Trad. Med. J., September-December 2023
Vol. 28(3), p 164-170
ISSN-p : 1410-5918 ISSN-e : 2406-9086

Submitted : 14-09-2022
Revised : 01-09-2023
Accepted : 26-10-2023

Comparing Antioxidant Activity of Extracts and Gel Preparations Combination of Buas-buas leaves (*Premna serratifolia* l.) and Secang Wood (*Caesalpinia sappan*)

Albianus Febri Tarung, Bella Cristin, Yuninta Maulidia, Insan Kamil, Regitha Rianda Syalsabila, Isnindar*
Faculty of Medicine, Universitas Tanjungpura, Pontianak Indonesia

ABSTRACT

Indonesia is a tropical country that has exposure to ultraviolet rays from the sun throughout the year. This causes susceptibility to skin problems for the Indonesian population, including the vulnerability to aging of the skin due to exposure to ultraviolet rays for a long time. In the prevention of degenerative diseases and aging, antioxidants play an important role. Buas-buas and secang, which are plants from the West Kalimantan region, have very high antioxidant activity. Gel preparations are typically formulated as moisturizers with the aim of increasing water content in the stratum corneum, thus hydrating and maintaining skin pH. Hence, this study aimed to create a gel preparation combining buas-buas leaf extract and secang, effective as a moisturizing and anti-aging gel. The results of measuring the antioxidant activity of the combination of crude ethanol extracts of buas-buas leaves and secang wood with the results of the three formulas have very strong antioxidant activity, namely F1 at 0.0329 mg/ml, F2 at 0.0246 mg/ml, and F2 at 0.0246 mg/ml. F3 of 0.2282 mg/ml, and IC_{50} results for gel preparations for F1 3,8677 mg/ml, F2 4,3953 mg/ml, F3 4,396 mg/ml. The gel preparations produced in this study have the appearance and fulfill the evaluation requirements, except for the evaluation of spreadability.
Keywords: Antioxidants; DPPH; Extract; Moisturaizer; Anti-aging

INTRODUCTION

Indonesia is a tropical country that has exposure to ultraviolet rays from the sun throughout the year. This causes susceptibility to skin problems for the Indonesian population, including the vulnerability to aging of the skin due to prolonged exposure to ultraviolet light (Ahmad and Damayanti, 2018). One effort to produce the effects of premature aging is to use products that contain antioxidants. Antioxidants play an important role in preventing degenerative diseases and aging. The need for antioxidants from outside the body is very important because the amount of antioxidants produced in the body can only counteract free radicals produced by the body itself from metabolic processes (Rani, 2017).

An antioxidant is any substance that delays, prevents or eliminates oxidative damage to target molecules. Antioxidants are an important component in the body's defense system against ROS (Rani, 2017). Antioxidants can inhibit the oxidation process, even at relatively small concentrations, therefore antioxidants have various physiological roles inside and outside the body. Research proves that antioxidants such as ascorbic acid, polyphenols and active tocopherols can counteract oxidative stress to restore aging skin. These substances can enhance the endogenous ROS elimination system in mammalian tissues and help in the possible management of skin infections and aging (Liguori et al., 2018). Natural antioxidants can be obtained from plants which generally contain antioxidants in the form of phenolic compounds. Antioxidant constituents from plant materials can act as: radical scavengers, and help convert radicals into less reactive types (Dhurhania and Novianto, 2019).

Premna serratifolia L. (figure 1) are wild plants that contain phenolic and flavonoid chemicals that play a role in very active antioxidant activity (Isnindar and Luliana., 2022). Based on the results of research Puspita, et al. in 2020 showed that the ethanol extract of *Premna serratifolia L.* leaves had an IC_{50} value of 20.66 g/mL which indicated a very high antioxidant activity. High antioxidant activity in *Premna serratifolia L.* leaves is due to the presence of phenolic compounds, especially the high flavonoid group with total flavonoid levels of 4.67 mg/g and 0.47% w/w (Puspita et al., 2020). There are also secang (*Caesalpinia sappan*.) plants that contain high antioxidants (figure 2). *Caesalpinia sappan*. wood contains saponins, flavonoids, and alkaloids that act as antioxidants (Sucita, et al., 2019).

