Standardization of Spray-Dried Powder of *Morinda citrifolia* Fruit Extract

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Abstract. *Morinda citrifolia L.* (Mengkudu) has been widely used in herbal medicine to prevent and treat various diseases. In the Indonesian Original Herbal Medicine Formularies list, Mengkudu is an ingredient for treating hypertension and dyslipidemia. The various efficacy activities are due to the great active compound content in the extract. All bioactive compounds in herbal extracts are highly susceptible to degradation by environmental and chemical factors that will affect the activation of compounds in the extract. The encapsulation method is a prospective strategy for maintaining the stability of the active compounds in herbal extracts. Spray drying encapsulation technology is one of the most efficient and widely used methods for encapsulating bioactive compounds from natural extract. The encapsulation of Mengkudu fruit extract can provide protection over the bioactive compounds in the extract and can mask the unpleasant taste. In the current study, Mengkudu fruit was extracted with 70% ethanol then encapsulated using spray drying using 50% (w/w) maltodextrin as encapsulate. This study aims to standardize spray-dried Mengkudu fruit extract powder. A variety of physicochemical parameters analysis e.g., total ash, water-soluble ash, and acid insoluble ash, moisture content, and loss on drying, were analyzed as per the standard methods including microbial contamination and heavy metal contaminant was measured by Inductively coupled plasma - optical emission spectrometry (ICP-OES). The fingerprinting of ethanolic extract and spray-dried powder of Mengkudu was also carried out using the CAMAG-HPTLC system to determine scopoletin as a marker compound. The encapsulation yield of the spray drying process was also measured. This process was carried out on a pilot-scale spray drying using maltodextrin as a coating material. The water content, total ash, acid insoluble ash, water-soluble ash, and encapsulation yield were 3.31, 0.6, 0.07, 0.53, and 60.53, respectively. The results of the ICP-OES analysis showed that no heavy metals were detected in the powder, while the pathogenic microbial contamination test also showed negative results. HPTLC analysis revealed that the scopoletin content was 1.43 mg/g of spray-dried powder. In conclusion, the results obtained from this study can be used to standardize spray-dried powder of noni fruit extract in the manufacture of standardized herbal medicine.

Keywords: Encapsulation · Mengkudu fruits extract · Spray-dried powder · Standardized extract

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1 Introduction

The World Health Organization's (WHO) strategy, 2014–2023, aims to strengthen the role of traditional medicine, emphasizing the importance of promoting and including the utilization of medicinal plants in the health systems both in developed and developing countries [1]. Indonesia has an enormous variety of plants with medicinal properties, and traditional herbal medicines used for promotive, preventive, and curative purposes such as *jamu* are still widely used by both urban and rural populations. *Jamu* is a national culture and asset that is need to be explored, researched, developed, and optimized for its utilization to become a competitive object [2]. Herbs as raw materials for treatment, clinically and empirically, are often used in the wider community. However, it is often found that it has not been standardized, so it does not guarantee the safety of the efficacy, and quality of the plant. Herbal simplicia is used for treatment that requires standardization to ensure the drug's quality, safety, and pharmacological efficacy [3, 4]. Moreover, it is necessary to validate the quality of traditional medicine to avoid any adverse effects [5]. According to the American Herbal Product Association, standardization is an information and control that must be carried out to obtain a product with a consistent composition of sustainable results and guaranteed quality and efficacy [6]. Standardization is crucial to do to develop drugs from natural ingredients that are widespread in Indonesia to ensure the quality and safety of these drug preparations, which can later be developed into standardized phytopharmaceuticals or herbal medicines.

