form of PDB files. (https://zlab.umassmed.edu/bu/rama/).

Epitope and allergen protein analysis

Apis Cerana Royal Jelly amino acids are included in the IEDB software to obtain epitope proteins. *Apis*

Cite this article: Mafruchati M, Makuwia J. Analysis of Royal Jelly *Apis Cerana* as Therapeutic Candidate in Cbavd Based on Bioinformatics Studies. Pharmacogn J. 2023;15(1): 175-182.

Cerana Royal Jelly amino acids are included in the Allerton software to get the allergen protein.

Analysis of proteins that are antigenic and toxin

Apis Cerana Royal Jelly amino acids are included in Vaxijen software to obtain antigenic proteins. *Apis Cerana* Royal Jelly amino acids are included in the Toxinpred software to obtain Toxic and Non-Toxic proteins.

RESULTS

The concept of Central dogma flow of genetic information consists of three main processes of using the information in cells. The first step is replication, which involves copying parent DNA to generate daughter DNA molecules with identical nucleotide sequences. The second phase is transcription, which involves copying DNA's genetic code into RNA molecules. The third step is translation, which converts the genetic message recorded in messenger RNA to a polypeptide with a specific amino acid sequence on the ribosome. Six amino acids are formed due to the translation of nucleotides into amino acids; the amino acid composition of *Apis Cerana* Royal Jelly bee is shown in table 1.

This study also carried out a three-dimensional structural analysis of the protein from *Apis Cerana* Royal Jelly bee and the Ramachandran plot. The research results obtained 6 three-dimensional structures of *Apis Cerana* Royal Jelly bee protein.

From the analysis results using the Ramachandran plot, it was found that the analysis of the three-dimensional structure of the *Apis Cerana* Royal Jelly bee protein no. 1 had a level of conformity with proteins that existed in nature by 95.4 percent and could be used as quality research data.

The Ramachandran plot analysis revealed that the three-dimensional structure of the *Apis Cerana* Royal Jelly bee protein number 2 conforms to 97.3 percent of the proteins found in nature and hence may be used as high-quality research data.

The analysis results using the Ramachandran plot found that the analysis of the three-dimensional structure of the *Apis Cerana* Royal

Table 1: Nucleotides of *Apis Cerana* royal jelly bee after conversion to amino acids.

