Indonesian J. Pharm. Vol. 25 No. 3 : 138 – 144
ISSN-p : 2338-9427
DOI: 10.14499/indonesianjpharm25iss3pp138

Research Article

BIOAVAILABILITY STUDY OF SAMBILOTO (*Andrographis paniculata*) HERBS INFUSION IN RABBIT

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Submitted: 04-02-2014
Revised: 20-04-2014
Accepted: 23-05-2014

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ABSTRACT

Andrographis paniculata or sambiloto is one of the most widely used medicinal herbs in Indonesia. The main bioactive chemical constituent, andrographolide, has been reported to have various pharmacological activities. Besides its function for medical purposes, the sambiloto herbs infusion is frequently taken to maintain health. This study was conducted to determine the bioavailability of sambiloto herbs infusion in rabbit plasma, stomach, and liver, calculated as total andrographolide. Fourteen male New Zealand white rabbits were used in this study. Sambiloto herbs infusion were administered orally at the dose 7.04mL/kg body weight to each rabbit. Blood samples were taken at intervals 0.0; 0.5; 1.5; 2.0; 3.0; and 5.0h after infusion administration. Sambiloto herbs infusion, which are calculated as andrographolide, levels in plasma, stomach, and liver were analyzed by high performance liquid chromatography using C-18 column as stationary phase and a mixture of methanol-double distilled water (60:40) as mobile phase. Bioavailability parameters obtained were C_{max} 0.5549µg/mL (in stomach), 0.2136µg/mL (in plasma), 0.0051µg/mL (in liver); while t_{max} 1h (in stomach), 1.5h (in plasma), 2h (in liver); and AUC 1.7451µg.h/mL (in stomach), 0.434µg.h/mL (in plasma), 0.0038µg.h/mL (in liver). These data showed that in healthy animals, sambiloto herbs infusion was fastly absorbed from the stomach, distributed in the circulation system, and metabolized in the liver, in subsequent process. Sambiloto herbs infusion showed good bioavailability in rabbit.

Key words: andrographolide, *Andrographis paniculata*, bioavailability, sambiloto

INTRODUCTION

Andrographis paniculata or sambiloto is one of the most widely used medicinal herbs in Indonesia. This plant also grows in many other Asian countries such as China, India, Thailand and Sri Lanka. It is particularly known for its extremely bitter properties and is used traditionally as a remedy against common cold, fever, inflammation, etc. The main bioactive chemical constituent, andrographolide, has been reported to have various pharmacological activities including anti-inflammatory *via* different mechanisms (Chiou *et al.,* 2000; Shen *et al.*, 2002; Satyanarayana *et al.,* 2004; Xia *et al.,* 2004; Abu-Ghefreh *et al.*, 2009; Levita, *et al*, 2010), anticancer and antitumour (Satyanarayana *et al.,* 2004; Shen *et al.,* 2009). Besides its function for medical purposes, sambiloto herbs infusion is frequently used to maintain health.

Andrographolide, an active component of *Andrographis paniculata*, is the major labdane diterpenoidal constituent in this plant, which has an α-alkylidene γ-butyrolactone, two olefin bonds at C8(17) and C12(13), and three hydroxyl groups at C3, C19, and C14 (Nanduri *et al.*, 2004).

Bioavailability of drugs refers to the extent and rate at which the active moiety (drug or metabolite) enters systemic circulation, thereby accessing the site of action. Pharmacological response is related with drug concentration at the receptor, therefore bioavailability of drug is an important element a clinical pharmaceutical effects (Chereson, 1996).

Previous study on the determination of bioavailability parameters of andrographolide

was performed by Budipramana and colleagues (2009). In their study, bioavailability of andrographolide in animal plasma was studied not from a single extract. Animals were treated with a mixture of ethanolic extracts of two plants (sambiloto and turmeric). They concluded that andrographolide was absorbed and distributed in blood within 60-90min and C_{max} 3.06-4.41ppm (Budipramana, 2009).

Other bioavailability study of andrographolide, didehydroandrographolide, and neoandrographolide in rats showed C_{max} 1.76μg/mL and t_{max} 2.04h after oral administration, while didehydroandrographolide was fastly absorbed and neoandrographolide was very slow (Pinthong *et al.,* 1991).

In this study, we determined the bioavailability of sambiloto herbs infusion, calculated as total andrographolide, the major component in the plant, using reversed-phase high performance liquid chromatography. Detection was set at ultraviolet wavelength, according to the compound's chromophores. Rabbit was chosen as tested animal. Measurement was carried out to rabbit plasma, stomach, and liver, calculated as total andrographolide.

