Journal of
Fundamental and Applied Pharmaceutical Science
https://journal.umy.ac.id/index.php/jfap

Formulation and Antioxidant Activity Analysis of Jotang Herb (*Acmella paniculata*) Extract Mask Cream

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Abstract

Pollution in the environment can increase the production of reactive oxygen species (ROS), which can reduce the amount of antioxidants in the skin. Antioxidants are compounds that can inhibit oxidation reactions by binding to free radicals and reactive molecules. Herbaceous plant jotang (*Acmella paniculata*) has secondary metabolites such as flavonoids, alkaloids, tannins, and saponins, which can protect the internal and external organs of the body's cells from damage caused by free radicals on the skin. One of the treatment preparations used for the skin is a cream mask, as the use or application at the final level of skin care can improve cleanliness and health and stimulate and repair skin cells. This study aims to determine a formula that has a good antioxidant value using the DPPH method (2,2-diphenyl-1-pikrilhidrazyl). In this study, preparations were made with 3 formulas, namely Formula I (1% w/w), Formula II (10%w/w), and Formula 0 (Control). Evaluation of physical properties included organoleptic tests, homogeneity tests, spreadability tests, pH tests, viscosity tests, cream-type tests, and antioxidant tests. The result showed that the organoleptic test had a cream mask dosage in the form of semi-solid, distinctive jotang odor, light and dark green, had a homogeneous dosage form, spreadability around 5.1-5.7 with an average pH value of 6, had a good viscosity value and had a type of cream M/A and a high antioxidant value in formula II (10% w/w) with an IC50 value of 21.959 µg/ml. Based on statistical tests, significant results were obtained of 0.000<p (0.05) so that it can be interpreted that there was a significant difference in each concentration of jotang herb extract to the resulting IC_{50} value.

Keywords: jotang; *Acmella paniculata*; mask cream; DPPH (2,2-diphenyl-1-pikrilhidrazil)

Data of article
Received : 28 Aug 2023
Reviewed : 19 Nov 2023
Accepted : 19 Feb 2024

DOI
10.18196/jfaps.v4i2.19341

Type of article:
Research

INTRODUCTION

Reactive Oxygen Species (ROS) are free radicals that play an important role in several physiological processes of the body's organs. ROS are known to also modulate vascular function by causing damage directly due to the influence of pollutants. In addition, pollutants can penetrate through the stratum corneum to deeper layers of the skin, causing damage to the skin.[1]

Antioxidants are compounds that can inhibit oxidation reactions by binding to free radicals and reactive molecules.

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Antioxidants are compounds that, in certain amounts, can slow down or inhibit the damage caused by the oxidation process. Antioxidant compounds are needed for the body to protect against exposure to free radicals, which work by donating electrons to compounds that have oxidant properties so that they easily inhibit oxidant activity.[2]

One of the plants that contain antioxidants and is often used is the jotang plant (*Acmella paniculata*), which has an IC_{50} value of 1.77 µg/ml. It is a herb that grows wild in gardens, along rice fields, river banks, and moist soils in tropical and subtropical regions. Jotang plants also contain several active compounds that are good for health, such as flavonoids, phenolic components, alkaloids, tannins, and saponins. Flavonoid compounds in plants can protect internal body cells and external organs from damage caused by free radicals on the skin.[3]

Facial skin is one of the parts most often exposed to UV rays. Ultraviolet rays can have adverse effects on the skin, such as causing skin cancer, darkening of the skin surface, sunburn, and signs of aging (Lailiyah et al., 2020). In addition, exposure to ultraviolet rays and air pollution contain free radicals. The skin care that most people use to treat their skin is mask preparations.[4]

Masks are cosmetics that are used at the last level in treating problematic facial skin,[5] improving skin hygiene, health, and beauty, and repairing and re-stimulating skin cell activities.[6] Based on the above background, the jotang plant has various properties, one of which is that it is an antioxidant for facial skin care. The research was carried out on the formulation and evaluation of the physical properties of cream mask preparations from the ethanol extract of the jotang herb (*Acmella paniculata*).

