



## *Crataegus Oxyacantha* Based on Bioinformatics Studies and Toxicity Test on The Chicken Embryo

Maslichah Mafruchati\*

Fakultas Kedokteran Hewan, Universitas Airlangga, Indonesia

Email : [maslichah-m@fkh.unair.ac.id](mailto:maslichah-m@fkh.unair.ac.id)

\*Penulis korespondensi: [maslichah-m@fkh.unair.ac.id](mailto:maslichah-m@fkh.unair.ac.id)

**Abstract.** Hawthorn extract has been used for ameliorating cardiac disorders and pulmonary hypertension. The main chemical constituents of hawthorn flavonoid extract (HFE) include flavonoids (1-2%), oligomeric proanthocyanidins (1-3%), and other bioactive components (e.g., triterpene acids, organic acids, sterols, and cardioactive amines). These compounds are reported to have many pharmacological effects, including neuroprotective, hepatoprotective, cardioprotective, and nephroprotective effects. This study was aimed the analysis *Crataegus oxyacantha* based on a bioinformatic study and toxicity test on the chicken embryo. This method consisted of analysis of the three-dimensional structure of *Crataegus oxyacantha* Protein, Epitope and Allergen Proteins, *Crataegus oxyacantha* Proteins that were antigens and toxins and toxicity test on the chicken embryo. The results of research conducted on 3 three-dimensional structures of *Crataegus oxyacantha* protein, GOME value and QmeanDisCo value. In addition, this study also obtained the results of proteins that are epitope, antigenic, non-allergenic and non-toxic and toxicity test on the chicken embryo was 250 ng/egg. Morphological description of the embryo on the 21st day after injection, at a concentration of 250 ng of *Crataegus oxyacantha* /egg product, an abnormal embryological picture was obtained. Chicken Embryo Weight and Body Length Measurements were carried out in chicken embryos. Need research for other species of plant.

**Keywords:** Bioinformatics, Chicken Embryo, Chicken Semicolon, *Crataegus Oxyacantha*, Toxicity Test.

### 1. BACKGROUND

There is some evidence that hawthorn extract may help alleviate heart conditions and pulmonary hypertension. Hawthorn flavonoid extract (HFE) primarily contains flavonoids (1-2%), oligomeric proanthocyanidins (1-3%), various bioactive components (such as triterpene acids, organic acids, sterols, and cardioactive amines), and other chemicals. There are several pharmacological effects that these chemicals are said to have, such as actions that protect the nervous system, liver, heart, and kidneys. In addition, hawthorn fruits may lower cardiovascular risk due to their tonic effects on the heart. One of hawthorn's natural secondary metabolites, flavonoids, is an antioxidant that helps reduce the effects of oxygen-derived free radicals, boosts cardiovascular health, regulates hypertension and heart conditions. The beneficial health benefits of hawthorn extract are thought to be caused by flavonoids and oligomeric proanthocyanidins. The flavonoids found in hawthorn (*Crataegus oxyacantha*) have antioxidant properties and may help reduce the impact of heart conditions, as well as avoid PHS and ascites (Ahmadipour et al., 2020; Jachimowicz et al., 2022; Jasemi, 2020; Nazhand, 2020; Zhang et al., 2020) Based on a bioinformatic analysis and toxicity testing on chicken embryos, this research aimed to analyze *Crataegus oxyacantha*. Collectively, the previous

studies have mainly been engaged in explaining the biochemical content of hawthorn along with its antioxidant effects and its overall mechanisms of cardioprotection.

## **2. THEORITICAL STUDY**

This is a feasible study because it combines bioinformatic analysis and in vivo toxicity experiment in chicken embryos to examine the possible therapeutic efficacy of *Crataegus oxyacantha* thoroughly. Although other studies have characterized the phytochemical content and overall cardiovascular efficacy of hawthorn extracts, not many studies have integrated the computational forecasting of interactions between bioactive compounds and experimental embryonic toxicity. The current paper gives a more mechanistic insight into the bioactive entities of hawthorn by revealing molecular targets by bioinformatics and validating safety profiles using embryo-based assays. This is an integration of the available literature as the blending of the two evidence (computational and biological) provided a strong scientific foundation on which the therapeutic applications of *Crataegus oxyacantha* can be applied in the future.

