Original Article

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Dosage Form of Moisturizer Gel and Evaluation of Antibacterial, Anti-inflammatory, Antioxidant of *Erythrina crista-galli* L. Flowers Extract

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Abstract:

Objective: This study aimed to determine the antioxidant, antibacterial, and anti-inflammatory activities of ethanol extracts from red Dadap flowers (*Erythrina crista-galli* L). In addition to formulate the extract into a moisturizing gel preparation of good physical quality.

Material and Methods: Antioxidant activity was determined using a 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity assay. Antibacterial activity was evaluated using the disc diffusion method. Anti-inflammatory activity was evaluated using carrageenan-induced paw edema in mice in vivo. Additionally, in silico studies were performed and the physical quality of the extract-gel formulation was evaluated.

Results: Antioxidant testing resulted in a half-maximal inhibitory concentration (IC_{50}) value of 79.1 ppm and an AAI value of 0.4. Antibacterial testing obtained the largest inhibition zone, with an average of 9.0 mm. Anti-inflammatory testing obtained the greatest anti-inflammatory effect at a dose of 300 mg/kg body weight in mice, with a percentage of inflammation inhibition that reached 59.32%. In silico testing, the smallest binding affinity values were obtained: respectively, at -10.3, -12.0, and -10.2 kcal/mol, by the specific compound Cyanidin-3-O-glucoside. Physical quality evaluation obtained values that are in accordance with the Indonesian National Standard.

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Conclusion: Ethanol extract of red Dadap flower (*Erythrina crista-galli* L.) has antioxidant and antibacterial activity in the weak category; however, in contrast it has anti-inflammatory activity in the strong category. In silico also obtained good activity in accordance with the results of in vivo testing. *Erythrina crista-galli* L. flower extract can be formulated into a moisturizer gel, this can be seen from the results of good physical stability.

Keywords: antibacterial, anti-inflammatory, antioxidant, *Erythrina crista-galli L.* flowers extract, moisturizer gel

Introduction

The skin is the sensory organ located on the surface of the body, and it plays a crucial role in providing protection to the body. Skin damage can disrupt both health and appearance[1]. Additionally, skin damage can occur due to free radicals from ultraviolet (UV) rays, air pollution, cigarette smoke, radiation sources, harmful chemicals, and unhealthy lifestyles. Free radicals have one or more unpaired electrons in their outer orbitals. This affects the process of attracting other electrons, leading to oxidation processes[2].

In addition to free radicals, various diseases can also be caused by pathogenic bacteria, such as acne, a common skin problem. Acne is a chronic, obstructive, and inflammatory skin disorder that often occurs during adolescence. Acne can occur due to excess oil, and one of the bacteria responsible for acne is Propionibacterium acnes[3]. The occurrence of an infection on the skin can be characterized by inflammation. Inflammation is a response provided by the body when tissue injury and infection within the body's cells occur in an effort of protection aimed at destroying or reducing damaged tissues[4,5].

The use of natural ingredients can help alleviate the effects of free radicals and acne caused by *P. acnes* bacteria, as well as address skin inflammation. *E. crista-galli* L or red Dadap is one type of plant that contains secondary metabolites of phenolic compounds, such as anthocyanins, alkaloids, flavonoids, and tannins[6,7].

The flower of the red Dadap plant, having a high content of secondary metabolite compounds, doesn't rule out the possibility of its bioactivity as an antioxidant, antibacterial, and anti-inflammatory[6,8,9]. Therefore, this research conducted antioxidant testing using the DPPH method, followed by analysis to determine the IC_{50} and AAI values. This method was chosen for its advantages in being easy, simple, sensitive to analysis, and requiring a short time. DPPH can also reflect the body's defense system against free radicals. In the antibacterial test, the disc diffusion method was used. This method was chosen due to it being one of the diffusion system tests that can be quickly performed in preparing disc papers, and is easy to execute. Furthermore, the anti-inflammatory test, using the method of inducing artificial edema on the paw of mice, was chosen because it is one of the simplest methods for testing anti-inflammatory activity and is frequently used in anti-inflammatory testing. Additionally, the extract of red Dadap flowers was formulated into a moisturizing gel preparation, which was then tested for its physical stability[10–12].

