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Antioxidant Evaluation of Facial Toner Formulations Containing Ethyl Acetate Fraction from Garcinia Mangostana L. Fruit using ABTS 2,2'-Azinobis 3-ethyl benzothiazoline 6-sulphonic Acid Method

[1]Sriyanty Sadsyam*, [1]Nielma Auliah, [1]Wa Ode Wisna Anto Uko, [1]Nasrawati Basir, [1]Andi Ulfiana Utari
Corresponding Author: *Sriyantisadsyam@unimerz.ac.id
[1] Megarezky University, Makassar, Indonesia

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ABSTRACT

The mangosteen fruit is rich in polyphenols, particularly xanthones, which have potent antioxidant effects. Given the various effects of facial toners and the growing demand for natural skincare products, mangosteen rind may serve as an alternative ingredient in facial toner formulations. This laboratory experimental study aimed to assess the antioxidant activity of facial toner preparations containing the ethyl acetate fraction of mangosteen rind (Garcinia mangostana L.) using the ABTS method at different concentrations (0.2%, 0.4%, and 0.6%). Four facial toner formulas were formulated, with Formula 1 being the control formula without any active substance and Formulas 2-4 containing the ethyl acetate fraction of mangosteen rind at different concentrations. The prepared formulas underwent physical and chemical stability tests, including organoleptic, homogeneity, pH, viscosity, and humidity tests, before and after six cycles of cycling. The antioxidant activity of the formulas was also evaluated using the ABTS method. The results showed no significant difference ($p>0.05$) in the organoleptic, viscosity, pH, and humidity tests before and after cycling. Formula 3 showed significant ($p<0.05$) antioxidant activity compared to the other formulas. Therefore, it can be concluded that the ethyl acetate fraction of mangosteen rind (Garcinia mangostana L.) can be formulated into a facial toner with potent antioxidant activity.

Keywords
ABTS
antioxidants
ethyl acetate fraction
mangosteen rind
toner

Introduction

With the advancement of technology and increased knowledge about beauty needs, cosmetics have become a top priority for women in supporting their daily appearance. However, women often make mistakes in choosing and using cosmetics without considering their skin conditions and environmental factors due to the excessive desire to beautify themselves [1]. Cosmetics are preparations or products that are routinely and continuously used by people of all ages, driven by the attractiveness of the cosmetics they buy. They are considered a necessity that plays an important role in the field of beauty and health for the human body [2].

Skincare, or skin care, refers to all types of care products whose primary function is to keep the skin well-cared-for, clean, and free from skin problems. Some common skincare products include cleansers, moisturizing creams, serums, sunscreens, facial washes, and facial toners. Facial toners are liquid formulas used to refresh and clean the face, while also balancing the pH of facial skin which tends to become alkaline due to cleansing soap. A pH below 4.5 or too acidic can cause skin irritation, while a pH that is too alkaline can cause dry or scaly skin. Therefore, the pH value of facial tonics must be in the range of the pH of facial skin, which is between 4.5 and 8.0 [3].

Mangosteen fruit (Garcinia mangostana L.) is a natural ingredient with potential as an antioxidant, which helps to prevent and suppress the working process of free radicals. Free radicals are foreign bodies that can damage the immune function of the body and cause various imbalances in the body. Therefore, using mangosteen fruit as an ingredient in facial toners can provide an effective way to protect the skin from damage [4].

The Mangosteen fruit (Garcinia mangostana L) is a plant known for its antioxidant activity and traditional medicinal uses. The highest secondary metabolites found in mangosteen rind are xanthones, which belong to the polyphenol class. Two alkaloid compounds, mangostin and α-mangostin, are found in the rind and can be used to produce natural dyes. The fruit skin also contains anthocyanins such as cyanidin 3-soforosida and cyanid 3-gluaoside, which contribute to its coloring. The skin is rich in xanthones including β-mangostin, 1-isomangostin, 3-isomangostin, 9-hydroxycalaba xanthone, 8-deoxygartanin,

demethylcalaba xanthone, garcinone B, garcinone D, garcinone E, gartanin, mangostanol, mangostanin, and mangostinone [5].

