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Research Article

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 254 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 14.4686 %. 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.

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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 antidiabetic and antihyperlipidemic,[4] anti-inflammatory,[5] anti-arthritic,[6] anticancer,[7] and hepatoprotective.[8]

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] antidiabetes,[13,14] antioxidant,[14,15] neuroprotective,[15] and treatment of cardiometabolic diseases.[10]

Caesalpinia sappan L. (family: Caesalpiniaceae)[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

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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 brazilin in the *C. sappan* wood extract.

MATERIALS AND METHOD

Reagent

Andrographolide (≥98%), myricetin (≥96%), and brazilin (≥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 (Jerman).

Plant materials

The *A. paniculata* herbs and sappan wood (*C. sappan*) were collected from "UD Herbal Jaya", Karanganyar-Surakarta, Central Java, Indonesia, whereas *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-809/IPH.3/KS/VII/2020). The samples were cleaned, impurities were removed, powdered using a blender, and stored in airtight container.

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) as much as 1 mg dissolved up to 10 mL with methanol. This solution was used for the quantification of andrographolide, myricetin, and brazilin by HPLC.

HPLC system and chromatographic condition

The andrographolide in APE, myricetin in SCE, and brazilin in CSE were determined using a Shimadzu 20 LC-AT HPLC System (Shimadzu, Japan). The system on the isocratic mode with LC-20AT pump and using an Inertsil ODS C18 reverse-phase column (150 x 4.6 mm; 5 µm). The elution was carried out with a binary solvent system of 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 at a flow rate of 1 mL/min and the temperature was set to 25°C for determination of andrographolide, myricetin, and brazilin, 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 brazilin 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 brazilin. 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 brazilin 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 brazilin in the samples were determined using the calibration curve.

RESULT AND DISCUSSION

Calibration curve of andrographolide, myricetin, and brazilin 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); brazilin 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]

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

HPLC	Description of the testing		
	APE	SCE	CSE
Instrument	HPLC Shimadzu LC-20AT		
Detector	UV-Vis		
Column	C-18 INERTSIL ODS-3 (4,6 x 150 mm, 5 µm particle size)		
Injected volume	20 µL		
Flow rate	1.0 mL/menit		
Mobile phase	methanol-water (50:50 v/v)	0.1% orthophosphoric acid-methanol (60:40 v/v)	0.3% acetic acid - acetonitrile (85.5:14.5 v/v)
Wavelength	254 nm	369 nm	280 nm

Table 2: The content of andrographolide in APE, myricetin in SCE, and brazilin in CSE.

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	Brazilin 2.1280

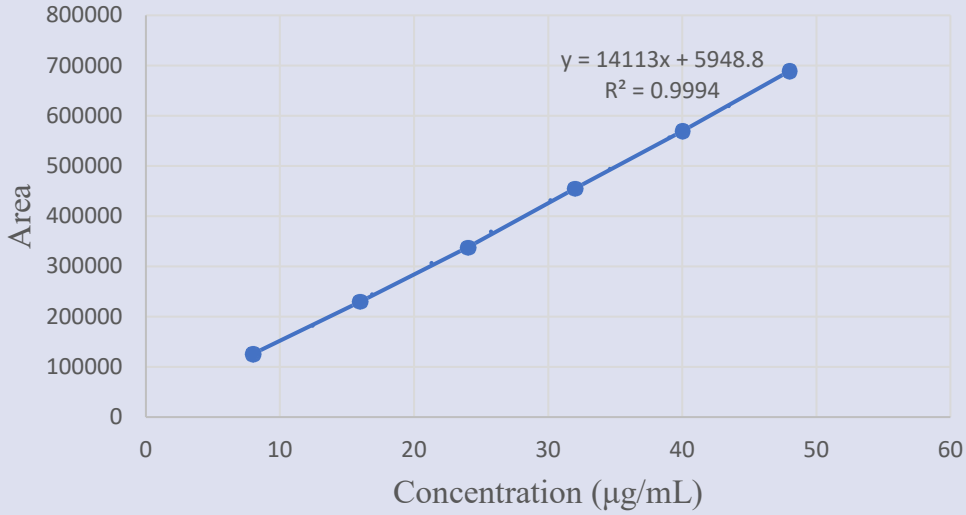

Figure 1: Calibration curve of andrographolide.

