Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2021; 10(4): 47-52
Received: 17-05-2021
Accepted: 19-06-2021

Greesty F Swandiny
Pancasila University Faculty of Pharmacy, Indonesia

Safira Nafisa
Pancasila University Faculty of Pharmacy, Indonesia

Erlindha Gangga
Pancasila University Faculty of Pharmacy, Indonesia

Muhammad Fauzi
Pancasila University Faculty of Pharmacy, Indonesia

Corresponding Author:
Greesty F Swandiny
Pancasila University Faculty of Pharmacy, Indonesia

Standardization of 70% ethanol extract and 96% lime leaves as antioxidants with DPPH and FRAP

Greesty F Swandiny, Safira Nafisa, Erlindha Gangga and Muhammad Fauzi

Abstract
The lime plant (*Citrus aurantifolium* S.) is a small tree, smells good. The fruit is slightly rounded with a slightly tapered tip. Lime has a characteristic taste that is soft, watery, and very sour with a sharp aroma. The use of lime and lime leaves is quite extensive, among others, as ingredients for traditional medicine, for beauty treatments, for flavoring food and for adding a fresh taste to drinks. The pharmacological effects of lime leaves include antioxidants properties. The ethanol extract of lime leaves (*Citrus aurantifolium* S.) was carried out by maceration using ethanol 70% and 96% which were characterized and standardized. The main target of this research is the production of standardized, characterized extracts that are safe, stable in their use.

Keywords: Lime leaves, 70% ethanol, 96% ethanol, antioxidants, DPPH, FRAP

Introduction
Nowadays, worsening air pollution can become a source of free radicals that cause premature aging and cause various degenerative diseases. Air pollution, cigarette smoke, motor vehicle gas emissions and excessive ultraviolet rays are all factors that trigger the growth of free radicals in the human body. In addition, most people do not get enough sources of antioxidants from the food they consume every day. Antioxidants are compounds that can inhibit or prevent the oxidation process caused by the presence of free radicals. The skin is an organ that lines the muscles and internal organs of humans, it can be said that the skin is a bulwark against viral and fungal bacteria. Lime (*Citrus aurantifolium* S.) is a plant that is widely grown in Indonesia, people use the fruit for cooking spices or refreshing drinks, removing the unpleasant smell (fishy) from fish and as antibacterial, while the leaves are used as a spice in the kitchen. The content of flavonoids and essential oil of lime leaves has antioxidant and antibacterial activity, so that lime leaf extract can be used as an active antioxidant / antiaging and antibacterial ingredient [3, 4]. Natural antioxidants are antioxidants extracted from natural ingredients derived from plants such as wood, bark, roots, leaves, fruit, flowers and seeds. Natural antioxidant compounds are generally phenolic or polyphenolic compounds which can be in the form of flavonoids, cinnamic acid derivatives, coumarin, and multifunctional organic acids.

Research methods
A. Preparation of lime leaf extract
The lime leaves are chopped and then dried in an oven at 40-50 ˚C for 48 - 72 hours, then mashed with a grinder machine, then sieved to obtain a simplicia powder with a size of 4/18. Then the simplicia powder is extracted by cold extraction, namely kinetic maceration using ethanol solvent, the extract obtained is filtered, collected and then thickened with a rotary evaporator at a temperature of 45 ˚C and vacuum pressure until all the ethanol is evaporated and an extract is obtained which is thickened with if necessary it is placed in a water bath until obtained dry extract.

B. DPPH method (1,1-diphenyl-2-picrylhydrazyl)
The DPPH method can be used for solid samples as well as in solution form. The principle is that the odd electrons in the DPPH molecule provide the maximum absorption at a purple wavelength of 517-521 nm. This color will change from purple to weak yellow when the odd electrons are paired with hydrogen atoms donated by antioxidant compounds. This change is based on chemical equilibrium. The parameter used to show antioxidant activity is IC50, namely the concentration of an antioxidant substance that can cause 50% of DPPH to

lose its radical character or the concentration of an antioxidant substance which provides 50% reduction [17]. The presence of antioxidant compounds can change the color of the DPPH solution from purple to yellow [19].

C. FRAP method (Ferric Reducing Antioxidant Power)

It is a method that refers to the reduction of Ferroin compounds (complexes of Ferric compounds (Fe +++) from Tripirildyltriazine, namely Fe (TPTZ) 3+. It becomes a Fe2 + complex, namely Fe (TPTZ) 2 + which is intense blue when combined with antioxidants in acidic conditions. Will be interpreted using an increase in absorbance at a wavelength of 593 nm, which is inferred to be the amount of Fe2 + equivalent to a standard antioxidant.

