Borneo Journal of Pharmacy
Vol 2 Issue 2 November 2019
Page 55 – 62

http://journal.umpalangkaraya.ac.id/index.php/bjop/article/view/898
DOI: https://doi.org/10.33084/bjop.v2i2.898
e-ISSN: 2621-4814

Simplicia and Extracts Standardization from Jualing Leaves (*Micromelum minutum* Wight & Arn.) from South Kalimantan

Sutomo[*1,2]

Herwina Dita Lestari [1]

Arnida [1]

Agung Sriyono [3]

[1]Department of Pharmacy, Universitas Lambung Mangkurat, Banjarbaru, South Kalimantan, Indonesia

[2]Center for Study of Natural Medicine, Universitas Lambung Mangkurat, Banjarbaru, South Kalimantan, Indonesia

[3]The Research and Development Agency of the Banua Botanical Gardens, South Kalimantan, Banjarbaru, South Kalimantan, Indonesia

*email: sutomo01@ulm.ac.id

Keywords:
Micromelum minutum
Non-specific parameter
Simplicia
Specific parameter
Standardization

Abstract

Jualing (*Micromelum minutum* Wight & Arn.) is a plant from South Kalimantan that has the potential to be developed as natural medicine. This study aims to establish standardization which includes specific and non-specific parameters of *M. minutum* leaf simplicia and extracts from three growing locations, namely Banua Botanical Garden, Sultan Adam Forest Park, and Forest Areas with Specific Objects of Rantau, South Kalimantan. The method of setting standardization parameters refers to the Indonesian Herbal Pharmacopoeia and General Standard Extracts Parameters. Organoleptic standardization of simplicia is brownish-green, has a distinctive odor, has a bitter and slightly spicy taste. Microscopic observations showed the presence of stomata, cell walls, cytoplasm, calcium oxalate crystals, upper epidermis, palisade tissue, spongy tissue, cortex, xylem, phloem, lower epidermis, and trichomes. Water content test showed the results of 21.9-22.07%; ethanol extract content of 12.87-13.17%; drying losses 4.64-4.84%; total ash content of 6.04-6.14%; acid insoluble ash content 1.13-1.19%; Pb levels of 0.022-0.025 mg/kg; Cd levels of 0.017-0.020 mg/kg; and Hg levels <0.0004 mg/kg. Extract standardization shows thick green-black, thick-smelling extract and bitter taste. *Micromelum minutum* leaves contain alkaloids, flavonoids, saponins, terpenoids, tannins, anthraquinones, phenols, and glycosides. Thin-layer chromatography profiles show the similarity of chemical compounds in each growth location. Other parameters are extract yield of 8.08-8.32%; moisture content of 6.07-6.27%; total ash content of 4.22-4.27%; and acid insoluble ash content 1.11-1.12%. All standardization parameters meet the requirements in the Media Materia Indonesia and the Republic of Indonesia Drug and Food Control Center.

Received: August 8th 2019
Accepted: October 12th 2019
Published: November 14th 2019

INTRODUCTION

Medicinal plants are parts or all parts of the plant that are believed to cure or reduce pain. Jualing (*Micromelum minutum* Wight & Arn.) is one of the medicinal plants with characteristics of oval-shaped compound leaves, wavy edges and spots on the upper surface (Suryanto & Syaifuddin, 2017; Uji, 2005). The people of South Kalimantan empirically use Micromelum minutum as traditional medicine for influenza, hypertension and stomachache. Carbazole alkaloids, coumarin, Mahanine compounds, and some terpenoid derivatives were found in *M. minutum*. Mahanine compounds have the potential to be anticancer, antioxidant, antimicrobial and have anti-inflammatory effects (Roy *et al*., 2017; Nooron *et al*., 2017). The compound was isolated from the simplicia extract of the leaves of *M. minutum*.

Simplicia is a dry material that has not undergone any processing intended for treatment (National Agency for Drug and Food Control Republic of Indonesia, 2014). Extracts are concentrated preparations which all or

almost all solvents have evaporated from active substances that are attracted during the extraction process (Ministry of Health Republic of Indonesia, 2014). The method that is often used in attracting chemical components in plants is the maceration method, which is the immersion of simplicia powder in a solvent at room temperature for several days (Sutomo *et al.*, 2017). The use of the maceration method can avoid damage to thermolabile compounds (not resistant to heating).

