

Determination of The Active Asiaticoside Content in *Centella asiatica* as Anti-Cellulite Agent

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Abstract

Centella asiatica accumulates large quantities of pentacyclic triterpenoid saponins, collectively known as centelloids. These terpenoids include asiaticoside, centelloside, madecassoside, brahmoside, brahminoside, thankunside, sceffoleoside, centellose, asiatic-, brahmic-, centellic- and madecassic acids. Preparations of *C. asiatica* are used in traditional and alternative medicine due to the wide spectrum of pharmacological activities associated with these secondary metabolites, such as anticellulite agent. Asiaticoside was found in *Centella asiatica*. In this present study, the asiaticoside was extracted using methanolic and ethanolic solvent. Determination of the asiaticoside content in the extract was conducted with High Performance Liquid Chromatography (HPLC), Thin Layer Chromatography (TLC), and Fourier Transform Infra Red (FTIR). Samples of *C. asiatica* used in this study came from three different plantation areas, Bogor, Lembang and Solo. Asiaticoside content in the methanolic extract from Bogor, Lembang, and Solo samples were 2.82%; 2.68%; and 2.8% respectively. Asiaticoside in ethanolic extract from Bogor, Lembang, and Solo samples were 2.79%; 2.75%; and 2.91% respectively. Two way ANOVA study showed that there was significant difference between types of solvent used in extraction and the asiaticoside content in the obtained extract, significant difference between varied plantation area and obtained asiaticoside content, and significant difference between interactions of different solvent with different plantation area.

Keywords: *Centella asiatica*, asiaticoside, anticellulite, medicine, metabolites

INTRODUCTION

Centella comprises some 50 species, inhabiting tropical and sub-tropical regions. This genus belongs to the plant family *Apiaceae* (*Umbelliferae*) and includes the most ubiquitous species *Centella asiatica*. This perennial creeper flourishes abundantly in moist areas and is a small, herbaceous annual plant of the subfamily Mackinlaya (Liu *et al.*, 2003), previously included in Hydrocotyle (Brinkhaus *et al.*, 2000), occurring in swampy areas of India, Sri Lanka, Madagascar, Africa, Australia (Schaneberg *et al.*, 2003), China, Indonesia, Malaysia, Australia and Southern and Central Africa (Verma *et al.*, 1999). The plant is clonally propagated by producing stolons that are characterized by long nodes and internodes which bear crowded cordate, obicular or reniform leaves and sessile flowers in simple umbels (Zheng and

Qin, 2007). Depending on environmental conditions, the form and shape of the *C. asiatica* plant can differ greatly (Adamson, 1950).

C. asiatica, also known as Gotu kola or Indian pennywort (Bruneton, 1995), is a medicinal plant that has probably been used since prehistoric times and has been reported to have been used for various medicinal and cosmetic purposes, thus becoming an important commercial product.

This plant is listed as a drug in the Indian Herbal Pharmacopoeia, the German Homeopathic Pharmacopoeia (GHP), the European Pharmacopoeia, and the Pharmacopoeia of the People's Republic of China (Schaneberg *et al.*, 2003).

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According to World Health Organisation (WHO) monographs, *Herbae Centellae* should not contain less than 2% of the triterpene ester glycosides asiaticoside and madecassoside (WHO, 1999).

This study would determine the asiaticoside content in *C. asiatica* from Indonesian plantation area. Samples were collected from three different *C. asiatica* plantation area in Indonesia that are Bogor, Lembang, and Solo. Quantitative determination of asiaticoside content was conducted with high performance liquid chromatography (HPLC).

MATERIALS AND METHODS

Samples of *Centella asiatica* (L.) Urban were obtained from three plantation area in Indonesia: Solo, Lembang, and Bogor. Species analyses of *C. asiatica* were confirmed by Research Center for Biology, Indonesian Institute of Sciences. Ethanol, methanol, acetonitrile, aquades, chloroform, Silika GF254 were used as received.

Extraction of *C. asiatica*

Extraction of *C. asiatica* was studied using methanol (70%) or ethanol (70%). Sample from each plantation area were extracted. Extraction was conducted with maceration technique by immersing 400g of *C. asiatica* sample in extraction solvent for 24 hours. Extract product was evaporated in rotary evaporator to obtain viscous extract.

