

## Formulation and Antioxidant Test of *Chromolaena odorata* Leaf Extract in Gel with DPPH Method (1,1-Diphenyl-2-Picril Hydrazil)

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**Abstract.** *Chromolaena odorata* leaf extract contains phenolic compounds that have activity as powerful antioxidants that can counteract free radicals. Free radicals are ions that are triggered by UV rays that can cause aging. The Antioxidant can stabilize free radicals so that overcome aging of the skin. The purpose of this study was to find out the most optimal formula of gel and to know its antioxidant activity. *Chromolaena odorata* leaf extract was obtained by maceration method by using ethanol 70%. This gel is formulated using CMC-Na as gelling agent and with dose variation of extract. The evaluation of gel were physical characteristic (spreadability and adhesivity) and tested for antioxidant activity using DPPH method. The results showed that percent inhibition of FI, FII, and FIII were  $8.77 \pm 0.39$ ,  $9.33 \pm 0.09$ , and  $56.6 \pm 0.13$  respectively. The antioxidant activity of FIII exceeded 1.5 times the vitamin C ( $p < 0.05$ ) which is a powerful antioxidant. This is supported by testing the physical properties of the preparation according to the pH of the skin which is  $5.93 \pm 0.17$  does not irritate the skin. Gel preparations also have good homogeneity, dispersion, and adhesion. The formulation of *Chromolaena odorata* leaf extract in gel contains powerful antioxidants that have capability as antiaging.

**Keywords:** *Chromolaena odorata*, leaf extract, gel, antioxidant

### 1. Introduction

The skin has the main function as a protector, including the skin from the rays of radiation and toxic substances. In carrying out its functions, there are several problems that can inhibit skin sustainability. The main and severe factors that occur in the body and can cause oxidative damage, commonly known as "Reactive Oxygen Stress" (ROS). The problem that can be ensnared by skin aging is the sun (photoaging), especially ultraviolet (UV) light which will increase ROS in cells. Skin exposed to sunlight will be at risk for aging skin aging, characterized by wrinkled skin, dry, rough, and round-line [6]. Use antioxidants as a preventative or best skin care effect with aging photos. Antioxidants used in synthetic or natural ingredients. Some anti-aging products use synthetic antioxidants, where the chemicals contained in antioxidant synthetic materials will provide a long-term adverse risk such as

butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) [19].

Thus, alternative solutions for antioxidant compounds from natural ingredients which are not harmful to the skin are needed. Phenolic antioxidants play a major role in fighting free radical species which are the main cause of various negative skin changes. Although isolated of leaf compounds have high potential for skin protection, herbal extracts show better potential because of the complex composition of herbal sources. Many studies have shown that green plants can improve skin damage due to UV exposure [14,20]. Phenolic is an effective source of antioxidants, which can be found in the leaves of the *Chomolaena odorata* plant, which is a very abundant plant population on Indonesian soil. This plant is just a weed plant that has not been utilized properly in increasing its use value. In

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the ethanolic extract of *Chomolaena odorata* leaves has a higher total phenolic content of 313.31mg/g phenolic compounds [1, 11].

The ethanol extract of *Chomolaena odorata* leaves can be further processed, namely making a gel preparation formula. Gel preparations are preparations that are kept in the community. The goal is to make good quality antioxidant gel preparations and have strong antioxidant activity. Antioxidant activity test using DPPH method. DPPH method has the advantage that it is easy and can be directly refined with antioxidant agents. *Chomolaena odorata* leaf extract gel can function as an aging therapy agent on the skin. In addition, the economic value of the plant is dehydrated.

## 2. Methodology

### 2.1 Tools

UV-Vis (UV-1700) Spectrophotometry, Halogen Moisturizer Analyzer (HB43 Metler Toledo), analytical balance (Ohaus), vacuum pump, buchner funnel, rotary evaporator, separating funnel, cuvette, aluminum foil, micropipette, filter paper, a set of maceration tools propipet, drop pipette, volume pipette, oven, glassware, and gel physical properties.

### 2.2 Materials

*Chomolaena odorata* leaves obtained from Kalasan, Sleman, DIY; DPPH 0.15 mM, 70% ethanol, aquadest, CMC-Na, glycerin, propylene glycol, methyl paraben, and ethanol.

### 2.3 Methods

#### 2.3.1 Raw material for *Chomolaena odorata* leaves

Fresh leaf extracts of 500 grams were taken, then the leaves were dried using an oven at 600 C. Simplicia

was smoothed using a blender and stratified with 50 and 60 mesh sizes [10].