***Corresponding author : Isnindar**
Email : isnindar@pharm.untan.ac.id

DOI: 10.22146/mot.77740 | *Traditional Medicine Journal, 28(3), 2023*

Figure 1. Buas-buas leaf (*Premna serratifolia L.*)

Figure 2. Secang wood (*Caesalpinia sappan*.)

Utari's research using the DPPH method proved that the secang extract has an IC_{50} value of 15.69, which means that the antioxidant activity is very strong if it has an IC_{50} value of less than 50 ppm (Utari, 2017).

Gel is one of the topical preparations that is easily applied to the skin and has a good physical appearance (Iskandar, et al., 2021). Gels are preferred because they provide a cooling, soothing, moisturizing effect, and are easily absorbed into the skin (Ak, 2019; Rahmat and Wirawan, 2020). Ordinary gel preparations are formulated as moisturizers, moisturizers are generally used for both normal and dry skin. skin pH to remain normal (Mawazi et al., 2022). In addition, moisturizers with certain antioxidant content can provide anti-aging effects (Nurhadianty et al., 2021). Therefore, it is necessary to carry out a new development in making a formulation of cosmetic preparations that utilize natural ingredients, so as to avoid the use of cosmetics containing chemical compounds, one of which is by making a gel preparation of a combination of *Premna serratifolia L.* leaf etanol extract and *Caesalpinia sappan*. etanol extract which is efficacious as a moisturizing and anti-aging gel.

The 96% ethanol extract of wild-buas leaf (*Premna serratifolia L.*) and 96% ethanol extract of secang wood (*Caesalpinia sappan*) were the plant extracts used in this study. The extract was obtained from the maceration of buas-buas leaves and secang wood. Plants obtained in the Pontianak area in June 2022, and have gone through a process of determination at the Biology Laboratory, Faculty of Mathematics and Natural Sciences, Tanjungpura University, Pontianak. The tools used in this research are glassware, pH meter, UV/Vis spectrophotometric instrument, hotplate, and analytical balance.

METHODOLOGY

Gel formulation of crude extract combination

Buas-Buas leaf and secang wood extracts were formulated in a gel dosage form whose formula refers to the research by Puspita et al in 2020 (table I) with modifications to the amount of extract and preservatives. In each of these formulas, the number of extract combinations varied in the ratio 1:1, 2:1, and 1:2. Gelling begins with developing a gelling agent, carbopal 940 in 10 milliliters of water at a temperature of 70 degrees Celsius, then added with TEA. After that, a combination of extracts in various ratios are added to the base. After that, propylene glycol, penoxyethanol, and oleum rose were added. The mixture was then added with distilled water up to 50 grams and stirred homogeneously.

Table I. Gel formulation of crude extract combination

Ingredient	F1(%)	F2 (%)	F3 (%)
Buas-buas leaf ethanol extract	1	1	2
Secang wood ethanol extract	1	2	1
Carbopol 940	0,75	0,75	0,75
TEA	0,5	0,5	0,5
Propilen glikol	10	10	10
Penoxyetanol	1	1	1
Oleum rose	Qs	Qs	Qs
Aquadest ad	100	100	100

Qs : Quantum satis

Evaluation of gel

Evaluation of gel preparation includes organoleptic test, homogeneity test, adhesion test, spreadability test, pH test, cycle test, and stability test.

Organoleptic test

The organoleptic test was carried out by observing the shape, color, and smell of the sample.

Homogeneity Test

Homogeneity testing is done by applying gel to a glass object and then covering it with another glass object on top. The preparation is observed and if there are no coarse grains in the preparation, it means that the preparation is homogeneous.

Adhesion Test

0.25 gram gel preparation is placed in the middle area, and then another glass object is placed. Glass objects that have been attached to each other are installed in the test equipment, then given a load of 80 grams. The time taken for the two slides to separate is recorded. A good adhesion test is not less than 4 seconds.