The mangosteen peel contains 82.50% carbohydrates, 3.02% protein, and 6.45% fat. Additionally, it is rich in antioxidants such as anthocyanins (5.7-6.2 mg/g) and xanthones and their derivatives (0.7-34.9 mg/g). Extracts from the mangosteen rind are effective as a cosmetic preparation for facial toner. Based on data and responses from respondents, the mangosteen rind facial toner can effectively remove bacteria, dirt, dust, and makeup from facial skin. This toner is particularly effective after traveling to clean the dust that accumulates on the face and remove makeup. It also moisturizes the skin, leaving it fresh and revitalized [6].

A previous study that optimized the extraction solvent using 96% ethanol, ethyl acetate, and methanol found that the strongest antioxidant activity was in the methanol extract with an IC50 value of 9.00 ± 0.048 µg/mL [3]. In this study, the ABTS method was used to test antioxidant activity. This method is more sensitive than DPPH and can be used to analyze antioxidants in foods and cosmetics. The ABTS method measures the ability of compounds to stabilize free radicals by donating proton radicals [7]. Previous studies that tested antioxidant activity using the ABTS method found that the sample had very strong antioxidant activity with an IC50 value of 32.1292 ppm, which was lower than the IC50 value of pure vitamin C (6 ppm) [8].

Method

The tools and materials used in this study included stir bars, blenders, spray bottles, evaporating cups, separating funnels, Erlenmeyer flasks, measuring cups, beakers, dropping pipettes, measuring pipettes, knives, filters, clamps, spectrophotometer UV-Vis spatula, test tubes, glass jars with lids, analytical balances, ovens, pH meters, pycnometers, vacuum rotary evaporators, vials, and containers. The study also used ABTS (2,2'-azinobis 3-ethyl benzothiazoline 6-sulphonic acid), aluminum foil, distilled water, 96% ethanol, ethyl acetate, glycerin, potassium persulfate, mangosteen rind (Garcinia mangostana L), methanol, sodium benzoate, n-hexane, rose oil, and vitamin C.

The mangosteen fruit used in the study was obtained from a market in Makassar, South Sulawesi Province. The skin of the fruit was washed thoroughly with running water and separated from the flesh. It was then cut into small pieces with a knife and dried at room temperature. After drying, the mangosteen rind was ground into a powder using a blender.

500 grams of mangosteen peel powder was dissolved in a methanol solution and allowed to stand for 3x24 hours with occasional stirring. The mixture was then filtered to obtain the filtrate. The sample dregs were re-macerated, and the filtrate was collected and evaporated at 40-45 0 C using a rotary evaporator (Buchi) until a thick extract was obtained.

10 grams of the viscous extract of mangosteen rind was dissolved in ethanol in a beaker, and then dissolved in 100 ml of distilled water. The mixture was then put into a separatory funnel and fractionated using a non-polar solvent, n-hexane. The size of the layers in the separatory funnel was compared until the solvent was completely extracted, resulting in a layer of aquadest and a layer of n-hexane. The aquadest fraction was added back into the separatory funnel, and a semi-polar solvent, ethyl acetate, was added by comparing the same volume. The mixture was then shaken and separated again to obtain the aquadest and ethyl acetate fractions. The ethyl acetate fraction was then concentrated using a rotary evaporator and evaporated over a water bath. Table 1 shows the formula design.

Table 1. The formula design.

Material Name	Utility	Concentration (%)				
		F1	F2	F3	K-	K+
Garcinia mangostana L.) Peel Fraction	Active substance	0.2	0.4	0.6	-	Viva *Face Tonic Green Tea*
Glycerin	humectants	10	10	10	10	
Sodium Benzoate	Preservative	0.02	0.02	0.02	0.02	
Rose oil	Aroma	Qs	qs	Qs	Qs	
Aquadest	Solvent	100 ml	100 ml	100 ml	100 ml	

The results of the ethyl acetate fraction of mangosteen rind (Garcinia mangostana L) were pipetted with concentrations of 0.2 % , 0.4 % and 0.6 % , then each formula is added with glycerin, sodium benzoate, rose oil to taste. After that, it is homogenized and then added distilled water up to 100 ml and then put into a container.

A. Evaluation of Facial Toner Preparations

Organoleptic test: This test involves identifying the facial toner's smell, color, and texture using human senses.