## **3. RESEARCH METHODS**

### **Conversion of nucleotides to amino acids**

The Expasy Translate Tool program was used to translate nucleotides from *Crataegus oxyacantha* found in the National Center for Biotechnology Information (NCBI), GenBank: HG764986.1, into amino acids.

### **Analysis of the three dimensional structure of *Achillea millefolium* Protein**

The study was conducted in accordance with the Protein Structure Homology Modeling Using SWISSMODEL Workspace approach in order to anticipate the three-dimensional structure of the *Crataegus oxyacantha* protein via homology:

### **Epitope and allergen protein analysis**

The IEDB program incorporates *Crataegus oxyacantha* amino acids to generate epitope-specific proteins. The Allertop program includes *Crataegus oxyacantha* amino acids to obtain allergy proteins.

### **Analysis of proteins that are antigenic and toxin**

In order to generate antigenic proteins, the Vaxijen program incorporates amino acids from *Crataegus oxyacantha*. The Toxinpred program incorporates *Crataegus oxyacantha* amino acids to generate proteins that are either toxic or non-toxic:

### Toxicity test of *Achillea millefolium* on the chicken embryo

An experiment was devised to study the impact of *Crataegus oxyacantha* on the development of chicken embryos. A number of sprouting chicken eggs were exposed to varying concentrations of *Crataegus oxyacantha*. In this instance, the therapy involves six different dose levels of *Crataegus oxyacantha* products, including.

1. “control (0 ng of *Crataegus oxyacantha* /eggs);
2. 15.6 ng of *Crataegus oxyacantha* /egg products;
3. 31.2 ng of *Crataegus oxyacantha* /egg products;
4. 62.5 ng *Crataegus oxyacantha* /egg product;
5. 125 ng *Crataegus oxyacantha* /egg product;
6. 250 ng *Crataegus oxyacantha* /egg.”

For each treatment dosage, 200 ul of solution was injected into an egg via the allantois space route. One hundred twelve-day-old brood chicken eggs were injected with 200 ul/egg of each treatment (treatments 1–6) using the air bag line. The first treatment, which served as a control, consisted of injecting 12-day-old sprouting chicken eggs with a 1 ml solution devoid of honey ingredients. After reinserting liquid paraffin into the injection hole, it was placed in an incubator and monitored daily using candling. At the 21-day mark, two eggs were removed from each treatment to examine the embryo morphology and any potential problems. It was necessary to measure and examine the newborn chicks (DOC) for any observable morphological defects.

## 4. RESULTS AND DISCUSSION

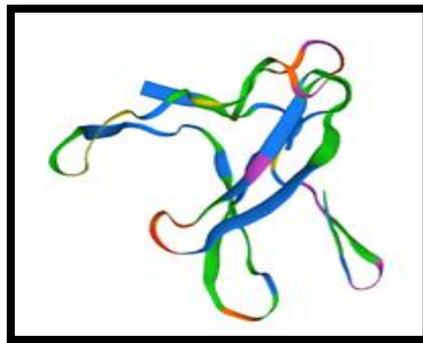
The amino acid composition of *Achillea millefolium* may be observed in table 1, which is derived from the results of translating nucleotides into amino acids. The amino acid composition of *Crataegus oxyacantha* may be observed in table 1, which is derived from the results of translating nucleotides into amino acids.

**Table 1** Nucleotides of *Crataegus oxyacantha* after conversion to amino acids.

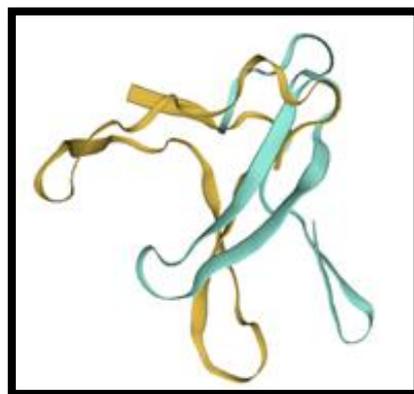
No	Protein Sequence
1	“GYWAALKAFSLGKSLSTGTSKSFFVRQTNKIKKVIKKRGKPNINRPDSKIEPF RFKKKFPNSISWINPEMEFFCLLPNSNKVFLTKKHKILDHLHFSIFSFDGSLIFPIN LFVLDLFLCTTFLLLDFLILISPYISGHEFTQKHLMKIFWINMCEFIINIFLFDKTYF WVVLFIKINQKRSIKKMFFGGGLIHSKTCWFYKSPSREESKIYNKNKTLNINEPS SIYLNKFLFIDLNLSYKILHSIEFLNLDDFLFIG