Spreadability Test

0.5 grams of the sample is placed onto the glass slide, followed by placing another glass on top for one minute. Measure the spread diameter of the gel in centimeters before applying an additional weight of 150 grams. Leave the weight for one minute, and then measure the diameter when it becomes constant. A desirable dispersion is between 5-7 cm, indicating a semisolid consistency that provides an ultimate comfort level for usage.

pH test

The pH measurement was conducted with a pH meter. To do this, 1 gram of the preparation was weighed, added to 10 mL of distilled water, and stirred until evenly distributed. The pH was then determined with a pH meter and the resulting value was recorded.

Cycling test

The cycling experiment entailed refrigerating the sample at 4°C and heating it in an oven set to 40°C. The test was conducted six times, with each cycle comprising the duration spent at both temperatures. Throughout each cycle, the physical properties of the sample were monitored for changes such as phase separation, phase inversion, and crystal formation.

Stability test

The stability test was carried out for 28 days and observed on the 0, 1, 7, 14, 21 and 28 days. Physical changes in the gel preparation at the beginning and end of the cycle were observed in terms of color, texture and scent.

Measurement of the antioxidant activity

The efficacy of DPPH free radical scavengers was tested by creating a master solution of 2000 ppm, which was subsequently diluted to concentrations of 25 ppm, 50 ppm, 100 ppm, 250 ppm, 500 ppm, 1000 ppm, and 1500 ppm. For each concentration series, 1 ml of the solution was added into a vial, followed by 2 ml of the DPPH solution which had been prepared by dissolving 2.5 mg of DPPH in 50 ml of methanol. Abbreviations are explained upon first use. The solution was incubated for 30 minutes and

measured at a wavelength of 516.80 nm with a UV/VIS spectrophotometer. After obtaining the absorbance, the percentage inhibition of free radical scavenging activity is calculated as a percentage of the reduced color DPPH, using the formula:

$$\%\ Inhibition = \frac{control\ absorbance - test\ absorbance}{control\ absorbance} x100\%$$

The measurement results were then plotted on the x and y axes, then entered into a linear regression equation. The equation is used to determine the IC50 value of each sample (Runtuwene et al., 2019).

RESULT AND DISCUSSION

Measurement of Antioxidant Activity of Crude Extract Combinations

The antioxidant activity of a sample is based on the IC_{50} value. Antioxidant activity can be said to be very strong if it is below 50 g/ml, strong if the IC_{50} value is around 50-100 g/ml, and said to be weak if the IC_{50} value is 100-200 g/ml (Mardhiyani, and Islami, 2022). In the research of Puspita et al. In 2020 the antioxidant activity of crude ethanol extract of buas-buas leaves showed an IC_{50} value of 20.66 g/ml. In addition, the antioxidant activity value of sappan wood shows a very strong IC_{50} value of 15.69 g/ml (Utari, 2017). The antioxidant activity was treated in 3 variations of the dosage formula with their respective ratios (1:1, 1:2, 2:1).

The antioxidant activity of the crude extract combination with the IC_{50} parameter was proven by the concentration of 3 variations of the formula, it was stated that all three had very strong activity.

Gel Dosage Formulation

The formulation of the gel preparation used a combination of buas-buas leaf extract and sappan wood which had been previously measured. In the manufacture of gel preparations, it is necessary to have a gelling agent. Carbopol is a gelling agent which is generally used in cosmetic preparations. In addition to carbopol as a gelling agent, other ingredients used are TEA as a pH stabilizer, propylene glycol as a humectant, phenoxyethanol as a preservative, oleum rose as a flavoring and aquades as a solvent. The ingredients used in the treatment of 3 variations of the same concentrated formulation, which differed only in the concentration of crude leaf extract and sappan wood were used in a ratio of F1 (1:1), F2 (1:2), F3 (2:1).

Evaluation of Gel Preparation

Evaluation results of crude extract gel preparation combination buas-buas leaf and sappan wood can be seen in the following table.