Qualitative determination of triterpenoid compound

5 mg of extract and asiaticoside standard were mixed with 5 ml methanol. The mixture was then heated in water bath for 2 minutes. These mixtures were cooled to room temperature, and filtered. Precipitate was washed with methanol to get 5 ml filtrate. Sample and asiaticoside standard were spotted in Silica gel GF254 plate and were developed in chloroform:methanol (8:2). The plate was then sprayed with H₂SO₄ 10% (in methanol), and incubated in oven for 10 minutes at 110°C. Spot was observed under 366 nm UV light to determine the R_f value of sample and asiaticoside standard.

Solvent partition of *C. asiaticoside* extract

Methanolic and ethanolic extract were further partitioned with dichloromethane-water

(1:1). Water phase from this dichloromethane-water were further partitioned with buthanol. Buthanol phase was separated, and the water phase was added with 100 ml of 1N NaOH. NaOH phase was separated and the water phase was added with aquadest until reaching pH of 7. This water phase was concentrated with rotary evaporator until viscous extract was obtained. Obtained extract was passed to column chromatography with n-buthanol:ethyl acetate:water:ammonia (60:40:10:5) as the eluent.

Quantitative analysis

Quantitative determination of asiaticoside was conducted with high performance liquid chromatography (HPLC) using acetonitrile-water (70:30) as the mobile phase and silica column as the stationary phase, with flow rate of 1.0 ml/minute. Light detector in 221 nm was used to analyze asiaticoside. Series of asiaticoside standard was prepared and used to obtain calibration curve.

RESULTS AND DISCUSSION

Qualitative determination of asiaticoside

Sample from Bogor, Solo, and Lembang was sent to Herbarium Bogoriense to identify sample species. Identification result confirmed that samples from Bogor, Solo, and Lembang were *Centella asiatica* (L.) Urb. Samples were macerated in ethanol or methanol for 24 hours. This extract was further partitioned with dichloromethane-water (1:1). The water phase was further partitioned with buthanol. Buthanol phase was separated, and the water phase was added with NaOH. The water phase was added with aquadest until the pH reach 7. After further concentrated and passed to column chromatography with n-buthanol-ethyl acetate-water-ammonia (60:40:10:5) as the eluent, isolate was used for further qualitative and quantitative determination. Extract from different plantation area and varied extraction solvent was identified in thin layer chromatography (TLC). Identification with (TLC) was based on glycoside content in *C. asiatica*. Plate used was Silica gel GF254 developed with chloroform-methanol (8:2). To visualize the spot, H₂SO₄ (10%) was sprayed on the plate. Fig. 1 showed the resulted spot observed under UV light (366 nm). The spots detected from every samples and standard were clear and detectable. The R_f value was shown in table I. Identification with TLC confirmed the presence of asiaticoside in

methanolic extract from every plantation area, having same R_f value with asiaticoside standard reference ($R_f = 0.2833$). Meanwhile, ethanolic extract gave spot with different R_f value with standard reference. This is due to different solubility of asiaticoside in ethanolic and

methanolic. Qualitative determination of asiaticoside in the extract was also conducted by infrared spectroscopy. Fig. 2 show the infrared spectra of asiaticoside standard reference and extract sample.

