INTRODUCTION

Ayapana triplinervis (Vahl) R.M.King and H. Rob. is a shrub belonging to the family Asteraceae, and it can be found in lowlands and at altitudes of up to 1600 m above sea level [1]. A. triplinervis contains essential oils, coumarin derivative compounds, namely, ayapin and ayapanin and terpene [2]. In addition, A. triplinervis contains saponins, flavonoids, quercetin, rutin, alkaloids, polyphenols, carotene, and Vitamin C [3-5]. A. triplinervis leaf extracts can be used as diuretics, antipyretics, and antidiarrheal [2,4]. Based on the previous studies by Matos et al. [14] and Melo et al. [13], A. triplinervis leaf extracts exhibit various pharmacological activities, including antianorexia, sedative, antifungal, antiparasitic, analgesic, anticoagulant, antibacterial, and antioxidant activities [6,7].

Maceration is a method of extracting simplicia at room temperature using a solvent with shaking several times. In the maceration method, the underlying principle applied is concentration on balance. Kinetic maceration is maceration with continuous stirring, while remaceration is maceration by adding solvent repeatedly after the screening of the first mass [8].

Standardization refers to a set of parameters, procedures, and measurement methods whose results are elements related to the paradigm of pharmaceutical quality, which means to conform to standard requirements (chemistry, biology, and pharmacy), including the assurance (limits) of stability of a general pharmaceutical product. Standardization is also the process of guaranteeing that a final product (medicine, extract, or extract product) has a constant parameter value (steady), which has to be determined (designed in a formula) first [8].

Simplicia standardization means that simplicia that would be used as medicine as a raw material has to conform to the requirements in the Ministry of Health Monograph (Materia Medica Indonesia). Extracts that are used as raw materials or pharmaceutical products must conform to the monograph of raw materials (simplicia) and standard parameters required for extract. The quality requirements for simplicia and extract consist of specific parameters and general (non-specific) parameters [8]. Therefore, the aim of this study was to investigate the specific and non-specific parameters of the simplicia and ethanol extract of A. triplinervis leaves.

MATERIALS AND METHODS

Materials

The prasman leaves used in this research were obtained from three different regions, namely, Bogor, Sragen, and Cikarang. All other chemicals and reagents were sourced commercially: Rutin from Sigma-Aldrich (Singapore), ethanol from Merck (Germany), n-hexane from Merck (Germany), ethyl acetate from Merck (Germany), methanol from Merck (Germany), formic acid from Merck (Germany), and acetic acid from Merck (Germany).

Preparation of extract

A. triplinervis samples were collected from Bogor, Sragen, and Cikarang, and identified based on microscopic and morphological characteristics at the Center for Plant Conservation Bogor Botanical Garden.

Extraction

Dried powdered prasman leaves (500 g) were macerated using 70% ethanol and then evaporated [9,9].

Standardization

Standardization was performed for simplicia and extracts. A parameter test for simplicia consists of two categories of parameters including specific parameters and non-specific parameters. Specific parameters...
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consist of macroscopic characteristics, organoleptic properties, microscopic features, water-soluble extracts, ethanol-soluble extracts, thin-layer chromatography, phytochemical composition, and chemical content, while the non-specific parameters consist of drying losses, total ash contents, and total acid insoluble ash contents [8].

A parameter test for extracts consists of two categories of parameters in the form of specific parameters and non-specific parameters. The specific parameters include organoleptic properties, phytochemical composition, and chemical content. The non-specific parameters consist of total water, total ash, and total acid insoluble ash contents [8].