Utilization of plants both in the form of simplicia and extracts requires standardization in order to guarantee the quality, efficacy and safety of medicinal raw materials following the requirements stipulated in the Indonesian Herbal Pharmacopoeia, Materia Medika Indonesia and Regulation of the Minister of Health of the Republic of Indonesia. Standardization consists of specific parameters and non-specific parameters. Specific parameters are a series of benchmarks that focus on compounds responsible for pharmacological effects including organoleptic, microscopic, soluble compounds in certain solvents, and others (Ministry of Health Republic of Indonesia, 2000), while non-specific parameters are parameters that focus on material stability and safety (Saefudin *et al.*, 2011). Non-specific parameters include water content, ash content, drying losses, heavy metal contamination, and others (Ministry of Health Republic of Indonesia, 2008). Based on this, this study aims to provide information on specific and non-specific parameters of *M. minutum* simplicia and leaf extracts.

MATERIALS AND METHODS

Tools and materials

The tools used in this study are glassware, sprayers, vaporizer cups, chambers, separating funnels, glass covers, desiccators, furnaces (Ney-Vulcan® D-550), hot plates, heater mantles, Atomic Absorption Spectroscopy (Shimadzu® ASC -7000), UV light sources 254 and 366 nm, maceration vessels, microscopes (Olympus®), glass objects, ovens, capillaries, distillation devices, analytical scales (Pioneers®), and water bath (Memmert®).

The materials used in this study were fresh leaves of *M. minutum*, acetic acid, aquadest, $Cd(NO_3)_2$, ethanol, ethyl acetate, $FeCl_3$, 1% gelatin, HCl, $HgCl_2$, HNO_3, H_2SO_4, KOH, filter paper, chloroform, methanol, n-hexane, NH_4OH, $Pb(NO_3)_2$, Dragendorff reagents, Liebermann-Burchard reagents, Mayer reagents, Mg powders, Thin-layer chromatography (TLC) with GF_{254} silica gel, and toluene.

Sample preparation

Simplicia *M. minutum* in powder form as much 500 g was macerated with 96% ethanol solvent for 3 x 24 hours with stirring every eight hours and every 24 hours a change of solvent of the same type and volume was carried out. The macerate is then filtered using filter paper and thickened on a water bath at 50°C. Evaporation is carried out to obtain extracts of *M. minutum* leaf with a fixed weight (Ministry of Health Republic of Indonesia, 2008).

Simplicia standardization

Specific parameters tests

a. *Organoleptic test*

   Organoleptic tests include examining the color, shape, aroma, and taste using the five senses without the aid of tools after simplicia in contact with air for 15 minutes (Ministry of Health Republic of Indonesia, 2008).

b. *Microscopic test*

   Microscopic testing involves observing the constituent fragments obtained from the cross and longitudinal section from simplicia of *M. minutum* leaves. Observations were carried out under an electric microscope with magnifications of 100x and 400x (Ministry of Health Republic of Indonesia, 2008). Observation at magnifications of 10x and 40x is generally rarely done

c. *Ethanol soluble extract and water-soluble extract*

A total of 5 g of *M. minutum* simplicia powder was macerated using 100 ml 96% ethanol solvent and chloroform saturated water. The extract is shaken in the first six hours and allowed to stand for the next 18 hours. A total of 20 ml of filtrate was filtered and evaporated on a water bath at 105°C until a constant weight was obtained. Levels are calculated in percent of compounds dissolved by the weight of the initial ingredients of simplicia (Ministry of Health Republic of Indonesia, 2008).