Morinda citrifolia L, commonly known as Noni in America and Mengkudu in Indonesia, has been widely used in traditional Polynesian medicine for over 2000 years for prevent and treat various diseases. Mengkudu is listed as an herb for the treatment of hypertension and dyslipidemia on the list of the Original Indonesian Herbal Medicines Formulary, which contains a list of medicinal plants that have been proven to be safe, efficacious, and of good quality [7]. The various efficacy activities are due to the great active compound from the mengkudu fruit such as: 3-chloro-8-methylthio11H-indol-[3,2-c] quinolone, 5-Pregnen-3β-ol-20 onetrifluoroacetate, α-amyrin, pinene, (2E) -3, 7, 11, 15-tetramethyl-2- hexadecen-1-ol, stigmasterol, lignan Americanin A, quercetin 3-O-β-D-glucopyranoside, octanoic acid, potassium, vitamin C, iridoids, terpenoid, alkaloid, anthraquinone, morindone, rubiadine and rubiadine-1-methyl ether, and the marker compound is scopoletin [8–10].

Plant secondary metabolites play an important role in determining of biological activities of medicinal plants used in traditional medicine. All bioactive compounds in herbal extracts are highly susceptible to degradation by environmental and chemical factors that will affect the activation of compounds in the extract. The encapsulation method is a prospective strategy for maintaining the stability of the active compounds in herbal extracts. Encapsulation has been widely used in the chemical, pharmaceutical, and food industries to protect bioactive compounds from environmental conditions such as temperature, pH, and light [11]. Encapsulation can also improve the characteristics of bioactive compounds, such as increased solubility in water. The main challenge of encapsulation is the selection of encapsulants that must have good binding properties, are non-hygroscopic, and do not easily aggregate [12] Maltodextrin is one commonly

used encapsulant, which has gelling and film-forming properties is a suitable binder, and is soluble in cold water [13]. Encapsulation can be done by spray drying process involves atomizing the extract dispersed in a polymer solution into droplets by spraying, followed by rapid evaporation, which is sprayed into a solid powder by hot air at a specific temperature and pressure [14]. Therefore, the objective of the present study is to standardize the spray-dried powder of Mengkudu extract based on physiochemical characterization and profiling of the active constituent.

2 Material and Methods

2.1 Chemical Reagents

Ethanol food grade were obtained from local supplier. Maltodextrin (DE10-12) from PT Natura Nuswantara Nirmala (Jakarta). Follin-Ciocalteu 2N were purchased from Sigma. Gallic acid, quercetin, vanillin, Na_2CO_3, $AlCl_3$, $NaNO_2$, NaOH, and H_2SO_4, were obtained from Merck.

2.2 Plant Materials

The Mengkudu fruit was collected from Sentul Bogor on February 2022. The fruit were washed with tap water followed by distilled water then drained and cut in to small species by Fomac VGC-J 23 A. After that the pieces were dried in a hot-air oven operated at 50 °C (Memmert - UF750), powdered and passed through sieve 14 to obtain uniform size powdered material.

2.3 Preparation of Mengkudu Fruit Extract and Spray Dried Powder

The dried powdered of mengkudu fruit (15 kg) was subjected to maceration with ethanol-water 70:30 (v/v) at pilot plant; solvent ratio of 1:10 (w/v), for 24 h at room temperature. The extracted process was continued by percolation using new solvent for 2 h at 50 °C. The extract was combined and concentrated under a vacuum until the total solid content was around 8–10%.

The spray-drying process was carried out on a lab scale and a factory scale. The lab-scale spray-drying process was done by dissolving the thick extract of the noni fruit in distilled water to obtain a TDS value of 10%. The filler material (maltodextrin) was then added in a particular ratio (1:1, 1:2, 2:1). Finally, the mixed solution was fed to a lab-plan spray dryer (Closed Cycle Spray Dryer; model: CL-8, Ohkawara Kakohki- Japan) with a feed flow rate of 500 ml/h and an inlet temperature of 150 °C. In comparison, the spray-drying process in the industrial process was carried out with an inlet temperature of 190 °C and an outlet of 90 °C with a feed flow rate of 300 ml/minute. At the end of the process, the powder formed is collected, weighed, packed in a closed container, and stored dry for further characterization i.e. encapsulation yield, size, and morphology of the Noni spray-dried powder.