Table 1: Parameter test results for simplicia from *Ayapana triplinervis* leaves

| Test                          | Bogor          | Sragen         | Cikarang        | Range of values          |
|-------------------------------|----------------|----------------|----------------|--------------------------|
| 1. Specific parameters        |                |                |                |                          |
| Water-soluble extract (%)     | 29.12          | 18.69          | 29.30          | 18.69–29.30             |
| Ethanol-soluble extract (%)   | 11.78          | 7.73           | 9.78           | 7.73–11.78              |
| Total flavonoid content       | 4.09 mg RE/g Simplicia | 3.31 mg RE/g Simplicia | 4.10 mg RE/g Simplicia | 3.31–4.10 mg RE/g Simplicia |
| 2. Non-specific parameters (%)|                |                |                |                          |
| Drying losses (%)             | 13.61          | 14.48          | 14.55          | 13.61–14.55             |
| Total ash (%)                 | 11.54          | 11.83          | 11.72          | 11.54–11.83             |
| Total insoluble ash (%)       | 2.66           | 2.22           | 1.90           | 1.90–2.66               |

Table 2: The chromatographic profile results of rutin and *Ayapana triplinervis* leaf simplicia in ethyl acetate-formic acid-water-acetic acid (100:15:17:0.6) mobile phase after spraying with sitroborate spraying reagent, heating, and viewing at a wavelength of 365 nm

| Spot number | Rf value of UV (366 nm) | Spot color |
|-------------|-------------------------|------------|
| Rutin       | 0.31                    | Bright yellow |
| 1           | 0.31                    | Light yellow |
| 2           | 0.36                    | Light yellow |
| 3           | 0.59                    | Blue        |
| 4           | 0.66                    | Bright yellow |

Fig. 1: Microscopic examination of *Ayapana triplinervis* leaf powder (×100): Lower epidermal fragments with corrugated walls, anisocytic stomata, and Compositae glandular hair (a), upper epidermal fragments with polygonal walls, oil drops (b), glandular hair (c), multicellular hair (d), vessels with spiral thickening (e)
RESULTS

Simplicia of *A. triplinervis* leaves from the three regions was similar in the form of single leaves, lanceolate-shaped leaves, leaf base and leaf tip pointed, flat-leaf edges, pinnae leaf bones, short leaf stalks, leaves 5–8 cm in length, leaves 1–2 cm wide, and brownish-green colored leaves. Organoleptic properties of *A. triplinervis* leaf powder from the three regions were similar in the form of dry powder, green colored powder, non-specific smell, and a fairly bitter taste. Microscopic examination of *A. triplinervis* leaf powder from the three regions revealed similarities in the form fragments consisting of lower epidermal fragments with corrugated walls, anisocytic stomata, Compositae glandular hair, upper epidermal fragments with polygonal walls, oil drops, glandular hair, multicellular hair, and vessels with spiral thickening (Fig. 1).

Simplicia of *A. triplinervis* leaves from the three regions was similarly based on their contents, including alkaloids, tannins, saponins, flavonoids, terpenoids, and glycosides. The yield extract from the three regions was 16.97–18.29%. In addition, the organoleptic properties of *A. triplinervis* leaf powder from the three regions were similarly based on the viscous extract, blackish green color, distinct odor, and bitter taste. Furthermore, the *A. triplinervis* leaf extracts from the three regions were similar in their contents, which included alkaloids, tannins, saponins, flavonoids, terpenoids, and glycosides.

DISCUSSION

*A. triplinervis* leaf simplicia was prepared from samples obtained from Bogor, Sragen, and Cikarang. The sampling regions were selected based on their altitudes. Samples were wet sorted to eliminate dirt and other foreign material from the simplicia, then washed with water to remove soil and other impurities. The sample was performed to accelerate the drying process and dried in a drying cabinet to obtain simplicia that would not degrade easily to facilitate storage for a longer time. Simplicia that had been dried was sorted to remove foreign materials such as unwanted plant parts impurities that still existed in the dry simplicia [9].

Specific parameters are associated with original plant constituents and compounds that are responsible for pharmacological activity [8]. Conversely, the non-specific parameters are linked to environmental factors, such as contamination with compounds such as pollutants or process additives, and compounds resulting from the interaction of constituent compounds with contaminants or compounds that can change and influence drug safety and stability [8,10].

