Standardization of Some Indonesian Medicinal Plants Used in “Scientific Jamu”

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Abstract. Jamu is Indonesian indigenous herbal medicine that has been used empirically to prevent and treat various diseases. To provide evidence on its safety and efficacy, Indonesian government has developed Jamu into Standardized Herbal Medicine and Phytopharmaca. Another strategy is development of Jamu into Scientific Jamu. This herbal medicine has assurance on safety and efficacy through health service-based research. Its raw material is various crude drugs. The problem of this type of raw material is that, depending on the environmental conditions, the quality can vary significantly. This study aimed to standardize crude drug of four medicinal plants included in the composition of Scientific Jamu. They are Orthosiphonis Staminei Folium, Centella Asiatica Herba, Curcumae Domesticae Rhizoma, and Curcumae Xanthorrhizae Rhizoma which were collected from three different origins in Indonesia, i.e.: Batu, Bogor, and Tawangmangu Districts. Standardization was conducted by determination of specific parameters (macroscopic, microscopic, total phenolics or flavonoids content, water and ethanol soluble extract) and non specific parameters (loss on drying, total ash, acid-insoluble ash). The results were then compared to Indonesian Herbal Pharmacopoeia to conclude whether the crude drugs have a good quality. Orthosiphonis Staminei Folium, Centella Asiatica Herba, Curcumae Domesticae Rhizoma, and Curcumae Xanthorrhizae Rhizoma from Batu, Tawangmangu, and from Bogor met specific parameters (macroscopic, microscopic, water and ethanol soluble extracts) as required by Indonesian Herbal Pharmacopoeia. However, they failed to comply with some non specific parameters especially ash content. All the results represented important information origin of the plant material and the crude drugs should be checked for their specific and non specific parameters before used to ensure their quality.

1. Introduction
Jamu is Indonesian indigenous herbal medicine that has been used empirically to prevent and treat various diseases. To provide evidence on its safety and efficacy, Indonesian government has developed Jamu into Standardized Herbal Medicine and Phytopharmaca. Another strategy is carried out through “Saintifikasi Jamu” to develop Scientific Jamu. Saintifikasi Jamu is an analysis of the use of jamu through health service-based research and it has been regulated under the Regulation of the Minister of Health No. 003/I/MENKES/2010 [1]. Until 2016, there have been 7 types of Scientific Jamu used in the Saintifikasi Jamu. They are mixture of various cut or chop crude drugs. Orthosiphonis Staminei Folium, Centella Asiatica Herba, Curcumae Domesticae Rhizoma, and
Curcumae Xanthorrhizae Rhizomae are four of crude drugs used in Scientific Jamu [2]. Moreover, these herbs are also become six of the 30 medicinal plants that are currently the focus of research and development program of traditional medicines raw materials supported by Indonesian Ministry of Health [3].

One of the objectives of Saintifikasi Jamu program is to increase the availability of herbal medicines that guarantee their safety and efficacy, and widely used both for self-medication and in health care facilities [4]. The availability of qualified crude drugs is a prerequisite for the availability of safe and efficacious herbal medicines. At the other hand, the availability of qualified and standardized crude drugs is still the main problem for physicians who want to participate in Saintifikasi Jamu program. This is caused by the main supplier of Scientific Jamu is B2P2TOOT Tawangmangu. Therefore, various efforts are needed to support the availability of qualified crude drugs in the diverse regions in Indonesia including cultivation and standardization.

Quality of the crude drugs is affected by various factors, such as: environment, soil, climate, seed quality, harvest time and method, preparation of crude drug including drying method and the storage condition [5,6]. In this study, we standardized four of nine plant materials used in Scientific Jamu as anti hyperuricemic and anti hypertension. Standardization was conducted on Orthosiphonis Stamini folium, Centella asiatica herba, Curcuma domesticae rhizomae, and Curcumae Xanthorrhizae Rhizomae collected from three different geographical locations in Indonesia. This is intended to ensure that the crude drugs meet the quality parameters required by Indonesian Herbal Pharmacopoeia [5].