METHOD

Extraction

The ethanol extract of jotang herb was made. The jotang herb was washed under running water until all the dirt was gone. The jotang herb was then dried in the air, crushed, and extracted. The jotang sample used was 300 g of Simplicia, and then 3000 ml of 96% ethanol solvent was added until the sample was submerged or a layer above the sample surface. The container was tightly closed and then stored for three days at room temperature, protected from light, with the daily treatment of stirring 3 times a day, namely in the morning and afternoon until the third day. Furthermore, the maceration was filtered, collected, and evaporated using a rotary evaporator or evaporated over a water bath at 70-100°C to obtain a thick extract.

Table 1. Preparation formulationof of Jotang Herb Ethanol Extract Cream Mask

Ingredients	Formula w/w (%)			Function
	F0	F1	F2	
Etanol Extract of herba jotang	-	1	10	Active Substance
Stearic acid	15	15	15	Emulsifier
Cetyl alcohol	1	1	1	Emollient
Glycerol	5	5	5	Humectant
TEA	2	2	2	Emulsifier
Nipagin	0.1	0.1	0.1	Preservative
Nipasol	0.05	0.05	0.05	Preservative
Aquadest	100	100	100	Solvent

Each ingredient was weighed, and then the additional ingredients in the formula were separated into two groups, namely the oil phase and the water phase. The oil phase, i.e. (stearic acid, cetyl alcohol, glycerol) was heated at 60°-70°C until melted. The water phase (TEA, nipagin. nipasol, and aquadest) was heated at 60°-70°C until melted. The oil phase was introduced slowly into the aqueous phase with stirring until a creamy mass was formed homogeneous, then the ethanol extract of jotang herbs was added according to the crushed concentration homogeneous. After the preparation was homogeneous, it was put in the appropriate container and tested for evaluation.

Evaluation of the Physical Properties of Cream Mask Preparations

Organoleptic Test

The organoleptic evaluation used the five senses, starting from the smell, color, and texture of the preparations. Observations were seen directly from the preparations made.[7]

Homogeneity Test

The amount of the preparation was applied to a piece of glass; the preparation must show a homogeneous composition, and there were no visible coarse grains.[7]

Spreadability Test

A sample of cream was placed in the center between two slides, where the top slide was loaded by placing weights until it reached a weight of 150 g. Measurements were made until the diameter of the spread of the cream was constant. This test was carried out for each formulation and replicated three times.[7]

The pH test

The pH test was carried out using a pH meter; weighing 1 gram of mask cream preparation and dissolving in 10 mL., then dipping the pH meter into the preparation. This test was performed on
each formulation and replicated three times.[7]

Viscosity Test

The viscosity of the cream mask preparation was carried out to find out from each mask preparation cream, measured using a Brookfield viscometer, and the respective formulas were replicated three times. The spindle and rotor were then installed. The viscosity result was recorded after the needle viscometer showed a stable number after five rounds.[3]

Cream Type Test

The cream preparation was taken sufficiently and then placed on the object

glass. 1 drop of methylene blue indicator was added. If the blue color of methylene blue can be mixed evenly in the cream preparation, then the cream is type M/A.[8]

Antioxidant Activity Testing of Jotang (Acmella paniculata) Herb (Acmella paniculata) Cream Mask Preparations[2]

Preparation of DPPH Solution

40 mg of DPPH powder was weighed and dissolved in 100 ml of 96% ethanol in a 100 ml volumetric flask, filling it to the mark. The solution was left to stand at a low temperature, protected from light. Next, 2 ml of the DPPH solution was taken and transferred into a test tube.

Determination of the Maximum Wavelength of DPPH

2 ml of a 96% ethanol solution was mixed thoroughly and shaken until homogeneous, and then 3 ml of the mixture was poured into a cuvette. The absorbance was measured at a wavelength ranging from 500 to 600 nm using a UV-Vis spectrophotometer, and the maximum wavelength was recorded at 517 nm.

Preparation of Blank Solution

In a test tube, 2 ml of DPPH solution was added, followed by the addition of 2 ml of 96% ethanol. The mixture was shaken until homogeneous and incubated in a dark room for 30 minutes. Afterward, the absorption was measured using a UV-Vis spectrophotometer at a wavelength of 500-600 nm to determine the maximum wavelength.