1	MTKWLLLMACLGIACQNIRGAVVRENSSRKKLTNTLNVIHEWKYVDYDFGSDEKRQAAIQSGEYDRTKNYPLDVDQWHDKTFVTMLRYDGVPSSLNVVSDKTGNGGPLLQPYPDWSFAKYEDCSGIVSANKIAIDEYERLWVLDSGLVNNIQPMCSPKLLAFDLTTSKLLKQVEIPHDVAVNATTGKGGLASLAVQAMDSVNTMVYMADNKDDALIVYQNADDSFHRLSSHISNHNFRSDKMSQENLTLKEVDNRVFGMALSSVTHNLYYSPLSSQNLYYVNTTSLMNSQNQGNDVQYESVQDVFSSQLSAKAVSKNGVLFFGFTNNTLGCWNEHQSLDRQNIDIVARNETLQMVVGMKIKQNLPQSGKVNNTQRNEHLLALTNKKQDVLNNDLNLEHVNFQILDANVNDLIRNSRCANSDNQDNNQHNYNHNQVRHSSKSDNQNNNQHNNQAYHSSKSDNWDNNNNQAHHSSKFDNQNNNQYNNVHHSSSNHVKSDNSFLDVSQIFKISLHYKTNKINIVFRIKKKKKKKKK
2	QNGCCWHALALVKILEVPLFEKIPREKNQIRTFTNGSMSIMISVATKKGKLRFNLANMIVRKIILLTSINGMIRLLSLCDTMVCLPLTWYLTKLATVDRFYNLIPIGHLLSMKIALESAPTKLLSTNMRDCGFWTRALSIIFNLCVLQNCLPLILLRNCSSKSRYRTMLPMPPQERADHLLFKLWILILWCTWQITKMMLLSTKMPMILSIDCLPTFPITTLDLTKCRKKISPKKTTEFLEWHLVPRIIFIIVLSLLRIYITLTQHRTRKIKEMTCSMKVSKTFSAVNYPLKQYRKMAYSFSDSRIILLVAGMSISHLTDKISILLEMRRFKWSLVRLSKTFHNLAKLIIHKEMNICWLPTKSRTCTTILISNMTSKFWMLMTTYGIVVAQILTIRIIINIIIIIKFVILQNLTIRITINITIKLIILQSLTIGITITIKLIIPQNLIIRITINITIRFIILHQIMLNLIINLFSMVKYFKKFHYIIKRIKISFFAKKKKKKKK
3	DKMVAVDGMPWHSLSKYRCRCSRKFLEKKINKYVERDSRMEVCRLFRRRKKASCDSIWRISYEKLSSRRSMADFCHYVKIRWCAFLFERGIQNWQRWTASTTLSRLVICVRLLWNRERQQNCYRRIEIVGSGLGPCQYSTYVFSKIACLFDYFEIAQASRDTARCCRKCHHRKGRISIFSCSSYGFCKYYGVHGRQRCFNCLPKCRFFPSIVFPHFQSQLIQNVARKSHLERSRQQSFWNGTFRDASLLSSLFSEFILRHNIVNELAKSRKRAVKCPRRFQQSIIRSSIEKWRTLFRIHEYSWLLEASVTQTKYRYCSSKDASNGRWYEDAKPSTIWQSYTKKTFVGFNQQKAGRAKQRSSRTCELPNFGCCKRLDTESLRKFQSGSTLSSSSSFFKIQSEQSTQSSLSFFKVQLGQQSSSSFLKISQSIQLGSSFFIKSCILIFSRCKSNILKNFITLNENKYRFSHKKKKKKKKK
4	FFFFFFFFFYAKNDIYFIRFIMNFLKYLTYIEKRLIIRFNMIRMMNLIVILIVILIIKFGMMSLIVIVIPIVRLRMISLIVMLIVILIVRFRMTNLIMIIIMLIIILIVRICATTIPYQVVYISIQNLEVHMFEIKIVVHVLLFVGSQQMFISLCIINFARLWKVLLNLHTNDHLKRLISSYNIDILSVKLMLIPATKSIIRESEKEYAIFRYCFSGLTAENVLDTFILHVISLILRVHRCCVNVIILRRERTIIKIMRHGTKCHSKNSVVYFFQGEIFLRHFVRSKVVIGNVGRQSMERIIGILVDNSIIFVICHVHHSIYRIHSLNSRCSALSCGGIYGNIVRYLDLLEQFRSSQIKGKQFWRTHRLNIIDKARVQNPQSLIFVDSNFVGAHDSRAIFILSKPIGIRLKRSTVASFVRYHVQRGRHTIVSHSDKSLIMPLIDVKRIIFRTIIFARLNRSLPFFVATEIIIDILPFVNHVQRICFFSRGIFSNNGTSNILTSYAKACHQQQPFCH
5	FFFFFFFFFMRKTIFILFVLCNEIFNILTSRKDLSDLTFDEETLLYLLFLSNFEEALLLLSQLSDFEEALLCLLFLSDFEERTLLLCLLSLSEFAQRLFRIKSFTLASKIWKFTCSRLRSLFSTSCFLLVKANKCSFLCVLLTLPDCGRFCLIFIPTTISVSFRATISIFCLSSDCSFQQPRVLFVNPKKSTPFFDTALADLLKTSWTLSYCTSFPFCEFINDVVLTYKFEERGLRLCVTELSAIPKTLLSTSFKVRFSCDILSDLKLLEMWEDNRWKESSAFWTIKASSLLSAMYTIVFTESIATAKDANPPFPVVAFTATSCGISTCLSNFEVVKSKASNFGEHIGILLTRPESRTHNLSYSSIAILLALTIPEQSSYLANDQSGGCRSGPPLPVLSDTTFKEEGTPSYLNIVTKVLSCHSTSRGFFVRSYSPDIAACLFSSLPKSSTYFHSITFNVFVNFFLEEFSRTTAPLIFQAMPRHAINSNHFV
6	FFFFFFFFLCEKRYLFYSFYNVMKFFKIFDLHREKINYQIHDLMKNDEPNCYIDCYSDYQILRNDELDCYCYPNCQTLKNDKLDCYVDCYSDCQILKNDELDYDYNYVDYYPDCQNLRNDYSVSSRLHHPKFGSSHVRDDRCLARPAFCWLKPTNVHFFVYYLCQIVEGFASSYQRPFEASHFELQYRYFVCQVTDAHSSNQEYYSIRKRVRHFSILLRIIDCKRLGHFHTARHFLDFASSLTMLCRNINSEKREDYNKDYASRNVPFQKLCCLLLSRDFLATFCQISCDWKCGKTIDGKNHRHFGRQLKHHLCYLPCTPYLQNPLEQLKMLIRPFLWWHLRQHRAVSRLAAISKSNQRQAILENTVEYYQGPSPEPTISHIRRQFCWRSRFQSNLHTQMTNRDKVVEAVHRCQFCQIPRSKRKAHHRILTQKSYHAIDRRQEDNFSYDHIRQIESQLAFFRRYRNHNRHTSIRESRSTYLLIFFSRNFLEQRHLYFDKLCQGMPSTATILS

Table 2: Characteristics of *Apis Cerana* royal jelly protein.

No	GQME Value	QmeanDisCoValue
1	0.13	0.43 ± 0.06
2	0.01	0.34 ± 0.09
3	0.01	0.24 ± 0.12
4	0.01	0.20 ± 0.12
5	0.02	0.31 ± 0.11
6	0.00	0.53 ± 0.12

Table 3: *Apis Cerana* royal jelly bee protein which is epitope and allergen.