MATERIALS AND METHODS

Andrographolide 98% 500mg CAS 5508-58-7 for R & D use (Aldrich), chloroform (Merck), double distilled water for HPLC (PT IPHA), methanol for HPLC (JT Baker), acetonitrile for HPLC (PT Bratachem), dried sambiloto herbs (Kebun Percobaan Manoko Lembang), New Zealand male rabbits (Faculty of Husbandry Universitas Padjadjaran).

Preparation of sambiloto herbs infusion

Prior to be used, sambiloto dried herbs was determined at Biology Department, Faculty of Mathematics and Natural Sciences Universitas Padjadjaran. Thirty grams of dried herbs were boiled in water for 15min 95°C (Hembing, 2008), filtered using cloth and kept in airtight container.

Thin layer chromatography

Thin layer chromatography was performed to detect andrographolide in sambiloto herbs infusion. A mixture of chloroform-methanol (9:1) was used as mobile phase. Sambiloto infusion was eluted on silica gel plate and detected under UV light at 254nm (Sukardiman, 2005).

HPLC

Optimization

Two hundred μg/L of andrographolide standard in methanol was injected into C-18 (250mm, particle size 5m) column, with a mixture of methanol: double distilled water (65:35) as mobile phase. Flow rate was 1mL/min and detection was set at 223nm. Parameters observed were resolution, time of retention and tailing factor (Kumaran *et al.*, 2002).

Validation of Bioanalytical Method

A mixture of rabbit plasma and methanol (1:5) was vortex-mixed for 2min and centrifugated for 30min at 3000rpm. It was used for validation assay.

Linearity

Linearity is obtained by plotting concentration of andrographolide against area under curve. Supernatant was used to dilute andrographolide standard solution to obtain 0.5ppm; 1ppm; 2ppm; 4ppm; 6ppm; 8ppm; and 10ppm concentrations. All solutions were milipore-filtered and 20μL of each solution was injected into the C-18 column.

Precision

A 20μL diluted solution of 4ppm concentration was injected into the C-18 column. The procedure was repeated 10times and percentage of RSD was calculated for precision.

Accuracy

Twenty μL diluted solutions with concentrations of 2ppm, 6ppm, and 10ppm were injected into the C-18 column. The procedure was repeated three times for each concentration and percentage of recovery was calculated for accuracy.

LOD and LOQ

LOD and LOQ were calculated as followed (Harmita, 2004)

$$SD = \sqrt{\frac{(Y - Yi)^2}{n - 2}}; \quad LOD = \frac{3 \times SD}{slope}$$

$$OQ = \frac{10 \times SD}{slope}$$

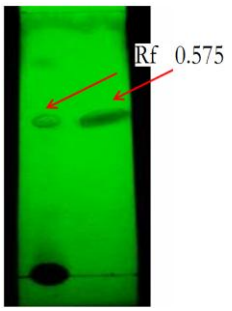

Figure 1. Thin layer chromatograms of andrographolide in sambiloto infusion (left) and andrographolide standard (right).

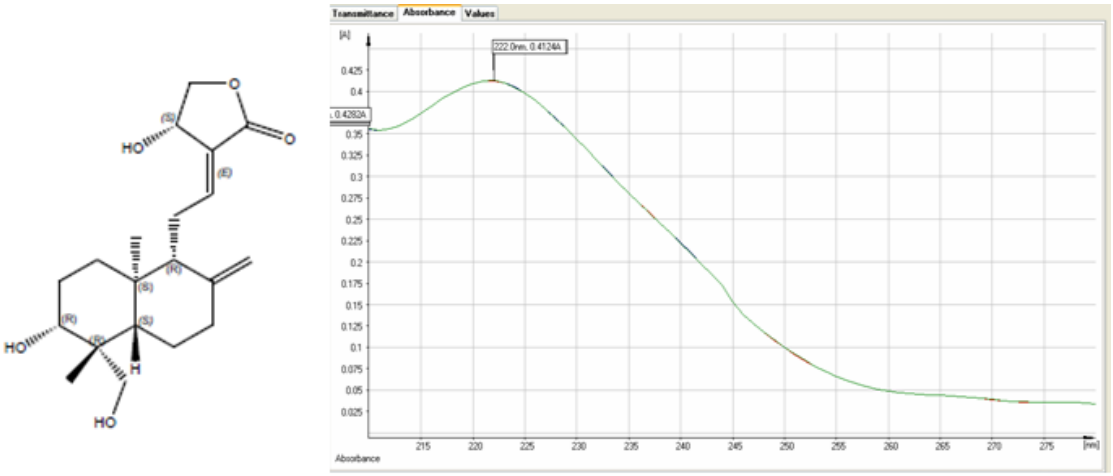

Figure 2. Andrographolide chemical structure (left) and UV spectrum in methanol (right)