- 
- 2 AIGRRKHFLGNLFLPGLPKVFLCDKQIKSKNKLLRNKGENLRTIIDLTQKLNPSV  
SKKNSPIPFPGELIHRKWNSSVYFDRIQTKFFQKNTRYISICILVYFLSRMGVLFPP  
STYLYYKIFFFVQLSYFWIFYKFLYRAHISLAMNSRKNTKYSGICVNFESKIFFCS  
LIKLIFFGLYFFKLIKNGQLKKCFLGGAFIVRLAGFTRVPVEKSLKSTIKIKPTKL  
MNLQVYITSFYLSIIFPTKYCTQLNSITISYS
- 3 LLGGVKSIFFREISFYRDFQKFFCATNKNQKISYEIKGKTLEQYKSTLKNTLFPQK  
KIPQFHFLVNSIGNGILLFTLTEFKQSFFNKKTDTRFAFYIFFPGWESYFSHPIC  
TIRFSFLYNFLTGFINSYIEPIYLWPIHAKTLNENILDKYVILNNLKYFFVHNLF  
LGCTFLNNKSKTVNKNVFWGGLNSDLLVLQESQSRRVNLQKNPKLNTFKYIFE  
QVFIYRFESFLQNALNILKSRRFLIHR”
- 

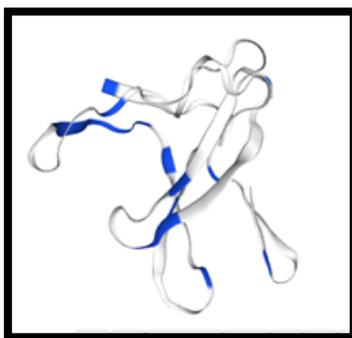
The research also included a three-dimensional structural investigation of a *Crataegus oxyacantha* protein, with the outcome being six different three-dimensional models of the protein.



**Figure 1.** Analysis of the three-dimensional structure of the *Crataegus oxyacantha* protein no 1.



**Figure 2.** Analysis of the three-dimensional structure of the *Crataegus oxyacantha* protein no 2.



**Figure 3.** Analysis of the three-dimensional structure of the *Crataegus oxyacantha* protein no 3.

The research also gathered information on the ligand characteristics, GQME, and Qmean values for each protein in *Crataegus oxyacantha*. *Crataegus oxyacantha* protein binding site not conserved ligand was more dominant, and GQME and Qmean values differed, according to the study.

**Table 2.** Characteristics of *Crataegus oxyacantha* Protein.

No	GQME Value	QmeanDisCo Value
1	0.21	0.13 ± 0.06
2	0.19	0.19 ± 0.09
3	0.05	0.17 ± 0.12

This research used the IEDB to examine the epitope-forming amino acids of *Crataegus oxyacantha*. Also, *Crataegus oxyacantha* was tested for allergens and non-allergens in its protein composition. Based on the study findings, it was discovered that most *Crataegus oxyacantha* proteins were not allergenic. These findings were derived from epitope studies.

**Table 3** *Crataegus oxyacantha* Protein which is Epitope and Allergen.

“ <i>Crataegus oxyacantha</i> ”	Peptides that are Epitopes	Position	Allergen
1	YKILHSIEFLNLDDF	241-255	PROBABLE ALLERGEN
2	FTRVPVEKSLKS	203-214	PROBABLE ALLERGEN
3	FFREISFYRDF	9-19	PROBABLE NON-ALLERGEN”

Antigenic and poisonous *Crataegus oxyacantha* proteins were also analyzed in this work using the software packages Vaxijen and ToxinPred. Research conducted utilizing

Vaxijen revealed that the vast majority of *Crataegus oxyacantha* proteins exhibited antigenic properties. Furthermore, study employing ToxinPred established that most *Crataegus oxyacantha* proteins are not hazardous. You can view the findings of study on the poisonous and antigenic proteins found in *Crataegus oxyacantha* in table 4.