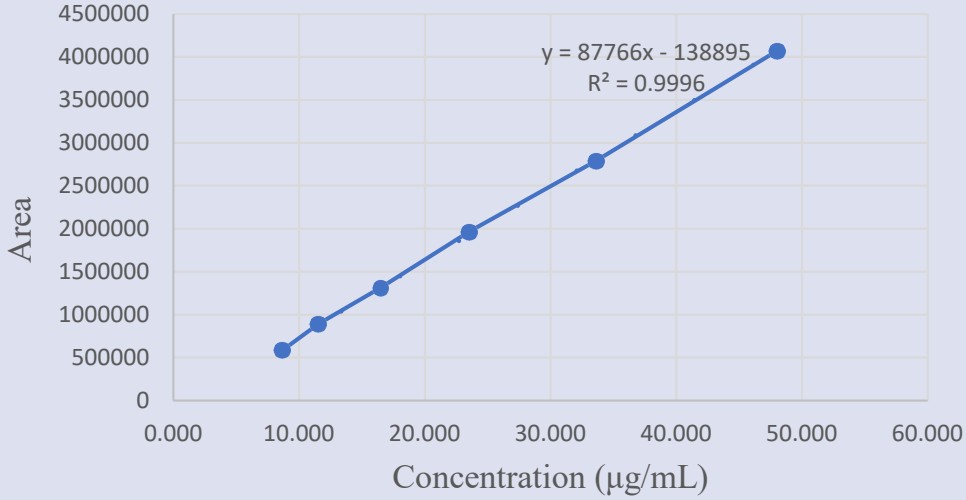

Figure 2: Calibration curve of myricetin.

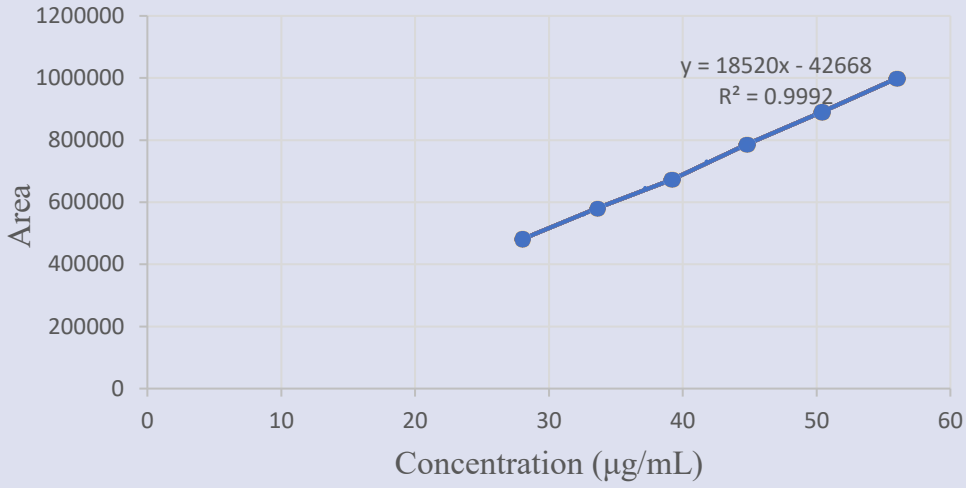

Figure 3: Calibration curve of brazilin.

