Standardization Raw Material and Ethanolic Extract of Andrographidis Herba (*Andrographis Paniculata* Nees) from District of Bogor and Tawangmangu

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Keyword: Standardization, raw material, ethanolic extract, Andrographis paniculata Nees, Andrographis herbs

Abstract: Sambiloto (Andrographis paniculata Nees) has a potency to be developed in phyto-pharmaceutical product, especially in this study focused on supporting anti-hyperglycemic research. This requires standardization can affect both the raw material or extract qualities that have been obtained based on the Indonesian Herbal Pharmacopeia method. Raw material for this study has been obtained from two traditional drug development institutions, there are BALITRO from Bogor and BPTO from Tawangmangu

1 INTRODUCTION

Sambiloto (*Andrographis paniculata* Nees) is one of medicinal plant that it has long existed and used as ingredient of traditional medicine. Sambiloto has bitter taste, contains flavonoids, tannins, and saponins [1].

Ethanolic extract of sambiloto can restore blood glucose level into normal in induced-rats, it indicated as anti-hyperglicemic activity [2]. Another study showed that combination curcumin and andrographolide (3:1) have role in reducing blood glucose level in rats by 47.945% was preprandial and 74.159% was postprandial [3]. The study of sambiloto as anti-hyperglicemic also showed in the mixture formula such as sambiloto and mahoni reduced blood glucose level in mice by 250.80 mg/dl [4].

Many studies have proven the efficacy of sambiloto as anti-diabetic agent and raises potential to be developed into phyto-pharmaceutical product as anti-diabetic. Therefore, it is necessary to do standardization. It can affect quality of both extract and raw material by methods that have been established by Ministry of Health through Indonesia Herbal Pharmacopeia. It is a guarantee for safety, effectiveness, and especially quality of plant material, raw material and ethanolic extract that developed into phyto-pharmaceutical preparation.

2 MATERIAL AND METHODS

2.1. Plant Material

In this research, the raw material was obtained from two different districts, there are Bogor (BALITRO, Balai Penelitian Tanaman Obat dan Aromatik) and Tawangmangu (BPTO, Balai Besar Penelitian dan Pengembangan Tanaman Obat dan Obat Tradisional). They prepared in dried raw material. The ethanolic extract was extracted by maceration method with 96% ethanol for 24 hours and performed up to three times [5]. The mixture extract was concentrate using rotary evaporator.

2.2. Determination of Specific and Non-specific Parameters of Dried Raw Material and Ethanolic Extract of Sambiloto

Determination of standard parameter was done using Ministry of Health Guidelines through Indonesian Herbal Pharmacopeia [6]. Specific parameters of raw material and extract were macroscopic, macroscopic, organoleptic, water-soluble extractive, ethanol soluble extractive, and andrographolide assay in raw material as well as ethanolic extract. While non-specific parameters encompassed loss on

Sukardiman, ., Rakhmawati, ., Prabowo, A. and Arifianti, L.
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DOI: 10.5220/0009846000002406
In *Proceedings of BROMO Conference (BROMO 2018) - Symposium on Natural Product and Biodiversity*, page 1
ISBN: 978-989-758-347-6

drying, total ash content, water content, and acid-insoluble ash content. In this case, the assay of andrographolide was based on previous study to prove andrographolide was major compound that had anti-hyperglicemic activity.

2.3. Determination of Andrographolide Content in Raw Material and Ethanolic Extract

Andrographolide content in raw material and extract determined based on the sambiloto monograph in the Indonesian Herbal Pharmacopeia. Andrographolide used to make calibration curve. Concentration of the andrographolide solution was 0.8 and 0.125 mg/ml. Andrographolide solution at 0.8 mg/ml was dropped with 1.2 and 3 µl, whereas the solution at 0.125 mg/ml was dropped with 2.4 and 6 µl of volume. The extract solution and ethanol soluble extractive of raw material were made at 1 mg/ml.

The thin layer chromatography system was silica-gel as stationary phase and the mobile phase system of chloroform:methanol (9:1) [7]. Then, sample eluted with mobile phase until the spot moved up to 8 cm. The spot was measured with TLC densitometry with lambda 310 nm.

3 RESULT AND DISCUSSION

Bogor district is representative city with high rainfall (4.086 mm/year). It has altitude at 190 – 330 m above of the sea level. The soil characteristic in the Bogor is yellowish-red latosol. Whereas Tawangmangu locations has altitude at 1236 m above of the sea level and the soil condition is dominated by brown litosol. Tawangmangu district was represented with high rainfall (3326 mm/year). The difference environment may influence component in the andrographis herbs.

3.1. Macroscopic Study

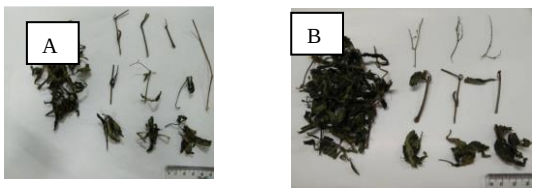

Figure 1: Dry herbs of sambiloto (A) from Bogor and (B) from Tawangmangu

3.2. Microscopic Study

There was eight identifier fragments in each sambiloto from two different places. Mesophyll tissues with leaves bone were identified (fig. 2A). Another fragment was discovered as stomata with diacytic type (fig. 2B) and xylem with dot type (fig. 2C) and ladder type (fig. 2D). Cystolith was discovered in the epidermal wall (fig. 2E). The trichoma was found in the leaves (fig. 2F) and chollenchyma was often found in the stems (fig. 2G).