Based on the background that has been described, the purpose of this study is to determine the antioxidant, anti-inflammatory, and antibacterial activities of the ethanol extract of the red Dadap flower (*Erythrina crista-galli* L.) in vitro, in vivo and in silico. In addition, this study also aimed to formulate the extract of the red Dadap flower into a moisturizing gel by testing its physical stability.

Material and Methods

Plant Samples

Fresh, red Dadap flowers were obtained from around the area of Samarinda City, East Kalimantan, Indonesian. The determination of plant samples was carried out to

ensure the suitability of the samples used. Taxonomists from Mulawarman University identified these plant samples.

Extraction

Extracts were prepared by the maceration method, using ethanol at 70% (Merck) as a solvent. Samples were washed and cleaned, then dried using an oven at 50 °C. The dried samples were then cut into small pieces. The dried samples, up to 30 grams, were placed into a tightly closed glass container, then 300 ml of 70% ethanol was added until the simplisia was completely submerged[13]. Maceration was carried out at room temperature (27±2 °C) for 3 days, while occasionally stirring. The extraction resulted in separation between the filtrate and the residue, then the residue was remacerated via the previous method. The results of maceration were then evaporated with a rotary evaporator (Buchi). After this, the thickening of the extract, using a waterbath (Faithful) at 50 ˚C, was performed until a thick extract was obtained[14].

Antioxidants

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution was made with 10 mg of DPPH powder (Sigma-Aldrich), and dissolved with methanol (Merck) in a 100 mL volumetric flask. Then, 35 mL was taken and put in a 100 mL volumetric flask, methanol was added to make a concentration of 35 µg/mL. DPPH 35 µg/ml solution was added to the cuvette, and the absorbance of the solution was measured with a UV-Vis Spectrometer (Thermo Fisher Scientific), with a maximum absorption wavelength of 515-520 nm methanol was used as a blank. The samples used were the red Dadap flower extract and Vitamin C (Merck), made with a concentration of 1,000 µg/mL. In the preparation of the parent solution, 50 mg of the sample was dissolved with methanol in a 50 mL volumetric flask and then homogenized. Then from the parent solution, a test sample solution was made having concentration variants of 20, 40, 60, 80 and 100 µg/mL for the red Dadap flower extract and 2, 4, 6, 8, 10 µg/mL for Vitamin C. From the sample solution each concentration was added into a test tube and 35 µg/mL DPPH array in a ratio of 1:2 was added. This was then homogenized and incubated in a dim place for 30 minutes. After this, the retention of the sample array was estimated using a UV-Vis Spectrophotometer, at the highest frequency of 515.87 nm.[15] The percentage of free radical capture in the test sample was calculated using the following formula:

$$\%\text{Antioxidant Activity: } \frac{(\text{Abs.Control} - \text{Abs.Sample})}{\text{Abs.Control}} \times 100\%$$

Calculation of antioxidant activity index (*AAI*) value used to determine the antioxidant index with the formula:

$$\text{AAI Value : } \frac{\text{Consentrasi DPPH (ppm)}}{\text{IC50 Sample (ppm)}}$$

Anti-inflammatory

Artificial edema was formed on the soles of the mice's feet using carrageenan (Sigma-Aldrich) solution as an edema inducer[16,17]. The volume of the mice's paw was measured using a plethysmometer (Orchid Scientific). Data obtained was the initial volume (Vo), which is the volume of the foot before being given the preparation and before being induced with carrageenan solution. Each test animal was induced with 1% carrageenan solution as much as 0.1 ml subplantar on their left hind paw[18]. After 30 minutes from the induction, the volume of the mice's paw was measured again by dipping it into the *Plethysmometer* (Orchid Scientific). The data obtained was recorded as the volume of the mice's legs (Vt), namely, the volume of the legs after being given the preparation and after being induced with carrageenan solution[19,20]. Anti-inflammatory power, using the calculation of percent inhibition, was noted to show how much the extract inhibit inflammation caused by the inflammatory process. Before calculating the percent inhibits, it is necessary to calculate the percentage of edema via this formula[21]:

Edema Percentage=$\frac{Vt-V0}{Vo}$ ×100

Vt=paw volume at time t (after carrageenan induction)