D. Standardization of simplicia (specific and non-specific)

1. Specific parameters

a. Determination of extract identity
b. Nomenclature description:

Extract name
Latin name for plants
Plant part used
Indonesian name for plants

c. Organoleptic

Using the senses to describe shapes, colors, smells.

d. Determination of the dissolved compound in a particular solvent
e. Content of water-soluble compounds.
f. Content of compounds that dissolve in ethanol.

2. Non-specific parameters

a. Gravimetric determination of drying losses
b. Determination of water content (titrimetric Karl Fischer method)
c. Gravimetric determination of total ash content
d. Determination of water soluble ash
e. Determination of the remaining solvent by gas chromatography Gas-liquid chromatography method:
f. Determination of heavy metal contamination by Atomic Absorption Spectrophotometry (AAS)
g. Determination of lead content
h. Cadmium assay
i. Determination of microbial contamination
a. Total Plate Figures
b. Yeast Mold Figures
j. Determination of total flavonoid levels

Results and Discussion

A. Determination of Foreign Organic Materials

The simplicia used is lime leaves (*Citrus aurantifolia*, Family: Rutaceae).

Table 1: Results of Foreign Organic Ingredients Determination

Weighing to-	Weight of simplicia (g)	Foreign Organic Ingredients (g)	Yield (%)	Requirements
I	100.13	1.73	1.73	≤2%
II	100.18	1.65	1.65	

Foreign organic material is part of the plant or the entire plant originating from simplicia, stated or the amount is limited in the description or description in the monograph concerned. Determination of foreign organic material is intended to limit the amount of organic material that comes from inside and outside the simplicia so that it affects the quality of the extract. In the determination, it was found that the average percentage of foreign organic matter in lime leaves was 1.73% and 1.65% of the yield fulfilled the requirements.

B. Determination of Degree of Refinement

The simplicia of lime which will be used first is pollinated then the degree of fineness is 4/18.

Table 2: Results of Determination of Simplicia Fine Degrees

Simplicia Powder	Total weight (g)	Sieve No.4 (g)	Sieve No. 18 (g)
Lime Leaves for 96% ethanol	1,002.09	1,002.09	225.93
Lime Leaves for 70% ethanol	1,000.34	1,000.34	207.00

Table 3: Results of determination of the fine degree of lime leaf simplicia powder

Simplicia Powder	Sieve No. 4	Sieve No. 18	Requirements
Lime leaves for 96% ethanol	100%	22.55%	▪ 100% through sieve no.4 ▪ Not more than 40% through sieve no.18
Lime leaves for 70% ethanol	100%	20.69	

The simplicia powder used in the maceration process uses a fine degree of 4/18. The fine degree is used to determine the optimum size which can produce a fine but not too fine simplicia powder. It is intended that the maceration process takes place optimally because the small particle size will make it easier for the powder to get wet with the solvent because the powder with a small surface area has a larger total surface area which complicates the filtering process because it can clog the pores of the filter paper. From the measurement results of 4/18 fine degrees, the results obtained are as big as the powder that meets the requirements for powder size 4/18 according to Materia Medika Indonesia, which is that all powders can 100% pass through sieve no, 4 and no more than 40% can pass through sieve no. 18.

C. Extract Yield and DER-Native

The yield of extract and DER-native was determined after 6 times maceration using ethanol solvent 70% and 96% with a total usage of 25L each. The obtained maserate was collected, then thickened using a rotary evaporator vacuum to obtain a thick extract of lime leaves.

Table 4: Yield (%) and DER-native extracts

No	Extract	Powder weight of simplicia (g)	Thick extract (g)	DER-native	Yield (%)
1	Ethanol Extract 70% Lime Leaves	1,000.34	80.4	12.44	8.04%
2	Ethanol Extract 96% Lime Leaves	1,002.09	53.8	18.62	5.37%

Extract yield was calculated by comparing the amount of extract obtained with the initial simplicia used. Maceration was chosen to obtain extracts containing secondary metabolites from lime leaf simplicia which are extracted in

large quantities and in a short time. In this study, 70% ethanol and 96% ethanol were used as solvents.