**Table 4** *Crataegus oxyacantha* Protein Which is Antigenic and Toxic.

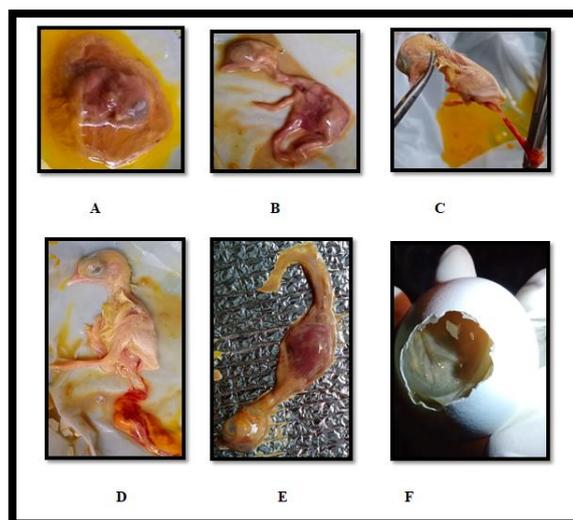
No.	Epitope Peptides	Antigenic Peptides	Toxic Peptides
1	YKILHSIEFLNLDDF	Probable NON ANTIGEN	Toxin
2	FTRVPVEKSLKS	Probable NON-ANTIGEN	Non-Toxin
3	FFREISFYRDF	Probable ANTIGEN	Non-Toxin

**Table 5** Chicken Embryo Weight after being injected with *Crataegus oxyacantha*.

“ <i>Crataegus oxyacantha</i> concentration	Chicken Embryo Weight (grams)
control 0 ng <i>Crataegus oxyacantha</i> /eggs	7.75±0.01
15.6 ng of <i>Crataegus oxyacantha</i> /egg product	7.71±0.03
31.2 ng of <i>Crataegus oxyacantha</i> /egg product	7.75±0.15
62.5 ng <i>Crataegus oxyacantha</i> /egg product	7.71±0.07
125 ng <i>Crataegus oxyacantha</i> /egg product	7.73±0.19
250 ng <i>Crataegus oxyacantha</i> /egg	7.71±0.11”

**Table 6** Body length of chicken embryo 21 days after injection with *Crataegus oxyacantha*.

“ <i>Crataegus oxyacantha</i> concentration	Embryo Length (cm)	Leg Length (cm)	Wing Length (cm)	Bust (cm)
control 0 ng <i>Crataegus oxyacantha</i> /eggs	7.17 ± 0.17	7.20 ± 0.37	4.26 ± 0.51	5.07 ± 0.72
15.6 ng of <i>Crataegus oxyacantha</i> /egg product	7.17 ± 0.14	6.39 ± 0.37	3.95 ± 0.59	5.16 ± 0.78
31.2 ng of <i>Crataegus oxyacantha</i> /egg product	7.67 ± 0.15	6.42 ± 0.34	4.09 ± 0.50	5.35 ± 0.07
62.5 ng <i>Crataegus oxyacantha</i> /egg product	8.27 ± 0.21	6.49 ± 0.32	4.80 ± 0.58	5.54 ± 0.79
125 ng <i>Crataegus oxyacantha</i> /egg product	7.27 ± 0.19	6.23 ± 0.34	4.05 ± 0.53	5.31 ± 0.75
250 ng <i>Crataegus oxyacantha</i> /egg	7.17 ± 1.10	6.20 ± 0.36	5.15 ± 0.55	5.25 ± 0.77”



**Figure 4** Observation of embryo morphology in each treatment. “A. control 0 ng *Crataegus oxyacantha* /eggs, B.15.6 ng of *Crataegus oxyacantha* /egg product, C.31.2 ng of *Crataegus oxyacantha* /egg product, D.62.5 ng *Crataegus oxyacantha* /egg product, E.125 ng *Crataegus oxyacantha* /egg product, F.250 ng *Crataegus oxyacantha* /egg”.

