SECONDARY METABOLITE CONTENT IN PEGAGAN(*Centella asiatica*) FROM NORTH SUMATERA

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ABSTRACT

One of wild plant that widely used from nature is Centella asiatica. Conservation efforts for environmental sustainability and utilization of medicinal plants has been existing but very limited. Medicinal plant, Centella asiatica as a biomass potential is not only harvested from the nature but also cultivated to obtain uniform quality. Some chemichal content has already known, among other saponins compound such as asiaticoside, madecassoside and asiatic acid. The study was conducted in lowland and highland of North Sumatera. The materials is pegagan accession,Labu Beach, Deli Serdang, Kabanjahe accession,Berastagi accession, and Samosir accession. Tools used are waters alliance type of HPLC system, auto sampler 2695, photodiode array detector 996, coulomadsorbosphere C_{18} *5μ, size 250 x 4.60 mm, UV absorbance, wavelength 210 nm, a flow rate 1.8 ml/min, injection volume 20 mL, and Empower Pro software. The results showed that the pattern of centelloside (asiaticoside, madecassoside and asiatic acid), when the content of one of bioactive compounds is high then otherwise be lower or biosynthetic pattern toward a compound needed. Age of Pegaganaffect the content of centelloside.Centelloside pattern is influenced by growing medium condition, very high phosphorus content, and centellosidebiosynthesis toward asiaticoside.*

Keywords: Centella asiatica, centelloside, asiaticoside, madecassoside and asiatic acid

INTRODUCTION

Knowledge of medicinal plants in Indonesia comes was inherited from generation to generation, and is constantly enriched with knowledge from outside the archipelago, particularly from China and India. Medicinal plants that have been domesticated for generations and maintained in the corners of garden began neglected, forgotten and cleaned. As a result, generally public does not recognize medicinal plants and their use as drugs. The same thing is not happening in our neighboring countries such as Japan, China, Taiwan, Hong Kong, Korea and other Eastern countries. Conservation efforts and the use of medicinal plants is existing but it is very limited and the impact is very small compared to the existing needs. This happens due to lack of community support. But in the last decade of the 20th century, there is a global tendency back to nature. In term of medicine field, such tendency means back to traditional medicinal plants (Januwati, Mariam and Conan, 2005).

Bioactive compound of *Centellaasiatica* is triterpenoidsaponin and sapogenin with ursanestructure, namely asiaticoside and madecassosideas well as madecassic acid and asiatic acid. Asiaticoside can accelerate the wound healing process and are useful in the treatment of leprosy and tuberculosis, while madecassoside have anti-inflammatory properties and can significantly increase the secretion of collagen III. Pegagan is sweet, cooling, has function to cleaning blood, smoothing blood circulation, urine laxative (diuretics), fever (antipyretic), stop bleeding (haemostatic), improve neurological memory, anti-bacterial, tonic, antispasmoic, anti-inflammatory, hypotension, insecticides, anti-allergic and stimulants. Saponins can inhibit the production of excessive scar tissue (inhibit keloids occurrence) (Mangas, et al., 2008).

Drug industry still requires the cultivation of medicinal plants using natural fertilizer; therefore it is necessary to know the effect of natural fertilizer to support growth, biomass production and bioactive ingredients of medicinal plants. One plant that is widely known throughout the world as drug is Pegagan or *Centella asiatica* L. (Urban). Pegagan is still categorized as wild plant that has not domesticated yet. Some chemical compound that already known, among other are saponinscompund such as asiaticoside, asiaticacid and madecassosideacid that stimulate the production of collagen I, thankunside, isothankunside, brahmoside, brahmikacid, madecassicacid, triterpeneacid, meso-inositol, centellosa, carotenoids, K salt, Na, Ca, Fe, phosphorus, vellarin, tannins, mucilago, resin, pectin, sugar, B vitamins, fatty oils, calcium oxalate, amigdalin (Matsuda, Morikawa, Ueda, 2001).

There are some constraints faced by agro medicine industry in Indonesia, such as cultivation, production processes, research and development, products marketing, and uniformity of material quality (Munif, Sandra and Nurliani, 2007). In agribusiness, Pegagan has a promising prospect and in turn open biopharmaceutical business opportunity where demand is increasing every year both domestically and export destination. In efforts to make Pegagan as domesticplants, early observations on the growth habits of the plant are needed. The objective is providing further information on Pegagan cultivation system.