Apis Cerana Royal Protein Jelly bees	Peptides that are Epitopes	Position	Allergen
1	WKYVDYDFGSDEKRQAAIQSGEYDRT	42-67	PROBABLE NON-ALLERGEN
	KTGNGGPLLQPYPDWSFAKYEDC	101-123	PROBABLE NON-ALLERGEN
	SHISNHNFRSDKMSQENLTLKEV	230-252	PROBABLE ALLERGEN
	MNSQNQGNDVQYESVQDVFSSQ	287-308	PROBABLE NON-ALLERGEN
	TLQMVVGMKIKQNLPQSGKVNNTQRN	351-376	PROBABLE ALLERGEN
2	VDRFYNLIPIG	97-107	PROBABLE ALLERGEN
	ESAPTKLLSTNMRDC	117-131	PROBABLE NON-ALLERGEN
	SSKSRYRTMLPMPPQERAD	160-178	PROBABLE NON-ALLERGEN
	PTFPITTLDLTKCRKKISPKKTTEFLE	215-241	PROBABLE NON-ALLERGEN
	VSKTFSAVNYPLKQYRKMAYSFS	280-302	PROBABLE NON-ALLERGEN
3	AVDGMPWHSLSKYRCRCSRKFLEKKINKYVERDSRME	5-41	PROBABLE ALLERGEN
	RRRKKASCDSIWRISYEKLSSRRSMA	47-72	PROBABLE ALLERGEN
	RFFPSIVFPHFQSQLIQNVARKSHLERSRQQSFWNGTFRDAS	206-247	PROBABLE ALLERGEN
	AKSRKRAVKCPRRFQQSIIRSSIEKWRTLFRIHEYSW	267-303	PROBABLE NON-ALLERGEN
	FGCCKRLDTESLRKFQSGSTLSSSSSFFKIQSEQSTQSSLSF	369-410	PROBABLE ALLERGEN
	VQLGQQSSSSFLKISQSIQLGS	413-434	PROBABLE NON-ALLERGEN
4	IYRIHSLNSRCSALSCGG	321-338	PROBABLE ALLERGEN
	LEQFRSSQIKGKQFWRTHRLNIIDKARVQNP	350-380	PROBABLE ALLERGEN
5	DLKLLEMWEDNRWKESSAF	265-283	PROBABLE ALLERGEN
	ISTCLSNFEVVKSKASNFG	326-344	PROBABLE NON-ALLERGEN
	EQSSYLANDQSGGCRSGPPLPVLSDTTFKEEGTPS	376-410	PROBABLE NON-ALLERGEN
6	SCHSTSRGFFVRSYSP	420-435	PROBABLE ALLERGEN
	IFDLHREKINYQIHDLMKNDE	28-48	PROBABLE NON-ALLERGEN
	ILKNDELDYDYNYVDYYPDCQNLRNDYSVSSRLHHPKFGSSHVRDDRC	95-142	PROBABLE NON-ALLERGEN
	AISKSNQRQAILENTVEYYQGPSPEPTI	352-379	PROBABLE NON-ALLERGEN
	IPRSKRKAHHRILTQKSYHAIDRRQEDNFSYDHIRQ	418-453	PROBABLE ALLERGEN
	FLEQRHLYFDKLCQGMPSTA	489-508	PROBABLE ALLERGEN

Table 4: *Apis Cerana* royal jelly bee protein which is antigenic and toxic.

Apis Cerana Royal Protein Jelly bees	Epitopes Peptides	Antigenic Peptides	Toxic Peptides
1	WKYVDYDFGSDEKRQAAIQSGEYDRT	Probable ANTIGEN	Non-Toxin
	KTGNGGPLLQPYPDWSFAKYEDC	Probable ANTIGEN	Non-Toxin
	SHISNHNFRSDKMSQENLTLKEV	Probable ANTIGEN	Non-Toxin
	MNSQNQGNDVQYESVQDVFSSQ	Probable ANTIGEN	Non-Toxin
	TLQMVVGMKIKQNLPQSGKVNNTQRN	Probable ANTIGEN	Non-Toxin
2	VDRFYNLIPIG	Probable NON-ANTIGEN	Non-Toxin
	ESAPTKLLSTNMRDC	Probable ANTIGEN	Non-Toxin
	SSKSRYRTMLPMPPQERAD	Probable ANTIGEN	Non-Toxin
	PTFPITTLDLTKCRKKISPKKTTEFLE	Probable ANTIGEN	Non-Toxin
	VSKTFSAVNYPLKQYRKMAYSFS	Probable NON-ANTIGEN	Non-Toxin
3	AVDGMPWHSLSKYRCRCSRKFLEKKINKYVERDSRME	Probable ANTIGEN	Non-Toxin
	RRRKKASCDSIWRISYEKLSSRRSMA	Probable ANTIGEN	Non-Toxin
	RFFPSIVFPHFQSQLIQNVARKSHLERSRQQSFWNGTFRDAS	Probable NON-ANTIGEN	Non-Toxin
	AKSRKRAVKCPRRFQQSIIRSSIEKWRTLFRIHEYSW	Probable NON-ANTIGEN	Non-Toxin
	FGCCKRLDTESLRKFQSGSTLSSSSSFFKIQSEQSTQSSLSF	Probable ANTIGEN	Toxin
	VQLGQQSSSSFLKISQSIQLGS	Probable ANTIGEN	
4	IYRIHSLNSRCSALSCGG	Probable ANTIGEN	Non-Toxin
	LEQFRSSQIKGKQFWRTHRLNIIDKARVQNP	Probable NON-ANTIGEN	Non-Toxin
5	DLKLLEMWEDNRWKESSAF	Probable NON-ANTIGEN	Non-Toxin
	ISTCLSNFEVVKSKASNFG	Probable NON-ANTIGEN	Non-Toxin
	EQSSYLANDQSGGCRSGPPLPVLSDTTFKEEGTPS	Probable ANTIGEN	Non-Toxin
6	SCHSTSRGFFVRSYSP	Probable NON-ANTIGEN	Non-Toxin
	IFDLHREKINYQIHDLMKNDE	Probable NON-ANTIGEN	Non-Toxin
	ILKNDELDYDYNYVDYYPDCQNLRNDYSVSSRLHHPKFGSSHVRDDRC	Probable ANTIGEN	Non-Toxin
	AISKSNQRQAILENTVEYYQGPSPEPTI	Probable NON-ANTIGEN	Non-Toxin
	IPRSKRKAHHRILTQKSYHAIDRRQEDNFSYDHIRQ	Probable NON-ANTIGEN	Non-Toxin
	FLEQRHLYFDKLCQGMPSTA	Probable NON-ANTIGEN	Non-Toxin

