JURNAL PHARMACOPOEIA
PHARMACEUTICAL SCIENCES & RESEARCH

ANTIOXIDANT ACTIVITY OF BODY LOTION FORMULA CONTAINING ROSELLA PETAL EXTRACT (*Hibiscus sabdariffa*) AND CALAMANSI ORANGE PEEL ESSENTIAL OIL (*Citrofortunella microcarpa*)

Suci Rahmawati*, Yendha Dwi Rolitta, Oky Hermansyah, Rose Intan Perma Sari, Dwi Kurnia Putri, Tri Danang Kurniawan
Diploma Program of Pharmacy, Faculty of Mathematic and Natural Science, The University of Bengkulu
*Corresponding author's email: srahmawati@unib.ac.id
DOI: 10.33088/jp.v5i1.1262

ABSTRACT
Rosella petals contain anthocyanins, and calamansi orange peel essential oil contains limonene, both of which are antioxidant compounds. Antioxidants capture free radicals, allowing them to serve as active ingredients in preventing premature aging in skincare products such as body lotions. This study was aimed to formulate and test of the antioxidant level from body lotion formula containing roselle petal extract (*Hibiscus sabdariffa* L.) and calamansi orange peel essential oil (Citrofortunella microcarpa). Roselle petal extract was extracted by maceration method using 96% ethanol and calamansi essential oil was obtained by distillation using aquades. Body lotion formula was designed in three formulas (F1, F2, and F3) with differing concentrations of roselle extract and calamansi essential oil, respectively: F1 (1% and 3%), F2 (2% and 5%), and F3 (4% and 7%). The formulations were evaluated their physical properties (organoleptic characteristics, homogeneity, pH, spread ability, viscosity, and stability) and also antioxidant activity. The evaluation results were indicated that all formulations met physical standards but were unstable under heat. F1, F2, and F3 were semi-solid, homogeneous, pink in color, and had a distinctive calamansi aroma. The antioxidant evaluation yielded IC_{50} values of 89.9 ppm, 81.7 ppm, and 69.7 ppm for F1, F2, and F3, respectively. In this study can be concluded that F3 formula had the highest antioxidant activity. This study was demonstrated that increasing the concentration both of roselle petal extract and essential oil of calamansi enhanced the antioxidant degree of body lotion formula.

Keywords: antioxidant, calamansi, essential-oil, lotion, Roselle-petals

INTRODUCTION

Free radicals are unstable molecules that have unpaired electrons in their outer orbit. They stabilize when they acquire electrons from other molecules to form bonds (Evrilia et al., 2014). Free radicals can impact skin's appearance by harming collagen and elastin, vital elements for skin's firmness and elasticity. This damage can lead to premature aging, the appearance of wrinkles, and dull, lackluster skin. Several treatments exist to help restore skin's appearance, including the use of antioxidants (Yusharyahya, 2021).

Antioxidants are compounds that can donate electrons to oxidants, inhibiting their activity. Antioxidants are essential for preventing degenerative diseases and ageing. Factors like sun exposure, smoking, and pollution generate free radicals, which can hasten aging. Antioxidants help to counteract these free radicals, thereby safeguarding cells from harm and contributing significantly to skin well-being (Afandi et al., 2021).

Roselle (*Hibiscus sabdariffa* L.) is a member of the Malvaceae family that grows abundantly in tropical regions such as Java and Kalimantan. Roselle flower petal contains anthocyanins, which act as natural antioxidants and can ward off free radicals. The bioactive compounds in roselle petals, such as anthocyanins, flavonoids, polyphenols, and ascorbic acid, help protect cells from the negative effects of free radicals, prevent premature aging, and maintain healthy skin (Mumpuni et al., 2021). Roselle petals contain anthocyanins, which have very strong antioxidant activity of 15,236 ppm (Adrianto, 2019).

The Calamansi orange (*Citrus microcarpa*) is a citrus fruit cultivated in Bengkulu Province. It is also known as calamondin, calamandarain, and kastuari lime. Calamansi oranges are used as a raw material for making syrup and other soft drinks (Rosalina et al., 2017). Calamansi orange peel has been studied for its antioxidant properties, with an IC50 value of 50.31 ppm (Septiani et al., 2024). Calamansi orange peel contains active compounds such as limonene, which can function as an antioxidant (Anggraini et al., 2021).

