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Research Article

DETERMINATION OF SPECIFIC AND NON-SPECIFIC PARAMETERS OF THE *SIMPLICIA* AND ETHANOL EXTRACT OF SANGKETAN (*ACHYRANTHES ASPERA* L.) LEAVES

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ABSTRACT

Objective: With recent advancements in traditional medicine, there is an increasing need for quality assurance assessments using standardized processes. In this study, we aimed to determine the specific and non-specific parameters of the *Simplicia* and 70% ethanol extract of *Achyranthes aspera* leaves from three different regions.

Methods: Samples were extracted using a maceration method with 70% ethanol as the solvent. The tests for the specific and non-specific parameters were based on the Herbal Pharmacopoeia of Indonesia.

Results: Our results of the specific parameters of the *Simplicia* showed that the ethanol-soluble extract contained 5.83–9.36% and the water-soluble extract contained 10.25–15.44%. The chromatogram pattern used β-sitosterol as the standard, and the total phenolic content was 0.93–1.15 mg gallic acid equivalent (GAE)/g *Simplicia*. The results of the non-specific parameters of *Simplicia* showed a loss on drying of 15.25–15.91%, a total ash content of 14.58–20.79%, and an acid-insoluble ash value of 1.75–2.19%.

Conclusion: Our results of the specific parameters of *A. aspera* extract showed a total phenolic content of 6.49–7.68% mg GAE/g extract. Our results of the non-specific parameters of the extract showed a total ash value of 13.18–14.52%, an acid-insoluble ash value of 0.14–0.29%, and a moisture content of 10.42–11.16%.

Keywords: *Simplicia*, Ethanol extract, *Achyranthes aspera* L., Traditional medicine, Specific, Non-specific.

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INTRODUCTION

According to the WHO, traditional medicine has a long history, representing the total of the knowledge, skill, and practices based on the theories, beliefs, and experiences indigenous to different cultures. Traditional medicine is used in the maintenance of health as well as in the prevention, diagnosis, and treatment of both physical and mental illness.

There are numerous institutions and investigators who research natural ingredients for their use in traditional medicine. Indonesia has inherited a rich traditional medicine culture with much variety, including traditional medicine herbal formulations, where the ingredients have been found to be written inside ancient texts, such as the "Pusaka Nusantara" [1,2]. The utilization and development of traditional medicine in its various aspects was initially based on empirical experience; however, many traditional medicines now have scientific evidence to justify their use and have been included in various standardized tests and preclinical and clinical trials. Historically, the field of traditional medicine, based on ancestral heritage and an empirical approach, was called herbal medicine. Then, if the traditional medicine had gone through further scientific testing, such as preclinical testing, it was called standardized herbal medicine. Finally, if a traditional medicine has gone through final scientific testing, such as a clinical trial, it is called phytopharmaca [1].

One of the medicinal plants currently being developed is Sangketan (*Achyranthes aspera* L.). The plant is reported to have several medicinal properties and is used as an emmenagogue, purgative, diuretic, antimalarial, antihyperlipidemic, estrogenic, antileprotic, antispasmodic, cardiotonic, antibacterial, and antiviral agent in various traditional systems of medicine [3-7]. However, the raw material (*Simplicia*) and the extract needed to undergo a thorough standardization process. Therefore, the aim of this study was to investigate the specific and non-specific parameters of the *Simplicia* and ethanol extract of Sangketan leaves.

MATERIALS AND METHODS

Materials

The Sangketan leaves (*A. aspera* L.) used in this research were obtained from three different areas, namely, Sragen, Klaten, and Boyolali. All other chemicals and reagents were sourced commercially. β-Sitosterol and gallic acid were obtained from Sigma-Aldrich (Singapore). Ethanol, n-hexane, ethyl acetate, methanol, toluene, and chloroform were purchased from Merck (Germany).

Preparation of extract

The Sangketan leaves were obtained from three different regions, namely, Sragen, Klaten, and Boyolali. The leaves were identified using microscopic and morphological characteristics at the Center for Plant Conservation – Bogor Botanical Gardens.

Extraction

A 500 g sample was extracted by maceration using 70% ethanol (in a 1:10 ratio) for 24 h and subsequently filtered. The residue was then re-extracted twice with the same method and solvent. The ethanol extract was then concentrated using a rotary vacuum evaporator followed by a water bath.

Standardization

Standardization was performed on the *Simplicia* and extract. Parameter testing for the *Simplicia* consisted of both specific and non-specific parameters. The specific parameters consisted of macroscopic, organoleptic, microscopic, water-soluble extract content, ethanol-soluble extract content, thin-layer chromatography, phytochemical

screening, and chemical content. The non-specific parameters consisted of the estimated loss on drying, total ash, and total acid-insoluble ash [8].