Bioavailability assay of sambiloto infusion herbs calculated as total andrographolide

Fourteen New Zealand male rabbits (*Oryctolagus cuniculus*) aged four months old and weighed 1-1.5kg were acclimatized for two weeks to adapt with the environment. Sambiloto herbs infusion with the dose of 7.04mL/kg BW was given orally at day 15th. The blood was taken at interval of 0.0; 0.5; 1.5; 2.0; 3.0; 5.0h after sambiloto administration. Blood samples were kept in vacuette tubes containing anticoagulant and were centrifugated for 20min 2000rpm to precipitate erythrocites and other proteins. Plasma was separated and diluted with methanol and milipore-filtered prior to be injected to the column. Rabbit stomaches were collected, washed and weighed, at interval 0.0; 0.5; 1.5; 2.0; 3.0; 5.0h after sambiloto administration. The organs were crushed and homogenized using methanol as solvent (2mL of methanol for 200mg of the organ) and centrugated for 30min 3000rpm. Supernatant was separated and milipore-filtered prior to be injected to the column. Rabbit livers were collected, washed and weighed, at interval of 0.0; 0.5; 1.5; 2.0; 3.0; 5.0h after sambiloto administration. The organs were crushed and homogenized using methanol as solvent (2mL of methanol for 200mg of the organ) and centrifugated for 30min 3000rpm. Supernatant was separated and milipore-filtered prior to be injected to the column.

RESULT AND DISCUSSION

Thin layer chromatography

Sambiloto infusion resulted purple spot similar to andrographolide at the Rf 0.575 (Figure 1).