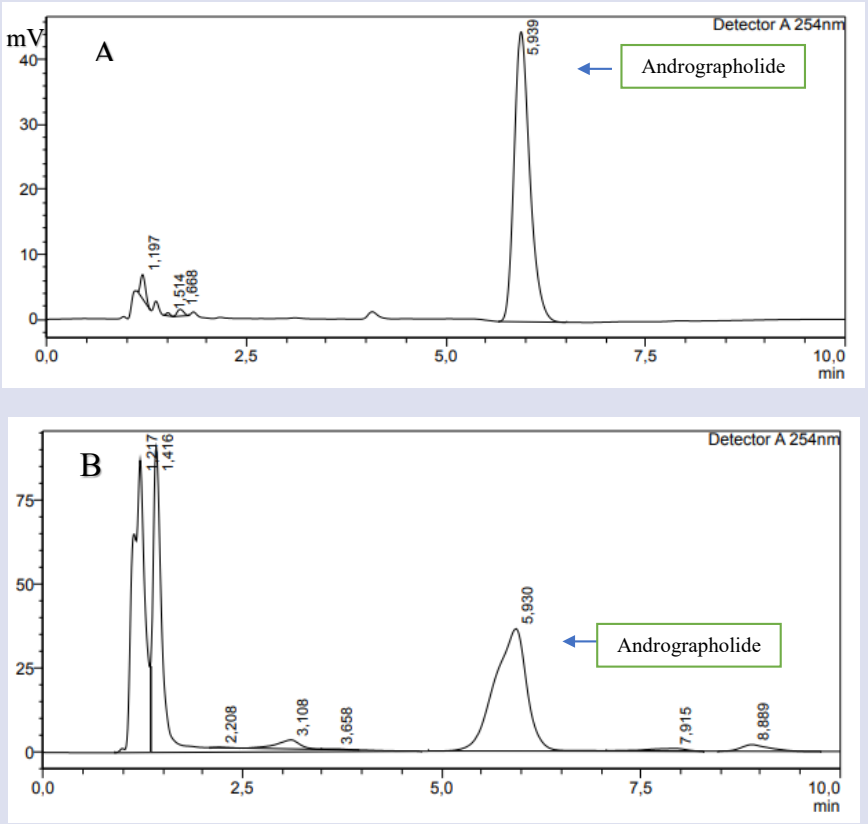

Figure 4: Representative HPLC Chromatogram of andrographolide standard (A) and APE (B). The mobile phase was methanol-water (50:50 v/v). Detection UV 254 nm.