V_0=paw volume at time 0 (before carrageenan induction)

Percentage of Inflammation Inhibition=$\frac{a-b}{a}$ ×100

a=percent of inflammation of the negative control group

b=percent treatment of test substance or comparator drug

Antibacterial

Agar media was made by dissolving 28 grams of Nutrient Agar (Himedia) in 1 liter of distilled water. This was then homogenized, heated using a hot plate, and sterilized by autoclave (Wafco) at 121 °C for 15 minutes, until a sterile nutrient agar media was obtained. The sterilized media was left at a temperature of ±40-45 °C, upon which NA media was poured into petri dishes and cooled to solidify. *Propionibacterium acnes* ATCC 11827 was taken using a sterile wire, then suspended in a test tube containing 10 ml of 0.9% NaCl (Otsuka). The turbidity of the test bacterial suspension was standardized using 0.5 of the McFarland standard solution (approximately $1.5x10^8$ CFU/mL). The test bacterial suspension was inoculated on the agar medium in a Petri dish: up to as much as 200 µL, then flattened with a sterile cotton swab, and left to dry. Paper discs soaked in samples of each concentration of 100%, 75%, and 50%, for at least 15 minutes, were placed on the surface of the media aseptically, then the media was incubated for 24 hours at 37 °C. Observations of the clear zone around the paper discs were made. The antibacterial inhibition zone was measured with a caliper for three repetitions at different positions, and the average value was measured[22].

In silico

Receptor Preparation

Receptor data that will be used was obtained from the Protein Data Bank (PDB), which can be accessed via the following link https://www.rcsb.org. The target protein used in this study was that produces fatty acid isomerase (PDB ID: 2B9W), this is a protein found in *P. acnes*. For antioxidants Crystal structure of human AKR1B10 complexed with NADP+ and the +and the synthetic retinoid UVI2008 B10 (PDB ID: 5M2F) was used, and for anti-inflammation the COX2 target protein (PDB ID: 1C2X). The data were then visualized using PyMol. The protein structure was then cleaned of additional data containing water molecules and ligands using PyMol. The cleaned protein file was then saved in a .pdb format for further analysis[23].

Ligand preparation

The compounds contained in *E. crista-galli* L, based on previous literature, were Cyanidin-3-O-glucoside, Cyanidin-3-O-galactoside, Pelargonidin-3-O-glucoside, Delphinidin 3-O-glucoside, Kaempferol 3-O-glucoside, Quercetin 3-O-glucoside and Isovixetin[7,22]. Then the structure and information related to the ligand were downloaded through the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). The results of the chemical components were then downloaded in the form of .pdb data. The data was then minimized using PyRX, for docking analysis[24].

Molecular docking

Molecular docking analysis, or pairing between ligand files and proteins, was performed using PyRx software, with the Autodock Vina wizard. In the program, energy minimization is carried out for all ligands to be tested. Furthermore, the determination of the grid box location for the docking location is done. The next step is to run the docking analysis. The results of docking analysis are the prediction of binding energy between ligands and proteins, along with the conformation and optimal location of interaction. This data is then exported into a .pdb format to be analyzed, using PyMol and Discovery Studio related to the type of bond formed at the interaction between the ligand and the target protein[25,26].

Formulation moisturizer gel

The formulation design of the moisturizing gel preparation of red Dadap flower extract can be seen in table 1[27,28]. Gel preparations with a Carbopol 940 (Sigma-Aldrich) base are made in this way Carbopol was developed, with a total of all remaining aqueous solutions in a glass beaker. This was left standing for a full day until it expanded. It was then homogenized after adding triethanolamine (Merck). Furthermore, propylene glycol (Merck) was added to the beaker and then homogenized. The extract was placed into a beaker and mixed with the base until homogeneous. Phenoxyethanol (Sigma-Aldrich) was dissolved with glycerin (Sigma-Aldrich), added to the mixture, and stirred until homogeneous. Furthermore, the preparation was evaluated for physical quality for three cycles, as well as irritation tests on test animals.