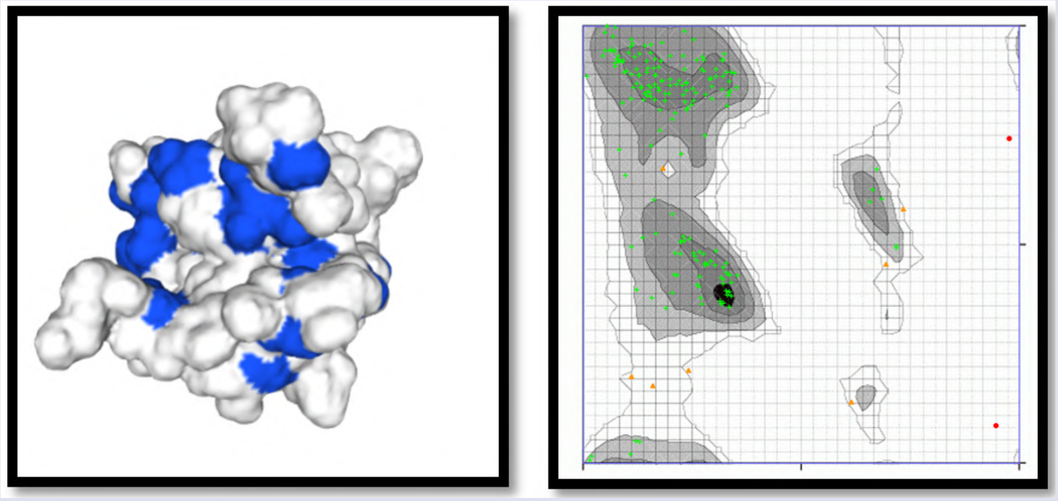


Figure 1: Analysis of the three-dimensional structure of the *Apis Cerana* Royal Jelly bee protein no 1 and the Ramachandran plot.

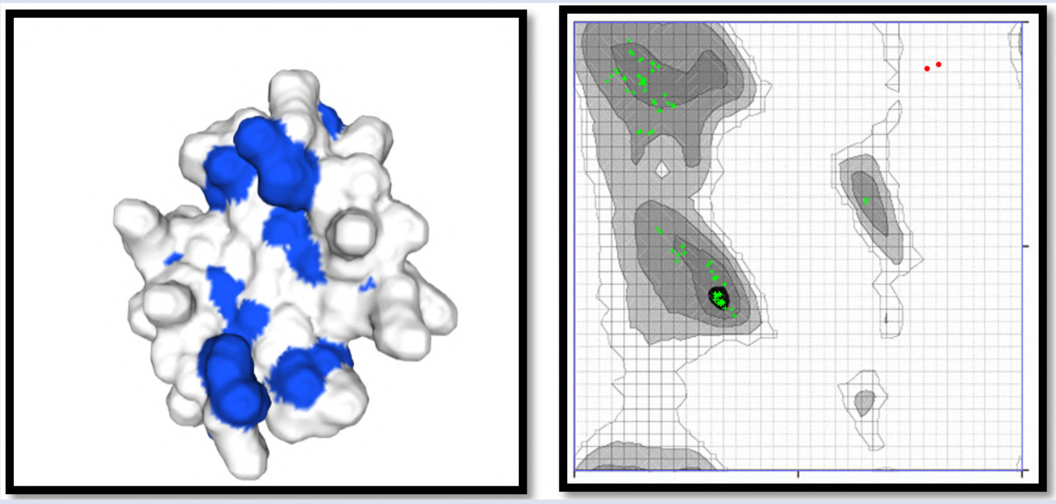


Figure 2: Analysis of the three-dimensional structure of the *Apis Cerana* Royal Jelly bee protein no 2 and the Ramachandran plot.

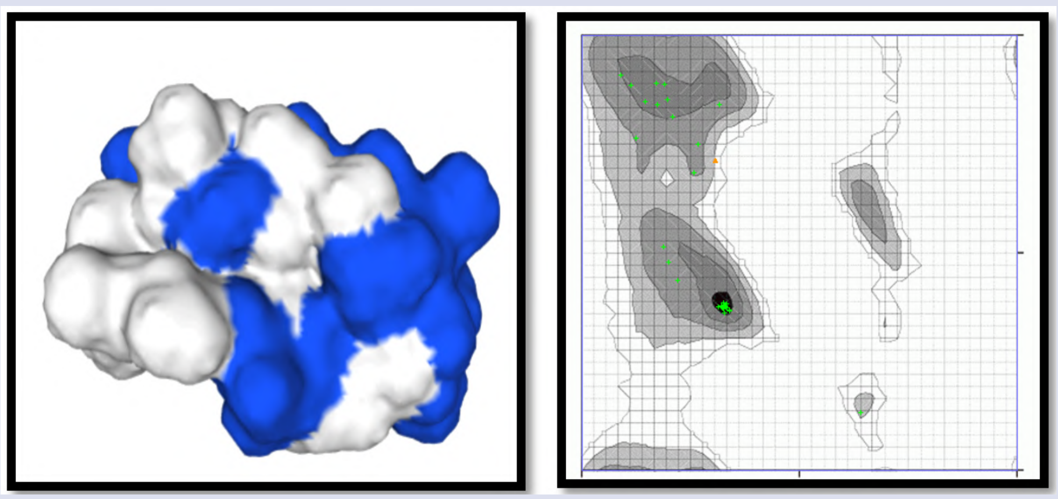


Figure 3: Analysis of the three-dimensional structure of the *Apis Cerana* Royal Jelly bee protein no 3 and the Ramachandran plot.