1. Homogeneity test: This test checks the homogeneity of the facial toner preparation by visually observing the presence of particles or coarse particles under light.
2. pH test: A pH meter is used to measure the pH of the facial toner by dipping it in the toner and waiting for the pH to stabilize. The pH meter should be calibrated using buffer solutions with a pH of 4 and pH 7.
3. Viscosity test: The facial toner is placed in a beaker glass, and its viscosity is determined using a Brookfield viscometer.
4. Moisture test: A skin analyzer is used to measure the humidity of the back of the hand before and after using the facial toner. The humidity level is measured over a period of 1 minute, 30 minutes, 60 minutes, and 120 minutes. The humidity level before and after using the facial toner is then compared [9].

5. Cycling test: The facial toner preparation is subjected to a cycling test by storing it at 4 0 C for 24 hours before transferring it to a 40 0 C oven for 24 hours. This process is repeated six times, and the organoleptic, homogeneity, pH, humidity, and viscosity of the facial toner are monitored during each cycle.

Those evaluation methods are commonly used to assess the quality of facial toner preparations.

B. Antioxidant Test

The ABTS method is a common method for measuring antioxidant activity [10]. In this method, the ABTS radical cation is generated by reacting ABTS with potassium persulfate. The antioxidant activity of the sample is measured by its ability to scavenge the ABTS radical cation, which results in a decrease in the absorption at 750 nm. The percentage inhibition of the ABTS radical cation by the sample is calculated by comparing the absorption of the sample with that of the control (without the sample). The higher the percentage inhibition, the higher the antioxidant activity of the sample.

The steps involved in the ABTS method for evaluating the antioxidant activity of facial toner preparations are as follows:

1. Preparation of facial toner stock solution of the ethyl acetate fraction of mangosteen rind (Garcinia mangostana L.): This involves preparing a stock solution of the facial toner preparation of the mangosteen rind fraction with a concentration of 0.2%, 0.4%, and 0.6% in ethanol.
2. Preparation of Vitamin C stock solutions: This involves preparing a stock solution of vitamin C in ethanol.
3. Preparation of ABTS stock solution: This involves preparing the ABTS stock solution by dissolving ABTS and potassium persulfate in distilled water or ethanol and incubating it in a dark room for 12-16 hours.
4. Measurement of the absorption of the Blank solution: This involves measuring the absorption of the ABTS solution without any sample.
5. Measurement of ABTS free radical scavenging activity with facial toner ethyl acetate fraction of mangosteen rind (Garcinia mangostana L.): This involves adding different volumes of the facial toner stock solution to the ABTS solution, homogenizing the mixture, and then measuring the absorption of the resulting solution.
6. Calculation of the antioxidant power: The antioxidant power is calculated using the formula: Antioxidant power = (Abs control - Abs sample) / Abs control × 100%.
7. Measurement of the antioxidant activity of vitamin C: This involves adding different volumes of the vitamin C stock solution to the ABTS solution, homogenizing the mixture, and then measuring the absorption of the resulting solution.

By comparing the percentage inhibition of the ABTS radical cation by the facial toner preparation with that of vitamin C, the antioxidant activity of the facial toner preparation can be evaluated. The higher the percentage inhibition, the higher the antioxidant activity of the facial toner preparation.

Results and Discussion

Mangosteen fruit is a source of natural chemicals known as polyphenols, which are produced through secondary metabolites and are considered to be very special. The fruit has black-red skin and clean white flesh with a sweet taste, while Donna fruit contains xanthones. Antioxidants are substances that protect cells from damage caused by free radicals, including singlet oxygen, superoxide, peroxide radicals, and hydroxyl radicals. Some foods contain compounds that have antioxidant properties, although they are not classified as nutrients.

Extracts are components or active ingredients that are separated from Simplisia using certain solvents. The extraction process aims to remove specific parts of the drug-containing material. There are two types of organic solvent extraction processes: cold (maceration and percolation) and hot (evaporation). The maceration method is a type of cold extraction that avoids damaging desired compounds during the extraction process. Percolation is also a cold extraction process, but it requires more solvent and uses a gradual process.

In this study, the sample used was the skin fraction of mangosteen fruit (Garcinia mangostana L), which was purchased as an antioxidant in Makassar. Prior to filtration, the mangosteen fruit sample was dried and blended to obtain mangosteen peel powder. The powder, weighing 500 grams, was dissolved using methanol as a solvent, as this solvent can dissolve both polar and non-polar compounds, making it ideal for extracting secondary metabolites [11]. The extract was allowed to stand for 3 x 24 hours with occasional stirring, after which it was filtered and evaporated using a rotary evaporator to obtain the desired concentration and facilitate storage. The resulting thick methanol extract from the mangosteen peel powder was 116.88 grams, with a yield value of 23.37%, indicating the effectiveness of the extraction process. The type of solvent used and the extraction time can affect the performance of the extraction process [12]. See Table 2 for the extraction results.