Data analysis

Data analysis on the phytochemical screening test was analyzed descriptively. Antioxidant test data were analyzed using Linear Regression curves. Anti-inflammatory test data were analyzed with Two-way ANOVA (Analysis of Variance) and continued with the Bonferroni Post Hoc test. Analysis of antibacterial and physical quality data with One-Way ANOVA (Analysis of Variance) statistics, and continued with the Post Hoc test. A p-value≤0.05 was considered statistically significant.

Results

Antioxidants

The antioxidant activity test of red Dadap flower extract and Vitamin C obtained the percentage inhibition value as presented in Figure 1. In the correlation results of linear regression, the regression obtained in the Vitamin c sample was: R^2=0.9827, with an IC_{50} value of 3.234 ppm and an AAI value of 10.9 (strong category). Then, in the sample of ethanol extract of red Dadap flower, the regression was: R^2=0.8048, with an IC_{50} value of 79.12 ppm and an AAI value of 0.4 (weak category).

Anti-inflammatory

The results of the percentage of inflammation inhibition can be seen in Table 2. Percent average inhibition of inflammation was to show the ability of each group in inhibiting inflammation caused by the inflammatory process[29]. The anti-inflammatory activity of each group was observed as increasing with each time interval. A statistical analysis revealed that extracts with doses of 150 and 300 mg/kg body weight (b.w.) exhibited superior anti-inflammatory efficacy compared to the positive control (p-value<0.05). This was further substantiated by the significant inhibition of inflammation (81.19%) observed at minute 120 in the 300 mg/kg b.w. extract.

Table 1 Gel preparation formulation of Ethanol extract of red Dadap flower

Material name	Usability	Concentration (%w/v)		
		F1	F2	F3
Red Dadap Flower Extaract	Active substance	0	0.7	1.2
Carbopol 940	*Gelling agent*	1.4	1.4	1.4
Triethanolamine	*Alkalizing agent*	1	1	1
Propylene Glycol	Humectant	30	30	30
Glycerin	Humectant	30	30	30
Phenoxyethanol	Preservative	1	1	1
Aquadest	Solvent	Ad 200	Ad 200	Ad 200

w/v=weight/volume, F=formula

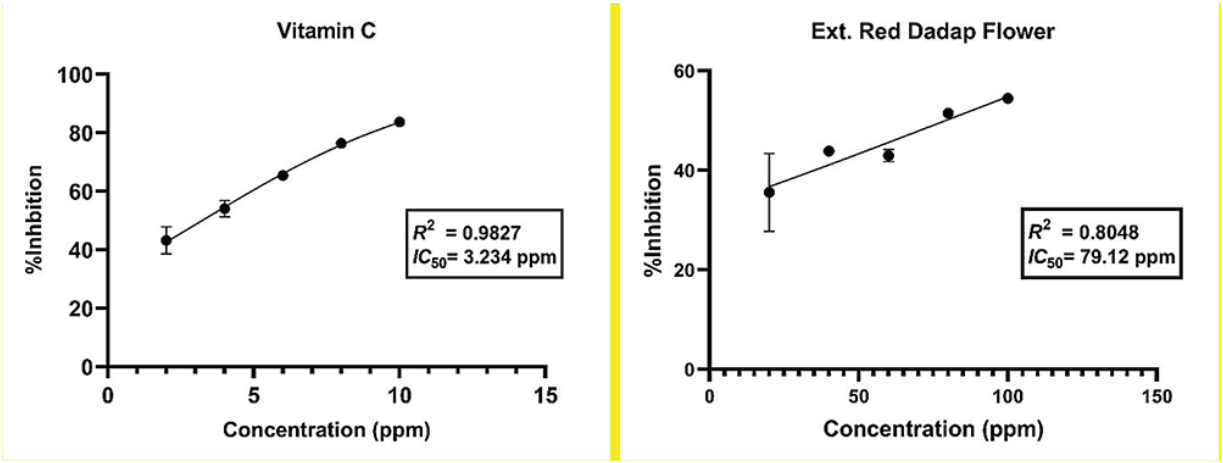


Figure 1 Antioxidant activity test results of red Dadap flower extract and Vitamin C