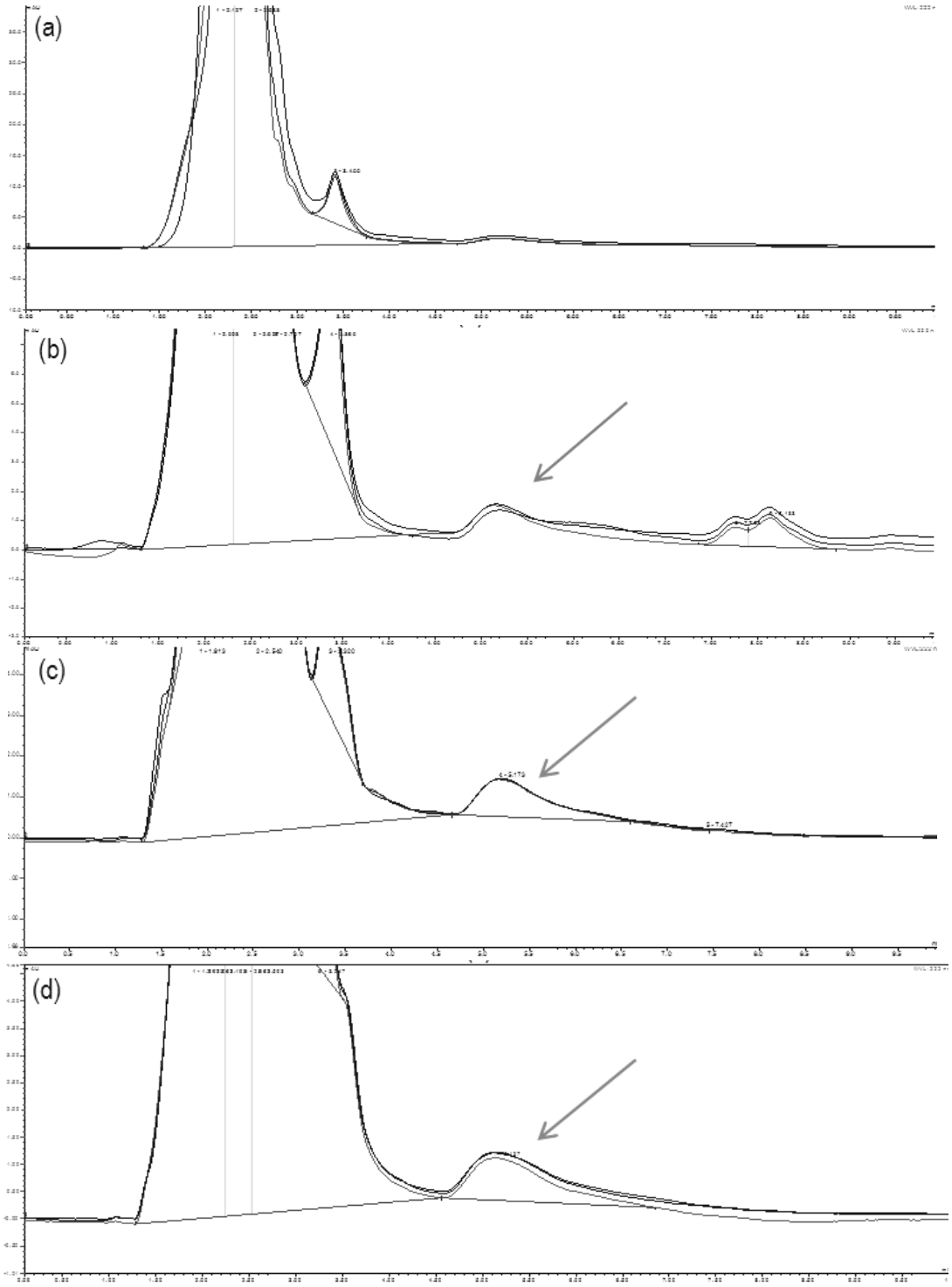

Figure 3. HPLC system of andrographolide in biological matrix using methanol-H_2O 60:40 (a) plasma blank (b) 1.5 hours after oral administration; (c) 3 hours after oral administration; (d) 5 hours after oral administration

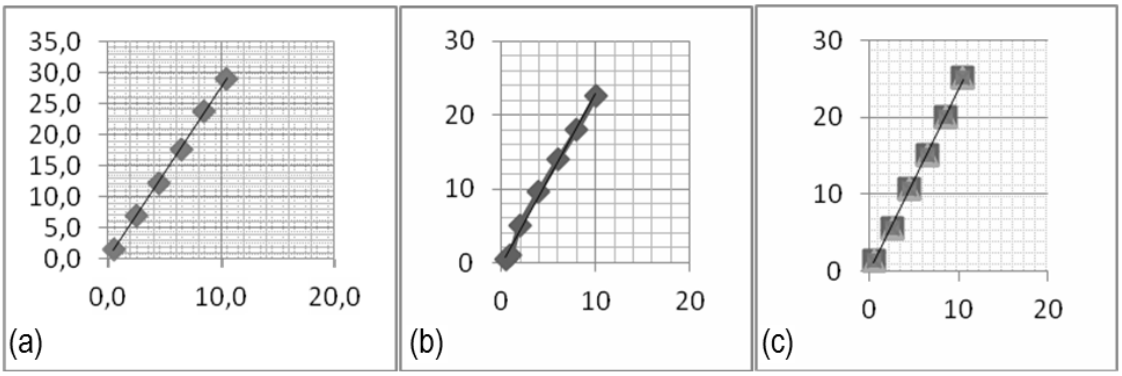

Figure 4. Linearity of andrographolide against detector response in rabbit plasma (a), stomach (b), and liver (c)

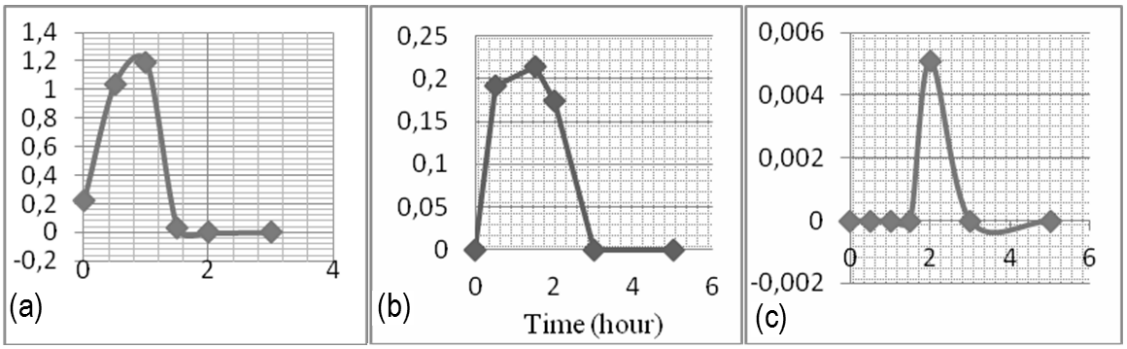

Figure 5. Pharmacokinetic profiles of andrographolide in rabbit plasma (a), stomach (b), liver (c)