Non-specific parameters tests

a. *Dry shrinkage*

A total of 1 g of *M. minutum* simplicia leaves were evenly inserted into the crucible which has been equalized. The crucible containing simplicia is put into an oven at 105°C for one hour in an open position. Crucible then weighed and reheated until a constant weight is obtained (Ministry of Health Republic of Indonesia, 2008).

b. *Total ash and insoluble acid ash*

A total of 3 g of *M. minutum* leaf simplicia powder was put into the crucibles which had been tapped and flattened in the furnace at 800°C, cooled and weighed. Ash obtained from the determination of total ash content, added 25 ml of HCl 1 N and boiled for five minutes. The acid-insoluble part is collected and filtered. The results are washed with hot water and incanded in a crucible, then weighed until a constant weight is obtained (Ministry of Health Republic of Indonesia, 2008).

c. *Heavy metal contamination (Pb, Cd, Hg)*

A total of 50 g of *M. minutum* simplicia was dissolved in the furnace at 450°C for 18 hours. Ash was added by 5 ml of HCl 6 M and evaporated to dryness, then 10 ml of HNO_3 was added by 10 ml. The test solution was put into a 50 ml measuring flask, and 0.1 M HNO_3 was added to the limit mark. The Pb level was measured using Atomic Absorption Spectroscopy at wavelength 217 nm, Cd content at wavelength 288.8 nm, and Hg was measured at a wavelength of 253.7 nm (Najib *et al*., 2017).

Extract standardization

Specific parameters tests

a. *Organoleptic extract*

Organoleptic extracts include an examination of color, shape, aroma, and taste using the five senses without the aid of tools after the extract has been in contact with air for 15 minutes (Ministry of Health Republic of Indonesia, 2008).

b. *Percentage of Yield*

Extract yield was obtained from the ratio of extract weight obtained to the weight of *M. minutum* leaf simplicia (Ministry of Health Republic of Indonesia, 2008).

c. *Phytochemical screening*

Phytochemical screening is carried out on compounds of alkaloids, terpenoids/steroids, flavonoids, saponins, tannins, anthraquinones, glycosides and phenolics. The ethanol extract of *M. minutum* leaves as much as 0.5 g was dissolved in 25 ml 96% ethanol and divided into eight tubes. Dragendorff and Mayer's reagents for identification of alkaloids, and Liebermann-Burchard reagents for terpenoids and steroids. The identification of flavonoids used concentrated Mg and HCl powders. Foam test with water is used for identification of saponins. Gelatin 1% for the tannin test and 10% methanolic KOH for the anthraquinone test. Identification of glycosides used concentrated chloroform and H_2SO_4. Identification of phenolic 10% was carried out using 10% $FeCl_3$.

d. *Thin-layer chromatography profile*

The ethanol extract of *M. minutum* leaves as much as 0.1 g was dissolved with 1 ml methanol. Test

solutions from the three different locations were made a stain on the GF_{254} TLC plate and eluted with n-hexane : ethyl acetate (8 : 2) v/v and chloroform : methanol (9 : 1) v/v. The stain observation was carried out with UV light of 254 nm, UV 366 nm, and 10% H_2SO_4 reagent.

Non-specific parameters tests

a. *Water content*

Determination of water content is processed by distillation. A total of 5 g of extract was put into a round bottom flask, and 200 ml of water-saturated toluene were added. Pumpkin is heated at 105°C for 150 minutes. The volume of water is measured and calculated against the weight of the initial extract in percent (Ministry of Health Republic of Indonesia, 2008).

b. *Total ash and insoluble acid ash*

The ethanol extract of *M. minutum* leaves as much as 3 g was put into a silicate crucible that had been equalized and incandescent in a furnace at 800°C, then cooled and weighed. The obtained ash was then added 25 ml of HCl 1 N and boiled for five minutes. The acid-insoluble part is collected and filtered. The results are washed with hot water and incanded in a crucible, then weighed until a constant weight is obtained (Ministry of Health Republic of Indonesia, 2008).

Data analysis

The data obtained in this study were quantitative and qualitative data analyzed descriptively. Quantitative data include water content, water-soluble extract, ethanol-soluble extract, total ash content, acid insoluble ash content, heavy metal contamination (Cd, Pb, and Hg), drying losses and yield. Qualitative data obtained included microscopic tests, organoleptic tests, chromatographic patterns and phytochemical screening.