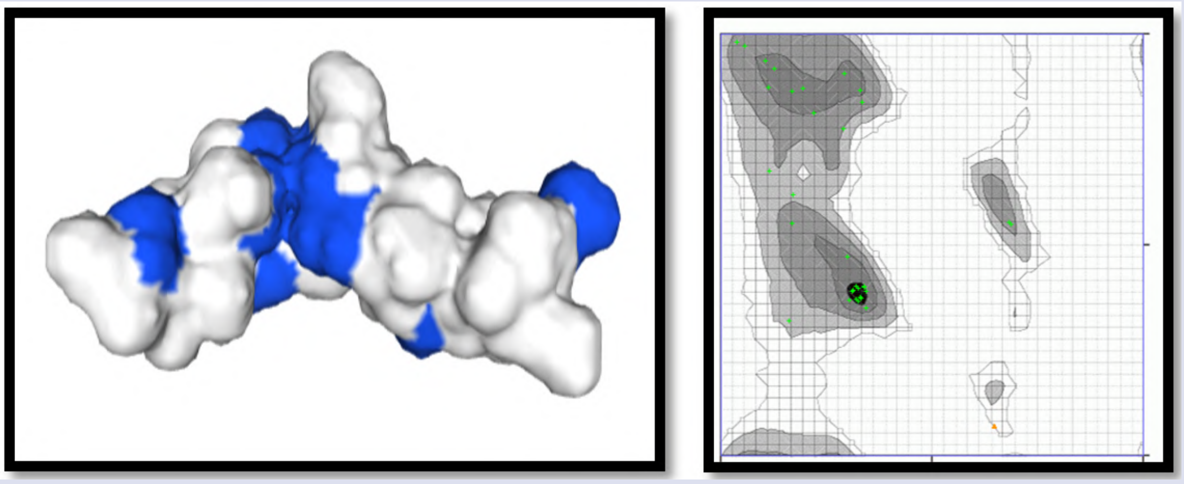


Figure 4: Analysis of the three-dimensional structure of the *Apis Cerana* Royal Jelly bee protein no 4 and the Ramachandran plot.

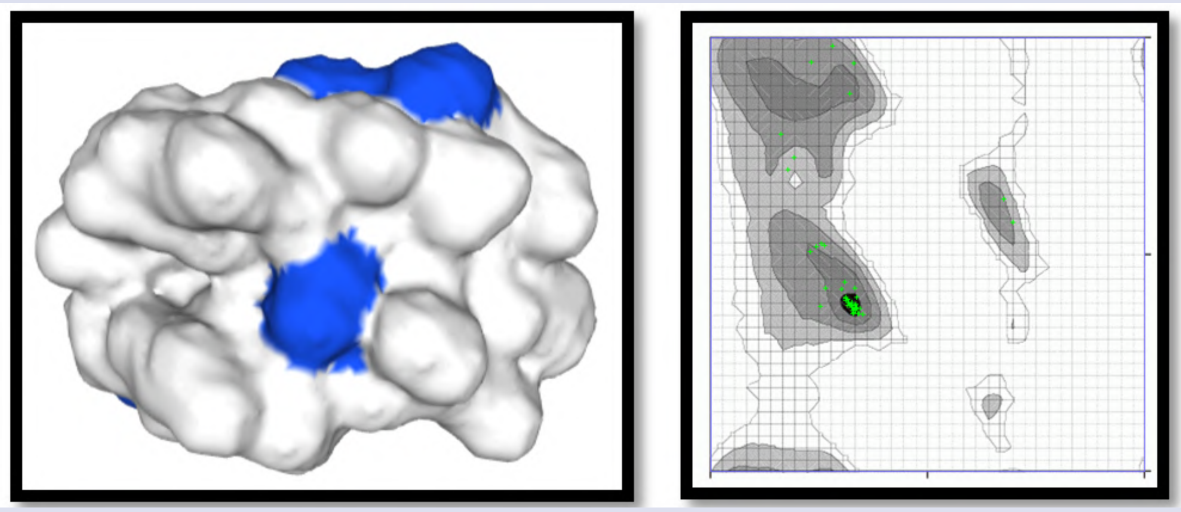


Figure 5: Analysis of the three-dimensional structure of the *Apis Cerana* Royal Jelly bee protein number 5 and the Ramachandran plot.