Table 2 Mean percentage of inflammation inhibition in each treatment group

Groups	Percentage of inflammation inhibition (%)				
	Time (min)				Average % inhibition±S.D.
	30	60	90	120	
Na-CMC (-)	0.00	0.00	0.00	0.00	0.00±0.00
Sodium Diclofenac (+)	34.64	36.59	54.18	63.63	47.26±14.01
Extract Dose 75 mg/kg b.w.	32.90	31.85	37.44	43.10	36.32±5.13
Extract Dose 150 mg/kg b.w.	40.18	41.16	56.14	69.17	51.66±13.77
Extract Dose 300 mg/kg b.w.	42.13	49.12	64.82	81.19	59.32±17.40

S.D=standard deviation, DMSO (-)=dimethyl sulfoxide (negative control), b.w.=body weight

Antibacterial

The presence of antibacterial activity is indicated by the formation of a clear zone around the disc paper. The vertical diameter and horizontal diameter of the clear zone were then measured. From the measurements that were made, it shows that the three concentrations of samples can inhibit the agar media that has been grown with P. acnes bacteria, as seen in Table 3. The 100% and 75% extracts exhibited inhibitory activity in the moderate category; however, this activity was not better than that of clindamycin (positive control) in the strong category.

In silico

Based on the results of the analysis, the compounds contained in the polar extract of red Dadap flowers are able to bind to receptors that cause bacterial infection, inflammation, and oxidation, as shown in table 4. Binding affinity is a measure of the compound's ability to bind to the receptor. The smaller the binding affinity value, the higher the affinity between the receptor and the ligand; conversely if the greater the binding affinity value, the lower the affinity[26]. From this statement, it can be determined that between the compounds in red Dadap flowers with receptors, these have high affinity; with the smallest binding affinity values of -10.3

Table 3 Measurement results of inhibition zone diameter (mm) of Ethanol extract of *E. crista-galli L.* against the growth of *P. acnes* Bacteria

Group	I	II	III	Mean±S.D. (mm)	Category
DMSO (-)	0	0	0	0	None
Clindamycin (+)	24.5	24.0	24.0	24.1±0.28	Strong
Extract 100% w/v	9.5	9.0	8.5	9.0±0.50	Medium
Extract 75% w/v	5.5	5.0	5.0	5.1±0.28	Medium
Extract 50% w/v	1.0	1.0	1.0	1.0±0.00	Weak

S.D=standard deviation, DMSO (-)=dimethyl sulfoxide (negative control), w/v=weight/volume

Table 4 The docking score of native ligands, test compounds, and comparative compound against antioxidant, antibacterial, and antiinflammation receptors

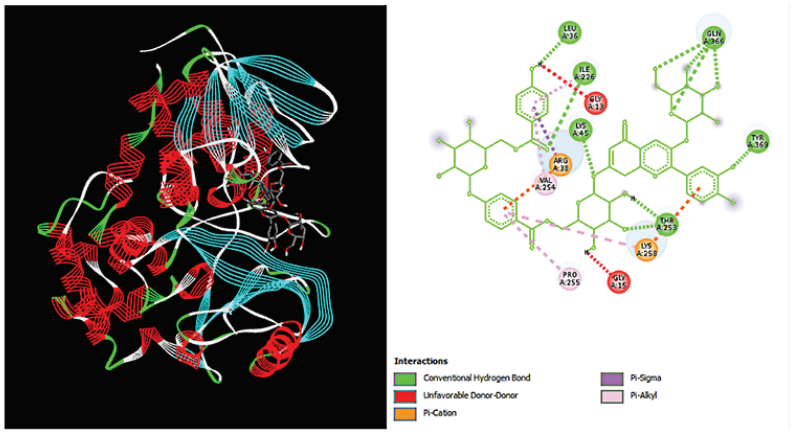
Compound	Docking score on receptors		
	Producing fatty acid isomerase	Human AKR1B10 complexed with NADP+ and the synthetic retinoid UVI2008	Prostaglandin selective inhibitor (COX-2)
Cyanidin-3-O-glucoside	-10.3	-12.0	-10.2
Cyanidin-3-O-galactoside	-8.5	-9.2	-8.1
Pelargonidin-3-O-glucoside	-8.9	-8.9	-8.0
Delphinidin-3-O-glucoside	-8.9	-9.2	-8.9
Kaempferol-3-O-glucoside	-8.9	-9.2	-7.7
Quercetin-3-O-glucoside	-9.8	-11.8	-8.9
Isovixetin	-9.2	-10.6	-9.8
Clindamisin	-7.9	-	-
Vitamin C	-	-6.2	-
Sodium diclofenac	-	-	-7.6