2. Material and methods
2.1. Plant materials and chemicals
The plant materials used in this study were Orthosiphonis Stamini Folium, Centella asiatica herba, Curcuma domesticae rhizomae, and Curcumae Xanthorrhizae Rhizoma. Crude drugs were obtained from UPT Materia Medica Batu; Biopharmaca Cultivation Conservation Unit, Studies Center for Tropical Biopharmaca IPB Bogor; and B2P2TOOT Tawangmangu, collected in March 2018. Determination of crude drugs was carried out by each of these institutions. The chemicals used include: chloral hydrate (Merck), HCl (Merck), chloroform (Mallinckrodt), ethanol 96% (Merck), H2SO4 (Merck), NaNO2 (Merck), AlCl3 (Merck), NaOH (Merck), CH3CO2K (Merck), quercetin (Sigma), and demineralized water.

2.2. Equipment
Equipment used included analytical balance (Ohaus), oven (Memmert), moisture content balance (Mettler Toledo), desiccator (Duran), binocular microscopes (Olympus), furnace (Memmert), ultrasonic cleaner (Branson 1200), spectrophotometer (Shimadzu), and laboratory glassware.

2.3. Crude drug preparation
All dried plant materials were ground using a blender, then sifted using sieve No. 40. Powder was then used for standardization process.

2.4. Macroscopic and microscopic determination
Macroscopic determination was carried out directly on whole crude drugs based on their shape, color, smell and taste. Microscopic observation was conducted on crude drugs powder. The powder was placed on a glass object, added with chloral hydrate, then covered with a glass cover and heated by using alcohol burner at a distance of ± 8 cm while being shaken. After being heated, it is observed under a microscope with 10x40 magnification and the fragments of the crude drugs were observed (5, 6).

2.5. Determination of total phenolics
Determination of the total phenolics content using Folin-Ciocalteu (FC) reagent was performed according to the established method [7, 8], customized for 96-well microplates. Gallic acid, prepared in 5 concentrations ranging from 5 to 15 μg/ml, was used as a standard. Each crude drug was extracted with 96% ethanol. Thirty μl of each extract or standard solution was added to 150 μl of 0.1 mol/l FC reagent and mixed with 120 μl of sodium carbonate (7.5%) after 10 min. Absorbance at 760 nm was read after 2 h. The phenolics concentration was determined by comparison with the standard calibration curve of gallic acid (y = 0.0399x + 0.0231, R² = 0.9995) and the results are presented as a mean value of triplicate tests. The total phenolics value was expressed as gram of gallic acid equivalents (GAE) per g of dry weight (DW).

2.6. Determination of total flavonoids
Determination of total flavonoids content was carried out using spectrophotometric methods as described by Amessis-Ouchemoukh et al. (9). Each crude drug was extracted with 96% ethanol. The extract was then piped 1 ml and put in a 10 ml volumetric flask, added with 4 ml of demineralized water and 0.3 ml of 5% NaNO₂, then left for 5 minutes. After that, 0.3 ml of 10% AlCl₃ was added. At the 6th minute, 2 ml of 1 M NaOH and demineralized water were added to exactly 10.0 ml. The absorbance of each sample solution was read using a spectrophotometer at 400.2 nm. Total flavonoids concentrations were deduced from a standard curve (y = 0.0271x + 0.0872 ; R² = 0.9981) and calculated in g quercetin equivalent (QE)/g dry weight (DW).

2.7. Determination of water and ethanol soluble extract
Five gram of crude drug powder was accurately weighed and placed in a glass-stoppered conical flask. This material was macerated with 100 ml of water (saturated with CHCl₃) or ethanol for 6 hours, shaken frequently, then allowed to stand for 18 hours. After 18 hours, it was quickly filtered into a 100.0 ml volumetric flask. Twenty ml of this extract was then transferred to a porcelain dish and then evaporated until dry. The residue was then heated using an oven at 105°C to constant weight. Replication was done 5 times and the soluble extract was calculated in g per g of air-dried material (5, 6).