#### 2.3.2 Extraction of phenolic compounds

Extraction of phenolic compounds from 100 grams of *Chomolaena odorata* leaf powder was carried out by solvent extraction method using 1000 mL 70% ethanol solvent, then stirred for 6 hours using an electric stirrer and allowed to stand for 12 hours. The ratio of *Chomolaena odorata* leaf powder to solvent is 1:10 (b / v). After the extraction process, separation of solids and liquids is carried out using a vacuum oven. The results are then evaporated with a rotary evaporator at a temperature of 400C. Then the extract was dried using Waterbath [7].

#### 2.3.3 Identification of phenolic compounds

Identification of phenolic compounds of ethanol extract 70% of *Chomolaena odorata* leaves was carried out using thin layer chromatography. This is done to confirm the presence of phenolic compounds which are efficacious as antioxidants in the extract. The test was carried out qualitatively using silica gel F254 nm as a stationary phase and toluene mixture: ethyl acetate: formic acid with a ratio (6:4:0.8) as the mobile phase. Comparators used gallic acid standards.<sup>15</sup> After the elution process is complete, the silica plate is sprayed using a DPPH 0.004% solution in ethanol. Positive extracts contain phenolic activity compounds when yellow spots with purple background on KLT plates are obtained [2].

#### 2.3.4 Determination of water content

Water content of *Chomolaena odorata* leaf ethanol extract was determined by a Halogen Moisture Analyzer. Extracts were tested as much as 1 grams with a temperature of 105°C for 15 minutes [12].

#### 2.3.5 Gel formulation

Table I. *Chomolaena odorata* leaf extract gel formula

Component	F1	F2	F3
<i>Chomolaena odorata</i> leaf ethanol extract	IC80	1.5 x IC80	3 x IC80
CMC-Na	0.3 grams	0.3 grams	0.3 grams
Glycerin	2 grams	2 grams	2 grams
Propylene glycol	1 grams	1 grams	1 grams
Methyl paraben	0.03 grams	0.03 grams	0.3 grams
Water	20 grams	20 grams	20 grams

\*Note: IC80 value = 28 mg/mL

Optimization of gel formula was carried out based on the effect of different concentrations of ethanol extract 70% of *Chomolaena odorata* leaves on the physical properties of the gel and antioxidant activity. The formula refers to the composition of the gelling ingredients that have optimal physical properties according to Maulina and Sugihartini (2015) with few changes (Table 2). Making the gel begins with developing CMC-Na in 10 mL of water at 70°C, then the extract is added according to concentration (Table 2). This mixture is called Mixture 1. Methyl paraben as a preservative is dissolved in a little water then a mixture of glycerin and propylene glycol is added as humectant. The mixture then it is called Mixture 2. The two mixtures are put together, after which they are stirred and water is added to 20 grams then the mixture is stirred for 5 minutes at speed 500/rpm until homogeneous.

### 2.3.6 Test the physical properties of the gel

- a. Organoleptic test and homogeneity  
Organoleptic tests are carried out by direct observation of the color, clarity, and odor of the gel. Homogeneity testing is done by applying gel on a piece of glass.
- b. PH test  
PH testing is done using a pH meter.
- c. Spread power test  
The testing of the dispersing power begins with as much as 0.5 grams of gel placed in a round glass, the other glass is placed on it, and left for 1 minute. After that, 150 grams of load were added, allowed to stand for 1 minute, and measured a constant diameter [8].
- d. Adhesion test  
Adhesion testing begins with a 0.25 grams gel sample placed between 2 glass objects in the sticky power test, then pressed using a 1 kg load for 5 minutes. The load is lifted and given a load of 80 grams on the tool then the gel release time is recorded [13].

### 2.3.7 Determination of antioxidant activity with DPPH

- a. Preparation of 0.15 mM DPPH solution

DPPH solution is made by DPPH stock solution (1 mM) diluted with ethanol solvent p.a. to obtain a concentration of 0.15 mM DPPH stock solution (1 mM) was made by weighing 9.8 mg of DPPH powder into a 25 mL measuring

flask and then adding p.a ethanol to the boundary markers.

- b. Making *Chomolaena odorata* leaf extract gel sample

Extraction of the gel extracts of *Chomolaena odorata* leaf ethanol extract was made by weighing 500 mg of extract gel from each formula dissolved in ethanol p.a to 50 mL to make a concentration of 10000 µg/mL. Then 2.5 mL of pipette is extracted from a solution of 10000 µg/mL into a 25 mL volumetric flask, and added to ethanol p.a to a boundary mark so that a concentration of 1000 µg/mL is obtained. Then pipette 3 mL from a solution of 1000 µg/mL and put in a 10 mL volumetric flask so that the concentration becomes 300 µg / mL. Each concentration in ad with ethanol p.a to make from formula 1, formula 2, and formula 3.

- c. Making negative controls

Negative control solution was made by mixing 1 mL DPPH (0.15 mM) with 1 mL ethanol p.a.