The encapsulation yield was calculated as the ratio of the mass of microcapsules obtained after spray drying to the total mass of the initial substance added (extract and maltodextrin) before spray drying [13]. All measurement were made in triplicate.

The particle size of the spray dried powder was measured by using Horiba Partica LA-960. All measurement were made in triplicate. The morphology of the microcapsules was analyzed by *Scanning Electron Microscope (SEM), a* small amount of sample was fixed on metallic stubs and coated with a thin layer of gold under vacuum using a sputter coater *(Hitachi SU-3500 and JSM-IT200).*

2.4 Physicochemical Analysis of Mengkudu Spray Dried Powder

The physicochemical standardization studies of the spray dried of Menkudu fruit extract were carried out according to Indonesian Pharmacopeia Herbal [7] guidelines on the quality control methods for medicinal plant materials and various other pharmacopeial procedures. The variety of physicochemical parameter such as moisture content, loss on drying, total ash content, acid insoluble and water-soluble ash contents. Analysis presence or absence of heavy metal (Hg, Pb, Cd, and as) and microbial contaminations were carried out as per standard methods.

2.5 Phytochemical Analysis of Mengkudu Spray Dried Powder

Phytochemical analysis to detect the presence of alkaloid, flavonoids, triterpenes/steroid, and saponin in the extract using standard methods was carried out using colorimetric methods according to previous study with slight modification [15]. The total phenolic content was estimated according to Folin-Ciocalteu method and total flavonoid was determined according to aluminums trichloride method using quercetin as the reference compound [16]. The total phenolic content in sample is expressed as gallic acid equivalent (GAE) (mg/g of dry powder extract). Whereas, the flavonoid content in the sample extract is reported as Quercetin equivalent (QE) (mg/g of dry powder extract).

2.6 HPTLC Fingerprinting of Mengkudu Spray Dried Powder

A quantitative HPTLC fingerprinting analysis (CAMAG HPTLC system with a Linomat 5 sample applicator, Switzerland) of spray-dried powder extract was carried out for development of characteristic fingerprint profile to confirm the presence and level of scopoletin as marker. Stock solution of standard scopoletin was prepared in methanol. The working solution of standard compound was subsequently diluted in methanol to afford a series of scopoletin solution of 10, 50, 100, 200 and 500 μg/mL. The spray-dried powder of mengkudu extracts (1 g) were dissolved in 50 mL methanol and then the mixture was centrifuged at 5000 rpm for 15 min. The supernatant was used as sample solution. Chromatographic development was carried out on 10x10 cm precoated silica gel 60 F_{254} aluminium plate (E. Merck) chamber with ethyl acetate–n-hexane (3:2, V/V) as mobile phase. Two (2) μL of standard compound and extracts were applied to the plate using of an automatic specimen applicator (CAMAG Linimoat 5, Switzerland), fitted with a Hamilton microliter syringe (Bonaduz, Switzerland). The conditions were

set at band length 8 mm. HPTLC plates: 20 × 10 cm, 0.2 mm thickness pre-coated with silica gel 60 F254; Merck. Band size: 6 mm, slit dimension: 5.00 × 0.45 mm. Scanning speed: 10 mm/s. Experimental conditions: temperature was 28 ± 2 °C; relative humidity was 40%. After developing, the TLC plate was dried using an air dryer and for post-chromatographic treatment sulfuric acid in methanol (5%) reagent was used as visualization agent. Quantification was conducted by using HPTLC Scanner 4 linked to Vision CATS basic version. Scanning of bands were performed at 366 nm Scanning speed: 10 mm/s, and source of radiation: deuterium lamp.

2.7 Statistical Analysis

Data were analyzed using Microsoft Excel and reported as mean ± standard deviation of triplicate determination.

3 Results

3.1 The Mengkudu Spray-Dried Powder Characterization

The Particle size analysis of pilot scale Mengkudu spray dried powder was 13.41 ± 0.15 μm.