Table 2. Extract and peel fraction results

Sample	Solvent Type	Dry Sample Weight (g)	Condensed Extract Weight (g)	Extract Render (%)	Extract Weight (g)	Fraction Weight (g)	Render Raction (%)
Mangosteen Peel	methanol	500 grams	116.88	23,376	60	48,43	80,71

After the extraction process, a fractionation process was performed using ethyl acetate to obtain yields of 48.43% and 80.71%. In this study, 10 grams of the concentrated methanol extract fraction was dissolved in 100 ml of distilled water and placed into a separatory funnel.

Then, 100 ml of n-hexane was added and mixed thoroughly until a clear colored n-hexane layer was obtained. The bottom layer was then added back into the separatory funnel, and 100 ml of ethyl acetate solution was added. The reason for using ethyl acetate was based on previous research which showed that this fraction had the highest antioxidant activity in inhibiting free radicals. The mixture was then evaporated using a rotary evaporator to obtain the thick ethyl acetate fraction. The principle of this method is based on the distribution of a new substance with a certain ratio between two solvents that do not mix with each other [11]. See Table 3 for the screening results.

Table 3. Screening results

Compound	Reactor	Test result	Ket
Flavonoids	Magnesium powder and concentrated HCL	Pink	+
Tannins	$FeCl_3$	Blackish blue	+
Saponins	HCL	Formed foam	+

For the facial tonic formulation, the ethyl acetate fraction of mangosteen rind (Garcinia mangostana L) was made with concentrations of 0.2%, 0.4%, and 0.6%. The negative control did not contain extract, and the positive control was Viva Face Tonic Green Tea. In this study, we used a basic toner formula consisting of glycerin, sodium benzoate, rose oil, and distilled water to fill the volume. Glycerin acts as a humectant and prevents skin dryness [13]. Sodium benzoate was used as a preservative since additional preservatives are allowed up to 0.5% in cosmetics. A concentration of 0.02% was used in this formula since the use of preservatives over time can irritate the skin. Rose oil was used as a fragrance. See Table 4 for the formula.

Table 4. Preparation formula

Material Name	**Utility**	**Concentration (%)**				
		1	2	3	-	K+
Garcinia mangostana L.) Peel Fraction	Active substance	0.2	0.4	0.6	-	
Glycerin	humectants	10	10	10	10	Viva *Face Tonic Green Tea*
Sodium Benzoate	Preservative	0.02	0.02	0.02	0.02	
Rose oil	Aroma	Qs	Qs	Qs	Qs	
Aquadest	Solvent	100 ml	100 ml	100 ml	100 ml	

A. Evaluation of Facial Toner Preparations

The evaluation of the preparation aimed to see if there were any changes in the physical and chemical form and the ability of the preparation to inhibit the antioxidant activity from the facial toner ethyl acetate fraction of mangosteen rind (Garcinia mangostana L). The antioxidant facial toner preparation of the ethyl acetate fraction of mangosteen rind (Garcinia mangostana L) was evaluated using the ABTS method (2,2`-azinobis 3-ethylbenzothiazolin 6-sulphonic acid). The physical changes in the preparation, such as its solution form, rose odor, and orange color, were observed by an organoleptic test. The organoleptic quality of the preparations

remained stable during storage without any changes in shape, color, or smell [14]. The result is showed by Table 5.

Table 5. Organoleptic test results

Formulas	Observation					
	Before cycling test			After cycling test		
	Form	Smell	Color	Form	Smell	Color
F1	Solution	Rose	Orange	Solution	Rose	Orange
F2	Solution	Rose	Old orange	Solution	Rose	Old orange
F3	Solution	Rose	Old orange	Solution	Rose	Old orange
K-		Rose	Clear	Solution	Rose	Clear

Homogeneity testing was performed to determine the homogeneity of the preparation at the time of manufacture and any changes in homogeneity that may occur during storage (Table 6). Homogeneity was indicated by the absence of coarse particles and differences in the preparations [15].