Table I. Optimization of HPLC system

Mobile phase	Parameter		
	Resolution	Time of retention (minutes)	Tailing factor
Methanol: H_2O (65:35)	2.46	5.98	0.70
Methanol: H_2O (60:40)	3.2	7.66	0.98
Methanol: H_2O (55:45)	5.85	10.78	1.13

Table 2. Bioavailability parameters of sambiloto infusion calculated as andrographolide

Organ	Parameter	Value	% absorbed
Stomach	C_{max}	1.1893µg/mL	28.28 %
	t_{max}	1 h	
	AUC	2.4824µg h/mL	
Plasma	C_{max}	0.2136µg/mL	2.93 %
	t_{max}	1.5h	
	AUC	0.4340µg h/mL	
Liver	C_{max}	0.0051 µg/mL	0.05 %
	t_{max}	2h	
	AUC	0.0038µg h/mL	

The same Rf value shown (Figure 1 green arrows) indicated that andrographolide was positively contained in the infusion. The polar groups of Si-O attached on the surface of silica gel, retained andrographolide on the stationary phase, because the three hydroxyl groups at C3, C19, and C14 in its structure (Figure 2) could form hydrogen bonds.

HPLC

UV spectrum of andrographolide standard in methanol showed one peak at 222nm. This peak was elicited by $\pi \rightarrow \pi^*$ electronic transition of two olefin bonds at position C8-C9 and C11-C12 (Figure.2). Methanol itself shows absorption band at 190nm due to $n \rightarrow \sigma^*$ electronic transition of nonbonding electron of oxygen.

Good resolution was showed by Rs>1.5 (Gandjar and Rohman, 2007), therefore all three mixtures fulfilled the criteria.

The best composition of mobile phase selected for this analysis was a mixture of methanol-water (60:40) as the tailing factor was the closest to 1 (Figure 3 and Table I), meaning that the peak's symmetrical level is the best (Gandjar and Rohman, 2007).

Validation of bioanalytical method

Linearity study (Figure 4) indicated that there were good correlation between concentration of andrographolide and detector response for all three matrices (plasma y=2.338x–0.404 and r=0.994; stomach y=2.761x+0.030 and r=0.999; liver y=2.364x–0.009 and r=0.999).

Accuracy and precision studies for all three matrices indicated that percentage of recovery fell in interval 97.06% to 102.25% while RSD value were 0.82 to 1.62%, which meant that the method had high accuracy and precision.

LOD and LOQ were 1.87ppm and 5.44ppm (plasma) and 0.272ppm and 0.906ppm (stomach).

Bioavailability assay of sambiloto infusion herbs calculated as total andrographolide

Andrographolide was detected and quantified in the stomach less than an hour after administration (Figure 5b t_{max} =1h) and distributed in the circulation after one hour (Figure 5a t_{max}=1.5h). It could be observed and quantified n the liver two hours after administration (Figure 5c t_{max}=2h). These data confirmed that andrographolide had good bioavailability because it was fastly absorbed from the stomach, directly distributed in the circulation and metabolized in the liver. These facts were probably caused by the three hydroxyls in andrographolide molecule (Figure 2) which contributed to its hydrophilic character, while the diterpenoid rings caused its lipophilicity. Drugs will be absorbed during two hours after administration depends on food intake and activity (Holford, 1998).

Andrographolide is a bicyclic diterpenoid which is slightly soluble in water (3.29±0.73 μg/mL). This compound has moderate lipophilicity (log P = 2.632±0.135), and easily hydrolyzed in weak base to neutral environment (Bothiraja *et al.*, 2009a, b). Previous study concluded that the percentage of andrographolide absorbed was 0.24% (Budipramana, 2009). In this work, the level of andrographolide in the stomach was the highest because the food (given 24h before the animals were treated with sambiloto infusion) in the rabbits' stomach were slowly metabolized. It seemed that the food slowered the passive transporttation of andrographolide through stomach.

CONCLUSION

Sambiloto infusion herbs was fastly absorbed from the stomach, distributed in the circulation system, and metabolized in the liver, in subsequent process. It showed good bioavailability in rabbit.

ACKNOWLEDGEMENT

The authors wish to thank DIKTI for funding this research in 2011 grant.

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