AKR1B10=aldo-keto reductase family 1 member B10, NADP+=nicotinamide adenine dinucleotide phosphate, COX-2=cyclooxygenase-2

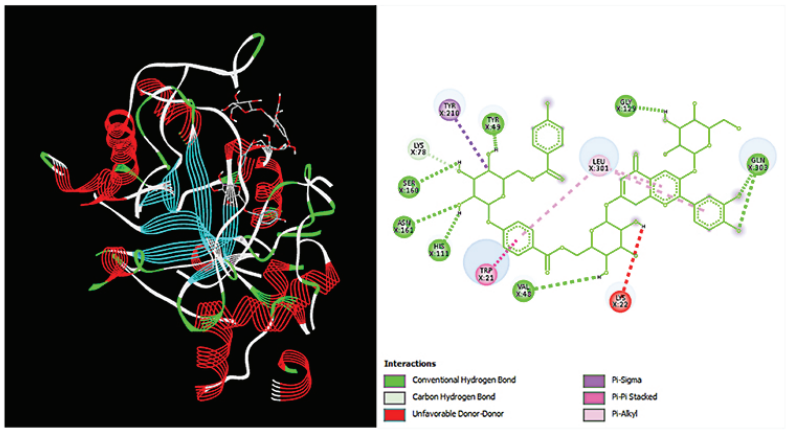
kcal/mol, -12.0 kcal/mol, and -10. 2 kcal/mol, produced by the specific compound Cyanidin-3-O-glucoside. This shows selectivity that exceeds clindamycin (-7.9 kcal/mol), vitamin C (6.2 kcal/mol) and na. diclofenac (-7.6 kcal/mol) as positive controls. The interaction between ligands and target proteins can be visualized in Figure 2.

Formulation physical quality evaluation

Evaluation of gel preparation includes physical observations consisting of: organoleptis, homogeneity, pH, adhesion, spreadability and viscosity. Results of the physical quality evaluation test of the moisturizer gel preparation are presented in Table 5. The physical quality of the three formulas has been thoroughly evaluated, demonstrating good physical stability during the three storage cycles and temperature changes that were implemented. The irritation test yielded a score of 0 for all three formulations, indicating no potential for irritancy.



(a)



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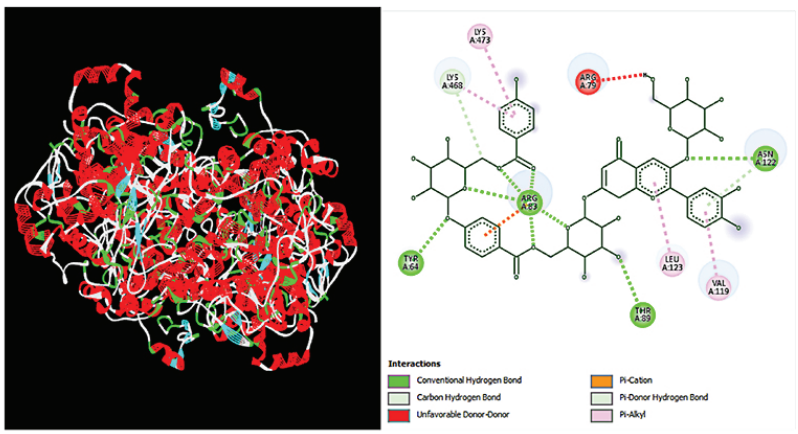

(c)

Figure 2 Visualization of the interaction between Cyanidin-3-O-glucoside in (a) producing fatty acid isomerase, (b) human AKR1B10 complexed with NADP+ and the synthetic retinoid UVI2008, (c) prostaglandin selective inhibitor (COX-2) receptor, using Discovery Studio software

Table 5 Test result of physical quality evaluation of moisturizer gel preparations