The combination of these two ingredients is expected to have synergistic antioxidant activity that can be applied as a body lotion to ward off free radicals. Body lotion is a type of cosmetic preparation belonging to the emollient (softener) group that contains a high water content (Mohiudin, 2019). The advantages of lotion include its ability to be used on both hands and body, its ease of application, its high spreadability and penetration, its non-greasy feel, its cooling effect, its ease of washing off with water, and its ability to moisturize the skin (Iskandar et al., 2021).

RESEARCH METHOD

Material and equipment

Rosella flower petals and Calamansi orange peel were collected in Bengkulu Tengah District, Bengkulu Province. The tools used include analytical scales, *rotary evaporator*, distillation, Separating funnel, *water bath, Brookfield viscometer (NDJ* ®), filter paper (Whatman®), 60 mesh sieve, slide, evaporating dish, pH meter. The materials used are rosella flower petals, calamansi orange peel, 96% ethanol (Jkcare®), glyceryl monostearate (Alfa®), cera alba (Making Cosmetics®), Polysorbate 80 (Micromaster®), glycerin (Palapa Muda Perkasa®), liquid paraffin (Asianoil®), benzyl alcohol (Nguyen BA®), distilled water (Bucofindo®).

Roselle Flower Petal Extraction

Rosella flower petals underwent extraction through the maceration technique utilizing a solvent of 96% ethanol at a proportion of 1:5. For this process,100 grams of the rosella flower petal material were combined with 500 milliliters of ethanol in a brown bottle and then a second maceration with the same proportions. The outcome of the maceration was subjected to filtration using filter paper to differentiate between the liquid extract and the solid remains. The remaining solids were concentrated using a rotary evaporator (Adrianto, 2019).

Calamansi Orange Peel Essential Oil Distillation

Distillation was used to extract the esse ntial oil from calamansi orange peel. A total of 8 kg of Calamansi orange peel was chopped and distilled with 8 L of distilled water in a steam distillation apparatus at 100ºC for 4 hours. The resulting mixture of water and essential oil was separated using a separating funnel (Septiani *et al*., 2024).

Preparation of Body Lotion

Table 1. Design of Body Lotion Formula Combining Rosella Flower Petal Extract and Calamansi Orange Peel Essential Oil.

Material	Ingredients Content in Formula (%)			Utility
	F1	F2	F3	
Calamansi Essential Oil	1	2	4	Active Ingredients
Roselle Flower Petal Extract	3	5	7	Active Ingredients
Glyceryl Monostearate	5.5	5.5	5.5	Emulsifier
Cera Alba	2.7	2.7	2.7	Stabilizer
Polysorbate 80	3.5	3.5	3.5	Surfactant
Glycerin	10	10	10	Humectant
Liquid Paraffin	10	10	10	Lubricant
Benzyl Alcohol	0.15	0.15	0.15	Preservative
Aquadest	Ad 100	Ad 100	Ad 100	Solvent

Oil phase ingredients such as glyceryl monostearate, cera alba, Polysorbate 80, Liquid paraffin and essential oils were melted at 75 ˚C in an evaporator dish. The aqueous

phase ingredients such as glycerin, benzyl alcohol, rosella petal extract, and distilled water were dissolved separately in a beaker glass. The oil and aqueous phases were mixed in a beaker glass at 50°C and homogenized using a magnetic stirrer until a homogeneous semi-solid mass was formed in each of Formulas 1, 2, and 3 (F1, F2, and F3). Each formula followed the formula design as shown in Table 1.

Physical Assessment of Body Lotio

Organoleptic evaluation, homogen eity, pH, spreadability, viscosity, and sta bility are all part of the physical assessm ent of body lotion, and each test is cond ucted three times.

Organoleptic Examination

The color, shape, and scent of the body lotion preparation were observed using organoleptic observations employing the five senses (Aisyah et.al., 2021).

Homogeneity Test

A glass slide is covered with 0.1 gram sample for the homogeneity test. Next, any inhomogeneity or the makeup of the coarse particles are noted. When a preparation is uniformlu combined with the lotion basis, it is said to be homogenous. No solid material may be felt on the glass when the active ingredient and lotion mass exhibit homogenous composition (Aisyah et.al., 2021).

Evaluation of Body Lotion pH

The pH was measured using a pH meter. The pH meter was calibrated with buffer solution pH 4 and pH 10. The electrode is then washed with distilled water and dried with tissue paper. The pH meters electrodes were dumped into an evaluation-prepared solution diluted with1 gram of lotionand10 mL of distilled water, and the indicators were observed. The safe pH value required for topical preparations is4.5–6.5 (Aisyah,2021).