The parameter tests for the extract consisted of both specific and non-specific parameters. The specific parameters consisted of organoleptic, phytochemical screening, and chemical content. The non-specific parameters consisted of total water content, total ash, and total acid-insoluble ash [8].

RESULTS AND DISCUSSION

Our results showed the specific parameters of the *Simplicia* of the leaves of *A. aspera*: The total water-soluble extract was 10.25–15.44%; the total ethanol-soluble extract was 5.83–9.36%; the chromatogram profile was obtained using thin-layer chromatography in a toluene-ethyl acetate-chloroform (5:1:4) mobile phase with β-sitosterol as the standard; and the total phenolic content was 0.90–1.15 expressed in mg gallic acid equivalent (GAE)/g *Simplicia*. The non-specific parameters of the *Simplicia* of the leaves of *A. aspera* were also determined. The total loss on drying was 15.25–15.91%; the total ash content was 14.58–20.79%; and the total acid-insoluble ash content was 1.75–2.19% (Table 1). Specific parameters of the extract of the leaves of *A. aspera* included the total phenolic content at 6.94–7.68% expressed in mg GAE/g extract. Non-specific parameters of the extract of the leaves of *A. aspera* included the total water content at 10.42–11.16%; the total ash content at 13.18–14.52%; and the total acid-insoluble ash content at 0.14–0.29% (Table 2).

The Sangketan leaves have a size of 1.5–5 cm in length and are 0.5–2 cm wide. The ends of the Sangketan leaves are slightly rounded, where the base of the leaf narrows slightly. The edges of the leaves have a slightly wavy shape, and both sides of the leaf are feathered. The Sangketan leaf powder is a dry powder with a grayish-green color and a distinctive and tasteless odor. Closing hair, thickened mesh vessels, spiral thickening vessels, anomocytic type stomata, and transverse palisade tissue were also observed (Fig. 1).

The level of the water-soluble extract of the Sangketan leaves was not <15.44%, whereas the level of the soluble extract of ethanol was not <9.36%. The chromatogram pattern was obtained using a mobile phase of toluene-ethyl acetate-chloroform (5:1:4) and the appearance of vanillin-sulfuric acid LP spots with β-sitosterol used as the standard (Fig. 2).

Our results of the *Simplicia* phytochemical screening of the Sangketan leaves indicated that the leaves contain alkaloids, tannins, saponins, flavonoids, terpenoids, and glycosides, with a total phenolic content level of not <1.15 mg GAE/g *Simplicia*. The non-specific parameters of the Sangketan leaf simplicial included a drying loss not more than 15.25%, a total ash content of not more than 14.58%, and an acid-insoluble ash content not more than 1.75%.

The extract yield was not <7.69%. The specific parameters of the Sangketan leaf extract included that the extract produced was thick and blackish-brown in color, odorless, and with a bitter taste. Phytochemical screening of the Sangketan leaf extract indicated that it contained alkaloids, saponins, tannins, terpenoids, glycosides, and flavonoids, with a total phenolic content not <7.68 mg GAE/g extract. The non-specific parameters of the Sangketan leaf extract included that the total ash content was no more than 13.18%, the acid-insoluble ash content was not more than 0.14%, and the water content was not <10.42%.

DISCUSSION

The materials used in this study were the leaves of *Achyranthes aspera* L. obtained from three different regions of Indonesia, namely, Sragen, Boyolali, and Klaten. The reason for choosing these three regions was because the distribution of most Sangketan plants is in the Central Java region. In addition, the three regions in Central Java have different altitudes. In Sragen, Boyolali, and Klaten, the altitude is ~109, ~516, and ~848 masl, respectively. Thus, we selected these regions to collect the Sangketan leaves in case, we found differences in the parameters of the leaves based on the altitude of the region. Plant samples were identified by microscopic and morphological characteristics at the Center for Plant Conservation – Bogor Botanical Gardens.

The processing of the *Simplicia* included wet sorting from dirt and other ingredients. Then, the samples were washed with water and air-dried. The leaves were dried in a drying cabinet at a drying temperature. The dried *Simplicia* was then subject to dry sorting to finish the *Simplicia* and separate it from impurities or other ingredients. After that, the leaves were homogenized using a blender.