Table 1: Result of phytochemical identification of raw material (dried herbs) and ethanolic extract of *Andrographis panciulata* Nees

Phytochemical parameters	Region	Sample form	Mean ± SD (n=3)
1. Water content (%)	Bogor	ethanol extract	12.28 ± 0.43
	Tawangmangu	ethanol extract	9.97 ± 0.01
2. Total ash (%)	Bogor	ethanol extract	3.12 ± 0.34
		dried herbs	11.81 ± 0.06
	Tawangmangu	ethanol extract	7.45 ± 1.45
		dried herbs	11.34 ± 0.52
3. Acid insoluble ash (%)	Bogor	ethanol extract	0.03 ± 0.01
		dried herbs	0.09 ± 0.08
	Tawangmangu	ethanol extract	0.04 ± 0.01
		dried herbs	0.53 ± 0.02
4. Loss on drying (%)	Bogor	dried herbs	8.66 ± 0.26
	Tawangmangu	dried herbs	7.91 ± 0.18
5. Ethanol soluble extractive (%)	Bogor	dried herbs	9.98 ± 0.09
	Tawangmangu	dried herbs	10.29 ± 0.33
6. Water soluble extractive (%)	Bogor	dried herbs	17.41 ± 0.43
	Tawangmangu	dried herbs	16.16 ± 0.99
7.Andrographolide content (%)	Bogor	ethanol extract	23.76 ± 1.06
		dried herbs	2.25 ± 0.02
	Tawangmangu	ethanol extract	15.61 ± 0.88
		dried herbs	1.95 ± 0.02

3.3. Physicochemical Identification

The results of physicochemical identification were showed in the table 1. They showed that several parameters such as water-soluble extractive loss on drying, and water content of extract from Bogor was higher than Tawangmangu, oppositely that ethanol soluble extractive of sample and insoluble acid ash from Tawangmangu higher than Bogor. Both ash content from Bogor or Tawangmangu showed higher than Indonesian Herbal Pharmacopeia

requirement. The water content should not more than 10% because it may affect stability during storage and production caused by enzymatic activity. The total ash content was higher than Indonesian Herbal Pharmacopeia requirement either from Bogor or Tawangmangu. The contaminations of total ash of the both samples were exceeded from limit requirement of Indonesia Herbal Pharmacopeia, but insoluble acid ash of both samples still at allowed level.

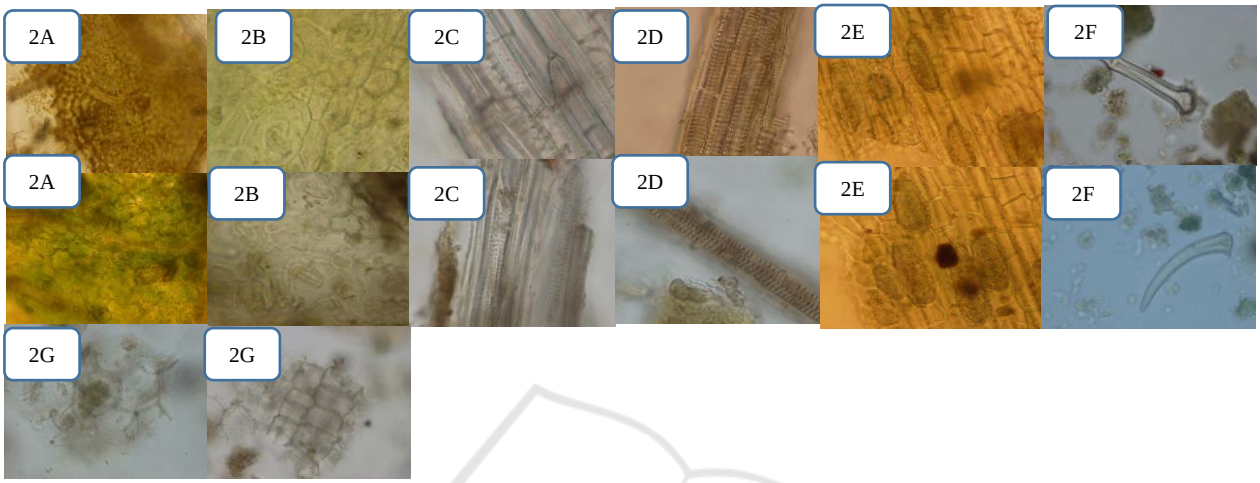

Figure 2 : Identifier fragments for sambiloto in the left side are from Bogor and right side are from Tawangmangu

3.4. Determination of the Andrographolide Content

Andrographolide ($C_{20}H_{30}O_5$) is a major diterpenoid in *A. paniculata* that presence about 4%, 0.8~1.2% and 0.5~6% in dried whole plant, stem and leaf extracts, respectively [2,3,5]. Their spot appeared at 0.37 of Rf value. The sample from Bogor had andrographolide higher than from Tawangmangu, not only as raw material but also as extract. Andrographolide content in ethanolic extract from Bogor was 23.76 ± 1.06 and from Tawangmangu was 15.61 ± 0.88, whereas andrographolide content in dried herbs was 2.25 ± 0.02 from Bogor district and 1.95 ± 0.02 from Tawangmangu district.

In conclusion, the results of standard parameters from Bogor and Tawangmangu allowed at level of Indonesia Herbal Pharmacopeia, except total ash content of ethanolic extract and dried herbs and water content of ethanolic extract sambiloto from Bogor was exceeded from Indonesian Herbal Pharmacopeia requirements.

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