Quantification of Andrographolide in *Andrographis paniculata* (Burm.f.) Nees, Myricetin in *Syzygium cumini* (L.) Skeels, and Brazilin in *Caesalpinia sappan* L. by HPLC Method

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**ABSTRACT**

**Introduction:** Andrographolide, myricetin, and brazilin are bioactive compounds from *Andrographis paniculata*, *Syzygium cumini*, and *Caesalpinia sappan* plants that have potential as medicinal ingredients. **Objectives:** To determine the levels of andrographolide in *A. paniculata* herb extract (APE), myricetin in *S. cumini* leaf extract (SCE), and brazilin in *C. sappan* wood extract (CSE) as marker compounds for extract quality control using the HPLC method. **Methods:** The separation was carried out on a reverse-phase C18 column (150 x 4.6 mm; 5 μm). The isocratic was prepared from methanol - water (50:50 v/v); 0.1% orthophosphoric acid - methanol (60:40 v/v); and 0.3% acetic acid - acetonitrile (85.5: 14.5 v/v) as mobile phase with flow rate 1 mL/min for andrographolide, myricetin, and brazilin determination, respectively and detection using UV detector at a wavelength of 284 nm, 369 nm, and 280 nm, respectively. **Results:** The linear regression for andrographolide was y = 14113x + 5948.8 ($r^2$= 0.9994); myricetin was y = 87766x – 138895 ($r^2$=0.9996); and brazilin was y = 18520x – 42668 ($r^2$=0.9992). The andrographolide content in APE was found to be 14.4868 %. The myricetin content in SCE was found to be 0.3190 %. The brazilin content in CSE was found to be 2.1280 %. **Conclusion:** The described HPLC method was successfully used for the analysis of the APE, SCE, and CSE. This method can be used for the identification and quantification of andrographolide, myricetin, and brazilin in herbal raw materials or herbal products containing these three extracts. **Key words:** Andrographis paniculata, Caesalpinia sappan, HPLC, Marker compounds, Syzygium cumini. Quality control.

**INTRODUCTION**

*Andrographis paniculata* (Burm.f.) Nees (family: Acanthaceae) is known as "Sambiloto" in Indonesia. It grows in South Asian countries and is used as traditional medicine in China, Hong Kong, the Philippines, Malaysia, Thailand, and Indonesia.1 The major constituents of *A. paniculata* are diterpenoids, flavonoids, and polyphenols.2 Typical contents are diterpene lactones, including andrographolide and its analogs, neoandrographolide, 14-deoxyandrographolide, and 14-deoxy-11-12-didehydroandrographolide.3 Andrographolide is an active component with a very bitter taste has many biological activities, including anti diabetic and antihyperlipidemic,4 anti-inflammatory,5 antiallergic,6 anticytokine,7 anticancer,8 and hepatoprotective.9

*Syzygium cumini* (L) Skeels (family: Myrtaceae) is known as "Jamblang" in Indonesia, is a tropical plant found across Southeast Asia, including Indonesia. It is known to have various medicinal properties, which have been attributed to the presence of bioactive compounds in various parts of the plant.9 This plant is reported rich in flavonoids and phenolic acids. The most flavonoid content was reported in the *S. cumini* leaves, especially quercetin, myricetin, myricitrin, kaempferol, and their glucoside derivatives, in addition to simple phenols such as ellagic acid, ferulic acid, chlorogenic acid, and gallic acid.10 Myricetin, as one of the bioactive markers in *S. cumini* leaf, has been reported to have various pharmacological activities including antiplatelet,11 antihyperglycemic,12 antioxidant,13,14 neuroprotective,15 and treatment of cardiometabolic diseases.16

*Caesalpinia sappan* L. (family: Caesalpiniaeae)16 is known as "Secang" in Indonesia. It grows and is widespread in Southeast Asia, including Indonesia. The chemical constituents contained in *C. sappan* are phenolic components including xanthones, coumarin, chalcones, flavones, homoisoflavonoids, and brazilin.17 Brazilin is the major phenolic compound contained in extracts of the *C. sappan* wood and has proven to have many pharmacological activities such as antidiabetic,17,18,19,20 antihypertensive, anti-inflammatory,19 antioxidant,21 and antibacterial.16