- d. Making positive controls

A positive control solution was prepared by dissolving 50 mg of vitamin C with ethanol p.a to 50.0 mL. Then from the mother's vitamin C solution a concentration of 8 µg / mL was made. Each concentration of ad up to 10 mL with ethanol p.a [5].

### 2.3.8 Reading antioxidant activity

- a. Determination of maximum DPPH

As much as 1 mL DPPH (0.15 mM) was reacted with 1 mL of ethanol p.a, then allowed to sit in a dark place for 30 minutes. After that the solution was measured at a wavelength of 450-650 nm using a UV-Vis spectrophotometer [17].

- b. Determination of operating time

Each extract gel sample and standard vitamin C solution were added with 1 mL DPPH solution (0.15 mM) then the absorbance was observed for 0-75 minutes at a wavelength of 517 nm [9].

- c. Testing of antioxidant activity

From each test solution and standard 1 mL pipetted then put into flakon, the solution was added 1 mL DPPH (0.15 mM) and shaken until homogeneous. The solution is left in a dark place during operating time. Then the absorbance test measured at the maximum wavelength. The blank solution used is ethanol p.a [16].

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### 2.3.9 Data analysis

The absorbance of *Chomolaena odorata* leaf extract gel and vitamin C was changed in the form of percent capture, namely the amount of DPPH radical captured by the sample. DPPH radical capture percentages can be calculated using the following formula:

$$\% \text{ DPPH Radical Capture} = \frac{(\text{Abs}_{\text{Control}} - \text{Abs}_{\text{Sample}})}{\text{Abs}_{\text{Control}}} \times 100 \quad (1)$$

Information:

$\text{Abs}_{\text{Control}}$  : Absorbance of controls

$\text{Abs}_{\text{Sample}}$  : Absorbance of test samples [3].

To see differences in the results of antioxidant activity and physical properties of gel between formulas an analysis was performed using one way ANOVA. The unpaired t test was used to see the difference in the results of antioxidant activity between the optimal gel formula and positive control of vitamin C.

## 3. Results and Discussion

*Chomolaena odorata* leaves were obtained in Purwomartani Village, Kalasan District, Sleman Regency, Yogyakarta Special Region. Leaves are taken from the same location to minimize variations in the active substance content which is strongly influenced by plant varieties, age, and plant location. The weight of the *Chomolaena odorata* leaf obtained was 4.8 kg. After 4.8 kg of wet leaves dried with an oven (60°C), the results of 880 grams of dried simplicia were obtained. Dry simplicia which has been mashed using a blender so that the resulting simplicia powder is 660 grams in the form of dry powder. The process of refining the dried simplicia using a blender aims to reduce the particle size, so that the surface area of the powder which is in contact with the solvent when extraction is greater. This will optimize the process of withdrawing the desired chemical compound.



Fig 1. *Chomolaena odorata* leaves

A simplicia is then extracted by maceration method and 70% ethanol solvent to attract phenolic compounds which are efficacious as antioxidants. After the macerate was evaporated, 121.61 grams of thick

ethanol extract were obtained. Qualitative analysis of phenolic compounds in the extract of thick leaves of *Chomolaena odorata* It is important to do this process to prove the presence of phenolic compounds in the extract. Identification of phenolic compounds was carried out using Thin Layer Chromatography (TLC) with comparable gallic acid. The results of the identification test found yellow spots with purple background on the TLC plate (Figure 2). This shows that the extract contains phenolic compounds. In addition to the qualitative analysis of the compound, water content was also measured in the extract. Water content must be below 10% to maintain the quality of the extract so that it is not easily contaminated with microbes when in storage [4]. The average water content of ethanol extract is 70% of *Chomolaena odorata* which is an average of 7.85%±0.38, so it is in accordance with the requirements of Indonesian Herbal Pharmacopoeia.



Fig 2. Identification of phenolic compounds

The thick leaf extract was then formulated in gel form with the formula listed in Table I. The extract dosage was based on the results of the study by Radava, *et al.*, (2011) which obtained IC80 values of 28 mg. IC80 is percent inhibition at 80%. Variation of dosage has been made based on the research, which is as much as 10xIC80, 15xIC80, and 30xIC80. The desired gel preparation of ethanolic extract of *Chomolaena odorata* leaves as antiaging was easily poured so that before making gel, CMC-Na optimization was carried out. The CMC-Na concentration needed in making gel preparations is 0.3 grams.