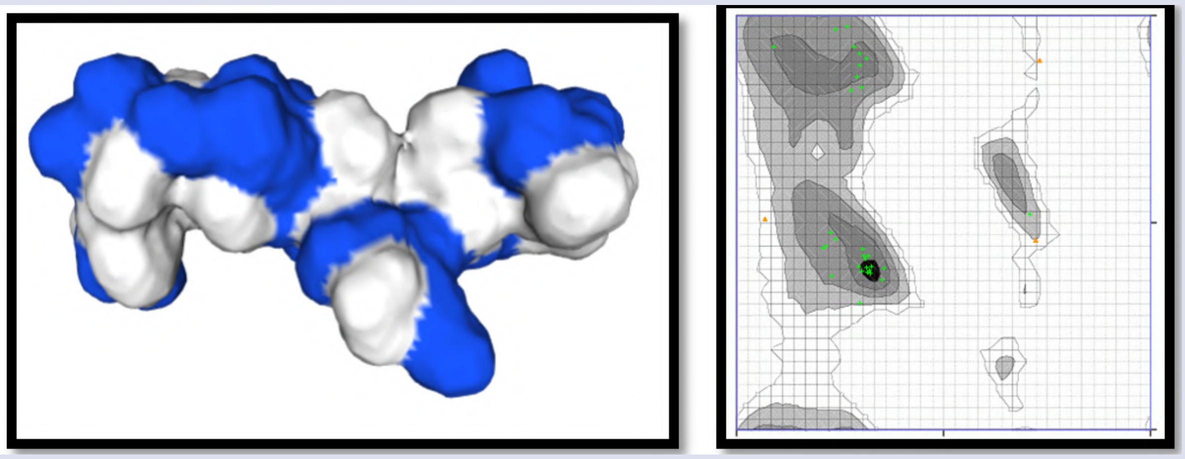


Figure 6: Analysis of the three-dimensional structure of the *Apis Cerana* Royal Jelly bee protein number 6 and the Ramachandran plot.

Jelly bee protein no 3 has a level of compatibility with proteins that exist in nature at 96.8 percent and can be used as quality research data.

From the analysis results using the Ramachandran plot, it was found that the analysis of the three-dimensional structure of the *Apis Cerana* Royal Jelly bee protein no 4 has a 96.9 percent level of conformity with proteins that exist in nature and can be used as quality research data. In addition, the analysis of the three-dimensional structure of the *Apis Cerana* Royal Jelly bee protein no 5 has a level of conformity with proteins that exist in nature by 100 percent and can be used as quality research data. Analysis of the three-dimensional structure of the protein *Apis Cerana* Royal Jelly bee no. 6 has a level of conformity with proteins that exist in nature of 90.9 percent and can be used as quality research data.

This study also obtained the results of GQME, Qmean value, and ligand properties in each *Apis Cerana* Royal Jelly bee protein. The research results found that the GQME and Qmean values varied, and the binding site not conserved ligand was more dominant in *Apis Cerana* Royal Jelly bee protein.

In this study, an analysis was carried out using the IEDB to analyze the amino acids of *Apis Cerana* Royal Jelly, which act as an epitope. In addition, the protein analysis of *Apis Cerana* Royal Jelly was also carried out, which was allergen and non-allergenic. The research results obtained 27 proteins of *Apis Cerana* Royal Jelly, which were epitopes, and found that the majority of *Apis Cerana* Royal Jelly proteins were non-allergenic.

In this study, analysis was also carried out using Vaxijen and ToxinPred software to analyze *Apis Cerana* Royal Jelly proteins which are antigenic and toxic. The results of research using Vaxijen found that the majority of *Apis Cerana* Royal Jelly proteins were antigenic. In addition, from the results of research conducted using ToxinPred, it was found that the majority of *Apis Cerana* Royal Jelly proteins are non-toxic. *Apis Cerana* Royal Jelly protein which is toxic has an amino acid composition of FGCCKRLDTESLRKFQSGSTLSSSSSFFKIQSEQSTQSSLSF. The results of research on *Apis Cerana* Royal Jelly proteins which are antigenic and toxic can be seen in table 4.

DISCUSSION

Traditional medicine, such as honey, is still utilized and trusted by the community. Since ancient times, honey has been used to treat wounds, fever, and internal heat and enhance bodily fitness when combined with food ingredients. Generally, honey effectively generates energy, increases endurance, and boosts stamina. Moreover, the magnesium mineral concentration of honey is identical to that of blood serum. In addition, the Fe content in honey can increase the number of erythrocytes in human blood and can increase hemoglobin levels.[6]

Royal jelly functions as a tonic to restore energy, get rid of pain, and improve appetite.[7] 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.[8]

Royal jelly also has the ability as a stimulant hormone whose ability 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.[9] 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.

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 very 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 newest research conducted by scientists and nutritionists demonstrates that the fundamental component of royal jelly is a perfect protein composed of 22 different amino acids classified 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 (H20): 65.00% - Minerals: 0.82% - Bio-Active Agent: 3-4%.[10,11]

Protein structures are classified as primary, secondary, tertiary, and quaternary. The main structure is a straightforward arrangement of amino acid sequences linearly akin to the order of letters in a word, with no chain branching. A protein's secondary structure is a two-dimensional structure composed of primary structures that are linearly stabilized by hydrogen bonds between =CO and =NH groups located along the polypeptide backbone. The -helix is an example of a secondary structure.[12] A protein's tertiary structure is an overlapping layer on top of a secondary structural pattern composed of an uneven twisting of the bonds between its side chains (R groups) of various amino acids. This three-dimensional conformation refers to the unique interaction between secondary structures.[13]

Three approaches are now available for modeling the three-dimensional structure of proteins: the homology/comparative method, the fold identification method, and the ab initio method.[14] Homology modeling is a technique for simulating the three-dimensional structure of a protein by aligning its amino acid sequence with that of a homologous protein whose three-dimensional structure is well-known in the laboratory (template).[15] The difficulty level of the process based on homology is lower than that of the fold recognition and ab initio methods. Furthermore, the homology technique is faster than working with other methods.[14]

