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Formulation and Test Antioxidant Activity of Gel Fraction Breadfruit Yellow Leaf (*Artocarpus Altilis* (Parkinson) Fosberg).

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ABSTRACT

One of the traditional medicines that are often found in Indonesia, especially in West Java, One of which has potential as an antioxidant is breadfruit (*Artocarpus altilis* (Parkinson) Fosberg). Breadfruit leaves contain a lot of active compounds as antioxidants. The purpose of this study was to determine most excellent antioxidant activity on the fraction of methanol, ethyl acetate and n-hexane derived from breadfruit leaf extract to be used as a gel formulation that has potential as an antioxidant. This study begins with the process of maceration using methanol, phytochemical screening, fractionation using liquid-liquid extraction, followed by testing the antioxidant activity using DPPH method, identification of antioxidant activity using UV-Visible by measuring the antioxidant activity carried out at a wavelength 516 nm is the wavelength absorption of DPPH as the reference standard[2]. On the antioxidant activity test results show that the highest antioxidant activity contained in ethyl acetate fraction. IC_{80} value of ethyl acetate fraction of 0.0053%. The results of the formulations tested to determine their antioxidant activity changes made before and after the gel formulation. The results showed that IC_{80} decreased slightly. Characteristics gel formulation of antioxidants observed consistency of shapes, colors, smells relatively stable, pH and viscosity during 28 days of storage showed pH and viscosity stable and did not change significantly and the result of irritation test on rabbit skin did not cause irritation so it is safe to use.

Keywords: Breadfruit Yellow leaves, Antioxidant, DPPH, *Artocarpus altilis* (Parkinson) Fosberg.

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INTRODUCTION

Advances in technology and modern science is rapidly increasing at this time, was not able to shift the traditional medicine. This is evidenced by many people who are interested in traditional medicine. Therefore, conducted research to explore the potential of traditional medicines on medicinal plants of good quality.

One of the higher plants as a potential Indonesian traditional medicine is breadfruit (*Artocarpus altilis* (Parkinson) Fosberg), is one of the plants that are readily available and have many uses. Plants belonging to the family Moraceae This contains efficacious compounds. Almost all parts of this plant has been used as medicine (leaves, fruit, root bark and sap). The root bark breadfruit efficacious as antiplatelet[1], his activity as antifungal and antioxidant[2], the sap is efficacious as a cure diarrhea and dysentery[3], the leaves are useful as antihypertensive, antidiabetic, antioxidant, and anticancer[3,4,5], compounds that have activity for the body such as saponins, polyphenols, tannins, hydrocyanic acid, acetylcholine, riboflavin and flavonoids. Breadfruit leaves contain flavonoids that are very effective as an antioxidant.

Antioxidant is a molecule that has the ability to inhibit or prevent other molecules become oxidized. Breadfruit leaves yellow empirically used as an antihypertensive and antidiabetic[3]. The ability of breadfruit leaves in treating some diseases were allegedly closely associated with antioxidant compounds in these plants[5]. From the results of previous studies conducted [6] declared breadfruit leaves yellow color that is still stuck in the tree (yellow sticks) has the highest antioxidant activity.

Skin care preparations in the form of a gel preparation is necessary to protect the skin because the skin is very sensitive to inflammation, cancer and premature aging caused by ultraviolet rays that have the effect of oxidative free radicals[7]. Free radicals are atoms that have one or more unpaired electrons in the outer shell so it is very reactive and capable of reacting with the lipid, protein, carbohydrate or DNA. Compounds include hydroxyl free radical, superoxide anion, hydrogen peroxide, hipoklorat acid, singlet oxygen, and peroxyl[8]. The negative effect of free radicals can be inhibited by the presence of antioxidants.

Gels are semisolid systems consisting of suspensions made of small inorganic particles or large organic molecules, penetrated by a liquid. Gel sometimes - sometimes called jelly[9]. Dosage form of gel rarely found in the market compared to a cream or lotion while the gel form has several advantages including non-sticky, does not contaminate clothing, easily applied, easily washable, does not leave an oily film on the skin, the viscosity of the gel did not change significantly during storage[10].

