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Analysis of Centelloside of Pegagan (*Centella asiatica*)

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Abstract. *Centella asiatica* is still categorized as a wild plant that has not undergone domestication. Sustainability of medicinal and aromatic plants for sustainable harvesting of medicinal plants so that it is not only in nature but also the harvest of cultivated so obtained uniform quality. Chemical constituents are already known: contain some saponin compounds, including asiaticoside, madeccasoside and asiatic acid. This study aims to determine the bioactive content; asiaticoside, madeccasoside and asiatic acid on the top (leaves and petiol) and bottom (roots and tendrils) with HPLC method. The experiment was conducted in growth chamber Department of Biological Sciences and School of Pharmacy Auburn University, USA. This study conducted in September-December 2011. The materials used are Centella asiatica of Deli Serdang accession, Kabanjahe accession, Berastagi accession and Samosir accession. Equipment needed to support the growth chamber study were digital scales, plastic pots, HPLC system types waters alliance 2695 auto sampler, 996 photodiode array detector, coulomb adsorbosphere C18 5µ, size 250 x 4.60 mm, UV absorbance, wavelength of 210 nm, flow rate 1.8 ml / min, injection volume of 20 mL, Empower Pro software, and others. The results that the pattern of centelloside (asiaticoside, madecassoside and asiatic acid), when the content of one of the high content of bioactive, the others bioactive will be lower or biosynthetic pattern toward a compound needed. Centelloside plant age affects the content of Centella asiatica. Centelloside pattern is influenced by the condition of the planting medium, very high levels of phosphorus, biosynthesis of centelloside more toward to asiaticoside.

Key words: *Centella asiatica*, centelloside, asiaticoside, madeccasoside, asiatic acid

Introduction

In the last decade of the 20th century there is a global tendency return to nature. Back to nature in the field of medicine is a return to the traditional medicinal plants. This trend is particularly strong in developed countries and major influence in developing countries. (Januwati and Yusron, 2005).

Centella asiatica harvested from nature until now. To support the development of large-scale Centella asiatica should be supported by the cultivation. Centella asiatica is still categorized as a wild plant that has not undergone domestication. Chemical constituents are already known, among other things: contain some saponin compounds (Mangas et al., 2009), including asiaticoside (Noverita, 2010; Noverita, Siregar and Napitupulu, 2012) madecasoside, asiatic acid that spurs production of collagen I, thankunside, isothankunside, brahmoside, brahmic acid, madeccasic acid, triterpene acid, meso-inositol, centellose, carotenoids, salt K, Na, Ca, Fe, phosphorus, vellarin, tannin (Noverita and Marline, 2012),

mucilago, resin, pectin, sugar, B vitamins, fatty oils, calcium oxalate, amigdalin (Matsuda, et al., 2001, Mangas et al., 2008).

Constraints faced by the industry of herbal medicines (agromedisin) Indonesia: cultivation, production processes, research and product development and marketing, quality of material variability issues have an impact on the quality of different products (Ghulamahdi, Sandra and Nurliani, 2007). The purpose of this study was to determine the content of centelloside (asiaticoside, madecasoside, asiatic acid) on several accessions of Centella asiatica in lowland Deli Serdang and highlands Berastagi, Kabanjahe and Samosir.

Materials and Methods

The experiment was conducted in the growth chamber Department of Biology Auburn University, USA. This study conducted in September-December 2011. The materials used are Centella asiatica of Deli Serdang accession, Kabanjahe accession, Berastagi accession, and Samosir accession.

Equipment needed to support the research were growth chamber, digital scales, plastic pots, digital camera, HPLC system types waters alliance 2695 auto sampler, 996 photodiode array detector, coulomb adsorbosphere C18 5µ, size 250 x 4.60 mm, UV absorbance, wavelength of 210 nm, flow rate 1.8 ml / min, injection volume of 20 mL, Empower Pro software, and others.

Test content ***of*** asiaticoside

Test content of asiaticoside C. asiatica leaves are done each harvest to determine asiaticoside, madecassoside and asiatic acid analysis in the leaves. Determination of asiaticoside levels are as follows: start with 0.2 g dry powder, add 4 ml of 90% methanol (90 methanol : 10 water), throughly mix and place on shaker for at least 5 hours. Filter using Whatman number 4 filter paper, filtrate can be stored at -20^{o}C. Evaporate liquid under fume hood at 50^{o}C, mix with 1 ml 90% methanol. It should completely dissolve. Filter the mixture using 0.22 µM filter adapter. Store the filtrate for HPLC analysis; will use 20 µl for HPLC injection (Jain and Ram, 2008).