Characterization testing of the *Simplicia* referred to the Indonesian Herbal Pharmacopoeia (FHI). The testing of the Sangketan leaves and simplicial consisted of both specific and non-specific parameters. Specific parameters carried out consisted of an organoleptic test, microscopic and macroscopic test, determination of water-soluble extracts, and determination of soluble ethanol content, screening of *Simplicia* phytochemicals, chromatogram patterns, and total phenol content determination, whereas for non-specific parameters carried

Table 1: Parameter test results for *Simplicia* from Sangketan leaves

Test	Sragen	Klaten	Boyolali	Range of values
1. Specific parameters				
Water-soluble extract (%)	11.18	15.44	10.25	10.25–15.44
Ethanol-soluble extract (%)	5.83	6.74	9.36	5.83–9.36
Total phenolic content	1.09 mg GAE/g *Simplicia*	0.93 mg GAE/g *Simplicia*	1.15 mg GAE/g *Simplicia*	0.93–1.15 mg GAE/g *Simplicia*
2. Non-specific parameters				
Drying losses (%)	15.25	15.91	15.46	15.25–15.91
Total ash (%)	14.58	16.83	20.79	14.58–20.79
Total insoluble ash (%)	1.75	1.85	2.19	1.75–2.19

Table 2: Parameter test results for Sangketan leaf extracts

Test	Bogor	Sragen	Cikarang	Range of values
1. Specific parameter				
Total phenolic content	7.33 mg GAE/g extract	6.94 mg GAE/g extract	7.68 mg GAE/g extract	6.94–7.68 mg GAE/g extract
2. Non-specific parameters				
Total water content (%)	10.99	11.16	10.42	10.42–11.16
Total ash (%)	13.18	13.89	14.52	13.18–14.52
Total acid-insoluble ash (%)	0.14	0.27	0.29	0.14–0.29

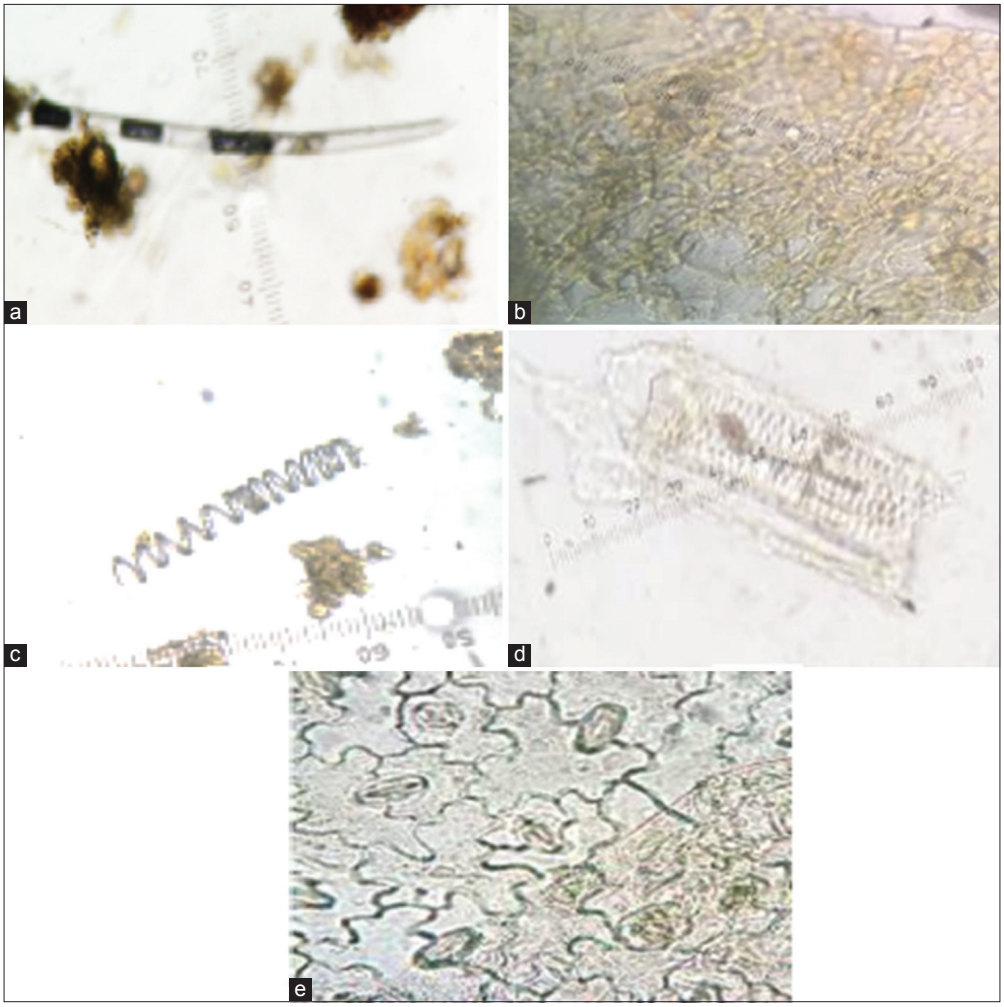

Fig. 1: Microscopic examination of Sangketan leaf powder (×100): Closing hair (a), thickened mesh vessels (b), spiral thickened vessels (c), transverse palisade tissue (d), anomocytic type stomata (e)