*A. paniculata*, *S. cumini*, and *C. sappan* can be combined to be developed into herbal products. The criteria for good herbal medicines are quality, safety, and efficacy. To meet these criteria, as a first step it is necessary to standardize raw materials as a guarantee of product quality.22 The raw materials in the form of plant extracts contain active substances with therapeutic levels. Determination of the concentration of substances in extracts requires a selective method with accuracy and precision that meets the requirement for a valid method. One such method is high-performance liquid chromatography (HPLC). It has been used to identify and determine levels of active compounds in a plant.23

Quantification of andrographolide, myricetin, and brazilin in plant parts is one of the initial steps in the standardization as quality control of herbal
Ingredients for their development as herbal products. Therefore, this study aimed to determine the content of the andrographolide in the herb extract of *A. paniculata*, myricetin in the *S. cumini* leaf extract, and brazilen in the *C. sappan* wood extract.

**MATERIALS AND METHOD**

**Reagent**

Andrographolide (≥98%), myricetin (≥96%), and brazilen (≥98%) standards were purchased from Sigma-Aldrich (St. Louis, MO, USA). All reagents, such as methanol and acetonitrile (HPLC-grade), analytical grade orthophosphoric acid, and concentrated acetic acid were obtained from Merck (Germany).

**Plant materials**

The *A. paniculata* herbs and sappan wood (*C. sappan*) were collected from "UD Herbal Jaya", Karanganyar-Surakarta, Central Java, Indonesia, whereas the *S. cumini* leaves were obtained from "Koperasi Bina Kimia LIPI", Serpong, West Java, Indonesia. The three samples were authenticated by the Indonesian Institute of Sciences, Research Center for Plant Conservation, Botanic Gardens, Bogor, West Java, Indonesia (voucher number: B-890/IPH.3/KS/VII/2020). The samples were cleaned, impurities were removed, powdered using a blender, and stored in airtight containers.

**Extraction**

The dried powder of *A. paniculata* herbs (500 g), *S. cumini* leaves (500 g), and *C. sappan* wood (500 g) was macerated using 70% ethanol (1:10 w/v) at room temperature for 24 hours, respectively. Subsequently, the filtrate was filtered and collected. The residue was macerated again using the same procedure. After 2 times re-maceration, all filtrate was collected, concentrated with a vacuum rotary evaporator, followed by a water bath to obtain a thick extract.

Each crude extract from *A. Paniculata* (APE), *S. Cumini* (SCE), and *C. Sappan* (CSE) was dissolved in HPLC-grade methanol to 10 mL. The solution was sonicated for 10 minutes, then filtered with Syringe Filter 0.45 μm PTFE. Subsequently, the sample solution was diluted with methanol-water (50:50 v/v); 0.1% orthophosphoric acid-methanol (60:40 v/v); and 0.3% acetic acid-acetonitrile (85:15 v/v) as mobile phase at a flow rate of 1 mL/min and the temperature was set to 25°C for determination of andrographolide, myricetin, and brazilen, respectively. The APE, SCE, and CSE injection volume was 20 μl, and the analyses were monitored with the UV-Vis detector at 254 nm, 369 nm, and 280 nm, respectively. The system and chromatographic conditions are presented in Table 1.

**Calibration curve of andrographolide, myricetin, and brazilen standards**

Each standard stock solution (100 μg/mL) were diluted with methanol (HPLC-grade) to 6 concentration series on the range of 8 - 48 μg/mL andrographolide; 8.7 - 48 μg/mL myricetin; and 28 - 56 μg/mL brazilen. Furthermore, the series solution concentration of the standard was injected and analyzed according to the chromatographic conditions of each sample and peak areas were recorded. Linearity was determined by three injections of six concentration series. The mean peak area was plotted against the concentration. Then the linearity was evaluated using a calibration curve to calculate the correlation coefficient, slope, and intercept.

**Quantification of andrographolide in APE, myricetin in SCE, and brazilen in CSE**

Five mg each extract of APE, SCE, and CSE dissolved in HPLC-grade methanol to 10 mL. The solution was sonicated for 10 minutes, then filtered with Syringe Filter 0.45 μm PTFE. Subsequently, the sample was injected into the HPLC system according to chromatographic conditions for each extract test (Table 1). The peak areas were recorded and the concentration of andrographolide, myricetin, and brazilen in the samples were determined using the calibration curve.