2.8. Determination of loss on drying
About 1-2 g of the crude drug powder was accurately weighed and placed in a previously tared crucible porcelain. The powder was spreaded in an even layer and put in an oven. The lid was opened and dried at 105°C for 60 minutes until the constant weight. Before each drying, the closed crucible was left to reach the room temperature in a desiccator. Replication was carried out 5 times and the LOD was calculated in g per g of air-dried material [5,6].

2.9. Determination of total ash
About 2-4 g of the crude drug powder was accurately weighed and placed in a previously ignited and tared crucible porcelain. The material was spreaded in an even layer and ignited by gradually increasing the heat to 500-600°C until it was white, indicating the absence of carbon. It was then cooled in a desiccator and weighed. If carbon-free ash cannot be obtained in this manner, the crucible was then cooled and the residue was moisten with ± 2 ml of water or a saturated solution of ammonium nitrate R. It was then dried on a water-bath, followed on a hot-plate and ignited to constant weight. The residue was allowed to cool in a suitable desiccator for 30 minutes, then weighed without delay. The content of total ash was calculated in g per g of air-dried material [5, 6].

2.10. Determination of acid-insoluble ash
To the crucible containing the total ash, 25 ml of hydrochloric acid (~70g/l) TS was added, covered with a watch-glass and boiled gently for 5 minutes. The watch-glass was rinsed with 5 ml of hot water and this liquid was added to the crucible. The insoluble matter was collected on an ashless filter-paper and washed with hot water until the filtrate was neutral. Filter-paper containing the insoluble matter
was transferred to the original crucible, dried on a hot-plate and ignited to constant weight. The residue was allowed to cool in a suitable desiccator for 30 minutes, then weighed without delay. The content of acid-insoluble ash was calculated in g per g of air-dried material [5, 6].

2.11. Data analysis
The results of all quality parameters evaluation on the four types of crude drugs collected from three different regions was compared to the quality standards of crude drug as listed in the Indonesian Herbal Pharmacopoeia Edition I [5].

3. Results and discussion
3.1. Macroscopic of the crude drugs
Macroscopic characterization is the first step towards establishing the identity and purity of a crude drug. Visual inspection provides the simplest and quickest means by which to establish identity, purity, and quality. This evaluation is followed by further tests to justify whether the crude drugs fulfill the pharmacopoeia standards.

In general, Orthosiphonis Staminei Folium (Figure 1a) is oval and elongated, brownish green, does not smell and tastes rather bitter. The tips of the leaves are thin and blunt, 2-7 cm long and 1-3 cm wide. Round and square stalks, slightly purple. The edges of the leaves are roughly serrated and roll down, the base of the leaves is tapered with pinnate leaf bones. *Centella asiatica* (Figure 1b): the leaves are rolled, wrinkled, kidney shaped, and slippery surface. The base of the leaf is grooved, the tip is rounded, the edges of the leaves are jagged, leaf bones are runny, and the lower surface is rather hairy. Leaf stalks are grayish brown, with fine hair. The color of the leaves is grayish green, weakly aromatic, at first it does not taste then rather bitter. *Curcuma Domesticae Rhizoma* (Figure 1c) are round to oval, sometimes branching, generally irregular curved, 2-6 cm long, 1-5 mm thick, and 0.5-3 cm wide. It is yellow, reddish orange to brownish yellow and has an aromatic odor. The taste is rather bitter, somewhat spicy, the more felt it causes a thick feeling on the tongue. *Curcumae Xanthorrhizae Rhizoma* (Figure 1d) is a thin, round or oval piece, yellow, orange to light brown with a distinctive smell, sharp and slightly bitter taste. Macroscopic analysis showed that four crude drugs from Batu, Tawangmangu, and Bogor are in accordance with the standard [5].