The evaluation results show that from 3 variations of gel formulation formulas, the combination of crude extracts of buas-buas leaves and secang wood has an organoleptic test value with a thick gel texture with a different color in each formula, F1 is orange, F2 is dark orange, and F3 is yellowish green. This color difference is based on the difference in the ratio of extract formulations used, F3 uses a more dominant concentration of crude extract so that it is yellowish green. The pH test of the 3 variations of the formula shows a pH value that is safe for the skin, because the appropriate pH requirements for topical preparations on the skin are in the pH range of 4-6.5 (Thomas et al., 2023). Based on the measurement of spreadability, the three formulas have different values and each value has not met the test requirements of 5-7 cm, but all formulas have adhesion that meets the requirements of more than 1 second (Sayuti, 2018; Yusuf et al., 2017). It can be seen that the 3 variations of the formula have a homogeneous texture, no foam and no coarse grains (table II). The cycling test is carried out to see the stability of the preparation if it is used or positioned at 2 temperatures including a cold temperature of 4ºC for the first 24 hours, and a hot temperature of 40ºC for the next 24 hours. The cycling test was carried out for 6 consecutive days with 6 treatment cycles and showed no significant changes in the preparation starting from the shape, color and aroma (Sani et al., 2021). The results of the cycling test (table III) on the three formulas show that the preparation is stable at extreme temperatures. In line with these results, based on stability testing for 28 days, the three formulas have good stability (table IV).

Measurement of Antioxidant Activity in Gel

Test of antioxidant activity in gel preparations of a combination of crude extract buas-buas leaf and sappan wood using DPPH mother liquor. DPPH solution was prepared by dissolving 2.5 mg of DPPH powder (1,1-diphenyl-2-2picrylhydrazyl) in 50 ml of methanol which was then incubated in the dark for 30 minutes at 37ºC measured with a maximum wavelength of 516.80 nm. Incubation in a dark place aims to prevent the DPPH solution from being exposed to direct sunlight which will result in the DPPH solution

Table II. Antioxidant activity of the combination crude extracts

Antioxidant activity of the combination crude extract		
F1	**F2**	**F3**
0.0329 mg/ml	0.0246 mg/ml	0.2282 mg/ml

Table III. Gel preparation evaluation results

Test	Formula	Evaluation result	
Organoleptic	F1	Texture	Gel
		Color	Orange
		Smell	Typical Oleum rose
	F2	Texture	Gel
		Color	Light orange
		Smell	Typical Oleum rose
	F3	Texture	Gel
		Color	Yellowfish green
		Smell	Typical Oleum rose
pH test	F1	6.2	
	F2	5.6	
	F3	6.1	
Spreadability test	F1	Loads 1 gram	2 cm
	F2	Loads 2 gram	3 cm
	F1	Loads 5 gram	3,8 cm
	F2	Loads 10 gram	4 cm
		Loads 30 gram	6 cm
	F3	Loads 1 gram	2 cm
	F1	Loads 2 gram	2,3 cm
	F2	Loads 5 gram	2,4 cm
	F1	Loads 10 gram	3 cm
		Loads 30 gram	4 cm
	F2	Loads 1 gram	2 cm
		Loads 2 gram	2 cm
		Loads 5 gram	4 cm
		Loads 10 gram	4,3 cm
		Loads 30 gram	4,5 cm
Homogeneity test	F1	Homogeneous preparation no coarse granules	
	F2	Homogeneous preparation no coarse granules	
	F3	Homogeneous preparation no coarse granules	
Adhesion test	F1	2.54 seconds	
	F2	2.58 seconds	
	F3	3.44 seconds	

being easily oxidized and affecting the absorbance reading (Islami and Nasution, 2022). The results of the antioxidant activity of the gel preparation are shown in the following table.