Based on the above, the purpose of this research is to develop a gel formulation which has a fraction of the antioxidant activity of the leaves of breadfruit (*Artocarpus altilis* (Parkinson) Fosberg) *yellow leaves.*

The novelty of study is to see the process of biosynthesis of breadfruit leaf yellow has tested antioxidant activity in extracts and fractions and then developed into a form sediian gel that can be used by communities to ward off free radicals on the skin.

MATERIALS AND METHODS

Plant materials:

Breadfruit plants determined at the Herbarium, School of Biological Sciences Technology (SITH), Bandung Institute of Technology. Yellow breadfruit leaves stuck collected, washed, sorted, 25-30 °C dried at room temperature, protected against direct sunlight and blend.

Tools used:

The tools used are maserator tubes, scales, rotary evaporator, test tubes, funnels, bowls crucible, measuring cups, glass beaker, a pipette, stirring rod, and a UV-Visible spectrophotometry (Shimadzu®), mortar & Stamper, vaporizer cup, spatula, watch glass, refrigerator (Polytron), pH meter (Mettler Taledo), viscometer (MYR-VP 1000**)**

Chemical material:

Materials used are ethyl acetate, methanol, n-hexane, fractions breadfruit leaves, Carbopol ultrez, triethanolamine, propilengikol, methyl paraben, propyl paraben, distilled water, DPPH.

Methods:

Procedure 1:

Crude simplicia weighed 600 grams, then extraction using maceration method using methanol (1:10) for 3x24 hours. The next stage is the process of thickening the extract using a rotary evaporatory. Followed by liquid-liquid extraction (LLC) in stages using the solvent n-hexane and ethyl acetate, then evaporated to obtain a thick fraction. Phytochemical screening performed on extracts and fractions yellow breadfruit leaf stuck to check for the presence of secondary metabolites. In general, these tests include alkaloids, flavonoids, tannins, polyphenols, triterpenoids, steroids, quinones, saponins, monoterpenes and sesquiterpenes. Fractionation is done in stages using the solvent water, ethyl acetate and n-hexane. Breadfruit leaf extract produced dissolved in distilled aqua then put into a separating funnel, fractionated by liquid-liquid extraction using a solvent n-hexane as much as 500ml Fractionation be repeated until translucent. Then the water fraction in the fractionation again using ethyl acetate solvent treatment of the same until the result becomes clear fractionation ethyl acetate. Fractionation results are collected and concentrated by rotary evaporator to obtain a thick fraction. Fraction of n-hexane, ethyl acetate fraction and water fraction.

Procedure 2:

In the early stages of testing it first made a standard curve for DPPH solution. A total of 2 mg DPPH put in a 50 ml flask and dissolved in methanol. DPPH solution that is made has a concentration of 40 ppm. Then dilution at a concentration of 20 ppm. Further uptake measured absorbance at 516nm λ^{11}.Sample fraction n-hexan, ethyl acetate and water each were made at a concentration of 40 ppm, 30 ppm, 20 ppm. Each concentration taken 1 ml was then added 2 ml of standard solution of DPPH shaken until homogeneous and incubated for 30 minutes. DPPH absorbance is measured by spectrophotometry at λ 516, each sample measured triplo.

Sub Procedure 2:

IC_{50} is calculated from a linear regression curve between % inhibition of uptake with various concentrations of the test solution. The highest results of IC_{50} measurements between fractions of n-hexane, ethyl acetate and water made gel formulation.

The highest results of IC_{50} measurements between fractions of n-hexane, ethyl acetate and water made gel formulation. The design of the dosage formula shown in Table 1.

Table1. Draft perfomed gel formula

Material	F1	F2	F3
Carbopol	0,5	0,5	0,5
TEA	0.5	0.5	0.5
Propilenglikol	10	10	10
Metil Paraben	0,075	0,075	0,075
Propil Paraben	0,025	0,025	0,025
Fraction	0,0053	0,0106	0,0159
Aquades	Ad 100	Ad 100	Ad 100

Preparations gel made with Carbopol sown way above 20 mL of hot water, let it rise for 60 minutes. Then stirred until homogeneous. Then TEA was added as a neutralizing, stirred until homogeneous and form a gel. Fraction breadfruit leaves with propilenglikol dissolved, stirring until blended homogeneous. Then put in Carbopol gel that has become, stirred again until homogenous. Methyl paraben and propyl paraben dissolved with propilenglikol, stirring until blended homogeneous. Then put in Carbopol which has become gel and water is added until the desired volume, stirred until homogeneous.