![Figure 1](image-url)  
**Figure 1.** Macroscopic profile of Orthosiphonis Staminei Folium (a), Centellae Asiaticae Herba (b), Curcumae Domesticae Rhizoma (c), and Curcumae Xanthorrhizae Rhizoma (d)

3.2. Microscopic of the crude drugs
Microscopic inspection of medicinal plant material is important for identification of powdered materials. Microscopic examination alone cannot always provide complete identification. However, when used in conjunction with other analytical methods, this method can often provide valuable supporting evidence.

Microscopic identification of Orthosiphonis Staminei Folium, Centellae Asiaticae Herba, Curcumae Domesticae Rhizoma, and Curcumae Xanthorrhizae Rhizoma are presented at Figure 2-5, respectively.
Figure 2. Microscopic fragments of Orthosiphonis Stamini Folium: trichome (a), lower epidermis with glandular trichome (b), diasitic stomata (c), xylem with spiral thickening (d)

Figure 3. Microscopic fragments of Centellae Asiaticae Herba: upper epidermis (a), leaf veins with rosette calcium oxalate crystal (b), mesophyle (c), lower epidermis with anomositic stomata (d), vascular tissue (e)

Figure 4. Microscopic fragments of Curcumae Domesticae Rhizomae: cork tissue (a), parenchyma tissue with yellow material (b), vascular tissue (c), trichome (d), amylum (e), parenchyma tissue with amylum (f)

Figure 5. Microscopic fragments of Curcumae Xanthorrhizae Rhizomae: vascular tissue (a), cork tissue (b), sclerenchyma tissue (c), parenchyma tissue cortex (d), amylum (e)

Figure 2-5 showed that the microscopic features of all crude drugs from the three regions meet the requirements of Indonesian Herbal Pharmacopoeia. This indicated that the identity of the crude drug is correct.

3.3. Total phenolics and flavonoids content
Total phenolics and flavonoids compounds in all samples were determined by the Folin-Ciocalteu assay and the aluminum chloride colorimetric method, respectively. Serial dilution of gallic acid and quercetin were used as standards to set the calibration curves, which were used to calculate the total phenolics or flavonoids contents for each crude drug. Presents total flavonoids and phenolics content calculated for each crude drug.
Table 1. Total phenolics and flavonoids content of crude drugs

| Origin          | Total flavonoids (%QE)* | Total phenolics content (%GAE)** |
|-----------------|-------------------------|----------------------------------|
| Orthosiphonis   | Centellae               | Curcumae                         | Curcumae                         |
| Staminei Folium | Asiatiae                | Domesticae                       | Xanthorrizae                     |
| Batu            | 1.42±0.02               | 0.46±0.01                        | 0.17±0.02                        | 0.28±0.01                       |
| Tawangmangu     | 2.12±0.09               | 0.35±0.01                        | 0.13±0.00                        | 0.28±0.01                       |
| Bogor           | 1.93±0.11               | 0.31±0.02                        | 0.16±0.01                        | 0.27±0.01                       |

Values are means ± SD of three determinations
*calculated as %Quercetin Equivalent
**calculated as %Gallic Acid Equivalent

3.4. Water and ethanol-soluble extracts, loss on drying, total ash content, acid-insoluble ash content

In addition to macroscopic, microscopic, and total phenolics or flavonoids content, the other specific parameters were determined, i.e.: water and ethanol-soluble extracts (Table 2). Total extract content has a correlation with the concentration of chemical compounds dissolved in certain solvents. Evaluation of this parameter is intended to provide an initial information of the total amount of the chemical compounds in an herbal material [10].

The results showed that water-soluble extracts of Orthosiphonis Staminei Folium, Centella Asiatiae Herba, Curcuma Domesticae Rhizoma, and Curcuma Xanthorrizae Rhizoma from the three origins were 12.6-15.59, 13.95-18.19, 11.28-13.54, and 10.98-18.36%, respectively. Meanwhile, ethanol-soluble extract of those crude drugs were 9.27-12.81, 14.85-16.44, 19.18-31.83, and 6.73-8.33%, respectively. Both water and ethanol-soluble extracts vary according to the origin and type of crude drug. This could be caused by various factors, including the age of the plant, harvest time, climate and geographical location.