D. Results of Phytochemical Screening

Antioxidant activity testing of lime leaf extract was carried out using the damping method with DPPH and FRAP and also the determination of total phenolic and total flavonoid levels was carried out using thick lime leaf extract as samples. However, before that, phytochemical screening was first carried out as a preliminary test which aims to see what compounds are contained in the lime leaf extract.

Table 5: Results of Phytochemical Screening

No.	Phytochemical screening	Extract 70%	Extract 96%
1	Coumarin	Positive (+)	Positive (+)
2	Flavonoids	Positive (+)	Positive (+)
3	Alkaloids	Negative (-)	Negative (-)
4	Saponins	Positive (+)	Positive (+)
5	Fault / catechoid tannins	+/-	+/-
6	Quinone	Negative (-)	Negative (-)
7	Steroids	Positive (+)	Positive (+)
8	Triterpenoids	Positive (+)	Positive (+)
9	Essential oil	Positive (+)	Positive (+)

In this study, phytochemical screening was carried out on the simplicia powder and plant viscous extracts tested. The results of phytochemical screening showed that the simplicia powder and thick extract from lime leaves contained secondary metabolites, including flavonoids, saponins, tannins, steroids, essential oil triterpenoids and coumarin.

E. Determination of Extract Quality Parameters

Specific quality parameters

1. Extract identity

Table 6: Extract Identity

No.	Extract identity	Check up result
1	Extract name	Citrus aurantifolia foliiextractum spissum
2	Latin name for the plant	Citrus aurantifolia S.
3	Plant parts used	Folium
4	Indonesian name of the plant	Lime

2. Organoleptic

Table 7: Organoleptic Examination Table

No.	Organoleptic	Check up result
1	Form	Extra thick
2	Color	Blackish green
3	Smell	Aromatic
4	Taste	Bitter

Non-specific quality parameters

a. Water soluble and ethanol soluble content

Table 8: Water soluble and ethanol content

Solvent	Result of Determination (%)		Requirements
	96%	70%	
Water	62.5	61.24	≥3%
Ethanol	48.22	42.11	≥1%

Determination of water soluble extract and ethanol content is a quantitative method to determine the content of compounds in extracts that can be contained in certain solvents. Determination of soluble extract content in ethanol is carried out to determine the amount of content of compounds dissolved in ethanol while determination of soluble juice content is carried out to determine the amount of content of compounds dissolved in water. The dissolved compound content indicates the number of secondary metabolite compounds dissolved in ethanol and water solvents. In this research, it was found that the content of soluble compounds in ethanol for 96% ethanol extract of lime leaves was 48.22% and for ethanol extract 70% of lime leaves was 42.11%.and water soluble compound sethanol for 96% ethanol extract of lime leaves was 62.85% and for ethanol extract of 70% lime leaves was 61.24%.

b. Total ash content and acid soluble ash content

Table 9: Total ash content, acid-soluble and water-soluble ash content

Non-specific parameters	Determination Result (%)	
	96%	70%
Total ash content	7.01	7.32
Acid insoluble ash content	0.42	0.49
Water soluble ash content	6.63	6.52

The determination of the ash content was carried out by the gravimetric method, an annealing process was carried out in a furnace at 450oC until ash was obtained. During the annealing process, organic matter will be digested and will only leave organic matter, namely ash. The determination of the ash content aims to determine the mineral content from the initial process until the extract is formed. The determination of the total ash content is carried out to determine the content of inorganic compounds (heavy metals such as Hg, Pb, Cd, Silicate), internal good mineral compounds such as K, Mg, Ca, or externals such as pollutants, dust, soil contained in the extract. Determination of acid insoluble ash content is carried out to determine the amount of inorganic substances (heavy metals such as Hg, Pb, Cd, silicates) or mineral content that cannot dissolve in acid.

c. Shrink drying and moisture content

Drying shrinkage was carried out to determine the moisture content and volatile compounds in the extract after gravimetric drying in an oven at 105oC. From the determination results, it was obtained that the drying shrinkage of 70% ethanol extract of lime leaves was 7.30% and ethanol extract of 96% lime leaves was 5.10% which was determined by the Karl-Fischer method.

Determination of the water content in the extract aims to provide a minimum limit or range of the amount of water content in the extract. The lower the water content in the extract, the better the quality of the extract. Because the extract is stable during storage. But if the water content of the extract has a high value. Then the extract will be susceptible to microbial contamination and can accelerate the growth of microorganisms.