Preparations Gel Stability Test

Gel formulation stability testing include observations organoleptic (Organoleptic observations conducted on gel preparation, including observation of the odor, color, shape, and homogeneity perfomed gel, which was observed during 28 days of storage. Observations carried out by visual observation on days 1, 3, 5, 7, 14, 21, and 28), pH gel preparation is done using a digital pH meter. Prior to use, the tool buffer pH meter is calibrated using pH 4, pH 7.00 and pH 10. Tests conducted by dipping the electrode into a gel preparation, so that the pH value can be read on the device. Observations were made on days 1, 3, 5, 7, 14, 21, and 28 and viscosity is done by means of a viscometer, using spindle rotor dialing numbers 1 and 62.5 rpm. Spindle 1 is dipped into the gel formulation will be evaluated, then the value of measurements taken when the number shown to have stabilized. The viscosity measurements performed on days 1, 3, 5, 7, 14, 21, 28.

Irritation Test

Irritation test performed on 3 rabbits (*Oryctolagus cuniculus*) with the method of Draize (1959). Rabbits used were adult rabbits, able-bodied, weighing 1.5 to 2 kg, rabbit fur sheared back is divided into 4 sections for testing base, Formula 1, Formula 2, Formula 3 of ethyl acetate fraction breadfruit yellow leaves. Each of these irritants as much as 0.5 gram sample smeared on the back of a rabbit that had been shaved, and covered with sterile gauze and then glued together with plaster and then wrapped with a bandage, and left for 24 hours. After 24 hours, plasters and bandages opened and left for 1 hour, then observed. Once observed, the section was closed again with the same plaster and observed after 72 hours. skin condition rated as follows:

1. Erythema
a. No erythema = 0
b. Very mild erythema = 1
c. Mild erythema = 2
d. Moderate erythema = 3
e. Severe erythema = 4
2. Edema
a. No edema = 0
b. Edema very light = 1
c. Mild edema = 2
d. Edema was = 3
e. Edema weight = 4

Irritation index is calculated by adding up the value of each rabbit after 24 hours and 72 hours sample irritant administration, Irritant assessment as follows:

0,00 = No irritation
0.04-.99 = Slight irritant
1.00 to 2.99 = Mild irritation
3.00 to 5.99 = Moderate irritation
6.00 to 8.00 = Severe irritation

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RESULTS AND DISCUSSION

Processing of Crude

Yellow breadfruit leaves stuck harvested, washed, drained, then dried to reduce the moisture content to obtain a water content of less than 10%. This is done to preserve the bulbs from the humid conditions which easily covered with mold and degrade the quality of crude drugs. Then cut into small pieces to match the size of the bulbs and to expand the surface area in contact with the liquid botanicals filter. It is important to seek maximum extraction process.

Extraction Results Breadfruit yellow leaves

The extraction method used is maceration because this is a method that is easy and simple to use tool, simply by soaking the samples in solvents and compounds suitable for heat resistant. The solvent used was methanol as solvent can dissolve almost all organic compounds that exist in the sample, both polar and nonpolar compounds. Methanol is volatile so easily freed from the methanol extract and tend to be less expensive compared to other organic solvents. All of the filtrate obtained from the extraction is evaporated at low temperature and pressure in order to obtain a thick extract is then yield calculated.

Table 2. Results of calculations% yield obtained

Breadfruit leaf	Weight Loss (grams)	extract botanicals condensed (grams)	% Yield
Yellow	600 gram	49.75 gram	8.29

Screening of phytochemical extract and fractions

Pytochemical screening performed on extracts and fractions of leaves of breadfruit. The purpose of the phytochemical screening is to determine what are the secondary metabolites contained in breadfruit leaves yellow simplicia stuck. The results of phytochemical screening positive breadfruit leaf extract contains flavonoids, tannins, phenolics, monoterpenes, sesquiterpene, steroid, and quinones. Such compounds are antioxidants. Results of phytochemical screening fraction breadfruit leaves stuck yellow indicates more specific compared with the results of phytochemical screening breadfruit leaf extract yellow stuck.