Table 2. Water and ethanol soluble extract of crude drugs

| Crude drug           | Origin       | Water soluble extract (%) | Ethanol soluble extract (%) |
|----------------------|--------------|---------------------------|-----------------------------|
| Orthosiphonis        | Batu         | 15.59±1.04                | 11.18±0.19                  |
| Staminei Folium      | Tawangmangu  | 13.02±0.19                | 9.27±0.41                   |
|                      | Bogor        | 12.60±0.36                | 12.81±0.39                  |
|                      | Standard     | NLT 10.2                  | NLT 3.2                     |
| Centellae Asiatiae   | Batu         | 13.95±0.74*               | 15.69±0.15                  |
| Herba                | Tawangmangu  | 22.01±0.73*               | 14.85±0.12                  |
|                      | Bogor        | 18.19±1.86*               | 16.44±0.30                  |
|                      | Standard     | NLT 28.3                  | NLT 2.1                     |
| Curcumae             | Batu         | 13.52±1.14                | 19.18±2.07                  |
| Domesticae           | Tawangmangu  | 13.54±1.24                | 31.83±0.31                  |
| Rhizoma              | Bogor        | 11.28±0.52                | 19.49±1.39                  |
|                      | Standard     | NLT 11.5                  | NLT 11.4                    |
| Curcumae             | Batu         | 18.36±1.07                | 8.33±0.14                   |
| Xanthorrizae         | Tawangmangu  | 17.15±1.42                | 6.73±0.49                   |
| Rhizoma              | Bogor        | 10.98±0.88                | 7.17±0.14                   |
|                      | Standard     | NLT 9.1                   | NLT 3.6                     |

Values are means ± SD of three determinations
NLT: not less than
*did not meet the quality standards according to the Indonesian Herbal Pharmacopoeia I
Loss on drying or LOD (Table 3) is residual substances after drying at 105°C for 30 minutes or until the constant weight. The purpose of this test is to provide maximum limits (in ranges) about the amount of compounds lost in the drying process, for example water and other volatile compounds such as essential oils [5]. All crude drugs obtained from 3 origins in this study met the requirements of LOD except Curcumae Xanthorrhizae Rhizoma from Bogor. LOD describes loss of water and volatile compounds [10]. High water content in the crude drugs will become a microbial growth medium during the storage as well as hydrolysis media which can cause decomposition of chemical compounds [11].

Total ash is designed to measure the total amount of material remaining after ignition. This includes both "physiological ash", which is derived from the plant tissue itself, and "non-physiological ash", which is the residue of the extraneous matter (e.g. sand and soil) adhering to the plant surface. At the other hand, acid-insoluble ash is the residue obtained after boiling the total ash with dilute hydrochloric acid, and igniting the remaining insoluble matter. This measures the amount of silica present, especially as sand and siliceous earth. Table 3 showed that total ash content and acid-insoluble ash of some crude drugs did not fulfill the requirement. High content of acid-insoluble ash or non-physiological ash could be caused by imperfect washing processes.