Spreadability Test

Spreadability of the lotion is measured by taking 0.5 grams of the lotion and placing it in the center of a 15 cm diameter petri disk. It placed a glass slide on top and stayed on for 1 minute. Subsequently, the diameter of the spread cream was measured, 50, 100, 150 and 200 grams were added to the slides, left for 1 minute, and then the spread diameter was recorded. The spread ability requirement for topical preparations is 5-7 cm (Aisyahand al.,2021).

Viscosity

A Brookfield viscometer with spindle number 7 was used to measure viscosity. One hundred grams of the sample were used to dip the spindle. The viscosity value was recorded once the instrument's reading achieved a steady state, and testing was carried out at a rotation speed of 20 rpm. Topical treatments have a viscosity value between 2,000 and 50,000 cps (Aisyah et al., 2021).

Stability

This test was conducted using the accelerated stability test method, which involved storing the lotion preparation at 4°C in the refrigerator for the first 1×24 hours, then removing it and storing it at 40°C in the oven for the second 1×24 hours. This treatment was done in one cycle, which was repeated six times over a 12-day period. The lotion preparation's physical characteristics, such as organoleptic, homogeneity, pH, spreadability, and viscosity, were then found to change with each cycle (Aisyah et al., 2021).

Antioxidant Activity Test of Body Lotion Formula

The DPPH (1,1-diphenyl-2-picrylhydrazyl) technique was used to calculate the combination extract's antioxidant value. A 125 µm DPPH stock solution, a sample solution, and a vitamin C solution were prepared before the antioxidant value test started. A UV-Vis spectrophotometer was then used to measure the sample solution and vitamin C at a maximum wavelength of 400–800 nm (Kholifah et al., 2024).

The absorbance of a standard vitamin C solution was measured in order to determine linear regression. Ten milligrams of vitamin C were weighed and dissolved in one hundred milliliters of distilled water to create a stock solution of 100 parts per million. solutions containing 2, 4, 6, 8, and 10 parts per million of vitamin C. 0.1 mM DPPH solution was added to 1 mL of each solution. A UV-Vis spectrophotometer was then used to measure the absorbance at the maximum wavelength after the solution had been incubated at room temperature (Kholifah et al., 2024).

The calculation of the IC50 completed the antioxidant activity testing of F1, F2 and F3. The various 100 ml volume tanks were filled with F1, F2 and F3 formulas. The extract was then mixed with 100 ml ethanol to obtain a concentration of 1 000 mg per ml. From the 1000 mg per ml stock solution, smaller quantities of 20, 40, 60, 80 and 100 mg per ml were prepared. Two milliliters of each concentration of the sample were then pipetted and mixed with two milliliters of the 40 ppm DPPH solution. After homogenisation the mixture was incubated in a dark room for half an hour. UV-Vis spectrophotometry was used to measure the maximum absorbance after incubation at the maximum wavelength. The following formula has been used to obtain the percentage inhibition:

% inhibition= ((Control Absorbance – Sample Absorbance): Control Absorbance) x 100%

The linear regression equation derived from the relationship between concentration and percentage inhibition, which expresses t he relationship between concentration (x) and percentage inhibition (y), is used to calculate the IC_{20} value (Kholifah et al. (2024).

RESULTS AND DISCUSSION

The material plants used in this study were verification in the Laboratory Biology Department Faculty Mathematics and Natural Science. The results verification based on document with number letter 66/UN30.12/BIO/TA.00.03/2025 stated plants used own family: Malvaceae, and species: *Hibiscus sabdariffa* L for Roselle sample in this. Family: Rutaceae and species : *Citrofortunella macrocarpa* Bunge for Calamansi orange sample. Plant verifications was done for determine authenticity of plants in this study.

The sample in this study was used the petals of rosella flowers and peel of calamnsi fruit orange were extracted with different method. The yield of the extract can be seen on Table 2.

Table 2. Yield extract petals rosella flowers and oil essential oils Calamansi

Sample	Yield (%)	Standard
Petals Rosella Flower Extract	20.33	≥ 19.1% (Ministry of Health . 2017)
Calamansi Peel Orange Essential Oil	1.29	-

Yield extracts were obtained as number 20.33%, based on Kemenkes RI (2017) yield extract rosella flowers was reach the standard (≥19.1%). Meanwhile, the yield of essential oils was produced in 1.73%, the yield more small from Saputri *et al ,.* (2022) study, namely 3.20%. That can caused by number of factors, such as condition beginning material, difference method or duration distillation, amount and ratio solvent was used.