Research's Problems

As so far, for the needs of industries, Pegagan is taken directly from nature without cultivation efforts, so the supply of raw materials and the quality is not guaranteed. With increased public interest in natural medicines, it is necessaryto supply raw material in consistently manner according to industrial quality through cultivation. To support such efforts, provision of high yield varieties is one way to produce top quality raw material. Cultivation is needed to support large scaledevelopment of Pegagan. Pegagan with high yield potency and high content of bioactive compounds is needed to produce qualified Pegaganproduct.

Research'sObjective

The research's objective is to determine the content of centelloside (asiaticoside, madecassoside, and asiatic acid) in some pegaganaccession namely lowland of Labu Beach, Deli Serdang and high land of Berastagi,Kabanjahe and Samosir.

RESEARCH METHODS

The study was conducted in *lowland and highland of North Sumatera*. The materials is pegagan accession, Labu Beach, Deli Serdang, Kabanjahe accession, Berastagi accession, and Samosir accession. Tools used are waters alliance type of HPLC system, auto sampler 2695, photodiode array detector 996, coulombadsorbosphere C_{18} 5μ, size 250 x 4.60 mm, UV absorbance, wavelength 210 nm, a flow rate 1.8 ml/min, injection volume 20 mL, and Empower Pro software.

Test of Asiaticoside Content

Testing for asiaticoside content of Pegagan leaf was conducted in every harvest to determine the stage of centelloside accumulation in leaves. Determination of centelloside is as follows:

Analysis stages of asiaticoside, madecassoside and asiatic acid are as follow:Pegagan powder either from leaves, petiole, roots and shoots are used 0.2 g respectively.

1. Add 4 ml of methanol 90% (90 methanol: 10 water).
2. For mixing, put in shaker for at least 5 hours.
3. The mixture is filtered using Whatman filter paper number 1.
4. The liquid evaporated using water bath at 50 °C.
5. Produced brown material is mixed with 1 ml of 90% methanol until completely dissolved.
6. Filter the mixture using PTFE filter 0.22 μM.
7. Save the filtrate for HPLC analysis; as many as 20 mL for each HPLC injection.

Mobile phase

All components of the mobile phase were filtered with a 0.2 μm membrane before use. Elution gradient is using 0.3% orthophosphoric acid (solution A) and acetonitrile (solvent B) as presented in Table 1. Wavelength is 210 nm, flow rate 1.8 ml/min, injection volume 20 uL.

Table 1. Mobile phase of Solution A and B on HPLC

No	Time (Minute)	Flow (ml)	% A	% B
1	0.00	1,80	95,0	5,0
2	1.00	1,80	95,0	5,0
3	5.00	1,80	80,0	20,0
4	15.00	1,80	50,0	50,0
5	20.00	1,80	20,0	80,0
6	23.00	1,80	20,0	80,0
7	30.00	1,80	50,0	50,0
8	35.00	1,80	80,0	20,0
9	40.00	1,80	95,0	5,0

RESULTS AND DISCUSSION

Results of HPLC analysis show content of centelloside for various pegagan accession harvested from nature (Table 2-6). Patterns of centelloside content show that when the content of one bioactive compoundis high then the otherwise be lower. As described in biosynthesis of triterpene saponins (Figure 1), the last stage of this biosynthetic pathway is not known yet, but centelloside (asiaticoside, madecassoside, asiatic acid and madecassic acid) will be synthesized in pegagan. The affecting factors is not clear (allegedly influenced by the age of harvested plant, the content of soil phosphorus and also there is a certain signal for the formation of secondary metabolites.

Table 2. Content of Centelloside in Various Pegagan Accession Harvested from Nature

Sample	Asiaticoside	Madecassoside	Asiatic Acid
		(μg/ml)	
Medan Accession	4,655	31,838	62,756
Deli SerdangAccession	42,307	125,711	350,814
BerastagiAccession	6,072	35,264	160,269
KabanjaheAccession	15,286	63,152	194,848
SamosirAccession	4,492	49,205	279,689

Table 3. PegaganSamples from Deli Serdang

Sample	Asiaticoside	Madecassoside	Asiatic acid
		(μg/ml)	
Leaf	53,603	23,082	31,027
Petiole	46,489	9,637	57,185
Root	103,906	24,640	70,308

According to centelloside content of the leaf, petiole and root (Table 3), it can be seen that the highest content of asiaticoside found in root, followed by leaf and petiole. The age of Pagagan affect the content of centelloside as shown at Tables 4 below. Centelloside pattern can be seen on the media conditions (soil analysis), that is the content of asiaticoside higher than madecassoside or asiatic acid both at 4 and 6 weeks after planting (WAP). Soil analysis shows that phosphorous levels is very high, namely 857.25 ppm. Table 4 shows that at 6 WAP, the pattern of Centelloside biosynthesis more towards asiaticoside.