RESULTS AND DISCUSSION

The results of the plant identification used for the study were *M. minutum* Wight & Arn of the Rutaceae tribe, according to a letter from the Bogor Indonesian Institute of Sciences No. B-145/IPH.3./KS/II/2019. The extraction carried out on simplicia showed similarities to the amount of extract obtained, namely for plants originating from the Banua Botanical Garden, Sultan Adam Forest Park, and Forest Areas with Specific Objects of Rantau, South Kalimantan respectively, 8.32%; 8.08%; and 8.15%.

Organoleptic simplicia examination aims to provide characteristics that make it easier to recognize the physical characteristics of *M. minutum* leaf simplicia. The results of organoleptic simplicia of *M. minutum* leaves are a brownish-green, bitter and slightly spicy and distinctive odor. Green color due to chlorophyll content. The bitter taste is possible from alkaloid compounds and saponins, while the spicy taste comes from the content of essential oils. Like most types of Rutaceae, *M. minutum* leaves have a strong, pungent odor.

The results of observations on the longitudinal cross-section of *M. minutum* leaf showed the presence of multi-faceted epidermal cell walls with straight and wavy anticlinal walls, cytoplasm, calcium oxalate crystals and anomocytic type stomata on the underside of the leaves. Cross-section of the leaves of *M. minutum* shows the presence of upper epidermis, palisade tissue, spongy tissue, cortex, xylem, phloem, lower epidermis, and trichomes. *M. minutum* leaves obtained from the Banua Botanical Gardens have a thicker palisade network than the other two samples which illustrate the amount of sun received by the *M. minutum* at the Banua Botanical Gardens more than the other two locations. The results of ethanol-soluble extract, water-soluble extract, drying shrinkage, ash content, and metal contamination (Pb, Cd,

and Hg) levels on the *M. minutum* leaves simplicia are presented in **Table I**.

Table I. The results of the standardization parameters of *M. minutum* leaf simplicia standardization

Parameters	Results			Standard*
	Banua Botanical Garden	Sultan Adam Forest Park	Forest Areas with Specific Objects of Rantau	
Ethanol soluble essence (%)	13.17	12.87	12.93	>16
Water-soluble essence (%)	22.07	21.95	21.93	>8
Dry shrinkage (%)	4.76	4.64	4.84	<10
Total ash content (%)	6.04	6.14	6.07	<16.6
Acid insoluble ash content (%)	1.13	1.19	1.17	<2
Pb level (mg/kg)	0.022	0.025	0.022	≤10
Cd level (mg/kg)	0.018	0.017	0.020	≤0.3
Hg level (mg/kg)	<0.00004	<0.00004	<0.00004	≤0.5

* According to Materia Medika Indonesia and National Agency for Drug and Food Control Republic of Indonesia

Data on ethanol-soluble extract levels are used as an initial description of the number of dissolved compounds in organic solvents. Determination of water-soluble extracts is useful as a data of the number of compounds that can be found in water if the simplicia of *M. minutum* leaves is used as a raw material for traditional medicine. Compounds dissolved in water include, among others, quaternary alkaloids, saponins, carotenoids, tannins, and others. Compounds dissolved in ethanol in the form of phenols, terpenoids, steroids, alkaloids, aglycones, glycosides, and volatile oils (Hardiana *et al*., 2012; Panjaitan *et al*., 2012; Zulharmita *et al*., 2012).

Determination of drying shrinkage aims to provide limits and ranges of compounds lost during the sample drying process (Ministry of Health Republic of Indonesia, 2000). The smaller the drying shrinkage value, the better the drying process is carried out so that, the smaller the risk of simplicia is easily overgrown with mold and fungus. If the water content in simplicia is high, then most of the weights that are weighted when weighing the simplicia for extracting are water weights so that the compound of interest is smaller (Isnawati *et al*., 2004).

Total ash content indicates the number of minerals present in the sample, both internal and external minerals. Internal minerals are minerals that come from plant tissue itself, such as calcium, phosphorus, and magnesium (Utami *et al*., 2017; World Health Organization, 1998). Guntarti *et al*. (2015) in his research stated that high levels of acid-insoluble ash are caused by external minerals in the form of silicate contamination such as soil and sand and metal elements during the processing of samples to form simplicia.