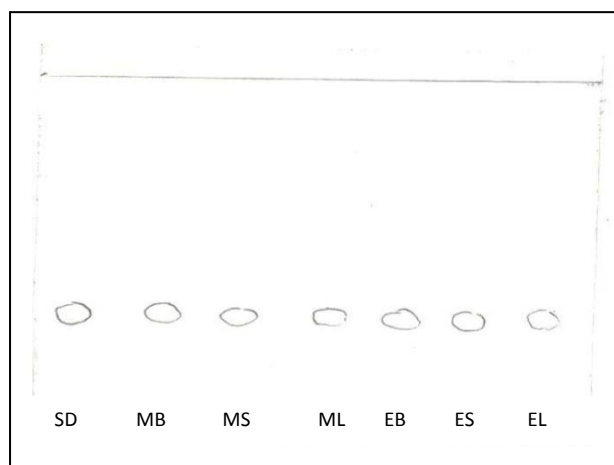


Figure 1. Identification of asiaticoside with TLC

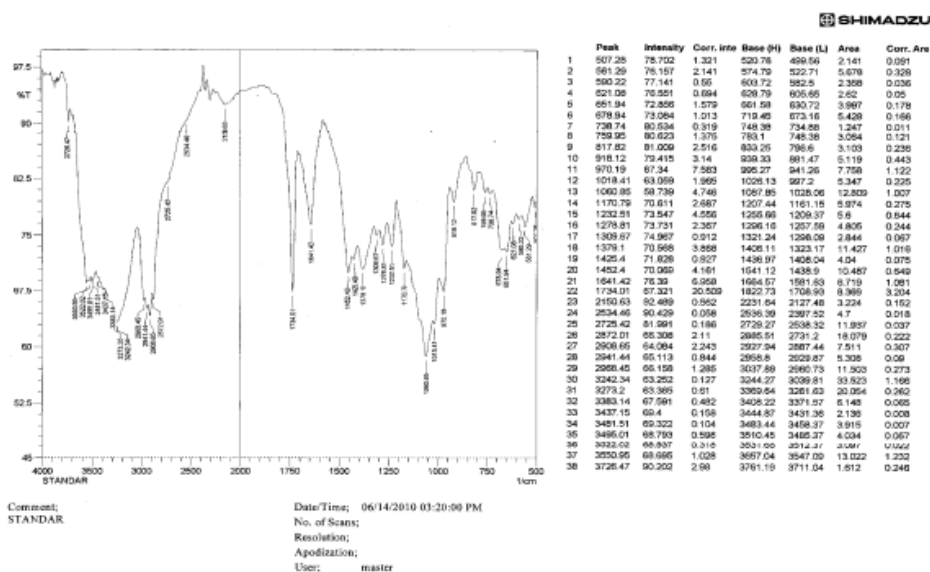
Table I. TLC Identification Result

Rf	Spot
0.2833	SD: asiaticoside standard reference
0.2833	MB: methanolic extract, Bogor plantation
0.2833	MS: methanolic extract, Solo plantation
0.2833	ML: methanolic extract, Lembang plantation
0.2666	EB: ethanolic extract, Bogor plantation
0.2666	ES: ethanolic extract, Solo plantation
0.2666	EL: ethanolic extract, Lembang plantation

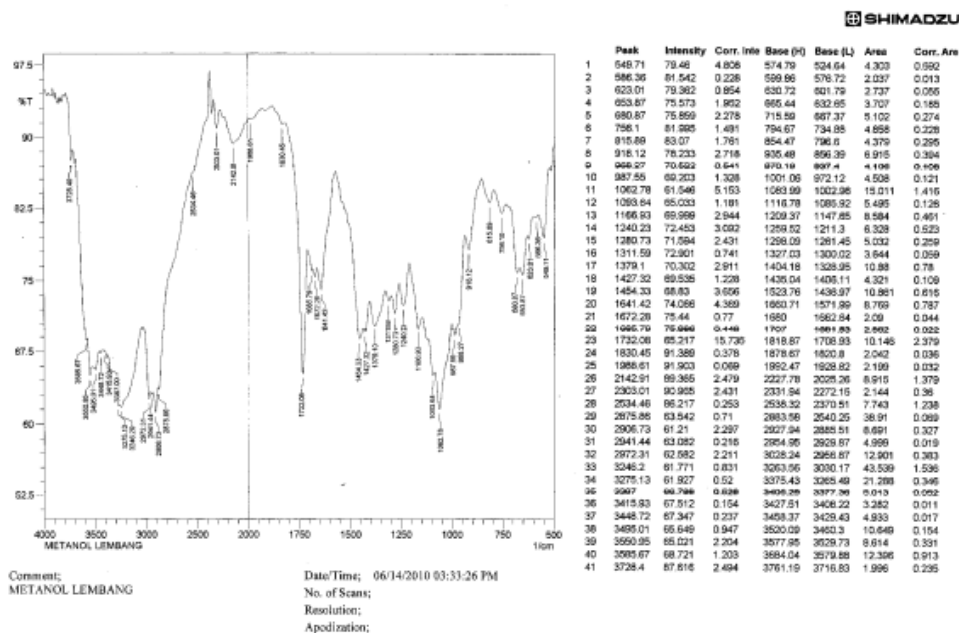
FTIR analysis of asiaticoside standard reference and methanolic extract from Lembang plantation