3.2 Physicochemical Analysis of Mengkudu Spray Dried Powder

A variety of physicochemical parameter according to PHI were analyzed to determine the quality of spray dried powder extract was listed in Table 2.

Table 1. Encapsulation yield of Mengkudu spray dried powder

Mengkudu spray dried powder	Encapsulation yield (%)	Scopoletin (mg/g)
Lab scale		
1:1	58.67 ± 10.21	1.04 ± 13.01
1:2	71,67 ± 5.27	0.84 ± 32,60
2:1	30.05 ± 7.64	1.16 ± 10,61
Pilot scale (1:1)	64.53 ± 5.01	1.16 ± 0.25
70% Ethanol extract	-	3.31 ± 0.27

Fig. 1. Mengkudu spray dried powder from lab and pilot scale process

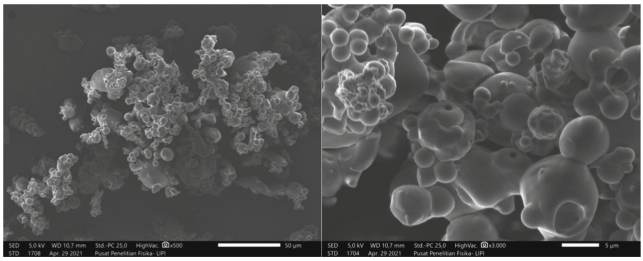

Fig. 2. Photomicrographs by SEM of spray-dried microcapsules of Mengkudu fruit extract with maltodextrin. Magnifications of 500 and 3000 X

Table 2. Physicochemical parameters of spray dried powder of Mengkudu extracts

Parameter	PHI	Mengkudu spray dried powder
Loss on drying	<10	2,52 ± 0,17
Moisture content	<10	3,70 ± 0,35
Total Ash (%)	<0.8	0.60 ± 0.05
Acid insoluble ash (%)	<0.1	0.07 ± 0.01
Heavy metal determination*		
Mercury (Hg)	Not detected	Not detected
Lead (Pb)	Not detected	Not detected
Cadmium (Cd)	Not detected	Not detected
Arsenic (As	Not detected	Not detected
Microbial contamination		
Test for *Escherichia coli*/g	Absent	Absent
Test for *Staphylococcus aureus*/g	Absent	Absent
Test for *Salmonella spp*/g	Absent	Absent
Test for *Pseudomonas aeruginosa*/g	Absent	Absent
Test for *Escherichia coli*/g	Absent	Absent

* Limit of detection (mg/kg): Pb ≤ 10, Cd ≤ 0.3, Hg ≤ 0.5, As ≤ 5

3.3 Phytochemical Analysis of Mengkudu Spray Dried Powder

The results of phytochemical analysis presented in Table 3.

3.4 HPTLC Fingerprinting of Mengkudu Spray Dried Powder

Table 3. Phytochemical analysis of Mengkudu spray dried

Parameters	Spray dried powder
Alkaloids	+
Flavonoids	+
Triterpenoids/steroids	−
Tannins	−
Saponins	++
Quinones	+
Total phenolic content (mg GAE/g)	4.84 ± 0.72
Total Flavonoid content (mg QE/g)	1.61 ± 0.63

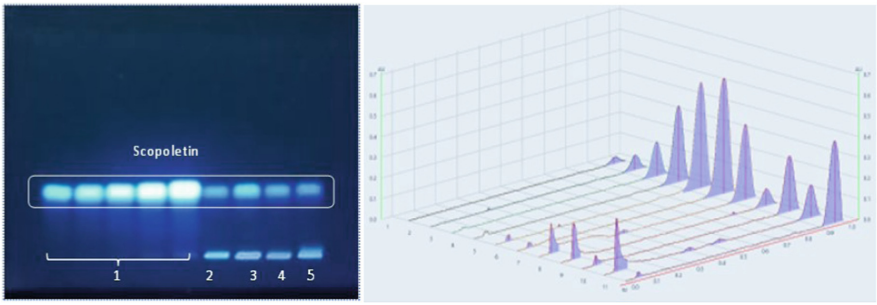

Fig. 3. HPTLC fingerprinting profile of Mengkudu slury extract and Mengkudu spray dried powder developed with hexane:ethyl acetate (3:1) as mobile phases and visualized under ultraviolet light $UV_{\lambda 366}$. Scopoletin (1), Spray dried powder lab scale (2; 2:1, 3; 1:1, 4;1:2), Spray dried pilot scale 1:1 [5]