The most accumulation of heavy metal is found in leaves through roots or from absorption by stomata (Heriyanto & Subiandono, 2011). The highest Pb level was obtained by simplicia from Sultan Adam Forest Park at 0.025 mg/kg. This is due to the distance between the locations growing *M. minutum* in Sultan Adam Forest Park is closer to the road than the other two locations. Pasaribu *et al*. (2017) mention that the largest source of Pb pollution comes from the combustion of vehicle engine fuels. The highest Cd level obtained was simplicia taken from Forest Areas with Specific Objects of Rantau was 0.020 mg/kg due to mining and distribution activities around the coal mine (Warni *et al*., 2017). Hg levels in the three growing sites are below the permitted level because the location of *M. minutum* grows in three places far from the source of Hg pollutants in the form of pesticides, industrial activities, household waste, and gold mining.

Organoleptic examination results of *M. minutum* leaf extract showed characteristics with a bitter taste, distinctive odor, and dark green color. Phytochemical screening results of *M. minutum* leaf extracts from the

Banua Botanical Garden, Sultan Adam Forest Park, and Forest Areas with Specific Objects of Rantau prove that the secondary metabolites contained in *M. minutum* leave that grow in different locations qualitatively have the same class of compounds. Phytochemical screening results from *M. minutum* leaves of ethanol extract can be seen in **Table II**.

Table II. Phytochemical screening results of *M. minutum* leaf ethanol extract

Compound group	Results		
	Banua Botanical Garden	Sultan Adam Forest Park	Forest Areas with Specific Objects of Rantau
Alkaloids	+	+	+
Terpenoids	+	+	+
Flavonoids	+	+	+
Saponins	+	+	+
Tannins	+	+	+
Anthraquinones	+	+	+
Glycosides	+	+	+
Phenolics	+	+	+

The observation of the TLC profile showed that the three samples taken from different locations showed almost a similar chromatogram pattern as seen from the Rf value of the spots on the sample. In the observation of spots with 10% H_2SO_4 reagents, eight spots were obtained on the n-hexane : ethyl acetate (8 : 2) v/v and nine spots on the chloroform : methanol (9 : 1) v/v. The Rf value of the *M. minutum* leaves of ethanol extract from the n-hexane : ethyl acetate (8 : 2) and chloroform : methanol (9 : 1) can be seen in **Table III** and **Table IV**, respectively.

Table III. The Rf value of ethanol extract of *M. minutum* leaves in H_2SO_4 spray reagents obtained on the n-hexane : ethyl acetate (8 : 2) v/v

No	Rf value		
	Banua Botanical Garden	Sultan Adam Forest Park	Forest Areas with Specific Objects of Rantau
1	0.23	0.22	0.20
2	0.26	0.25	0.25
3	0.29	0.30	0.29
4	0.38	0.39	0.38
5	0.48	0.47	0.47
6	0.59	0.58	0.58
7	0.89	0.89	0.89
8	0.98	0.98	0.98

Table IV. The Rf value of ethanol extract of *M. minutum* leaves in H_2SO_4 spray reagents obtained on the chloroform : methanol (9 : 1) v/v

No	Rf value		
	Banua Botanical Garden	Sultan Adam Forest Park	Forest Areas with Specific Objects of Rantau
1	0.20	0.20	0.20
2	0.34	0.34	0.34
3	0.47	0.47	0.50
4	0.55	0.55	0.59
5	0.66	0.65	0.71
6	0.73	0.71	0.75
7	0.82	0.82	0.84
8	0.89	0.89	0.91
9	0.97	0.97	0.97

The moisture content of the *M. minutum* leaves ethanol obtained from three locations fulfills the requirements set in the National Agency for Drug and Food Control Republic of Indonesia (2014), which is less than 10%. High levels of water cause low shelf life of extracts because they are prone to fungi (Setyorini *et al.*, 2016). One of them is *Aspergillus flavus* which can produce aflatoxins which are harmful to health (Ratnani *et al.*, 2017). The results of determining the yield, water content, total ash content, and acid insoluble ash content of the ethanol extract of *M. minutum* leaves are presented in **Table V**.