Table 6. Homogeneity test results

Formulas	Observation	
	Before cycling test	After cycling test
F1	Homogeneous	Homogeneous
F2	Homogeneous	Homogeneous
F3	Homogeneous	Homogeneous
K-	Homogeneous	Homogeneous

During pH testing, the facial freshener preparation was found to meet the standard range of 5.4-5.8. The pH test aims to determine the acidity level of the product and its suitability for use on the skin. It is crucial to ensure the safety of the preparation since it will be applied to the facial skin. Although there were variations in pH before and after the cycling test due to temperature changes and gas entering the preparation, the pH remained stable and within the range suitable for the skin. See Table 7 for the results. Preparations with pH levels that are too acidic can irritate the skin, while those that are too alkaline can cause dryness [16].

Table 7. pH test results

Formulas	Observation		Range
	Before cycling test	After cycling test	
F1	5,6	5,7	4.5-6.5
F2	5,7	5,7	
F3	5,8	5,6	
K-	5,7	5,7	

Viscosity is an important criterion in determining the ideal consistency of facial fresheners. The viscosity of this formulation remained stable before and after cycling and varied depending on the section. A low viscosity formulation will not have enough contact time with the skin, rendering the active ingredients ineffective, while high viscosities may reduce

dispersion within the 5-50 mPas range. Table 8 shoes the results. This facial freshener has an optimal thick consistency, making it easy and comfortable to use [16]. Water is used as a standard calculation for viscosity, which is ± 1 mPa.s [17].

Table 8. Viscosity test results

Formulas	Observation		Range
	Before cycling test	After cycling test	
F1	21.0	27.0	5-50 mPas
F2	25.0	27,3	
F3	28.0	35.0	
K-	15.0	25.0	

The humidity test, which met the standard range of 44.0-58.0, aimed to determine skin moisture levels before and after using the facial freshener at 1 minute, 30 minutes, 60 minutes, and 120 minutes. The results showed an increase and decrease in skin moisture levels, which were influenced by temperature and weather. However, the skin moisture values remained within the range of the moisture test, which is <45 for dry, 46-55 for normal or moist, and 56-100 for very moist [18]. The cycling test aimed to determine the stability of the preparation under varying temperature conditions during storage. The preparations were stored in an oven at 40°C for 24 hours and then in a refrigerator at 4°C for 24 hours, for a total of 6 cycles. Physical changes were observed from the beginning to the end of the test, including organoleptic, homogeneity, pH, and humidity. The cycling test aimed to see the physical stability of the preparation, particularly its pH, viscosity, and organoleptic properties. The research results showed no significant changes in organoleptic examination, but the pH of the preparation decreased slightly due to the solution form having a shorter shelf life than solid dosage forms. This decrease was still within the range of standard mouthwash preparations and not significant [17]. Table 9 shows the result before cycling test.

Table 9. Humidity results before cycling test

Formulas	Humidity %					
	Before the show	1 minute	30 minutes	60 minutes	120 minutes	Range
F1	13,6	48,3	52,3	54,2	56,4	<45
F2	17,9	47,9	50.0	53,3	59,1	46-55
F3	18,7	50,4	52,7	55,4	55,9	56-100
K-	17,3	46,6	48,3	48.0	49,4	

Table 10 shows the result after cycling test.

Table 10. Humidity results after cycling test

Formulas	Humidity%				
	Before the show	1 minute	30 minutes	60 minutes	120 minutes
F1	12,3	42,6	44.0	48.5	54.0
F2	18,2	41,2	42.8	46.0	48,2
F3	27.5	49,6	47.0	58,3	59.5
K-	30.0	47,6	50.0	51,2	54.0

B. Antioxidant Test

The ABTS method is used to determine the antioxidant activity of mangosteen peel (Garcinia mangostana L) preparations. This method involves the oxidation of potassium persulfate with gram diammonium ABTS at a wavelength of 750 nm. Results (Table 11) show that each concentration of the three formulas (I, II, and III) exhibited antioxidant activity, with IC50 values of 48.82 µg/mL, 37.10 µg/mL, and 22.74µg/mL, respectively. The vitamin C reference solution showed an IC50 value of 16.57 µg/mL. The research findings indicate that the higher the concentration of the preparation, the lower the absorbance value, indicating higher antioxidant activity. This is also supported by the % inhibition value.

According to the IC50 classification, the preparations and vitamin C are classified as very strong antioxidants, with values below 50 ppm. The presence of strong antioxidant activity is attributed to the presence of bioactive flavonoid compounds in the mangosteen fruit rind (Garcinia mangostana L), as well as the sample maceration time.