Characterization of Crude Simplicia

Simplicia characterization performed on breadfruit leaves yellow bulbs stuck covering ash, water soluble extract content, and the content of ethanol soluble extract. Results from simplicia characterization, assay results showed 25.5% ash, ash content determination gives an overview and inorganic mineral element content contained in crude drugs. Sari assay aims to provide an initial overview of the amount of the compound content. Results of the assay of ethanol soluble extract was 2.2% and the water soluble extract content is 3%, the results suggest more compounds are attracted by water than ethanol, it is estimated that water soluble compound is a compound having polarity such as water, the compound the possibilities are polar compounds such as flavonoids are bound to glucose.

Antioxidant Activity Testing

The method used in testing the antioxidant activity with DPPH spectrophotometry is because the method is simple, easy, and using samples in small amounts with a short time.

Measurement of antioxidant activity carried out at a wavelength of 516 nm which is the wavelength of absorption of DPPH as standard on testing the antioxidant activity. The fraction reacted with DPPH solution, instantly change the color purple to yellow DPPH solution. According to [11], the antioxidant activity of the sample results in a color change in the solution of the original DPPH purple to yellow. Change color intensity

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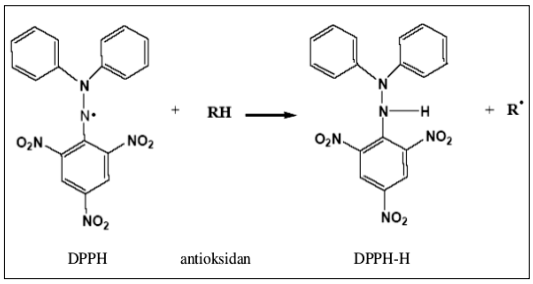
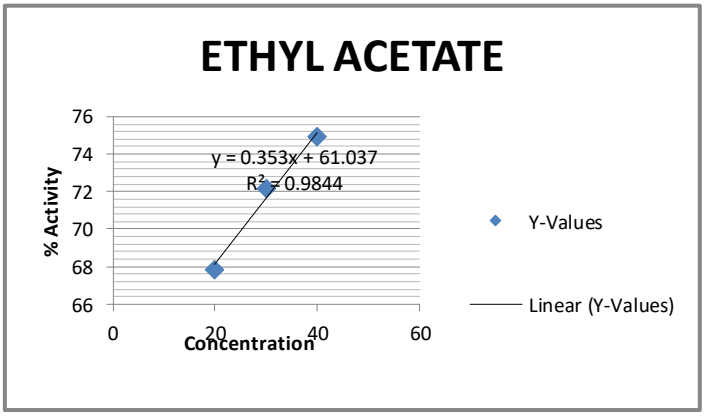
caused by a reduction in the DPPH conjugated double bonds, because electrons in the DPPH radical pairs with the hydrogen atoms of the antioxidant that becomes DPPH + H which is an unstable radical.



From the data below that obtained the highest antioxidant activity% ethyl acetate fraction contained on, so that the fraction of ethyl acetate gel formulation of antioxidants.

Determination of fraction dose of antioxidants used in the formulation is IC_{50}, calculation by inserting concentration as % inhibition as x and y, the obtained regression equation.

Table 3.Graph the regression equation ethyl acetate fraction



In the present study used % inhibition using the IC_{80} and IC_{80} values from ethyl acetate fraction obtained at 53.73 ppm. With such calculations below:

IC_{80} = 80-61.03 = 57.73ppm=0.0053%

0.353

The number of fractions of ethyl acetate are added to the gel preparation

F1=1xIC80=53.73ppm=0.0053%
F2=2xIC80=107.46ppm=0.0107%
F 3 = 3 x IC80 = 161.19 ppm = 0.0161%

From the formulations tested antioxidant activity to determine the change in antioxidant activity before and after created gel formulation. The result is IC_{80} decreased slightly.

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Table 4. Testing the antioxidant activity of gel preparation

Antioxidant Activity		
F1	F2	F3
0,0073%	0,0059%	0,0027%

The greater the dose the greater the added antioxidant activity generated on the test results proved the antioxidant activity in the F2 and F3.