The FTIR spectra of asiaticoside standard reference and asiaticoside with methanolic extract from Lembang plantation are showed in Fig. 2. Comparing with the spectrum of asiaticoside standard reference (Fig. 2(a)), the absorption band at 918 and 970 cm^{-1} ascribed to $-(\text{CH}_2)_n$, 1060 cm^{-1} , 1641 cm^{-1} , 2908 cm^{-1} , and 3273 cm^{-1} are ascribed to $-\text{CO}$, $-\text{C}=\text{O}$ of , C-H and R-OH of

Asiatic acid and Madecassid acid. As showed in (Fig. 2b), the absorption bands at $3275 - 3550$ cm^{-1} ascribed to C-H, the absorption bands of $\text{C}=\text{O}$ (1732 cm^{-1}) and absorption bands at 2906 cm^{-1} ascribed to R-OH, the absorption 1062 cm^{-1} and $653-680$ cm^{-1} are ascribed to C-O and $-(\text{CH}_2)_n$ bands. This information FTIR spectra of asiaticoside with extract methanolic from Lembang plantation suitable with asiaticoside standard reference.



a) Standard



b) Methanolic extract, Lembang

Figure 2. FTIR spectra of a) asiaticoside standard reference and b) methanolic extract from Lembang plantation

Quantitative determination of asiaticoside

Quantitative determination was conducted with HPLC. The HPLC detector used in this study was light detector. Prior to HPLC analysis, we determined the λ_{max} of asiaticoside standard

reference and found that asiaticoside gave maximum absorbance in 221 nm. We used this λ_{max} for HPLC analysis of asiaticoside.

HPLC analysis was conducted using stationary phase silica, mobile phase acetonitrile-

water (70:30), and flow rate of 1.0 ml/minute. System suitability test for this system resulted in standard deviation of 0.53%. It meets the requirement from Farmakope Indonesia VI edition,

which is minimal 2%. Table II and III show the result of quantitative determination of asiaticoside content in ethanolic and methanolic extract.

Table II. Asiaticoside content of methanolic extract

Sample	RT (mnt)	Area under curve	Asiaticoside content (%)	Average content (%)
Bogor	7.372	25981.89	2.8172	2.82
	7.378	25967.47	2.8157	
Lembang	7.374	24688.88	2.6740	2.68
	7.374	24773.79	2.6833	
Solo	7.376	25465.27	2.7788	2.80
	7.381	25914.11	2.8278	

Table III. Asiaticoside content of ethanolic extract

Sample	RT (mnt)	Area under curve	Asiaticoside content (%)	Average content (%)
Bogor	7.380	25773.79	2.7947	2.79
	7.380	25725.19	2.7894	
Lembang	7.371	25365.27	2.7473	2.75
	7.372	25327.66	2.7433	
Solo	7.373	26395.25	2.8803	2.91
	7.375	26962.66	2.9422	

We can see that asiaticoside content between plantations areas were not differing largely. To see any significant difference of asiaticoside content between different plantation area and different extraction solvent, we conducted 2 ways ANOVA analysis. ANOVA analysis with $\alpha = 0.05$ showed that:

1. there was significant difference between types of solvent used in extraction and the asiaticoside content in the obtained extract,
2. there is significant difference between varied plantation area and obtained asiaticoside content, and

3. there is significant difference between interactions of different solvent with different plantation area.

CONCLUSION

Asiaticoside content in the methanolic extract from Bogor, Lembang, and Solo samples were 2.82%; 2.68% and 2.8% respectively. Asiaticoside in ethanolic extract from Bogor, Lembang, and Solo samples were 2.79%; 2.75%; and 2.91% respectively. Two way ANOVA study showed that there was significant difference between types of solvent used in extraction and the asiaticoside content in the obtained extract,

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