Table V. The results of the standardization parameters of the ethanol extract of *M. minutum* leaves

Parameters	Results			Standard*
	Banua Botanical Garden	Sultan Adam Forest Park	Forest Areas with Specific Objects of Rantau	
Yield (%)	8.32	8.08	8.15	-
Water content (%)	6.07	6.30	6.27	<10
Total ash content (%)	4.22	4.27	4.23	<8.9
Acid insoluble ash content (%)	0.11	0.12	0.11	<0.7

* According to Materia Medika Indonesia and National Agency for Drug and Food Control Republic of Indonesia

Ash content and an insoluble ash content of extract acid have a relationship with ash content of simplicia. The content of total ash and acid insoluble ash extract of *M. minutum* leaf has a lower value compared to the *M.*

minutum leaf simplicia ash content level. Based on these data, it can be said that during the extraction process, some minerals, both internal and external such as sand and soil, did not come along with the extract. Total ash content and an insoluble ash content of the ethanol extract of *M. minutum* leaves meet the maximum limit of ash content regulated in Materia Medika Indonesia.

CONCLUSION

The results of the determination of the standardization parameters of simplicia and extracts of *M. minutum* leaves obtained at Banua Botanical Garden, Sultan Adam Forest Park, and Forest Areas with Specific Objects of Rantau, South Kalimantan have met the quality requirements of raw materials for traditional medicines stipulated by the Government.

ACKNOWLEDGMENT

Authors would like to thank the Banua Botanical Gardens for research funding as well as to all parties involved in completing this research. Authors also thanked Mr Mohammad Rizki Fadhil Pratama from Universitas Muhammadiyah Palangkaraya for his assistance in translating this article.

REFERENCES

Guntarti, A., Sholehah, K., Irna, N., Fistianingrum, W. 2016. Penentuan Parameter Non Spesifik Ekstrak Etanol Kulit Buah Manggis (Garcinia mangostana) Pada Variasi Asal Daerah. *Farmasains*. **2**(5):1-6.

Hardiana, R., Rudiyansyah, Zaharah, T.A. 2012. Aktivitas Antioksidan Senyawa Golongan Fenol dari Beberapa Jenis Tumbuhan Famili Malvaceae. *Jurnal Kimia Khatulistiwa*. **1**(1): 8-13.

Heriyanto, N.M., Subiandono, E. 2011. Penyerapan Polutan Logam berat (Hg, Pb dan Cu) Oleh Jenis-Jenis Mangrove. *Jurnal Penelitian Hutan dan Konservasi Alam*. **8**(2):177-188. https://doi.org/10.20886/jphka.2011.8.2.177-188

Isnawati, A., Alegantina, S., Raini, M., Nikmah, B. 2004. Karakterisasi Simplisia dan Ekstrak Daun Strobilanthus crispus. *Media Penelitian dan Pengembangan Kesehatan*. **14**(2):20-25.

Ministry of Health Republic of Indonesia. 2014. *Farmakope Indonesia Edisi Kelima*. Jakarta: Ministry of Health Republic of Indonesia.

Ministry of Health Republic of Indonesia. 2008. *Farmakope Herbal Indonesia Edisi Pertama*. Jakarta: Ministry of Health Republic of Indonesia.

Ministry of Health Republic of Indonesia. 2000. *Parameter Standar Umum Ekstrak Tumbuhan Obat Edisi I*. Jakarta: Directorate General of Drug and Food Control and Directorate of Traditional Medicines Control.

Najib, A., Malik, A. Ahmad, A.R., Handayani, V., Syarif, R.A., Waris, R. 2017. Standarisasi Ekstrak Air Daun Jati Belanda dan Teh Hijau. *Jurnal Fitofarmaka Indonesia*. **4**(2):241-245. https://doi.org/10.33096/jffi.v4i2.268

National Agency for Drug and Food Control Republic of Indonesia. 2014. *Peraturan Kepala Badan Pengawas Obat dan Makanan Republik Indonesia. Nomor 12 Tahun 2014 tentang Persyaratan Mutu Obat Tradisional*. Jakarta: National Agency for Drug and Food Control Republic of Indonesia.