If no template protein exists in the database, the ab initio approach is used to model the protein. The ab initio method is the most demanding and sophisticated method available and takes the longest to complete. The ab initio method is based on simulating the three-dimensional structure of proteins using the energy function. This approach is only applicable to tiny proteins, and the resulting accuracy is also quite low.[16]

Fold recognition modeling is a reasonably simple technique compared to ab initio but more challenging than homology.[14] The fold recognition method works by comparing the target sequence to the template structure in the library to generate the structural model with the highest fold value.[17] Thus, in all circumstances, when a template protein structure is available, the homology modeling method is the optimal way for generating a three-dimensional protein structure model *in silico*.[16]

Additionally, homology modeling is commonly utilized in virtual screening, the design of mutagenesis experiments, and the investigation of the impacts of sequence variation.[16,18] According to the study's findings, there were six three-dimensional configurations of the *Apis Cerana* Royal Jelly protein, each with its unique properties. Additionally, this investigation yielded the Ramachandran plot results. The Ramachandran plot is critical since it is one method for validating the three-dimensional structure of a protein.

GMQE (Global Model Quality Estimate) is a quality estimate that considers both the alignment of the template to the target and the structure of the template. They were merged to predict the IDDT scores from the resulting model using a trained multilayer perceptron.

GMQE is available prior to the actual model construction and aids in selecting the appropriate template for the modeling task. After developing the model, the investigation results produced values.[19] This study demonstrates that the three-dimensional structure of the six *Apis Cerana* Royal Jelly bee proteins meets the specifications for use, with values ranging from 0.00 to 0.13.

The global QMEANDisCo score is the median QMEANDisCo score for all residues, highly correlated with the IDDT score. The offered error estimates are based on the global QMEANDisCo scores determined for many models and represent the difference in the root mean squares (i.e., standard deviation) of the global QMEANDisCo and IDDT scores (basic truth). Because the prediction's dependability is proportional to the model's size, a given error estimate is generated using a model of the same size as the input (Studer, G et al., 2020; Benkert P et al., 2011). The values found from the research are 0,20 ± 0,12 to 0.53 ± 0.12. This finding demonstrates the low mistake rate of the six *Apis Cerana* Royal Jelly bee proteins.

This study carried out an immunoinformatics analysis on the protein Royal Jelly *Apis Cerana* Royal Jelly. This method can help the discovery of peptide vaccines, namely vaccines that consist of a minimal portion of antigen (8–15 amino acids) that can induce the immune system.[20] From the results of the research conducted, it was obtained an overview of immunogenic proteins. In addition, the results of this study also obtained proteins from *Apis Cerana* Royal Jelly, which are non-allergenic and non-toxic. Frequently allergenic compounds are water-soluble glycoproteins with molecular weights ranging between 10-70 KDa.[21] Allergies can trigger mild symptoms such as itching, runny nose and eyes, and swelling. Allergies can also cause severe reactions such as anaphylaxis which can cause death.

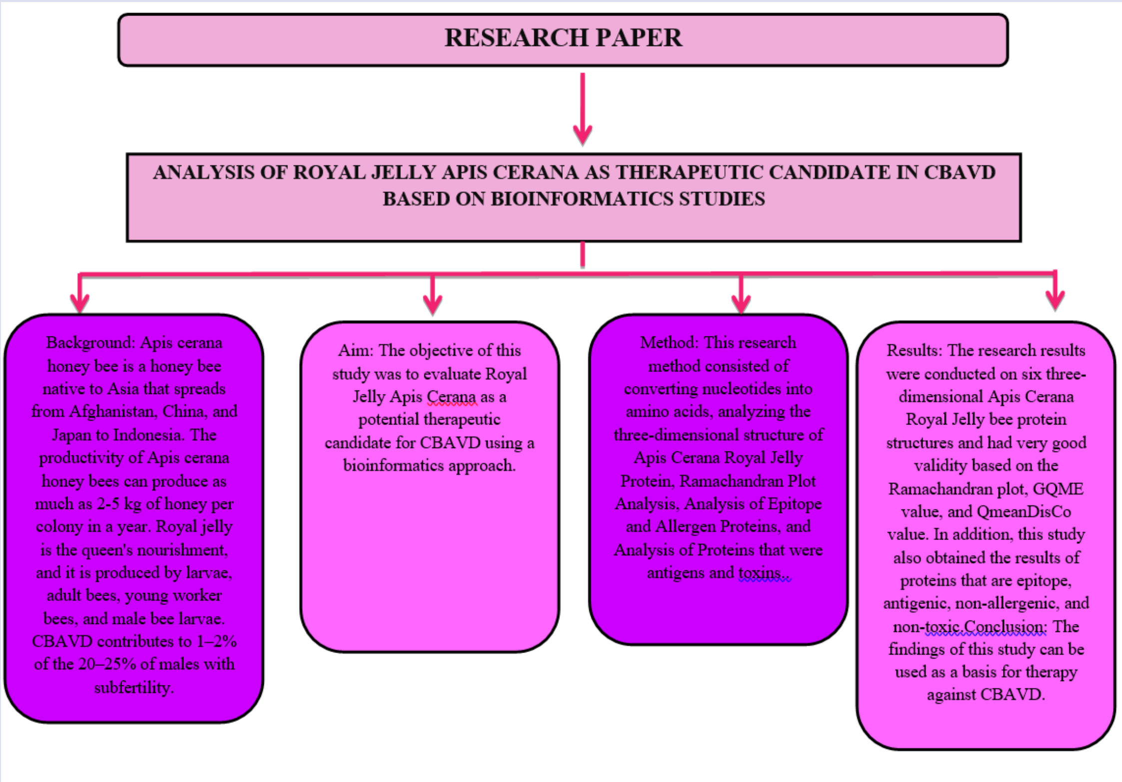
CONCLUSION

The Ramachandran plot, GQME value, and QmeanDisCo value indicate that the six three-dimensional structures of *Apis Cerana* Royal Jelly bee protein have a high degree of validity. Additionally, this study determined proteins' epitope, antigenic, non-allergenic, and non-toxic properties. The findings of this study can be used as a basis for therapy against CBAVD.