4 Discussion

In this study, Mengkudu fruit extract obtained from 70% ethanol extraction was spray-dried to obtain the encapsulation of the bioactive components with 1:1 ratio between extract and maltodextrin as encapsulate. The spray drying process was carried out on a pilot scale with a predetermined operating system with an inlet temperature of 190 °C and an outlet of 90 °C, a feed flow rate of 300 ml/minute.

Three extracts and maltodextrin comparison formulas were carried out on a lab-scale (1–2 L): 2:1; 1; 1, and 1;2. Table 1 and Fig. 1 show the highest encapsulation yield of the 1:2 formula due to the concentration of maltodextrin, which can absorb moisture from the extract. However, the active compound level is low, while at a ratio of 2:1, the scopoletin content was slightly higher than powder 1:1. However, the yield was low due to the hygroscopic nature of the extract, causing the powders to get stuck on the chamber walls, reducing the encapsulation yield. Therefore, the drying process on a factory scale

was chosen with a 1:1 formula, with an efficiency of >60%, producing a uniform fine powder with a light brown color.

The morphology of the spray-dried Mengkudu powder obtained at pilot scale conditions was presented in Fig. 2. The microcapsules have a relatively spherical shape and varied particle sizes due to the presence of maltodextrin as a coating material which causes surface irregularities [11]. However, the lack of wall cracks and cavities on the microcapsule surface confirmed that encapsulation was successful. The Particle size analysis of pilot scale Mengkudu spray dried powder was 13.41 ± 0.15 μm.

In order to improve the quality, safety, and benefits of traditional medicines, one of the steps taken is the standardization of raw materials used in the production of traditional medicines, including the standardization of extracts. Standardization of a traditional medicinal preparation is a requirement that must be met in order to achieve reproducibility of the formula and therapeutic quality. The physicochemical parameter of Mengkudu spray dried powder was presented in Table 2 in agreement with Indonesian Herbal Pharmacopeia [7].

Standardization of herbal product/extracts is more challenging than synthetic drug. Herbal extracts contain number of constituents of complex chemical nature and are inconsistent in composition. In most of the cases the biological activity is not exclusive dependent on the active constituents, but is due to synergistic effect of all chemical constituents of the plant [17]. Phytochemical screening revealed the presence of phenolic compound, flavonoid, alkaloid, quinone, and saponin in the Mengkudu spray dried powder (Table 3). These results also show that the spray drying process qualitatively does not damage the content of active compounds in the extract. However, it still has to be analyzed further, for example, by comparing the LCMS-MS profile of the extract before and after the spar drying process.

HPTLC technique is an important analytical tool for identification, detection, and separation, and some other assessment of plant and their product [18]. The HPTLC of Mengkudu spray-dried powder was carried out by using Hexan: Ethy acetate solvent system. According to PHI [7], scopoletin has been used as biomarker for the standardization of Mengkudu raw drug and extract. Scopoletin, a coumarine compound which shows emits blue fluorescence spot on TLC plate under UV irradiation at 365 nm. As shown in Fig. 3, scopoletin presence in all Mengkudu spray dried powder is in accordance to standard marker. The scopoletin content was 1.43 ± 0.72 mg/g Mengkudu spray dried powder.

5 Conclusion

In conclusion, the standardization analysis of Mengkudu spray dry powder meets the requirements for herbal medicine. Furthermore, this spray drying process can protect the extract's bioactive compounds and mask the unpleasant taste of the Mengkudu fruit extract. The resulting powder will facilitate the final preparation of Mengkudu fruit extract.

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