Preparation of Cream Mask Solution from Jotang Herb Extract[9]

Preparation of 100 ppm concentration main solution

Mask Cream from Jotang Herba Extract has weighed as much as 10 mg and was added with 96% ethanol, then put into a 100 ml measuring flask. The volume was filled with 96% ethanol up to the mark.

Pre Conc preparation of centration Series Test Solutions

The main solution of Mask Cream from Jotang Herb Extract was made in a series of concentrations of 2,4,8,16 and 32 ppm. From the main solution of 100 ppm, 0.2 ml, 0.4 ml, 0.8 ml, 1.6 ml, and 3.2 ml were pipetted into a 10 ml volumetric flask and then made up.

Absorption Measurement of UV-Vis Spectrophotometer

A total of 2 ml of the jotang herb ethanol extract cream mask test solution was put into a test tube, and 2 ml of DPPH solution was added and shaken until homogeneous, incubated for 30 minutes in a dark room, then the absorption was measured at wavelength.

Preparation of Comparison Solution

Preparation of 100 ppm concentration main solution

As a comparison, the mudwort cream mask weighed 10 mg and was dissolved in 96% ethanol, then put into a 100 ml volumetric flask. The volume was made up of 96% ethanol up to the mark mark.

Concentration Series of Test Preparation

Mugwort cream mask solution was compared with concentrations of 2, 4, 8, 16, and 32 ppm. The solution mains 100 ppm of pipette 0.2 ml, 0.4 ml, 0.8 ml, 1.6 ml, and 3.2 ml were put into a 10 volumetric flask ml.

Absorption Measurement of UV-Vis Spectrophotometer

The test was carried out by pipetting 2 ml of various concentrations of the mugwort cream mask solution (2,4,8,16 and 32 ppm), then adding 2 ml of DPPH until it was completely mixed. The mixture was stored for 30 minutes in a dark room, and then the absorbance was measured using UV-Vis spectrophotometry at the maximum wavelength.

RESULTS AND DISCUSSION

Extraction

In the extraction process, the jotang herb (*Acmella paniculata*) was extracted using the maceration method with 96% ethanol solvent for 3 days and 24 hours, which was carried out 2 times.[10] The resulting filtrate was then concentrated using a rotary evaporator at 50°C, aiming to concentrate the extract and be able to separate between ethanol solvents and compounds active herb jotang. The results of the thick extract obtained were then weighed, and the extract yield was calculated.

Table 2. Extract yield results

Sample	Sample Weight	Solvent Volume	Extract Weight (gram)
Herb Jotang	300 gram	3000 ml	13.2 gram

The yield of jotang herb extract was 4.4%. The yield obtained was lower than that of Kholifah and Insani (2023), who obtained a thick extract of 5.12%.[10] The yield percentage obtained was influenced by the effectiveness of the extraction process and several factors, namely the stirring process during maceration, the type of solvent, the amount of Simplicia, and the time and temperature used during the extraction process.[11]

Evaluation of Physical Properties of Preparations

Organoleptic

Organoleptic tests carried out aim to observe the physical properties of the preparations, including shape, color, and smell. An organoleptic examination was carried out to describe the consistency, color, and smell of the cream that had been mixed with several bases. The resulting preparation should have an attractive color, a pleasant odor, and sufficient consistency to be comfortable to use.[12]

The organoleptic test results obtained from the cream mask preparation of the ethanol extract of the jotang herb (*Acmella paniculata*) for Formula 0, Formula 1, and Formula 2 have the same form, namely semi-solid. The cream mask preparation Formula 0 (without extract) had a white color and was odorless, while Formula 1 with an extract concentration of 1 gram had a light green color and a distinctive jotang smell. In contrast, Formula 2, with an extract concentration of 10 grams, had a dark green color and a distinctive jotang odor.[8]

Table 3. Organoleptic test results

Formula	Form	Color	Smell
Formulas 0	Semi-solid	White	No smell
Formulas 1	Semi-solid	Light green	Typical jotang smell
Formulas 2	Semi-solid	Dark green	Typical jotang smell