Table 4. Sample of Pegagan Leaf at 4 and 6 WAP (I)

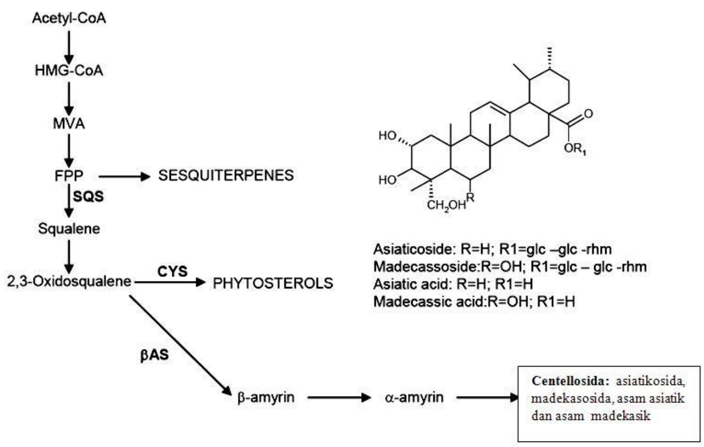
Sample	Asiaticoside	Madecassoside	Asiatic acid
		(μg/ml)	
Leaf 4 WAP	146,916	27,665	29,169
Leaf 6 WAP	1663,928	229,736	21,691

Noverita (2010) and Noverita, Siregar and Napitupulu (2012) confirm that soil phosphorus levels affect asiaticoside for some observed accession. Phosphorus levels in the growing medium are shown at Appendix. Here are presented some stage in the biosynthesis of triterpene saponins.

Centelloside is triterpenoid compounds that biosynthesized through mevalonate pathway in the cytoplasm. Biosynthesis can be divided into three stages:

1. Universal precursor synthesis of all terpenoids, isopentenyl diphosphate (IPP).
2. First Synthesis of triterpenes, squalene.

3. Centelloside Synthesis.



Keterangan: SQS = squalene synthase, CYS = cycloartenol synthase, βAS = β-amyrin synthase

The last step of such biosynthetic pathway is not yet known.Centelloside (asiaticoside, madecassoside, asiatic acid and madecassicacid) specifies bioactive that will be synthesized.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSION

1. The pattern of centelloside (asiaticoside, madecassoside and asiatic acid) is when the content of one of bioactive compounds is high then otherwise be lower or biosynthetic pattern toward a compound needed.
2. Age of pegagan affect the content of centelloside.
3. Centelloside patternis influenced by growing medium condition, very high phosphorus content, and centelloside biosynthesis toward asiaticoside.

Recommendation

Further research in the field is needed to study the effect of phosphorus, plant age or harvesting and the provision of elicitor on the patterns of cetelloside or plant responses in the biosynthesis of secondary metabolites with the treatment given.