Nooron, N., Athipornchai, A., Suksamrarn, A., Chiabchalard, A. 2017. Mahanine Enhances the Glucose-Lowering Mechanisms in Skeletal Muscle and Adipocyte Cells. *Biochemical and Biophysical Research Communications*. **494**(1-2):101-106. https://doi.org/10.1016/j.bbrc.2017.10.075

Panjaitan, E.N., Saragih, A., Purba, D. 2012. Formulasi Gel dari Ekstrak Rimpang Jahe Merah (Zingiber officinale Roscoe). *Journal of Pharmaceutics and Pharmacology*. **1**(1):9-20.

Pasaribu, C.A., Sarifuddin, S., Marbun, P. 2017. Kandungan Logam Berat Pb Pada Kol Dan Tomat Di Beberapa Kecamatan Kabupaten Karo. *Agroekoteknologi*. **5**(2):355-361.

Ratnani, R.D., Hartati, I., Anas, Y., Endah, D.P., Khilyati, D.D. 2017. Standardisasi Spesifik dan Non Spesifik Ekstraksi Hidrotropi Andrographolid dari Sambiloto (Andrographis paniculata). In *Prosiding Seminar Nasional Peluang Herbal sebagai Alternative Medicine, Fakultas Farmasi, Universitas Wahid Hasyim*. Semarang: Universitas Wahid Hasyim, 147-155.

Roy, S., Dutta, D., Satyavarapu, E.M., Yadav, P.K., Mandal, C., Kar, S., Mandal, C. 2017. Mahanine Exerts In Vitro and In Vivo Antileishmanial Activity by Modulation of Redox Homeostasis. *Scientific reports*. **7**(4141): 1-16. https://doi.org/10.1038/s41598-017-03943-y

Saefudin, A., Rahayu, A., Teruna, H. Y. 2011. *Standarisasi Bahan Obat Alam 2*. Yogyakarta: Graha Ilmu.

Setyorini, H.A., Kurniatri, A.A., Adelina, R., Adelina, A. 2016. Karakterisasi Mutu Ekstrak Daun Sirsak (Annona muricata L.) dari Tiga Tempat Tumbuh. *Buletin Penelitian Kesehatan*. **44**(4): 279-286. http://dx.doi.org/10.22435/bpk.v44i4.5184.279-286

Suryanto, E., Syaifuddin. 2017. *Tumbuhan Obat di KHDTK Rantau, Kalimantan Selatan*. Bogor: Forda Press.

Sutomo, S., Azhari, H., Arnida, A., Fadlilaturrahmah, F., Yunus, R. 2017. Isolasi Dan Identifikasi Senyawa Antioksidan dari Buah Kasturi (Mangifera casturi Kosterm.). *Jurnal Pharmascience*. **4**(2):246-254. http://dx.doi.org/10.20527/jps.v4i2.5778

Uji, T. 2005. Studi Taksonomi Micrommelum blume (Rutaceae) di Indonesia. *Biodiversitas*. **6**(2): 100-102. http://doi.org/10.13057/biodiv/d060206

Utami, Y. P., Umar, A.H., Syahruni, R., Kadullah, I. 2017. Standardisasi Simplisia dan Ekstrak Etanol Daun Leilem (Clerodendrum minahassae Teisjm. & Binn). *Journal of Pharmaceutical and Medicinal Sciences*. **2**(1):32-39.

Warni, D., Karina, S., Nurfadillah, N. 2017. Analisis Logam Pb, Mn, Cu dan Cd Pada Sedimen di Pelabuhan Jetty Meulaboh, Aceh Barat. *Jurnal Ilmiah Mahasiswa Kelautan Perikanan Unsyiah*. **2**(2):246-253.

World Health Organization. 1998. *Quality Control Methods for Medicinal Plant Materials*. Switzerland: World Health Organization.

Zulharmitta, Z., Kasypiah, U., Rivai, H. 2017. Pembuatan dan Karakterisasi Ekstrak Kering Daun Jambu Biji (Psidium guajava L.). *Jurnal Farmasi Higea*. **4**(2):147-157.