Table 10: Drying losses and water content of 96% ethanol extract and 70% lime leaves

Solvent	Result of Determination (%)	
	96%	70%
Drying Shrinkage	5,10	7.30
Water content	3.19	5.33

d. Heavy Metal Contamination

The results of the examination of heavy metal contamination in 70% ethanol extract and 96% of lime leaves.

Table 11: Heavy metal contamination of 70% ethanol extract & 96% of lime leaves

No.	Metal	Check-up result (mg / kg)		Extract Monograph Requirements (mg /kg)
		Ethanol extract 70% lime leaves	96% ethanol extract of lime leaves	
1	Pb	0.3270	0.0388	≤10
2	CD	0.5538	0.0554	≤0.3

The results of the examination of Pb and Cd metal levels in the ethanol extract of 70% and 96% of lime leaves contained Pb levels of 0.3270 mg / kg and 0.0388 mg / kg, respectively, and contained Cd metal respectively of 0, 05538 mg / kg and 0.0554 mg / kg. Pb and Cd metals are heavy metals whose numbers in the body must be limited because they can cause poisoning and are neurotoxic.

If these heavy metals accumulate in the body it can cause damage to the kidneys, nerves and lungs. Extracts can contain Pb and Cd metals which come from the environment where the original plant was grown and the production process. The maximum limit of Pb and Cd content in the extract according to volume 2 of Indonesian Medicinal Plant Extract Monographs is for Pb of ≤10 mg / kg and for Cd of ≤0.3 mg / kg so that the resulting extract meets the quality requirements of the extract.

e. Microbial contamination

The results of microbial contamination examination in 70% ethanol extract and 96% lime leaves.

Table 12: The results of examination of levels of bacteria and mold contaminants

No.	Checking type	Examination Result (colony / g)	
		70% ethanol extract of lime leaves	96% ethanol extract of lime leaves
1	Total Plate Figures	TSUD	TSUD
2	Yeast Mold Figures	TSUD	TSUD

Slope: TSUD (Too Difficult to Calculate)

The test for microbial contamination is carried out to provide assurance that the extract must not contain pathogenic microbes and non-pathogenic microbes beyond the specified limits.The microbes contained in the extract can be caused due to the high water content and the unhygienic processing and storage of extracts. The high levels of microbial contamination in the extract can be dangerous for its use on the body, and affect the stability of the extract. The results of checking the levels of microbial contamination in the ethanol extract of 70% and 96% of lime leaves have total plate values and the yeast mold numbers are too little to count.

F. Determination of Total Flavonoid Levels

In determining total flavonoid levels, quercetin was used as a comparison flavonoid. The maximum absorption wavelength of quercetin which has been reacted with aluminum chloride is 428 nm.

Table 13: Total Flavonoid Levels

No.	Ethanol extract	Total Flavonoid Level (%)	Average total flavonoid levels (%)
1	Lime leaves 96%	1.54	1.54
		1.48	
		1.61	
2	Lime leaves 70%	1.46	1.48
		1.50	
		1.48	

Quercetin is used to determine total flavonoid levels as a reference standard that will react with AlCl3 so that a shift in the maximum wavelength to a larger wavelength occurs, the maximum wavelength is determined and the result is 425.0 nm. Determination of total flavonoid levels was carried out to determine the number of flavonoids contained in the 70% ethanol extract and 96% of lime leaves. The total flavonoid content in the ethanol extract of 96% lime leaves was 1.54%, and the 70% ethanol extract of lime leaves was obtained at 1.48%. From the results of total flavonoid levels obtained, 96% ethanol extract of lime leaves has the highest total flavonoid levels.

G. Determination of Total Phenolic Content

Determination of total phenolic compound levels in this study using the Folin-Ciocalteau method. This method is the most common method used to determine the content of total phenolic compounds in plants with the consideration that with this technique the process is simpler and uses the Folin-Ciocalteau reagent because the Folin-Ciocalteu reagent contains phosphotungstic compounds and phosphomolibdic acid which will form complexes through redox reactions with phenolic compounds present in the measured sample. Determination of the total phenolic compounds content was carried out to determine the levels of phenolic compounds in the sample used with gallic acid solution. Gallic acid is used as a solution because it is a natural and stable phenolic, and is relatively inexpensive compared to others. Gallic acid is included in the phenolic compounds derived from hydroxy benzoic acids which are classified as simple phenolic acids. Gallic acid is reacted with Follin Ciocalteu's reagent to produce a yellow color which indicates that it contains phenolic compounds, after which Na2CO3 solution is added to give an alkaline atmosphere and produce a blue color.