Observations Gel Formulation Stability Test

The observation of changes in gel dosage forms

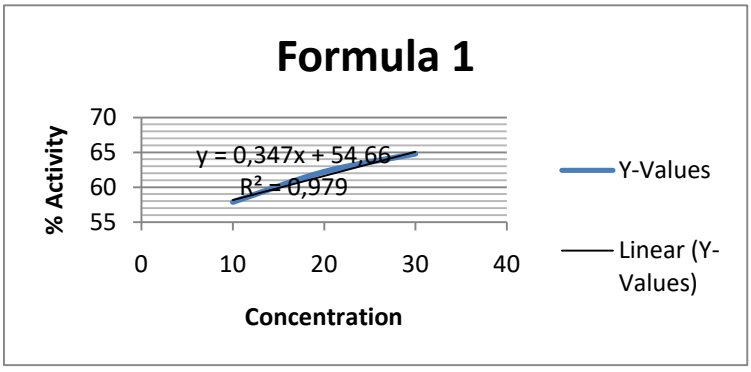
Table 5. Results of observation of changes in gel dosage form ethyl acetate fraction breadfruit yellow leaves.

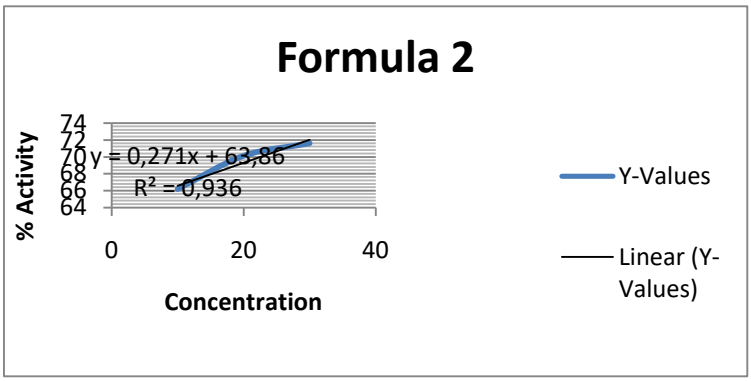
Formula	Gel Form on the day		
	F1	F2	F3
1	VG	VG	VG
3	VG	VG	VG
5	VG	VG	VG
7	VG	VG	VG
14	VG	VG	VG
21	VG	VG	VG
28	VG	VG	VG

Description: VG : Viscous Gel

From the table above it can be seen that each gel formula that is made has a good shape and is stable for 28 days of storage. Charts gel formulation of the test can be seen in Table 6

Table 6: Gel Formulation Stability Test Graph





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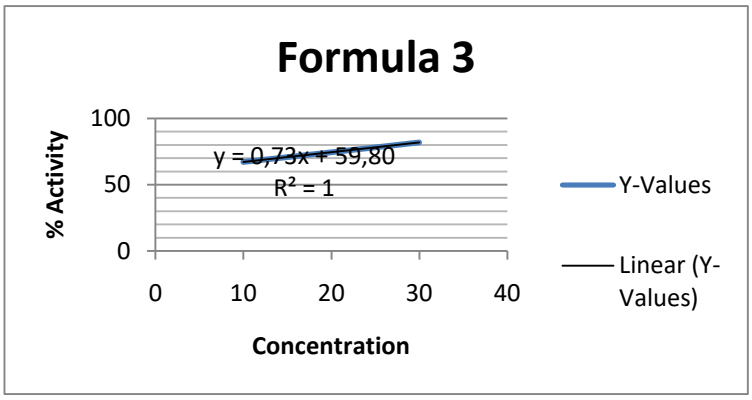


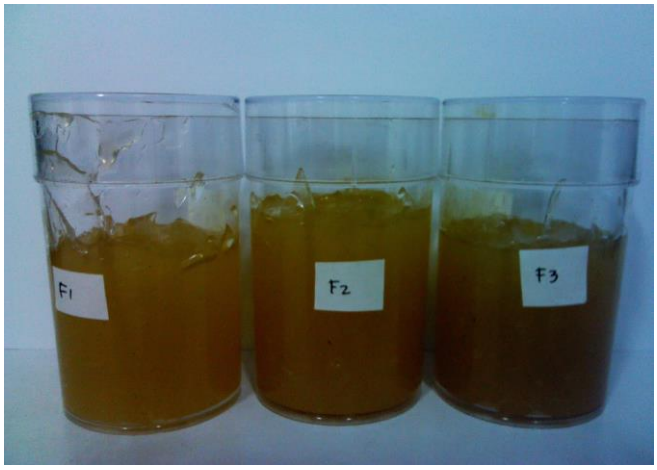
Observations discoloration

Table 7. Observations discoloration gel formulation of ethyl acetate fraction breadfruit yellow leaves.