**RESULT AND DISCUSSION**

**Calibration curve of andrographolide, myricetin, and brazilen standards**

The linear regression equation of calibration curve for andrographolide was $y = 14113x + 5948.8$ ($r^2 = 0.9994$) (Figure 1); myricetin was $y = 87766x - 138895$ ($r^2 = 0.9996$) (Figure 2); brazilen was $y = 18520x - 42668$ ($r^2 = 0.9992$) (Figure 3). The linear regression equation obtained has good linearity with correlation coefficient value ($r^2$) > 0.998 was considered as evidence that the assay method used is quite sensitive.24

| Sample | Injected concentration (μg/mL) | Area | Slope | Intercept | Concentration obtained (%) |
|--------|-------------------------------|------|-------|-----------|---------------------------|
| APE    | 500                           | 1026926 | 5948.8 | 14113     | Andrographolide 14.4686   |
| SCE    | 500                           | 1079  | -138895 | 87766     | Myricetin 0.3190          |
| CSE    | 500                           | 154383 | -42668 | 18520     | Brazilen 2.1280           |

**Table 1**: HPLC condition for determination of andrographolide in the APE, myricetin in the SCE, and brazilen in the CSE.

**Table 2**: The content of andrographolide in APE, myricetin in SCE, and brazilen in CSE.
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**Figure 1:** Calibration curve of andrographolide.

\[ y = 14113x + 5948.8 \]
\[ R^2 = 0.9994 \]

**Figure 2:** Calibration curve of myricetin.

\[ y = 87766x - 138895 \]
\[ R^2 = 0.9996 \]

**Figure 3:** Calibration curve of brazilin.

\[ y = 18520x - 42668 \]
\[ R^2 = 0.9992 \]
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**Figure 4:** Representative HPLC Chromatogram of andrographolide standard (A) and APE (B). The mobile phase was methanol-water (50:50 v/v). Detection UV 254 nm.

**Figure 5:** Representative HPLC Chromatogram of myricetin standard (A) and SCE (B). The mobile phase was 0.1% ortho phosphoric acid-methanol (60:40 v/v). Detection UV 369 nm.
Quantification of andrographolide in APE, myricetin in SCE, and brazilin in CSE

In this study, 70% ethanol was successfully used to extract the andrographolide, myricetin, and brazilin compounds contained in A. paniculata herb; S. cumini leaf; and C. sappan wood, respectively. The retention times obtained for andrographolide, myricetin, and brazilin were 5.930; 10.492, and 5.533 min, respectively. The representative HPLC-chromatogram of andrographolide standard and APE was shown in Figure 4, myricetin standard and SCE was shown in Figure 5, and brazilin standard and CSE was shown in Figure 6.

The andrographolide content in APE was found to be 14.4686 %. The myricetin content in SCE was found to be 0.3190 %. The brazilin content in CSE was found to be 2.1280 %. The result of andrographolide, myricetin, and brazilin determination are shown in Table 2.

The varying levels of bioactive compounds obtained are influenced by several factors, including the area where the plant grows, harvesting and post-harvest processing, as well as the extraction method and solvent used to extract the active compounds. 23,25,26

CONCLUSION

In this study, andrographolide in A. paniculata herb extract, myricetin in S. cumini leaf extract, and brazilin in C. sappan wood extract could be detected and quantified using the HPLC method. The andrographolide, myricetin, and brazilin content was 14.4686 %; 0.3190 %; and 2.1280 %, respectively. The quantification data obtained can be used to assess the biological activity of the raw materials of A. paniculata, S. cumini, and C. sappan singly or in combination. In addition, as a guarantee of quality control of herbal products containing the ingredients of these three extracts.

CONFLICTS OF INTEREST

There is no conflicts of interest.

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ABBREVIATIONS

APE: Andrographis paniculata herb extract; CSE: Caesalpinia sappan wood extract; HPLC: High-Performance Liquid Chromatography; SCE: Syzygium cumini leaf extract; UV: Ultraviolet.
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GRAPHICAL ABSTRACT

The dried powder of (a) *A. paniculata* herbs, (b) *S. cumini* leaves, and (c) *C. sappan* wood were macerated using 70% ethanol (1:10 w/v) at room temperature. Quantitative analysis by HPLC method.

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