In determining the total phenol content, the maximum wavelength was determined and obtained 769.0 nm. Determination of total phenol content was carried out to determine the amount of phenolic compounds contained in the 70% ethanol extract and 96% of lime leaves.

Table 14: Total Phenol Test Results

Sample	Repetition	Weighing Weight (mg)	Volume End	Dilution Factor	Absorption	Levels (%)	Average Grade (%)
Ethanol Extract 96% Lime Leaves	I	25.3	50	25/10	0.6726	4.08	4.09
	II	25.1	50	25/10	0.6737	4.12	
	III	25.4	50	25/10	0.6735	4.07	
Ethanol Extract 70% Lime Leaves	I	25.4	50	25/10	0.5769	3.40	3.40
	II	25.3	50	25/10	0.5763	3.41	
	III	25.6	50	25/10	0.5768	3.40	

In table V.13. It can be seen that the total phenolic content contained in the ethanol extract of 70% lime leaves is 3.40% and in the ethanol extract of 96% lime leaves is 4.09%. The high levels of phenolic compounds also increase the antioxidant power of the ethanol extract of lime leaves. The levels of total phenolic compounds in each plant also vary according to where the plant is grown.

H. Antioxidant activity test of 70% ethanol extract and 96% lime leaves using the DPPH method

Antioxidant activity tests were carried out on 70% ethanol extract and 96% of lime leaves using the DPPH method. With vitamin C as a positive control.

Table 15: DPPH test results for 96% ethanol extract of lime leaves

Sample	Concentration (ppm)	Ab	US	% Inhibition (%)	IC50(bpj)	Average IC50(bpj)
1	25	0.7337	0.5143	29.90	83.63	83.39
	50		0.4820	34.31		
	75		0.3777	48.52		
	100		0.3095	57.82		
	125		0.2601	64.55		
2	25	0.7284	0.5124	29.65	83.47	
	50		0.4781	34.36		
	75		0.3698	49.23		
	100		0.3095	57.51		
	125		0.2637	63.78		
3	25	0.7334	0.5118	30.21	83.06	
	50		0.4815	34.35		
	75		0.3755	48.80		
	100		0.3125	57.39		
	125		0.2605	64.48		

Table 16: DPPH Test Results 70% ethanol extract of lime leaves

Sample	Concentration (ppm)	Ab	US	% Inhibition (%)	IC50(bpj)	Average IC50(bpj)
1	25	0.7664	0.6488	15.34	87.12	88.02
	50		0.5365	29.99		
	75		0.4270	44.28		
	100		0.3251	57.58		
	125		0.2318	69.75		
2	25	0.7660	0.6465	15.60	87.34	
	50		0.5359	30.04		
	75		0.4288	44.02		
	100		0.3266	57.36		
	125		0.2330	69.58		
3	25	0.7459	0.6486	13.28	89.59	
	50		0.5368	28.63		
	75		0.4271	42.74		
	100		0.3259	56.31		
	125		0.2319	68.91		

Based on the data above, the results of testing the antioxidant activity of the ethanol extract of 70% and 96% of lime leaves resulted in an IC50 value of 83.89 bpj, and 96% ethanol extract of lime leaves resulted in an IC50 value of 88.02 bpj. This shows that the activity. The antioxidants possessed by the ethanol extract of 70% and 96% of lime leaves are strong. The strong antioxidant activity is due to the presence of secondary metabolite compounds such as flavonoids contained in the lime leaf extract.

I. Antioxidant activity test using FRAP method from 70% ethanol extract and 96% lime leaves

In this study, antioxidant activity was measured using the FRAP test. The FRAP test was chosen because the procedure is simple, the method is cheap, fast and the reagent used is quite simple and does not use special tools to calculate total antioxidants. Testing of antioxidant activity using the FRAP (ferric reducing antioxidantpower) method is based on the ability of antioxidant compounds to reduce iron (III) -tripiridyl-triazine compounds to iron (II) -tripiridyl triazine at pH 3.6.