Homogeneity Test

The homogeneity test is one of the physical property evaluation tests that aims to identify the mixing of the ingredients of the cream preparation so that the entire cream preparation does not show any coarse grains when the preparation is smeared on the slide. The cream is considered homogeneous when there is an even color equation and no particles are found in the cream.[12]

The results obtained from the homogeneity test of the jotang herb extract cream mask from Formula 0, Formula 1, and Formula 2 showed a homogeneous composition. The active substance properties of the ethanol extract of the jotang herb were mixed with the M/A base so that phase collection and separation did not occur. It demonstrated that a homogeneous cream mask preparation indicates the ingredients used in the cream mask preparation were perfectly mixed.[7]

Spreading Test

A spreadability test was carried out to determine the quality of the spreading power of the cream preparation when applied to the skin. The greater the spreading power is, the better the physical properties of the cream preparation will be.[12]

The results of the spreading power of the cream mask from the ethanol extract of jotang herb from the Table above showed an average value obtained for FO of 5.1 cm, for F1 of 5.4 cm, and F2 of 6.2 cm. These results indicated that the spreadability of the cream mask preparation met the standard requirements for a good spread, which is around 5-7 cm.[13]

Table 4. Spreadability test results

Formula	Power Spread (cm) Replication			Average
	1	2	3	
Fo	5	5	5.5	5.1
F1	5	5.2	6	5.4
F2	5	6	6.2	5.7

pH Test

The pH test aims to determine whether the preparation is acidic, alkaline, or neutral. For topical preparations, it must meet the requirements. It is because if the pH is too alkaline, it will result in dry skin. Otherwise, if the pH is too acidic, it can trigger irritation to the skin.[14] The results of the table data above show that Fo has a pH of 6.06, Fl has a pH value of 6.04, and F2 has a pH value of 6.09. The ideal pH of cream preparations is preferably according to the physiological pH of the skin, which is around 4.5-6.5.[13]

Table 5. pH Test Result

Formula	pH test Replication			Average
	1	2	3	
Fo	5.85	6.14	6.19	6.06
F1	6.08	6.04	6.01	6.04
F2	5.99	6.08	6.29	6.09

Viscosity Test

The viscosity test was carried out with the aim of determining the level of thickness of the mask preparation and the resulting cream. The higher the viscosity is, the greater the resistance will be. On the viscosity test, a Brookfield viscometer was used to determine the viscosity of a

preparation. Measurement Viscosity was determined by attaching the sample in a Brookfield viscometer to the submerged spindle and then letting the spindle rotate at the specified speed. The viscosity measurement was carried out using spindle number 4 and speed 60 rpm. The value of the viscosity range was located in the range of 2,000-50,000 centipoise viscosity values.[15]

Table 6. Results of Viscosity Testing

Formula	Viscosity test (cps) Replication			Average
	1	2	3	
F0	9990	9990	9990	9990
F1	9820	9970	9990	9927
F2	6020	6020	6020	6020

Test of Cream type

The cream type test aims to determine the type of cream mask preparation from herbal extracts jotang that has been made. The results of microscope observations showed that the cream mask preparation was a type M/A. It is indicated by the blue color visible on the outer phase due to methylene blue-soluble in water.[8] There is a combination of ingredients: stearate sour cream and triethanolamine will form a stable M/A cream preparation.[16]