The hawthorn tree, scientifically known as *Crataegus pinnatifida* Bge. (Rosaceae), is a common evergreen ornamental shrub in China (Nazhand, 2020). Not only is hawthorn a famous Chinese herbal remedy, but it also has a long history of use as an edible food. Not only is hawthorn a good source of carbohydrates, organic acids, vitamins, and minerals, but it also has many different biological uses, such as warding off bacteria, protecting the nervous system from damage, and regulating blood sugar levels (Ahmadipour et al., 2020; Zhang et al., 2020). Hawthorn is rich in polyphenols, which are known to have bioactive properties. The plant contains over 150 phenolic compounds, such as procyanidins (C1, B2, and B5), flavonoids (E., hyperoside, quercetin, rutin, and isoquercitrin), and triterpenoids acid (ursolic, corosolic, oleanolic, and maslinic acid) (Ahmadipour et al., 2020; Zhang et al., 2020). Historically, hawthorn fruits have been eaten raw or transformed into a variety of sweet treats including jams, jellies, wine, juice, and more. Recently, hawthorn extracts have found extensive application as a kind of nutritional enhancer, thanks to the flourishing functional and health care business. antioxidant activity and phenolic content of three hawthorn species extracted with 80% acetone. The only solvents tested that inhibited LDL oxidation were the ethyl acetate extracts. Among the chemical compositions and antioxidant activities of hawthorn extracts derived from various solvents, the results demonstrated that the extract derived from 80% acetone contained the most total polyphenols and flavonoids, while the extract derived from deionized water exhibited the highest capacity to scavenge DPPH and ferric reducing power.

Hence, several variables, including extraction processes, origins, and variations, may substantially impact the biological activities and health consequences of hawthorn extracts. The polarity of the extraction solvents had the most impact on the phytochemical compositions and bioactivities of the material, according to many studies (Semjon et al., 2020; Sorour et al., 2021)

The four main structural components of a protein are the amino acids, peptide bonds, and quaternary ammonia. Amino acid sequences are organized in a linear fashion, much like the arrangement of letters in a word, and there is no branching in the fundamental structure. Hydrogen bonds between =CO and =NH groups along the polypeptide backbone linearly stabilize the combined primary and secondary structures of a protein, which is a two-dimensional structure. According to Taylor et al. (2001), the  $\alpha$ -helix is a kind of secondary structure (Taylor et al., 2001). A protein's tertiary structure is an extra layer on top of its secondary structure, which is a pattern of twisted bonds between the R groups (side chains) of different amino acids. According to Sobolev et al. (2020) and Tam et al. (2020), this structure is a three-dimensional conformation that describes the unique connection between secondary components (Sobolev et al., 2020; Tam et al., 2020). So far, the homology/comparative approach, the fold recognition method, and the ab initio method have all been used to predict the three-dimensional structure of proteins (Waterhouse et al., 2018; Zaki & Bystroff, 2008). The target protein's amino acid sequence is aligned with a comparable protein whose three-dimensional structure is known in the lab (the template) in order to model the target protein's three-dimensional structure using the homology modeling approach (Hung & Samudrala, 2003; Rose, 2019; Sali & Blundell, 1993). When compared to the fold identification and ab initio methods, the computing complexity of the homology process is lower. Additionally, compared to other methodologies, working with the homology method is quicker (Benkert et al., 2011; Zaki & Bystroff, 2008). The ab initio approach is used to model proteins when there is no template protein in the database. When compared to other approaches, the ab initio method is the most complicated, time-consuming, and challenging. Protein three-dimensional structures are modeled using the energy function as the basis of the ab initio technique (Hardin et al., 2002). Both the method's applicability and the precision it produces are limited to proteins with modest sizes (Kopp et al., 2007; Ramana & Mehla, 2020). According to Zaki and Bystroff (2008), fold recognition modeling is more challenging than homology but simpler than ab initio (Zaki & Bystroff, 2008). To create a structural model with the optimal fold value, the fold recognition technique compares the target sequence to a library of template structures (Hung & Samudrala, 2003). So, if you have a template protein