The specific parameters of the simplicia consist of microscopic tests performed to establish morphological specificity, including size and color of the simplicia. The organoleptic properties of the powder are to recognize the material simply and objectively as possible. Macroscopic features can be used to determine key characteristics of the tissues or anatomy, which could be used to identify simplicia types, as non-specific identification fragments. The water-soluble extract refers to the amount of compounds that could be absorbed by water solvents. Higher yields of the water-soluble extract compared with those of ethanol-soluble extracts indicate that simplicia contains more polar compounds so that they are more soluble in water [8,11]. The ethanol-soluble extract refers to the amount of compounds that can be absorbed using ethanol solvents. Phytochemical screening in *A. triplinervis* simplicia is performed the same as extract. A thin-layer chromatogram profile provides an initial overview of the chemical components (Fig. 2), data shown in Table 2 [8]. Flavonoids have been reported previously in *A. triplinervis*. Total flavonoid content determination indicated the flavonoid concentrations in the simplicia and the leaf extracts [3].

The non-specific parameters of the simplicia consist of the total drying losses, which indicate the maximum limits of the amounts of compounds lost during drying processes. Compounds that could be lost after heating at 105°C include water and volatile compounds such as essential oils [8,12]. The total ash content indicates the internal and external mineral content in simplicia, if the value of the total ash content is high, there are minerals in the simplicia [8,13]. The total acid insoluble ash content reveals the amount of mineral content that is not soluble in acid. A high value of the total acid insoluble ash content indicates that the product under examination has silicate components associated with soil or sand, silver, lead, or mercury [8,14].

The maceration method was selected for the present study due to its relative ease and simplicity of application [15–17]. Maceration was performed using 70% ethanol as a solvent to attract all the chemical components because ethanol is a universal solvent that can attract soluble compounds in both polar and nonpolar solvents [16]. Parameter tests for the *A. triplinervis* leaf extracts consisted of two parameters including specific parameters and non-specific parameters. The specific parameters of the extract consisted of an organoleptic test for initial examination in a simple and objective manner. The results of phytochemical screening from simplicia is more reliable than extracts data shown in Table 1 [8].

The non-specific parameters of the extract consist of water content, which refer to the minimum range amount of water content in the

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**Fig. 2: The chromatogram profile of rutin and Ayapana triplinervis leaf simplicia in an ethyl acetate-formic acid-water-acetic acid (100:15:17:0.6) mobile phase after spraying with sitroborate spraying reagent, heating, and viewing at a wavelength of 365 nm: Rutin (P). A. triplinervis leaf simplicia from Bogor (S1), A. triplinervis leaf simplicia from Sragen (S2), A. triplinervis leaf simplicia from Cikarang (S3)**

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**Table 3: Parameter test results for Ayapana triplinervis leaf extracts**

| Test                        | Bogor                  | Sragen                  | Cikarang                  | Range of values                  |
|-----------------------------|------------------------|-------------------------|---------------------------|----------------------------------|
| 1. Specific parameter       |                        |                         |                           |                                  |
| Total flavonoid content     | 17.92% mg RE/g extract | 14.94% mg RE/g extract | 22.41% mg RE/g extract    | 14.94–22.41% mg RE/g extract     |
| Total water content         | 11.07                  | 11.28                   | 12.66                     | 11.07–12.66                      |
| Total ash                   | 10.89                  | 10.70                   | 10.55                     | 10.55–10.89                      |
| Total acid insoluble ash    | 0.32                   | 0.26                    | 0.25                      | 0.25–0.32                        |

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extract. High yields may be observed due to the water absorbed from humidity in the environment during storage and absorption of moisture from the atmosphere during storage before inspection [8]. High water content may be due to water absorbed from the atmosphere during storage before inspection [8]. High water content can facilitate the growth of microbes, which would reduce the stability of an extract [17]. Total ash content illustrates internal and external mineral content in simplicia. If the value of the total ash content is high, there high mineral content in the material examined [8,13]. Total acid insoluble ash content illustrates the amounts of minerals that are insoluble in acid. A high indicates that a compound has high silicate content, potentially from soil or sand, or silicate, lead, or mercury, data shown in Table 3 [8,14].

CONCLUSION
The present study presents specific and non-specific parameters from simplicia and leaf extract of *A. triplinervis*. The parameters identified could be used to determine the safety and stability of extracts and simplicial before administration to treat various ailments, considering environmental factors such as humidity could influence the quality and stability of products in storage, in addition to their cultivation conditions.

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CONFLICTS OF INTEREST
The authors report no conflicts of interest.

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