Formula	Forms a gel at day						
	1	3	5	7	14	21	28
F1	Y	Y	Y	Y	Y	Y	Y
F2	Y	Y	Y	Y	Y	Y	Y
F3	Y	Y	Y	Y	Y	Y	Y

Description: Y: Yellow

From the table above it can be seen that each gel formula that is made has a stable color because it does not change color during the 28 days of storage. Picture of gel formulation can be seen in picture



Monitoring changes the odor of gel performed.

Table 8. Results of observation changes the smell of ethyl acetate fraction gel preparation breadfruit yellow leaves

Formula	The forms a gel at day						
	1	3	5	7	14	21	28
F1	Do	Do	Do	Do	Do	Do	Do
F2	Do	Do	Do	Do	Do	Do	Do
F3	Do	Do	Do	Do	Do	Do	Do

Description: Do(Distinctive odor): All the typical odor

Gel formulation has not changed during the 28 days of storage smell, the smell remains the same, namely the distinctive smell stuck breadfruit yellow leaves.

Observation of changes in pH gel preparation

Table 9. observed changes in pH gel fraction of ethyl acetate breadfruit yellow leaves.

F	pH on day....						
	1	3	5	7	14	21	28
F1	6.58	6.63	6.73	6.75	6.71	6.58	6.58
F2	6.38	6.41	6.41	6.40	6.39	6.31	6.31
F3	6.25	6.26	6.31	6.31	6.37	6.30	6.28

Results of testing the pH range of gel formulation containing fraction breadfruit leaves yellow stuck during 28 days of storage showed that the pH preparations meet the requirements which limit skin admission ranges from pH 5.5-10.

Carbopol soluble in water, ethanol 95%, and the glycerin. If Carbopol is dispersed in water, it forms a colloidal acidic and has a low viscosity. However, after neutralization with a base, it will increase the viscosity. Amino acids, potassium hydroxide, trietranolamin can be used as carbopol a neutralizer

Observation of changes in viscosity gel formulation breadfruit yellow leaves.

Table 10.Change in viscosity gel formulation breadfruit yellow leaves

F	**Viscosity gel at day**						
	1	3	5	7	14	21	28
F1	131.5	131.5	131.4	129.6	129.6	129.6	127.9
F2	131.8	131.5	130.2	129.6	129.3	129.3	127.8
F3	131.4	131.1	130.6	130.2	129.6	128.2	128.2

The test results change viscosity gel formulation containing fraction breadfruit yellow leaves during 28 days of storage showed that the stocks did not change significantly. The viscosity of such preparations are still included in the dosage range of topical gel with a gel comparison of the market that pirofel gel.

Observations of irritation test

Table 11. Results irritation index calculation

Test group	Irritation index
F1	0
F2	0.3
F3	0.3
Basis	0

From the observation of irritation test gel formulation containing ethyl acetate fraction yellow breadfruit leaf stuck on rabbits showed slight irritation irritating (range 0.04 to 0.99), while the base and showed no reaction formula 1 (0).

Irritation index values shown antioxidant gel is slightly irritating. These results are not classified as dangerous, because basically animal skin sensitivity is slightly different from human skin. To the irritation index values shown are mild irritation.

CONCLUSION

Results of phytochemical screening of extracts and fractions stuck yellow leaves of breadfruit contains flavonoids, tannins, phenolics, monoterpenes and sesquiterpene, a steroid and quinones IC_{80} test results with DPPH, the ethyl acetate fraction of the nicest. The test results comparing the antioxidant activity of the antioxidant activity before and after the gel formulation. Results IC_{80} ethyl acetate fraction perfomed better than gel ethyl acetate decreased activity. The observation of form, color, odor, pH, viscosity gel and irritation overall no significant change so it is safe to use.

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