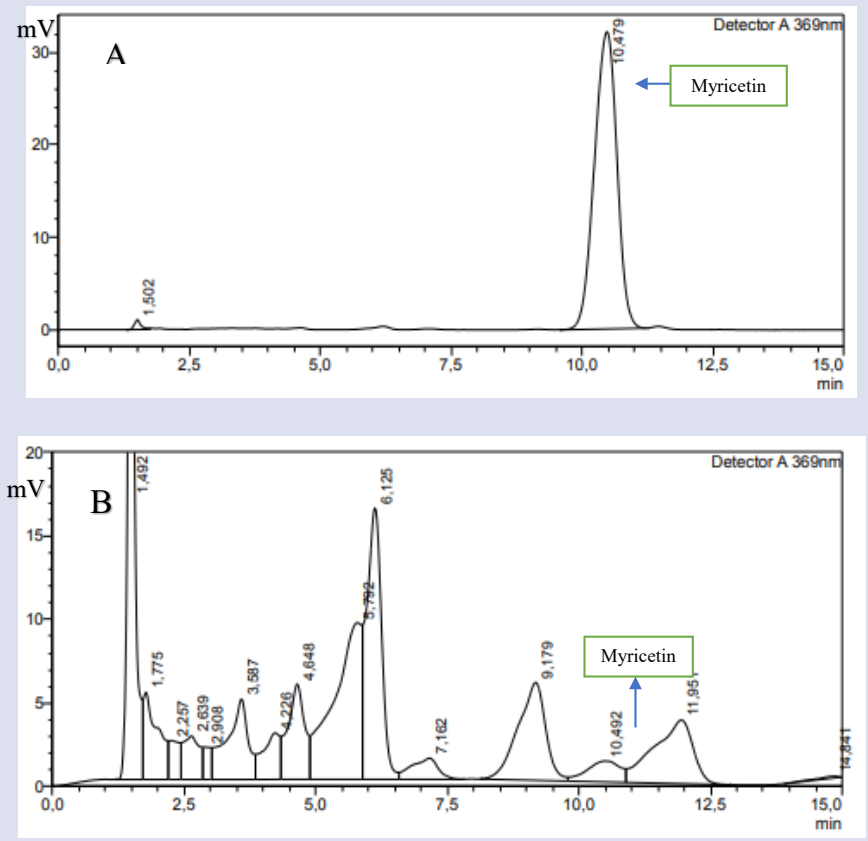

Figure 5: Representative HPLC Chromatogram of myricetin standard (A) and SCE (B). The mobile phase was 0.1% ortho phosphoric acid-methanol (60:40 v/v). Detection UV 369 nm.

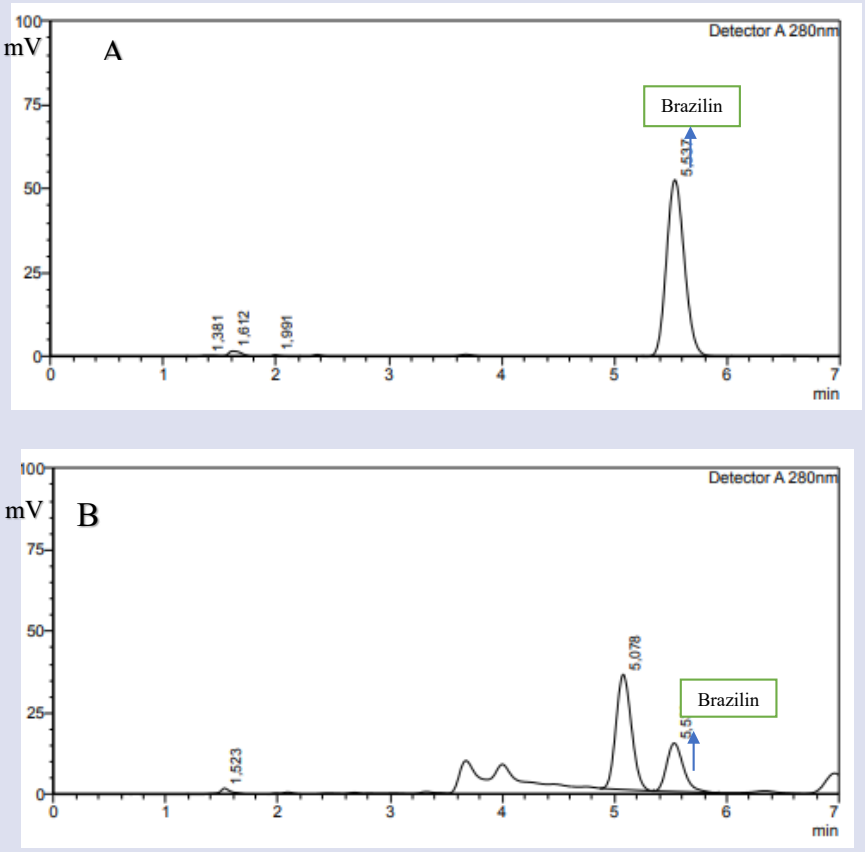

Figure 6: Representative HPLC Chromatogram of brazilin standard (A) and CSE (B). The mobile phase was 0.3% acetic acid-acetonitrile (85.5:14.5 v/v). Detection UV 280 nm.

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 quantification of andrographolide, myricetin, and brazilin found in 70% ethanolic extracts of *A. paniculata* herb, *S. cumini* leaf, and *C. sappan* wood varied with the results of previous studies. Sharma's research reported that the highest andrographolide content was in the leaves of *A. paniculata* 4.686%.[27] Jadhao's study reported that the content of andrographolide in the dried powder *A. paniculata* was 1.12%, using an isocratic solvent system consisting of isopropyl alcohol, formic acid, and water (70:10:20 v/v) was monitored at UV 223 nm.[28] The content of myricetin in *S cumini* plant powder was 1.19% with 0.1% orthophosphoric acid and methanol 65:35 (v/v) as mobile phase, was detected at UV 220 nm.[9] The content of brazilin in 95% ethanolic extract of sappan wood was 7.70% with gradient elution methanol and 2.5% acetic acid was detected at UV 280 nm.[29]