Table 11. Antioxidant test result

Concentration (µg/mL)	Blank absorbance	Sample absorbance	% Inhibition	IC $_{50}$ (µg/mL)
Vitamin C				
10	0.1174	0.0657	42.7201	
20	0.1174	0.0547	52.3103	
30	0.1174	0.0387	66.2598	16.57
40	0.1174	0.0245	78.6399	
50	0.1174	0.0155	89.4864	
Formula I (0.2%)				
10	0.1174	0.0767	33.1299	
20	0.1174	0.0735	35.9197	
30	0.1174	0.0683	40.4533	48,82
40	0.1174	0.0636	44.5510	
50	0.1174	0.0549	52.1360	
Formula II (0.4%)				
10	0.1174	0.0783	31.6860	
20	0.1174	0.0736	35.8430	
30	0.1174	0.0627	45.3488	37,10
40	0.1174	0.0553	53.5174	
50	0.1174	0.0477	58.3721	
Formula III (0.6%)				
10	0.1174	0.0653	43.0233	
20	0.1174	0.0626	45.6977	
30	0.1174	0.0534	53.4593	22.68
40	0.1174	0.0414	63.9244	
50	0.1174	0.0332	71.0756	

In summary, the ABTS method is a reliable method for evaluating the antioxidant activity of mangosteen peel preparations, and the high concentration of bioactive compounds in mangosteen peel makes it a potent source of natural antioxidants.

Conclusions and Recommendations

Based on the research findings, it can be concluded that the ethyl acetate fraction of mangosteen rind can be used to make a stable facial toner with concentrations of 0.2%, 0.4%,

and 0.6%. The facial toner preparations also showed significant antioxidant activity, with each concentration showing a very strong antioxidant effect, comparable to vitamin C. Further research should be conducted to explore the possibility of creating preparations with higher concentrations of the ethyl acetate fraction. Additionally, it would be beneficial to employ other methods to determine the antioxidant activity of each preparation. This will provide a more comprehensive understanding of the potential benefits of using mangosteen rind in skincare products.

Conflict of Interest

The authors declare that there is no conflict of interest.

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Authors

Sriyanty Sadsyam is an educational scientist and a lecturer in the Pharmacy Study Program at the Faculty of Pharmacy, Megarezky University Makassar, Indonesia. She earned her Bachelor's degree from Universitas Islam Negeri Alauddin Makassar and her Master's degree from Universitas Hasanuddin, Indonesia. Her research interests lie in post-incidence therapy, and she is active in various professional associations. (email: sriyantisadsyam@unimerz.ac.id).

Nielma Auliah is a dedicated lecturer at the Pharmacy Department of the Faculty of Pharmacy, Megarezky University Makassar, Indonesia. She completed her Bachelor's degree at Universitas Hasanuddin Makassar and continued her academic journey by pursuing a Master's degree at the same university. She is particularly interested in drug development and has conducted extensive research on the topic. Her expertise in the field has earned her respect among her colleagues and students alike. (email: nielmaauliah@gmail.com).

Wa Ode Wisna Anto Uko is a dedicated student in the Pharmacy Study Program at the Faculty of Pharmacy, Megarezky University Makassar, which is located on Jalan Antang Raya No.4 in Makassar, Indonesia. She is passionate about expanding her knowledge of the field of pharmacy and is committed to using her education to make a positive impact on her community. (email: Wisnauko@gmail.com).

Nasrawati Basir is a lecturer at the Pharmacy Department, Faculty of Pharmacy, Megarezky University Makassar, Indonesia. She earned her Bachelor's degree from Universitas Islam Negeri Alauddin Makassar and her Master's degree from Universitas Hasanuddin Makassar, Indonesia. With her expertise in pharmacy, she is committed to promoting excellence in education and research in the field of pharmacy. (email: nasrawatibasir@unimerz.ac.id).

Andi Ulfiana Utari is a lecturer in the Pharmacy Department at the Faculty of Pharmacy, Megarezky University Makassar, Indonesia. She obtained her bachelor's degree from Universitas Islam Negeri Alauddin Makassar and her master's degree from Universitas Hasanuddin Makassar, Indonesia. Her research interests include pharmacology, drug delivery systems, and herbal medicine. (email: andiulfianautari@unimerz.ac.id).