Parameters	Cycle 1			Cycle 2			Cycle 3		
	F1	F2	F3	F1	F2	F3	F1	F2	F3
Organoleptics									
Color	Clear white	Brownish red	Chocolate	Clear white	Brownish red	Chocolate	Clear white	Brownish red	Chocolate
Smell	None	Fragrant extract	Fragrant extract	None	Fragrant extract	Fragrant extract	None	Fragrant extract	Fragrant extract
Shape	Semi solid	Semi solid	Semi solid	Semi solid	Semi solid	Semi solid	Semi solid	Semi solid	Semi solid
Homogeneity	Homogenous	Homogenous	Homogenous	Homogenous	Homogenous	Homogenous	Homogenous	Homogenous	Homogenous
pH	5.2	5.5	6	5.6	6	6.5	6	6.5	6.5
Stickiness (seconds)	3	2.65	2	4	3	4	2	2	1.42
Spreadability (cm)	5	6	6	6	6	6	6	6	6
Viscosity (P=cP)	211.3	294.3	205.5	200.3	237	187.1	265.7	212.8	175.4

Discussion

Antioxidant activity testing uses five concentration variants for each sample. The percentage inhibition value of Vitamin C is greater than the inhibition value of red curcuma flower extract, because Vitamin C is an excellent antioxidant in counteracting free radicals. The IC_{50} value produced by Vitamin C is categorized as very strong in counteracting free radicals, with an IC_{50} value of 3.2, while the ethanol extract of red curcuma flower shows its activity as a strong antioxidant category, having a value of 79.1. Furthermore, the IC_{50} value is evaluated using the AAI value to see the antioxidant capacity of an extract or compound obtained. AAI can be divided into 4 categories: (i) AAI<0.5 (low), (ii) AAI 0.5–1 (medium), (iii) AAI 1–2 (strong), and (iv) AAI>2 (very strong)[30]. From the results obtained, it shows that Vitamin C has a very strong antioxidant activity capacity, with an AAI value of 10.9, while the red Dadap flower extract obtained an AAI value of 0.4, placing it in the weak category. The antioxidant activity may be attributed to the presence of anthocyanins, alkaloids, and flavonoids in *E. crista-galli* L., which have been observed to capture free radicals through the provision of hydrogen atoms or single electron transfer[31–33].

From the anti-inflammatory obtained test results, the percentage value of inflammation inhibition successively from doses of 75 mg/kg b.w. mice, 150 mg/kg b.w. mice and 300 mg/kg b.w. mice was 36.32%, 51.66% and 59.32%. This shows that the greater the percentage value of inflammation inhibition indicates a better the anti-inflammatory effect given. Thus the dose with the greatest anti-inflammatory effect is a dose of 300 mg / kg b.w. mice, with a percentage of inflammation inhibition at 59.32%. Earlier studies found that a dose of 300 mg/kg of *E. crista-galli* L. had a strong anti-inflammatory effect[34]. It is hypothesised that anthocyanins, alkaloids and flavonoids in *E. crista-galli* L may play a role in inhibiting inflammatory pathways by reducing the expression of inflammatory mediators; such as COX-2, TNF-α, IL-1β and IL-6. This also allows for a reduction in the number of inflammatory cells (neutrophils) that accumulate at the site of inflammation[34–37].

In testing antibacterial activity, it can be seen that the average value of the diameter of the largest inhibition zone of 24.1 mm was produced by the positive control group, namely, Clindamycin 1.2%. Then, successively 100% concentration of 9.0 mm, 75% concentration of 5.1 mm, and 50% concentration of 1.0 mm; while the negative control group, DMSO, did not produce an inhibition zone. From the average diameter of the inhibition zone, there is a significant difference in the ability of antibacterial activity between the three extract concentrations. This is statistically supported by the results of data analysis that was performed, wherein a p-value>0.05 was obtained. There was also a significant difference in antibacterial activity between clindamycin as a positive control, DMSO as a negative control, and the three extract concentrations. Again, this is further supported statistically by a p-value<0.05. Therefore, this study showed that the ethanol extract of red Dadap flower (*E. crista-galli* L.) can provide antibacterial activity against the growth of *P. acnes.* There are no previously documented findings linking *P. acnes*; however, it is conceivable that the antibacterial activity of alkaloid compounds in *E. crista-galli* L may involve a mechanism of action that encompasses the inhibition of bacterial cell wall synthesis, alterations in cell membrane permeability, suppression of bacterial metabolism, and inhibition of nucleic acid and protein synthesis[38,39].