Table 3. LOD, total ash and acid-insoluble ash of crude drugs

| Crude drug                  | Origin        | LOD (%)     | Total ash (%) | Acid-insoluble ash (%) |
|----------------------------|---------------|-------------|---------------|------------------------|
| Orthosiphonis Staminei Folium | Batu          | 10,22±0,11  | 10,20±0,35    | 0,64±0,10              |
|                            | Tawangmangu   | 10,26±0,12  | 10,66±0,16*   | 0,43±0,04              |
|                            | Bogor         | 9,97±0,18   | 7,88±0,27     | 0,60±0,11              |
|                            | Standard      | NMT 12      | NMT 10.2      | NMT 3.4                |
| Centella asiatica Herba    | Batu          | 9,23±0,20   | 12,28±0,22*   | 2,36±0,19              |
|                            | Tawangmangu   | 8,57±0,22   | 12,71±0,35*   | 0,25±0,02              |
|                            | Bogor         | 7,31±0,18   | 10,47±0,21*   | 0,06±0,01              |
|                            | Standard      | NMT 11      | NMT 18.05     | NMT 4.9                |
| Curcumae Domesticae Rhizoma | Batu          | 7,78±0,97   | 8,18±0,77     | 1,23±0,11*             |
|                            | Tawangmangu   | 8,39±0,95   | 7,25±0,02     | 1,47±0,31*             |
|                            | Bogor         | 7,36±0,42   | 11,22±0,41*   | 0,63±0,11              |
|                            | Standard      | NMT 12      | NMT 8.2       | NMT 0.9                |
| Curcumae Xanthorrhizae Rhizoma | Batu          | 11,56±0,03  | 9,28±0,11*    | 1,31±0,24*             |
|                            | Tawangmangu   | 12,59±0,35  | 5,37±0,05*    | 1,36±0,06*             |
|                            | Bogor         | 13,50±0,21* | 4,13±0,07     | 0,58±0,04              |
|                            | Standard      | NMT 13      | NMT 4.8       | NMT 0.7                |

Values are means ± SD of three determinations
NMT: not more than
*did not meet the quality standards according to the Indonesian Herbal Pharmacopoeia I

4. Conclusion
Orthosiphonis Staminei Folium, Centella asiatica Herba, Curcumae Domesticae Rhizoma, and Curcumae Xanthorrhizae Rhizoma from Batu, Tawangmangu, and from Bogor met specific parameters (macroscopic, microscopic, water and ethanol soluble extracts) as required by Indonesian Herbal Pharmacopoeia. However, they failed to comply some non specific parameters especially ash content. All the results represented important information origin of the plant material and the crude drugs should be checked for their specific and non specific parameters before used to ensure their quality.
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References
[1] Purwadianto A, Poerwaningsih E, Widiyastuti Y, Neilwan A, Sukasediati N. Pedoman Penelitian Jamu Berbasis Pelayanan Kesehatan. Jakarta: Lembaga Penerbit Badan Penelitian dan Pengembangan Kesehatan; 2017.
[2] Triyono A, et a. Tujuh Ramuan Jamu Saintifik - Pemanfaatan Mandiri Oleh Masyarakat. Jakarta: Lembaga Penerbit Badan Penelitian dan Pengembangan Kesehatan; 2016.
[3] Rencana induk pengembangan bahan baku obat tradisional, Permenkes RI No. 88 (2013).
[4] RI KK. Peraturan Menteri Kesehatan Republik Indonesia Nomor 003. MENKES/PER/2010 tentang saintifikasi jamu dalam penelitian berbasis pelayanan kesehatan, 2010.
[5] RI D. Farmakope Herbal Indonesia Edisi 1. Jakarta: Departemen Kesehatan Republik Indonesia. 2008.
[6] WHO. Quality control methods for medicinal plant materials. 1998.
[7] Bera IN, Lesjak MM, Jovin ED, Balog KJ, Anačkov GT, Orčić DZ, et al. Plantain (Plantago L.) species as novel sources of flavonoid antioxidants. Journal of agricultural and food chemistry. 2009;57(19):9268-73.
[8] Singleton VL, Orthofer R, Lamuela-Raventos RM. [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods in enzymology. 1999(299C):152-78.
[9] Amessis-Ouchemoukh N, Madani K, Falé PLV, Serralheiro ML, Araújo MEM. Antioxidant capacity and phenolic contents of some Mediterranean medicinal plants and their potential role in the inhibition of cyclooxygenase-1 and acetylcholinesterase activities. Industrial Crops and Products. 2014;53(0):6-15.
[10] Indonesi DKR. Parameter standar umum ekstrak tumbuhan obat. Jakarta: Departemen Kesehatan Republik Indonesia. 2000:9-12.
[11] Krisyanella K, Susilawati N, Rivai H. Pembuatan Dan Karakterisasi Serta Penentuan Kadar Flavonoid Dari Ekstrak Kering Herba Meniran (Phyllanthus niruri L.). Jurnal Farmasi Higea. 2017;5(1):9-19.