Figure 1. The Results of Body Lotion Formula F1, F2, and F3

Extract and essential oil were obtained formulated in accordance with formula design on Table 1 and evaluated their physique characteristics and antioxidant activity. The results of physique evaluation and antioxidant activity from 3 formulas can be seen on Table 3.

Table 3. Results evaluation of body lotion containing combination petal rosella extract and calamansi essential oils.

Evaluation	Observation		
	F1	F2	F3
Organoleptic Smell Color	Semi-solid Calamansi aroma Pink	Semi-solid Calamansi aroma Pink	Semi-solid Calamansi aroma Hard pink
Homogeneity test	F1, F2, F3 Homogeneous		
pH test	6.10 ± 0.01	6.06 ± 0.00	6.00 ± 0.00
Spread ability	5.44 – 6.44cm	5.59 – 6.62cm	5.93 – 6.82cm
Viscosity test	12333.3cP	11000cP	10333.3cP
Stability test	Unstable	Unstable	Unstable
Antioxidant activity (IC_{50} Value)	89.9 ppm	81.7 ppm	69.7 ppm

Body lotions were formulated with comparison concentration substance active extract petals rosella flowers and Calamansi essential oil each of them is F1 (1 % : 3%), F2 (2% : 5%), and F3 (4% : 7%). The difference concentration substance active was aimed to know the best formula of body *lotion* that provides high antioxidants activity.

Results evaluation seen that all formulas meet standard physical, but unstable in hight temperature. F1, F2 and F3 is semi- solid homogeneous, the colored were pink with a distinctive calamansi aroma, as well as homogeneous. The organoleptic is step beginning in evaluation stock topical purpose for evaluate physical characteristics such as color, smell and consistency through visual observation and smell. F1 and F2 with the soft pink color and F3 were showed in dark pink color. All formulas had distinctive calamansi aroma, which were showed that essential oils still stable in all formula. Difference color between the formulas were affected by variation of roselle extract concentration in formula, more concentration were produced dark color (Hamsinah *et al,.* 2023).

The preadibility test was evaluated to show the ability of the lotion to spread on the surface skin, which is an important parameter to determine comfortable and effectiveness when the formula will use on to skin (Latifah *et al.,* 2023). Results were showed that all formulas (F1, F2 and F3) have spread ability that was in appropriate range of 5.1-6.82 cm with the standard namely between 5–7 cm. However, there are trend that improvement concentration essential oils in formula.

The viscosity test were determine to measure the level viscosity availability, comfort in applicate, and stability during storage. Based on Indonesian National Standard/SNI (2016), viscosity is considered in accordance for topical product the range between 2,000 - 50,000 cP. High viscosity value can make the lotion difficult to applied in the skin.

pH test was performed with objective for ensure that formulated lotion preparation is at on appropriate pH range with physiological pH skin. The ideal pH value for product maintenance skin generally is at in range 4.5 up to 6.5, adjust with natural pH skin (Latifah *et al.,* 2023). This very important for ensure comfort moment used as well as for prevent risk occurrence irritation or disturbance skin other consequence mismatch pH. Based on results pH research on F1, F2, and F3 showed

that the pH ranged from between 6.0-6.56. Which Still is at in range that meets mark standard. Will remain on test stability use cycling test method shows stock No stable during storage (14 days) with change. The stability test was shows there is physical changes on lotion fomula due to influence temperature and time, High temperatures in product storage can affect the stability of active ingredients or excipients in storage (Malik *et al.,* 2020).

The DPPH solution was used as a free radical in an antioxidant activity test using UV-Vis spectrophotometry. method, which will interact like an antioxidant compound and change into the non-radical compound 1,1-diphenyl-2-picrylhydrazine (Molyneux, 2004).

In this study, Vitamin C was used as standard solution in determination equality regression with IC_{50} value 5.15 ppm (Table 4) which measurement with maximum wavelength in 517 nm. Antioxidant activities that are carried out on maximum wavelength in 517 nm where obtained IC_{50} value respectively of F1, F2, F3 formulas are 89.9 ppm, 81.7 ppm, and 69.7 ppm. IC50 value show mark antioxidants with category strong (50-100 ppm). In this result than can be see high concentration of extract can give antioxidant value increasingly approach mark very strong (< 50 ppm) (Molyneux, 2004).