Table 17: FRAP Antioxidant Test Results 96% ethanol extract of lime leaves

Concentration (ppm)	Sample	Ab	US	levels (mg AaE / g)	Average levels (mg AaE / g)	SD
50	1	0.1923	0.2860	166.0	164,799	2.81
	2		0.3545	166.81		
	3		0.4314	161,588		
100	1		0.2851	192,063	191,851	1.12
	2		0.3550	192,857		
	3		0.4321	190,635		
150	1		0.2844	209,417	209,593	0.49
	2		0.3536	210,158		
	3		0.4312	209,206		
	$\bar{x}$				188.74	1.47

Table 18: FRAP Antioxidant Test Results 70% ethanol extract of lime leaves

Concentration (ppm)	Sample	Ab	US	Levels (mg AaE / g)	Average levels (mg AaE / g)	SD
50	1	0.1923	0.2815	152,380	150,369	1.74
	2		0.2806	149,522		
	3		0.2805	149,206		
100	1		0.3521	188,253	187,407	0.73
	2		0.3513	186,984		
	3		0.3513	186,984		
150	1		0.4280	205,820	205,326	0.68
	2		0.4268	204,550		
	3		0.4278	205,608		
	$\bar{x}$				181,034	1.05

In this study, the FRAP test results obtained from the ethanol extract of 96% 50 bpj obtained 188.74 mg AaE / g and for the 70% ethanol extract of lime leaves 181.034 mg AaE / g extract. It can be seen that the greater the equality contained in the extract, the better the antioxidant activity.

Conclusion

The ethanol extracts of 70% and 96% of lime leaves meet the quality standards of simplicia and extracts. Both extracts have antioxidant activity as measured by DPPH and FRAP, so they can be developed into standardized natural drug candidates.

Reference

1. Dalimartha S. Atlas Tumbuhan Obat Indonesisa. Edisi 2. Jakarta: Trubus agriwidya 1999, 85-92.
2. Malee T. Chemical Constituents From the Leaves and Stems of *Citrus aurantifolia* Swingle (thesis). Bangkok: Prince of Songkla University, 2011.
3. Departemen Kesehatan Republik Indonesia. Farmakope Indonesia. Edisi IV. Jakarta: Ditjen POM, 1995. H. 63-64, 508, 551, 589, 713, 999, 1086, 1039-1040.
4. Departemen Kesehatan Republik Indonesia. Panduan Teknologi ekstrak. Jakarta: Direktorat Jendral Pengawasan Obat dan Makanan, 2000, H.1- 2, 6-7, 13-4.
5. Departemen Kesehatan Republik Indonesia. Formularium Kosmetika Indonesia. Jakarta: Direktorat jenderal Pengawasan Obat dan Makanan, 1985, 70-77.
6. Tan HT, Kirana R. Obat- obat Penting. Edisi 5. Jakarta: Elex Media Komputindo 2002, 228-236.
7. Departemen Kesehatan Republik Indonesia. Kodeks Kosmetika Indonesia.. Jakarta: Departemen Kesehatan Republik Indonesia; 1993, 1, 254, 255, 326, 578, 591.
8. Kibbe VC. Standars Textbook of Cosmetology. New York: Milady Publishing corporation 434, 435.
9. Balsam MS, Gershon SD, Rieger MM, Edward Sagarin Strianse SJ. Cosmetic Science and Technology. 2nd ed. Newyork: Willey Interscience 1998;2:103-9.
10. Aulton ME. Pharmaceutics the science of dosage form design. 2nd ed. Churchill Livingstone 2002, 59-63
11. Raymon Rowe C, Paul Sheskey J. Handbook of Pharmaceutical Excipents.6th ed. London: Pharmaceutical Press; 2009, 7,47,167-168, 176-178, 229-232, 310, 355-361, 411-414.
12. Barel AO, Paye M dan Maibach HI, editors. Handbook of cosmetic science and technology 3rd ed. New York: Informa Healthcare 2009, 473-75.
13. Brown RG, Burns T. Lectures notes on dermatology. Ed 8.Jakarta: Erlangga; h.1-9.
14. DepKes RI, 2000. Panduan Teknologi Ekstrak. Jakarta: Ditjen POM; h. 2005, 6-14.
15. United States Pharmacopeia 25/National Formulary 20 (USP25/NF 20),
16. Heyne K. 1987. Tumbuhan Berguna Indonesia. Jilid II. Terjemahan: Badan Litbang Jakarta. Yayasan Sarana Wana Jaya, 1987, 753.
17. Martin A, Swarbrick J, Cammarata A. Farmasi Fisik. Edisi 3. Diterjemahkan
18. oleh Jayadisastra Y. Jakarta: UI press; 1993, 1077-1109, 1154.
19. Winlkinson JB, Moore RJ. Harry's Cosmeticology. 8th Edition. London: George Godwin, 2000.