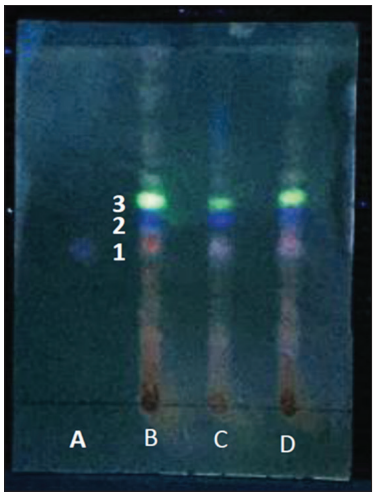

Fig. 2: The chromatogram profile of β-sitosterol and Sangketan leaf *Simplicia* in an toluene-ethyl acetate-chloroform (5:4:1) mobile phase after spraying with vanillin-sulfuric acid reagent, heating, and viewing at a wavelength of 366 nm: β-Sitosterol (a), Sangketan leaf *Simplicia* from Sragen (b), Sangketan leaf *Simplicia* from Klaten (c), Sangketan leaf *Simplicia* from Boyolali (d)

out consisted of drying losses, total ash content, and acid-insoluble ash content. Each of these parameters is carried out 3 times.

The extraction of the Sangketan leaves (*A. aspera* L.) was carried out by the maceration method. The maceration method was chosen because this method is the simplest method to perform. The solvent used in this method was 70% ethanol based on the method specified in the Indonesian Herbal Pharmacopoeia (FHI). In addition, ethanol solvents are considered non-toxic; thus, using ethanol as a solvent makes sense because later the extract produced will be used as the raw material for traditional medicine.

CONCLUSION

Based on the results of this study, it can be concluded that specific and non-specific parameters can be obtained from *Simplicia* and ethanol extract of Sangketan leaves. This testing process was conducted using samples from three different regions to see the accuracy of the results obtained. The findings of the specific parameters of *Achyranthes aspera* extract showed a total phenolic content of 6.49–7.68% mg GAE/g extract. The findings of the non-specific parameters of the extract showed a total ash value of 13.18–14.52%, acid-insoluble ash value of 0.14–0.29%, and moisture content of 10.42–11.16%.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES

1. Department of Health Republic of Indonesia. National Traditional Medicine Policy 2017. Jakarta: Department of Health Republic of Indonesia; 2007.
2. Benzie I, Wachtel-Galor S. Herbal Medicine: Biomolecular and Clinical Aspects. Boca Raton: Taylor and Francis 2011;2.
3. Bhosale UA, Yegnanarayan R, Pophale P, Somani R. Effect of aqueous extracts of *Achyranthes aspera* Linn. on experimental animal model for inflammation. Anc Sci Life 2012;31:202-6.
4. Badan Penelitian dan Pengembangan Kesehatan, Departemen Kesehatan. Inventaris Tanaman Obat Indonesia (IV). Jakarta: Departemen Kesehatan RI; 1997.
5. Alam MA, Slahin N, Uddin R, Hasan SM. Analgesic and neuropharmacological investigations of the aerial part of *Achyranthes aspera* Linn. Stam J Pharma Sci 2008;1:44-50.
6. Barua CC, Talukdar A, Begum SA, Buragohain B, Roy JD, Borah RS, *et al*. Antidepressant-like effects of the methanolic extract of *Achyranthes aspera* Linn. In animal models of depression. Pharmacologyonline 2009;2:587-94.
7. Chakraborty A, Brantner A, Mukainaka T, Nobukuni Y, Kuchide M, Konoshima T, *et al*. Cancer chemoprotective activity of *Achyranthes aspera* leaves on Epstein-Barr virus activation and two-stage mouse skin carcinogenesis. Cancer Lett 2002;177:1-5.
8. Department of Health, Republic of Indonesia. Common Standard Parameters of Medical Plant Extracts. Jakarta: Department of Health, Republic of Indonesia; 2000.