Table 4. Calculation of IC_{50} Vitamin C

Concentration (ppm)	Absorbent	% Inhibition	IC_{50} (ppm)	Concentration	% Inhibition
2	0.222	0		0	0
4	0.178	20		2	20
6	0.140	37	5.15	4	37
8	0.089	60		6	60
10	0.052	77		8	77

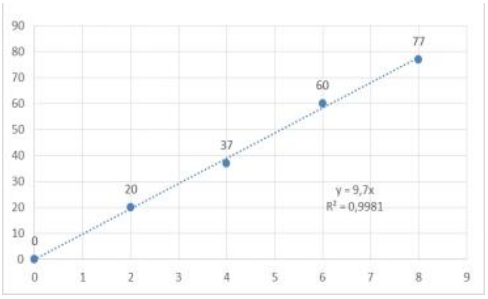

Figure 2. The Chart of Concentration vs percent inhibition in Vitamin C Standard Solution

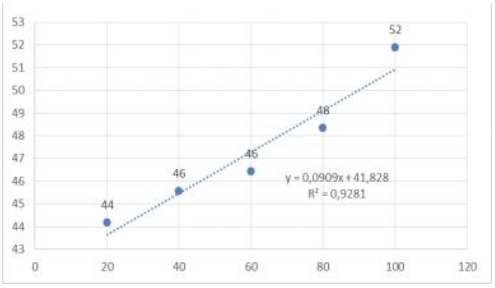

Figure 3. The Chart of Concentration vs percent inhibition in F1

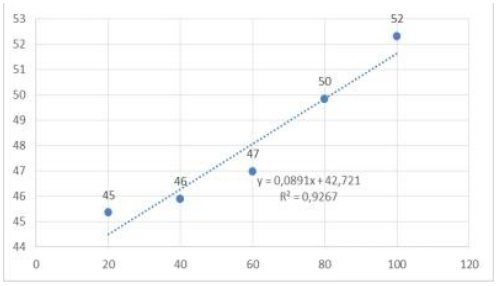

Figure 4. The Chart of Concentration vs percent inhibition in F2

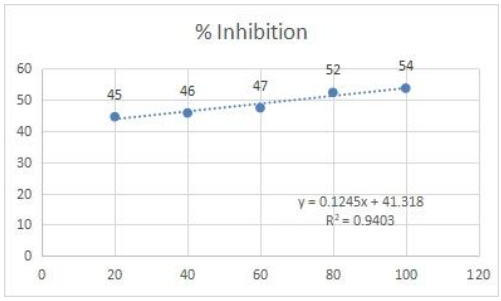

Figure 5. The Chart of Concentration vs percent inhibition in F3

CONCLUTION

According to the study's findings, a body lotion preparation with physical qualities that satisfied the requirements could be created by combining rosella flower petal extract with calamansi orange peel essential oil at different concentrations of F1 (1 % and 3%), F2 (2% and 5%), and F3 (4% and 7%). The body preparation's F3 had the highest antioxidant activity, according to the test results.

ACKNOWLEDGEMENTS

This research was funded by LPPM of Bengkulu University under the 2025 fundamental grant scheme.

REFERENCES

Adrianto. A. (2019). Skrining Fitokimia Metabolit Sekunder Ekstrak Bunga Rosella Dengan Perbandingan Pelarut Etanol 96% Dan 70% Serta Uji Aktivitas Skrining Fitokimia Metabolit Sekunder Ekstrak Bunga Rosella Dengan Perbandingan Pelarut Etanol 96% Dan 70% Serta Uji Aktivitas Antioksidan Menggunakan Metode Dpph (1.1-Diphenyl-2 Picrylhydrazyl). *Skripsi*. Universitas Ngudi Waluyo

Afandi. M. R. Z.. Iswandi. I.. & Safitri. C. I. N. H. (2021). Formulasi Dan Stabilitas Mutu Fisik Ekstrak Temu Ireng (*Curcuma Aeruginosa* Roxb.) Sebagai Body Butter. *Prosiding Snpbs (Seminar Nasional Pendidikan Biologi Dan Saintek)*. 359–365.