The gel preparation's antioxidant activity is expressed through the parameter IC_{50} value. After measuring absorbance through UV/Vis spectrophotometry, the subsequent step is to calculate the percent inhibition of free radical scavenging activity as a reduction in DPPH color, using the formula:

$$\%\ Inhibition = \frac{control\ absorbance - test\ absorbance}{control\ absorbance} x100\%$$

The concentration of the sample and the inhibition percentage were plotted on the x and y axes, respectively, using the linear regression equation. The equation is utilized for calculating the IC_{50} value for each sample, with a y value of 50 and an x value that corresponds to IC_{50} (Runtuwene et al., 2019). Table II shows the value of antioxidant activity in crude extract

Table IV. Measurement results of cycling test

Formula	Parameter	Before test	After test
F1	**Texture**	Gel	Gel
	Color	Orange	Orange
	Smell	Typical Oleum rose	Typical Oleum rose
F2	**Texture**	Gel	Gel
	Color	Light orange	Light orange
	Smell	Typical Oleum rose	Typical Oleum rose
F3	**Texture**	Gel	Gel
	Color	Yellowfish green	Yellowfish green
	Smell	Typical Oleum rose	Typical Oleum rose

Table V. Measurement results of stability test

Formula	Parameter	Day				
		1	**7**	**14**	**21**	**28**
F1	**Texture**	Gel	Gel	Gel	Gel	Gel
	Color	Orange	Orange	Orange	Orange	Orange
	Smell	Typical Oleum rose	Typical Oleum rose	Typical Oleum rose	Typical Oleum rose	Typical Oleum rose
F2	**Texture**	Gel	Gel	Gel	Gel	Gel
	Color	Light orange	Light orange	Light orange	Light orange	Light orange
	Smell	Typical Oleum rose	Typical Oleum rose	Typical Oleum rose	Typical Oleum rose	Typical Oleum rose
F3	**Texture**	Gel	Gel	Gel	Gel	Gel
	Color	Yellowfish green	Yellowfish green	Yellowfish green	Yellowfish green	Yellowfish green
	Smell	Typical Oleum rose	Typical Oleum rose	Typical Oleum rose	Typical Oleum rose	Typical Oleum rose

Table VI. Antioxidant activity of gel combination

Antioxidant activity of gel combination of buas-buas and secang		
F1	F2	F3
3.8677 mg/ml	4.3953 mg/ml	4,396 mg/ml

preparations that have been formulated in gel preparations with antioxidant activity values using only a combination of crude extract in table I shows a potential value, this is also due to the influence of the excipients used in determining a value of the antioxidant activity of the preparation.

CONCLUSION

According this investigation, it can be concluded that crude extracts have high antioxidant activity and gel preparations. The comparison study of the extract and gel combination of buas-buas leaves and secang wood is that IC50 results in crude extract preparations are F1 0.0329 mg/ml, F2 0.0246 mg/ml, F3 0.2282 mg/ml, and IC50 results for gel preparations at F1 3,8677 mg/ml, F2 4,3953 mg/ml, F3 4,396 mg/ml.

REFERENCES

Ahmad, Z. & Damayanti. 2018, 'Skin Aging: Pathophysiology And Clinical Manifestation', Periodical Of Dermatology And Venereology. 30 (3) : 208-215.

Ak, M, 2019, 'Skin Care Creams: Formulation And Use', OSP Journal Of Clinical Trials. 1(1):1-22.

Dhurhania, C.E. & Novianto, A, 2019, 'Uji Kandungan Fenolik Total Dan Pengaruhnya

Terhadap Aktivitas Antioksidan Dari Berbagai Bentuk Sediaan Sarang Semut (Myrmecodia Pendens)', Jurnal Farmasi Dan Ilmu Kefarmasian Indonesia. 5(2) : 62-68.

Iskandar, B., Dian, Z.P. & Renovita, F., 2021, 'Formulasi Dan Evaluasi Gel Lidah Buaya (Aloe Vera Linn) Sebagai Pelembab Kulit Dengan Penggunaan Carbopol Sebagai Gelling Agent', Health Science and Pharmacy Journal. 5(1):1-8.

Islami N & Nasution P., 2022, 'Uji aktivitas antioksidan ekstrak etanol pada buah kurma safawi (Phoenix dactylifera L.) dengan metode DPPH', Jurnal Farmasi, Sains dan Kesehatan. 1(2) : 149-152

Isnindar & Luliana S., 2022, 'Aktivitas Antioksidan Kombinasi Seduhan Bebuas (Premna Serratifolia Linn.), Meniran (Phyllanthus Niruri L.), Secang (Caesalpinia Sappan Linn.) Dan Rosela (Hibiscus Sabdarifa) Dengan Metode Dpph (2,2-Difenil-1-Pikrilhidrazil)', Media Farmasi;18(1):25-29.