structure, the homology modeling approach is the way to go for building an in silico three-dimensional model of a protein (Kopp et al., 2007). According to Hillisch et al. (2004) and Kopp (2007), homology modeling is extensively used in virtual screening, mutagenesis experiment design, and sequence variation impact analysis (Hillisch et al., 2004; Kopp et al., 2007). Three distinct three-dimensional structures of the *Crataegus oxyacantha* protein were identified based on the study's findings (Rose, 2019; Sobolev et al., 2020; Tam et al., 2020). The IDDT score and the global QMEANDisCo score, which is the average of the two, showed a strong correlation. The offered error estimates are derived from the root mean of squares (i.e. standard deviation) disparity between the global QMEANDisCo and IDDT scores (basic truth), which in turn are based on the global QMEANDisCo scores determined for several models. According to Studer et al. (2020) and Benkert et al. (2011), a given error estimate is determined using a model that is the same size as the input, as the reliability of the prediction is dependent on the size of the model (Benkert et al., 2011; Studer et al., 2020). The values that were acquired from the investigation are  $0.13 \pm 0.06$ ,  $0.19 \pm 0.09$ , and  $0.17 \pm 0.12$ . This demonstrates that the mistake rate of the three *Crataegus oxyacantha* bee proteins is modest. One such quality estimate is the Global Model Quality Estimate, or GMQE. It takes into account both the template-target alignment and template structural features. In order to forecast IDDT scores, they were fused using a trained multilayer perceptron. In order to choose the best template for the current modeling challenge, GMQE is accessible before the actual model is built. Research findings yielded values after model creation (Studer et al., 2020; Waterhouse et al., 2018). The *Crataegus oxyacantha* protein was the subject of an immunoinformatics analysis in this investigation. This approach may be useful in the search for peptide vaccines, which are those that stimulate the immune system with just a little amount of antigen (about 8–15 amino acids) (Chakraborty et al., 2014; He et al., 2010; Oli et al., 2020; Vita et al., 2018). A survey of immunogenic proteins was compiled from the investigations that were undertaken. Furthermore, this study's findings also derived non-toxic and allergenic proteins from *Crataegus oxyacantha*. Molecular weights ranging from 10 to 70 KDa are characteristic of water-soluble glycoproteins, which are often allergenic compounds (Coskun et al., 2014). Mild symptoms including itching, watery eyes and nose, and swelling might be triggered by allergies. According to Oli et al. (2020), Ramana and Mehla (2020), and Chakraborty et al. (2021), severe responses like anaphylaxis may be fatal when caused by allergies (Chakraborty et al., 2014; Oli et al., 2020; Ramana & Mehla, 2020). Injecting eggs with amino acids, hormones, carbohydrates, or pollen extracts did not impact hatchability, according to Bhanja & Mandal (2005), Nowaczewski et al. (2012), Moore et al. (1994), Shafey et al. (2012), Saritas

et al. (2011), and Coskun et al. (2014), respectively (Bhanja & Mandal, 2005; Coskun et al., 2014; Moore et al., 1994; Nowaczewski et al., 2012; Saritas et al., 2011; Shafey et al., 2012). Researchers found no significant influence on embryonic mortality from in-ovo injections of vitamin C, ascorbic acid, carbs, or glucose (Ipek et al., 2004; Nowaczewski et al., 2012; Sgavioli et al., 2015; Shafey et al., 2012). Results showed that both the weight and length of the embryonic body at 21 days post-honey injection were significantly different between treatments in this research. When certain nutrients are injected into the eggs during incubation, it might lead to nutritional imbalances and, as a result, restrict the embryo's maximum growth and development. There was no statistically significant change in weight between the control group and the group that received in-ovo vitamin E injections, according to Shirakawa et al. (2020). Taha et al. (2019) reported that RJ injection had a good impact on all metrics (Shirakawa et al., 2020; Taha et al., 2019). The investigated parameters were unaffected by the various chicken strains. Researchers found that different chicken strains had different reactions to RJ inoculation. The fundamental objective of the novel in ovo technique is to introduce bioactive chemicals into eggs while the bird is still an embryo. As a result, we need to figure out how to put in ovo feeding technologies to good use in the poultry industry. This exploratory work has the potential to pave the way for further investigations into the optimal location for manipulating nutritional solutions and carriers for in ovo injection of embryos in laying hens (El-Tarabany et al., 2021; Pawłowska et al., 2022)). The reproductive tract shape, egg production rate, and internal egg quality features of old laying hens may be improved by RJ therapy, according to (Bălan et al., 2020; El-Tarabany et al., 2021; Fathi Najafi et al., 2021; Kunugi & Mohammed Ali, 2019; Li et al., 2021; Pan et al., 2019; Shirakawa et al., 2020; Soejima et al., 2022; Taha et al., 2019; Yamaga et al., 2021)

## **5. CONCLUSIONS AND SUGGESTIONS**

Based on the GQME and QmeanDisCo values, the validity of the six *Achillea millefolium* three-dimensional structures studied is high. Proteins that are epitope-specific, antigenic, hypoallergenic, and non-toxic were also identified in this research. On the twenty-first day after injection, a morphological analysis revealed an anomalous embryological image at a concentration of 250 ng of *Achillea millefolium*/egg product. Researchers measured the length and weight of chicken embryos. Research on other plant species is necessary.

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