Aisyah. N.. Naya. L.. & Mardiyanti. S. (2021). Uji Stabilitas Krim Ekstrak Daun Kemangi (*Ocimum Americanum* L.) Dan Uji Antibakteri Terhadap Propionibacterium Acnes Penyebab Jerawat. *Journal Of Pharmacy. Medical And Health Science*. *2*. Https://Journal.Unsika.Ac.Id/

Anggraini. D. K.. Irnameria. D.. Khasanah. H. R. . M. Z.. & Jatiningsih. S. (2021). Uji Aktivitas Antibakteri Ekstrak Etanol Kulit Jeruk Calamansi (Citrus Microcarpa) Terhadap Bakteri (Propionibacterium Acnes). *Skripsi.*

Dewan Standardisasi Nasional 2016. *Standar Nasional Indonesia 16 - 4399 - 1996*.

Evrilia Sr. Hana N. & Sri Y. (2014). Pemanfaatan Limbah Kulit Buah Manggis (*Garcinia Mangostana* L.) Dalam Sediaan *Masker Peel Off* Sebagai Antioksidan. . *Bimfi*. *2*(2). 94–100.

Hamsinah. Vina Purnamasari M. Amelia Meylinda. dan Aminah. (2023). Formulasi Sediaan Lotion Ekstrak Daun Tanaman Porang (*Amorphophallus muelleri Blume*) Yang Stabil Secara Farmaseutik. *Pharmacoscript 6(2)*

Iskandar. B.. & Eni Br Sidabutar. S. (2021). Formulasi Dan Evaluasi Lotion Ekstrak Alpukat (*Persea Americana*) Sebagai Pelembab Kulit. *Journal Islamic Pharm* (Vol. 6. Issue 1).

Kemenkes RI. (2017). *Farmakope Herbal Indonesia Edisi II.* Kementerian Kesehatan Republik Indonesia

Kholifah, E., Fitriani, V., & Shobah, A. N. (2024). Formulation and antioxidant activity analysis of Jotang Herb (Acmella paniculata) extract mask cream. *Journal of Fundamental and Applied Pharmaceutical Science*, *4*(2), 70–80. https://doi.org/10.18196/jfaps.v4i2.19341

Latifah F., Sugihartini N., and Yuwono T. (2023). Evaluation Of Physical Properties And Irritation Index Of Lotion Containing *Syzigium Aromaticum* Clove Essential Oil At Various Concentration. *Traditional Medicine Journal* 21(1)

Malik, F., Suryani., S. Ihsan., E. Meilany, dan R. Hamsidi. (2020). Formulation Of Cream Body Scrub From Ethanol Extract Of Cassava Leaves (Manihot esculenta) As Antioxidant. *Journal of Vocational Health Studies.* 04: 21- 28.

Mohiudin. A. (2019). Skin Care : Formulation And Use. *American Journal Of Dermatological Research And Reviews*. *2*(8).

Molyneux, P. (2004). The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. In *Songklanakarin J. sci. technol*. academia.edu. https://www.academia.edu/download/39013361/Molineux_07-DPPH.pdf

Mumpuni. N. C.. Triwahyuni. I. E.. & Lestari. P. E. (2021). Efektivitas Ekstrak Bunga Rosella (*Hibiscus sabdariffa* L.) Sistemik Terhadap Penyembuhan Ulser Pada Tikus Wistar (*Rattus norvegicus*). *Stomatognatic – Jurnal Kedokteran Gigi.* . *18*(2). 56.

Rosalina. Y.. Susanti. L.. & Karo. N. B. (2017). Kajian Ekstraksi Pektin Dari Limbah Jeruk Rimau Gerga Lebong (Jeruk Rgl) Dan Jeruk Calamansi. *Agrointek*. *11*(2). 68. Https://Doi.Org/10.21107/Agrointek.V11i2.3174

Saputri. Yeni and Fitriani. Dyah dan Eni. Widiyati. (2022). Karakteristik Fisikokimia Dan Komponen Minyak Atsiri Kulit Jeruk Calamansi (*Citrus microcarpa*) Asal Padang Serai Kampung Melayu Kota Bengkulu. *Undergraduated thesis*. Universitas Bengkulu.

Septiani. N.. Maryanti. E.. Hermansyah. O.. & Putri. M. W. J. (2024). Uji AktivitasAntioksidan Minyak Atsiri Kulit Buah Jeruk Calamansi (*Citrus Microcarpa* Bunge) Menggunakan Spektrofotometri Uv-Vis. *Jurnal Ilmiah Pharmacy*. *11*(1).