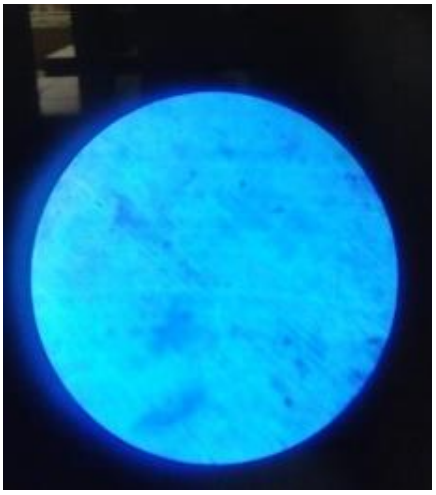
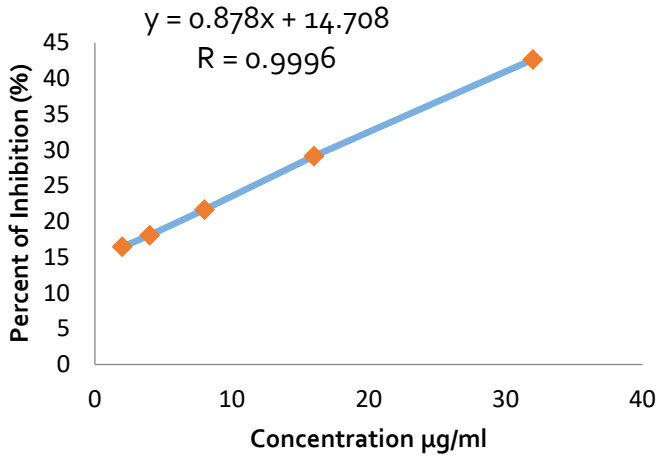


Figure 1. cream type test

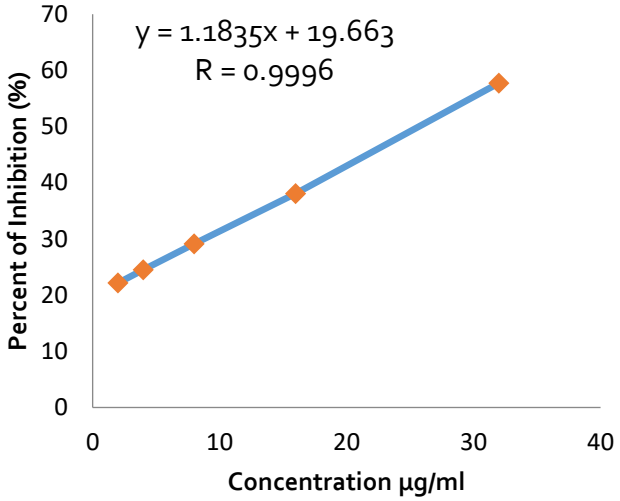
Antioxidant Activity Test of Jotang Herb (Acmella paniculata) Ethanol Extract Cream Mask

The determination of the value of antioxidant activity in this study used the DPPH method. The antioxidant activity test method with DPPH (2,2-diphenyl-1-fikrylhidrazyl) was chosen because it is an easy, simple, fast method that only requires a few samples to evaluate the antioxidant activity of natural product compounds.[17] This antioxidant is a quantitative measurement of antioxidant activity, namely by measuring DPPH radical scavenging by a compound that has antioxidant activity using a UV-Vis spectrophotometer so that it can identify the value of free radical scavenging activity expressed by the value of IC50 (Inhibitory Concentration).[18]

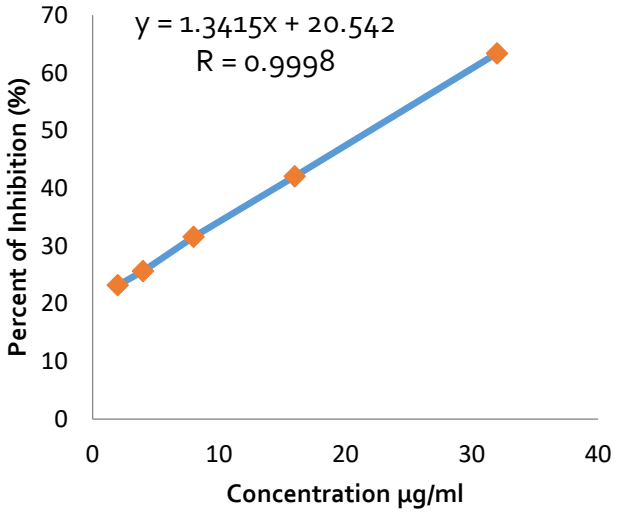
In this study, the antioxidant activity test was carried out on 3 cream mask preparation formulas from the ethanol extract of jotang herbs in each formula, with the aim of knowing the IC 50 value in each formula for the preparation of jotang herb extract cream masks with extract ratios as much as 1 gram and 10 grams and to find out the comparison of mugwort cream masks. Measurement of the absorbance of cream mask samples was carried out with various concentrations (2, 4, 8, 16, and 32 ppm) and then measured at a wavelength of 517 nm.