The positive control used is clindamycin, which is a broad-spectrum antibiotic that can inhibit the growth of gram-positive and negative bacteria. The mechanism of action of clindamycin is by cutting the elongation of the peptide chain; thus, inhibiting the formation of proteins in bacteria[28]. The average diameter of the inhibition zone decreased as the concentration of the sample decreased; this is thought to be because the decrease in concentration is in line with the decrease in the amount of active substance content: hence, the resulting antibacterial activity is weaker.

In silico testing between the interaction of the test compound Cyanidin-3-O-glucoside against the comparison compound clindamycin on the protein producing fatty acid isomerase resulted in the similarity of amino acid residues, namely, Pro255, Lys45, and Thr253. This is also in line with the small binding affinity value produced (-10.3), indicating that there is inhibitory activity of the compound against proteins, and the presence of antibacterial activity by the red curcuma flower. Similarly, the interaction of the test compound Cyanidin-3-O-galactoside with the Vitamin C comparator compound on the human AKR1B10 protein complexed with NADP+ and the synthetic retinoid produces similar amino acid residues; namely, TYR210, TYR49, LYS78, and TRP21. Again, this is in line with the small binding affinity value produced (-12.0), indicating that the inhibitory activity of the compound against proteins, and the presence of antioxidant activity and the interaction of the test compound Cyanidin-3-O-galactoside with the comparator compound sodium diclofenac on Prostaglandin selective inhibitor (COX-2) protein resulted in the similarity of amino acid residues; namely, Pro538, Leu224, and Asn375. Once more, this is in line with the small binding affinity value produced (-8.1), indicating that there is inhibitory activity of the compound against the protein, and indicating the presence of anti-inflammatory activity by the red dadap flower[26].

The first physical evaluation test carried out was the organoleptic test. The color produced in F2 and F3 comes from the red Dadap extract, which is dark red due to the use of a small concentration The resulting color then becomes brownish red and then clear brown. In this gel preparation, there is an absence of fragrance addition. The scent produced by the gel does not modify the inherent scent of red curcuma flowers. In the observation during 3 cycles of storage, there were no changes to the three formulations. The homogeneity of the three gels for three cycles resulted in a homogeneous preparation, devoid of any discernible granules or lumps. In the context of our study, the preparation exhibited a homogeneous composition, characterized by the absence of visible coarse granules.

The pH measurements from the three gel sample preparations ranged from 5.2-6.5. Based on SNI 16-4399-1996, the optimal pH value should range from 4.5-6.5 (p-value>0.05)[40], The adhesion obtained from the three formulations ranged from 1.42 - 4 seconds (p-value>0.05), indicating that the adhesion of the gel preparation meets the requirements: because the gel sticks well. The spreadability of the three formulations ranged from 5-6 cm (p-value>0.05. Based on the value contained in SNI 06-2588-1992, a good spreadability is 5-7 cm[41]. The viscosity results showed that the three formulations still met the requirements of SNI 16-4399-1996, ranging from 2,000-50,000 cps (p-value>0.05)[42]. The irritation test aims to see the presence of symptoms that occur on the skin of test animals, such as redness (*erythema*) and swelling reactions. The results of the irritation test yielded a score of 0 for all three formulations, at both 24 and 48 hours. This finding indicates that the test animals did not experience irritation. Therefore, it can be concluded that the preparation of red Dadap flower extract formulations does not cause irritation in animal testing.

Conclusion

As a conclusion from the results of this research, the ethanol extract of red Dadap flowers has good anti-inflammatory activity, even though antibacterial and antioxidant activities are still lacking. These results have also been confirmed through in silico studies that obtained good interactions of compounds owned by *E. crista-galli* L flowers with target receptors/enzymes, indicating that there is activity in accordance with the results of in vivo tests. The *E. crista-galli* L flower extract in the aforementioned formulation produced good physical stability, which indicates that the sample can be formed into a gel moisturizer preparation.

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Conflict of Interest

The authors declare that they have no conflicts of interest

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