Liguori, I. et al., 2018, 'Oxidative Stress, Aging, And Diseases', Clinical Interventions In Aging. 13 : 757–772.

Mardhiyani D., & Islami D., 2022, 'Foemulasi masker peel-off ekstrak daun bidara (*Ziziphus Spinachristi* L.) dan biji Chia (*Salvia Hispanica*) sebagai antioksidan', Jurnal Farmasi Tinctura. 3(2): 48.

Mawazi, S.M. et al., 2022, 'A Review Of Moisturizers; History, Preparation, Characterization And Applications', Cosmetics. 9(3); 1-19.

Nurhadianty et al., 2021, 'Formulasi Krim Anti-Aging Pada Kulit Daerah Tropis Berbasis Ekstrak Daun Kelor, Minyak Kenanga Dan Minyak Lemon Sebagai Bioaktif', Journal Of Innovation And Applied Technology. 7(1):1166–1172.

Puspita, W., Puspasari, H., & Restanti, N.A., 2020, 'Formulation And Physical Properties Test Of Spray Gel From Ethanol Extract Of Buas Buas Leaf (*Premna Serratifolia L.*)', Jurnal Ilmiah Farmako Bahari. 11(2) :145-152.

Puspita, W., Yuspita Sari, & D., Ristia Rahman, I., 2020, 'Uji Aktivitas Antioksidan Ekstrak Etanol Daun Buas-buas (Premna Serratifolia L.) Asal Kabupaten Melawi Provinsi Kalimantan Barat Dengan Metode Dpph', J. Insan Farm. Indones. 3, 405–412.

Rahmat, D. & Wirawan, D., 2020, 'Formulasi Gel Nanopartikel Ekstrak Temulawak (Curcuma Xantohrriza Roxb.) Berbasis Kitosan Na-Tripolifosfat Sebagai Antiacne', Majalah Farmasetika. 4(1) :107-112.

Rani, K., 2017, 'Role Of Antioxidants In Prevention Of Diseases', Journal Of Applied Biotechnology & Bioengineering. 4(1) :495-496.

Runtuwene, K.N., Yamlean, P.V.Y., & Yudistira, A., 2019, 'Formulasi,Uji Stabilitas Dan Uji Efektivitas Antioksidan Sediaan Gel Dari Ekstrak Etanol Daun Sesewanua (*Clerodendron Squamatum Vahl*) Dengan Menggunakan Metode DPPH', Pharmacon. 8(2):298-305.

Sani, L.M.M., Subaidah, W.A., & Andayani, Y., 2021, 'Formulasi Dan Evaluasi Karakter Fisik Sediaan Gel Ekstrak Etanol Daun Salam (Syzygium Polyanthum)', Sasambo J. Pharm. 2(1):16-22.

Sayuti, Nutrisia A, 2015, 'Formulasi Dan Uji Stabilitas Fisik Sediaan Gel Ekstrak Daun Ketepeng Cina (Cassia Alaata L.)', Jurnal Kefarmasian Indonesia. 5(2): 74-82.

Thomas, N.A, Tungadi, R., Latif, M.S, Sukmawati, M.E, 2023, 'Pengaruh Konsentrasi Carbopol 940 Sebagai Gelling Agent Terhadap Stabilitas Fisik Sediaan Gel Lidah Buaya (*Aloe Vera*)', Indonesian Journal of Pharmaceutical Education. 3 (2): 316 – 324.

Utari, F.D., 2017, 'Produksi Antioksidan Dari Ekstrak Kayu Secang (*Caesalpinia Sappan L.*) Menggunakan Pengering Berkelembaban Rendah', J. Apl. Teknol. Pangan. 6.

Yusuf, A.L., Nurawaliah, E., & Harun, N., 2017, 'Uji Efektivitas Gel Ekstrak Etanol Daun Kelor (Moringa oleifera L.) sebagai Antijamur Malassezia furfur', Kartika: Jurnal Ilmiah Farmasi. 5 (2):62-67.