Results and Discussion

HPLC analysis of the results obtained with the content of centelloside of the various accessions of Centella asiatica which are harvested from the wild (see Table 1). Centelloside content patterns can be seen that when the content of one bioactive higher than others will be lower than others. As explained in the biosynthesis of triterpene saponins (Figure 1.)

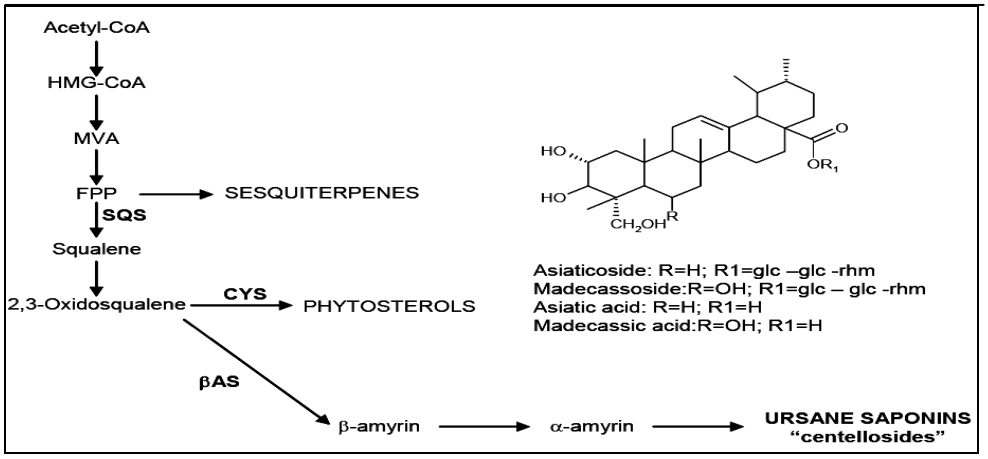

Figure 1. Biosynthesis of triterpene saponins

The last step biosynthetic pathway is not yet known, but centelloside (asiaticoside, madecassoside, asiatic acid and madeccasic acid) will be synthesized in Centella asiatica. The factors that influence it is not yet so clear (allegedly influenced by the age of the plant when harvested, the soil content of phosphorus and also there is a specific signal for the formation of secondary metabolites.

Table 1. The Centelloside content of Centella asiatica in various accession harvested from nature

Sample (accession)	Asiaticoside	Madeccasoside	Asiatic Asid
	(µg/ml)		
Medan	4.655	31.838	62.756
Deli Serdang	42.307	125.711	350.814
Berastagi	6.072	35.264	160.269
Kabanjahe	15.286	63.152	194.848
Samosir	4.492	49.205	279.689

Table 2. Centella asiatica samples from Deli Serdang

Sample	Asiaticoside	Madeccasoside	Asiatic Asid
	(µg/ml)		
Leaves	53.603	23.082	31.027
Petiole	46.489	9.637	57.185
Root	103.906	24.640	70.308

Table 3. Centella asiatica leaves samples Ages 4 and 6 weeks after planting

Sample	Asiaticoside	Madeccasoside	Asiatic Asid
	(µg/ml)		
Leaves (4)	146.916	27.665	29.169
Leaves (6)	1663.928	229.736	21.691

Table 4. Centella asiatica leaves samples 6 weeks after planting

Sample (accession)	Asiaticoside	Madeccasoside	Asiatic Asid
	(µg/ml)		
Kabanjahe	994.581	393.464	77.652
Berastagi	250.550	620.530	50.624
Deli Serdang	2226.351	411.082	43.759

Centelloside content of the leaves and roots petiol (Table 2.) It can be seen that the highest asiaticoside content contained in the roots followed leaves and petiole. As already mentioned above that the age of the plant affects centellosida content of Centella asiatica. It can be seen from the data presented in Table 3. Centelloside pattern can be seen in the condition of the media (can be seen in the results of analysis of soil) that content of asiaticoside is higher than madeccasoside or asiatic acid at the age of 4 and 6 week after planting. The results of soil analysis, there is a very high content of phosphorus is 857.25 ppm. Similarly in Table 4. it can be seen that the age of 6 week after planting, centelloside biosynthesis pattern more toward to asiaticoside.

Conclusions

Centelloside pattern (asiaticoside, madeccasoside and asiatic acid), when the content of one bioactive is higher the others bioactive will be lower or biosynthetic pattern toward to a compound needed. Centelloside pattern is influenced by the condition of the planting medium and very high levels of phosphorus. Plant age affects the centelloside content of Centella asiatica.

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