REFERENCES

1. Hadisoesilo S. The diversity of indigenous honey bee species of Indonesia. Biodiversitas J Biol Diversity. 2001;2(1).
2. Anwar APM, Maruddin F, Yuliati FN, Wangdi JT, Dagong MIA. The physiochemical properties of kefir using honey concentrations. Canrea Journal: Food Technol Nutritions Culinary J. 2021;8-16.
3. Mafruchati M, Makuwia J. Protein Analysis of Royal Jelly Bee Apis Mellifera Carpatica as Candidates for Immunotherapy in Reproductive Disorders. Pharmacogn J. 2022;14(4).
4. Organization WH. Towards more objectivity in diagnosis and management of male infertility. Results of a World Health Organization multicenter study. Int J Androl. 1987;7.
5. Hussein TM, Zakaria NH, Zahran AM. Clinical, laboratory and genetic assessment of patients with congenital bilateral absent vas deferens. Andrologia. 2011;43(1):16-22.
6. Lestari I, Lestari AE. Effectiveness of Honey in Increasing Hemoglobin Levels of Mothers Post Sectio Caesarea. Humanistic Network for Sci Technol. 2019.
7. Jabde PV. Text Book of Applied Zoology. Discovery Publishing House. 2005.
8. Chillemi S. The complete herbal guide: A natural approach to healing the body. Lulu Press, Inc. 2013.
9. Elkins R. Bee pollen, royal jelly, propolis and honey: an extraordinary energy and health-promoting ensemble. Houghton Mifflin Harcourt. 1996.
10. Elnagar SA, Elghalid OA, Abd-Elhady AM. Royal jelly: can it reduce physiological strain of growing rabbits under Egyptian summer conditions? Animal. 2010;4(9):1547-52.
11. Saritas N, Yildiz K, Büyükipekci S, Coskun B. Effect of different levels of royal jelly on biochemical parameters of swimmers. Afr J Biotechnol. 2011;10(52):10718-23.
12. Taylor WR, May ACW, Brown NP, Aszódi A. Protein structure: geometry, topology and classification. Rep Progress Phys. 2001;64(4):517.
13. Nelson DL, Cox MM. Lehninger principles of biochemistry lecture notebook. Macmillan. 2004.
14. Zaki M, Bystroff C. Protein structure prediction (Vol. 413). Springer Science & Business Media. 2007.
15. Šali A, Blundell TL. Comparative protein modelling by satisfaction of spatial restraints. J Molecular Biol. 1993;234(3):779-815.
16. Kopp J, Bordoli L, Battey JND, Kiefer F, Schwede T. Assessment of CASP7 predictions for template-based modeling targets. Proteins: Struct Funct Bioinform. 2007;69(S8):38-56.
17. Hung LH, Samudrala R. PROTINFO: secondary and tertiary protein structure prediction. Nucleic Acids Res. 2003;31(13):3296-9.
18. Hillisch A, Pineda LF, Hilgenfeld R. Utility of homology models in the drug discovery process. Drug Discovery Today. 2004;9(15):659-69.
19. Waterhouse A, Bertoni M, Bienert S, Studer G, Tauriello G, Gumienny R, *et al*. SWISS-MODEL: homology modelling of protein structures and complexes. Nucleic Acids Res. 2018;46(W1):W296-W303.
20. Vita R, Mahajan S, Overton JA, Dhanda SK, Martini S, Cantrell JR, *et al*. The immune epitope database (IEDB): 2018 update. Nucleic Acids Res. 2019;47(D1):D339-43.
21. Cianferoni A, Spergel JM. Food allergy: review, classification and diagnosis. Allergol Int. 2009;8(4):457-66.

GRAPHICAL ABSTRACT



ABOUT AUTHORS

Professor Jonathan Makuwira hold a position as the Deputy Vice Chancellor of the Malawi University of Science and Technology. Professor Makuwira holds a Diploma in Education from University of Malawi (Chancellor College), Bachelor of Education with Honours (BEd Hons.) and Master of Philosophy (MPhil.) both from University of Nottingham, UK; and Doctor of Philosophy (PhD) in International Development Studies from University of New England, Australia.

Dr. Maslichah Mafruchati is senior lecturer from Faculty of Veterinary Medicine of Universitas Airlangga (UNAIR), Indonesia. She specializes not only in embryology as her foundation from former education, but also in herbs and medicine. She also teaches in postgraduate school of UNAIR as lecturer for medical major.

Cite this article: Mafruchati M, Makuwia J. Analysis of Royal Jelly Apis Cerana as Therapeutic Candidate in Cbavd Based on Bioinformatics Studies. Pharmacogn J. 2023;15(1): 175-182.