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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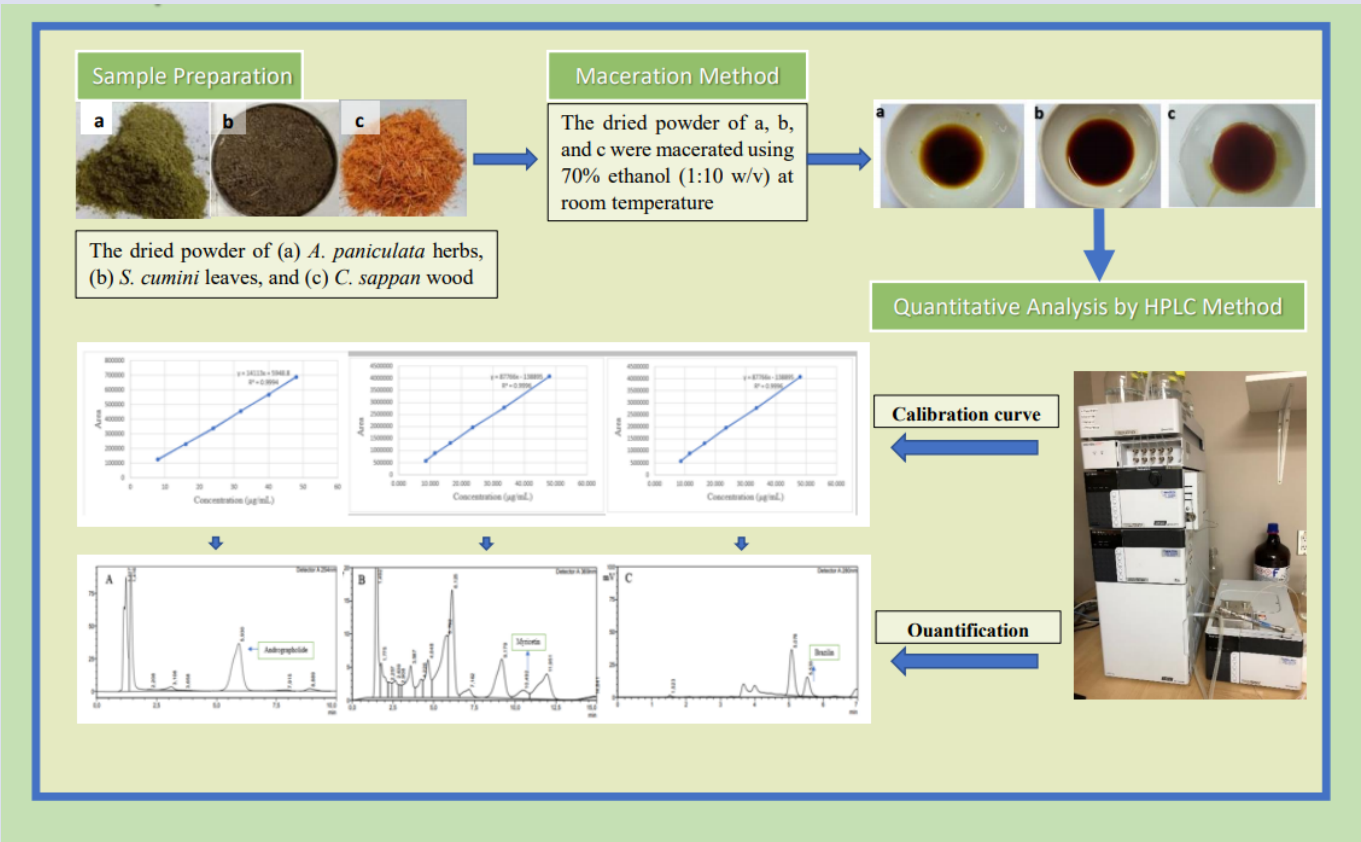
GRAPHICAL ABSTRACT



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