Before testing the physical properties and further processing, the gel antioxidant activity was tested by DPPH method to really ensure that the gel had good activity. Determination of OT is a step to find out the reaction time between the active substance and the radical agent, DPPH. Whereas the max lamda is done to find out the lamda from DPPH solution which has 517±2 nm value. DPPH is a stable free radical and is used to evaluate the reduction of free radicals in natural ingredients. The principle of the reaction of this method is DPPH will be reduced by the hydrogen or electron donation process so that the color changes from violet to yellow with changes in color intensity

which is proportional to the number of electron donations followed by DPPH absorbance [18]. The greater the decrease in DPPH absorbance, the stronger

the antioxidant activity. The results of the measurement of antioxidant activity can be seen in table II.

**Table II.** Results Measurement of antioxidant activity of gel of ethanol extract of *Chomolaena odorata* leaves using DPPH method

Formula	Replication	Absorbance Of DPPH Control	Sample Absorbance	% Inhibition	Average % Inhibition
I (10 x IC80)	1	0.836	0.765	8.49	8.77±0.39
	2		0.758	9.33	
	3		0.765	8.49	
II (15 x IC80)	1	0.836	0.758	9.33	9.33±0.09
	2		0.759	9.21	
	3		0.757	9.45	
III (30 x IC80)	1	0.836	0.367	56.1	56.6±0.13
	2		0.364	56.5	

The measurement results (Table II) show that with an increase in the dose of *Chomolaena odorata* leaf extract in gel preparations, the absorbance value produced decreases so that % inhibition increases and is significantly different ( $p<0.05$ ;  $p=0.00$ ). The results of the formula III were the best results which had %

inhibition of free radicals of 1.5x ( $p<0.05$ ) significantly greater than a strong antioxidant of vitamin C. These results proved great potential for *Chomolaena odorata* leaf extract gel as a product that has strong antioxidant activity.

**Table III.** The results of measuring the antioxidant activity of vitamin C

Vitamin C Concentration	Replication	Absorbance of DPPH Control	Sample Absorbance	% Inhibition	The Average of % Inhibition
8 µg/ml	1	0,836	0.581	30.50	35.29±3.41
	2		0.517	38.16	
	3		0.525	37.20	

To guarantee the good quality of the gel physical properties were tested including organoleptic,

homogeneity, pH, dispersion, stickiness. Organoleptic test results. Homogeneity, and pH are stated in table IV.

**Table IV.** Organoleptic test and homogeneity

Formula	Organoleptic Test			pH test
	Color	Smell	Homogeneity	
F1	Greenish brown	Typical extract	Homogeneous	6.09
F2	Greenish brown	Typical extract	Homogeneous	5.95
F3	Greenish brown	Typical extract	Homogeneous	5.74
				Average : 5.93±0.17

The results of the third organoleptic test consistency formula, namely greenish brown color, characteristic of extracts and have good homogeneity. The pH of the gel preparation meets the requirements of 5.93 so that it does

not cause irritation to the skin, because the pH of the skin is normal at 5-6. The pH value produced so that the pH of the gel meets the requirements.



**Table V.** Results of the gel dispersion test

Formula	Replication I		Replication II		Replication III		Average	
	D (cm)	L (cm <sup>2</sup> )	D (cm)	L (cm <sup>2</sup> )	D (cm)	L (cm <sup>2</sup> )	D (cm)	L (cm <sup>2</sup> )
F1	5.7	27.86	5.76	26.04	5.72	25.68	5.7±0.03	26.47±1.07
F2	5.74	25.86	5.46	23.40	5.8	26.41	5.6±0.18	25.22±1.60
F3	5.4	22.89	5.5	23.75	5.36	22.55	5.4±0.07	23.06±0.61

\*Note: D = Diameter ; L = Large

**Table VI.** The test results of the gel adhesion strength

Formula	Time (second)			
	Replication I	Replication II	Replication III	Average
F1	1.70	1.75	1.75	1.73±0.03
F2	1.56	1.55	1.59	1.56±0.02
F3	1.21	1.24	1.48	1.31±0.14

The good dispersion of gel preparations is gels that have a diameter of 5-7 cm [21]. Statistical analysis of scattering power shows the difference in results between F1, F2, and F3. This is shown by statistics with one way ANOVA having a significance of 0.03 (<0.05), so that H<sub>0</sub> is rejected which means that there are significant differences with the use of different doses that can affect the physical properties of gel preparations. Increasing doses show a decrease in

adhesion while a slightly higher dose increases. The results of statistical tests showed that the data were normally distributed 0.68 (>0.05) and not homogeneous 0.03 (<0.05) so that it used the kruskal wallis statistical test which obtained a significance value of p <0.05. These results indicate that there are significant differences in the effect of different doses used on the sticky power of the gel ethanol extract gel leaf extract.

## 4. Conclusion

Preparation of ethanol extract gel formula III *Chomolaena odorata* leaves (*Chromolaena odorata*) can be formulated in gel form. gel preparations have good physical properties. gel especially formula 3 has strong antioxidant activity compared to vitamin C so gel preparations can be an alternative to aging treatment.

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