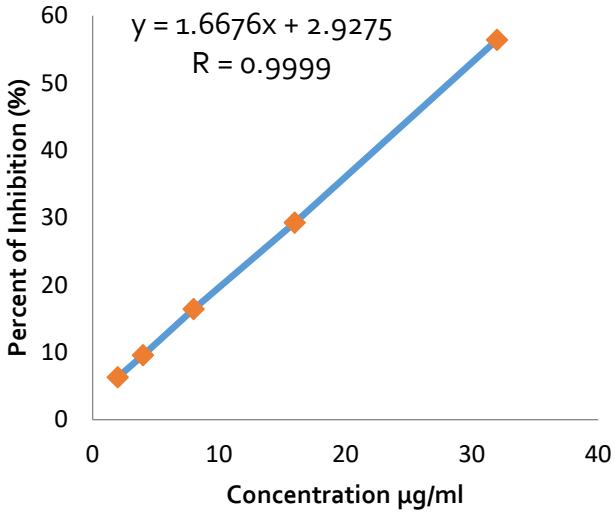
a. Linearity curve Fo



b. Linearity curve F1



c. Linearity curve F2



d. Linearity curve Positive control

Table 7. Antioxidant Test Results

Sample	IC_{50}
Formula 0	40.1959 µg/ml
Formula 1	25.663 µg/ml
Formula 2	21.959 µg/ml
Positive control	28.228 µg/ml

The results of the IC_{50} value of the antioxidant test for cream mask preparations from the ethanol extract of the jotang herb (*Acmella paniculata*) showed that Formula 0 cream masks had very strong antioxidants (40.1959 µg/ml) and Formula I had very strong antioxidant value (25.663 µg/ml). Formula II had antioxidants of (21.959 µg/ml) while the mugwort comparator mask had an antioxidant value of (28.228 µg/ml). Preparation of cream masks from the ethanol extract of jotang herbs was categorized into strong antioxidant activity. Formula 0 did not contain extract but had very strong antioxidant activity. It is due to the presence of a cream base that contains antioxidant activity, namely those found in stearic acid and glycerol.[4]

Stearic acid is one of the fats, namely nonessential fatty acids, resulting in a greater amount of oil phase in the preparation. The greater number of oil phases in the jotang herbal cream mask formulation (*Acmella paniculata*) causes the need for antioxidants to protect the stability of cream mask preparations against a greater oxidation,[19] as well as the presence of a glycerol base which affects activity of antioxidants. There is an OH group which is the mechanism of action of antioxidants in the propagation stage of the formation of free radicals (R) which are very reactive.[20] Another factor that caused Fo to have antioxidant activity is due to the pipetting process;[21] the tools used were less clean because Formula 0 had antioxidants, and should not have antioxidants. Meanwhile, formulas I and II contained jotang herb extract so, it has a very strong antioxidant value.[22]

Based on the results of the data analysis above, the statistical test was continued with the Post Hoc Test to identify honestly significant difference (HSD). It aims to identify the difference in the results of each substance concentration. The Table above shows that there are significant differences between the treatments of jotang herb ethanol extracts. The difference lied in FO, F1, F2, and Comparator mugwort mask (Skintific), that there was a significant difference because all formulas had a Sig value 0.00

<0.05. It incated there were differences in each cream mask formula from herbal ethanol extract jotang as an antioxidant. It stated that the provision of variations in the concentration of herbal extracts jotang affected the IC50 values obtained.[23]

CONCLUSION

The ethanol extract of jotang herb (*Acmella paniculata*) could be formulated in a mask preparation cream and had a good physical evaluation test with a homogeneous dosage form. Power spreaded with an average value of FO 5.1, FI 5.4 and F2 5.7, an average pH value of 6, a viscosity value of 6020-9990 cps, and the type of cream M/A. Formula 2 cream mask preparation had a higher antioxidant value of 21.959 µg/ml.

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