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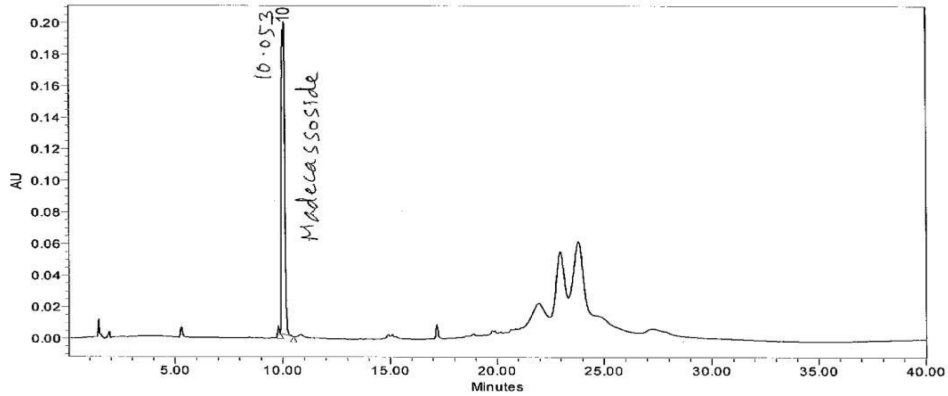
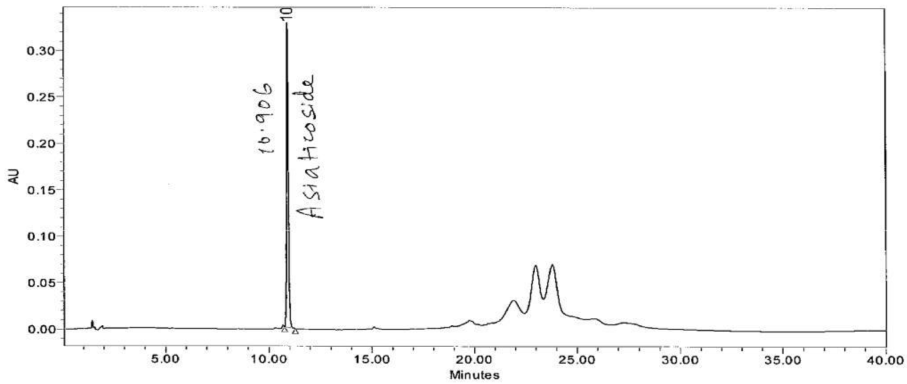
APPENDIXS



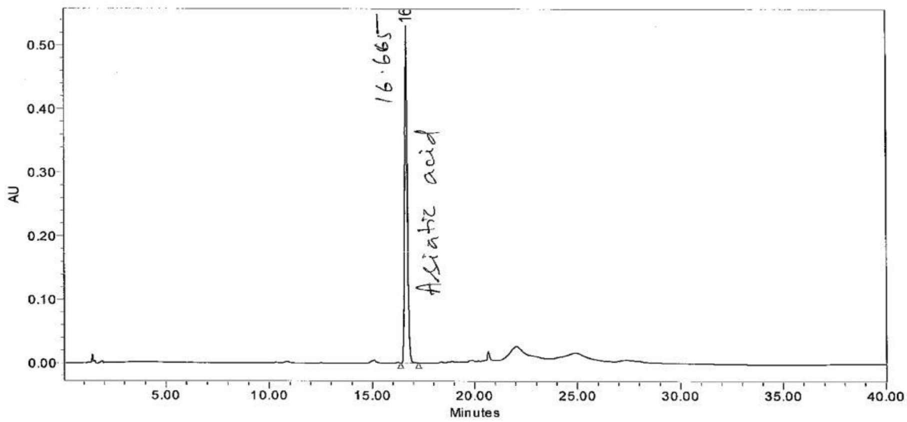
	RT	Area	% Area	Height
1	10.053	2051008	100.00	197654

Appendix 1. Standard chromatogram of Madecassoside



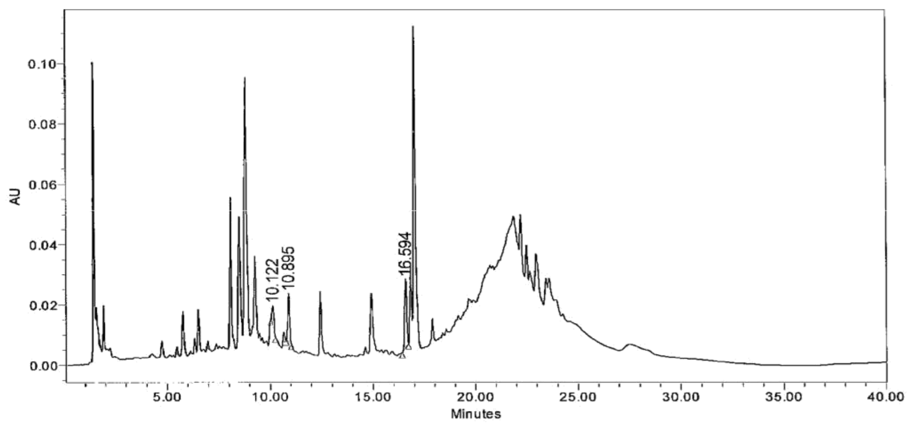
	RT	Area	% Area	Height
1	10.906	1926807	100.00	324990

Appendix 2. Standard chromatogram of asiaticoside

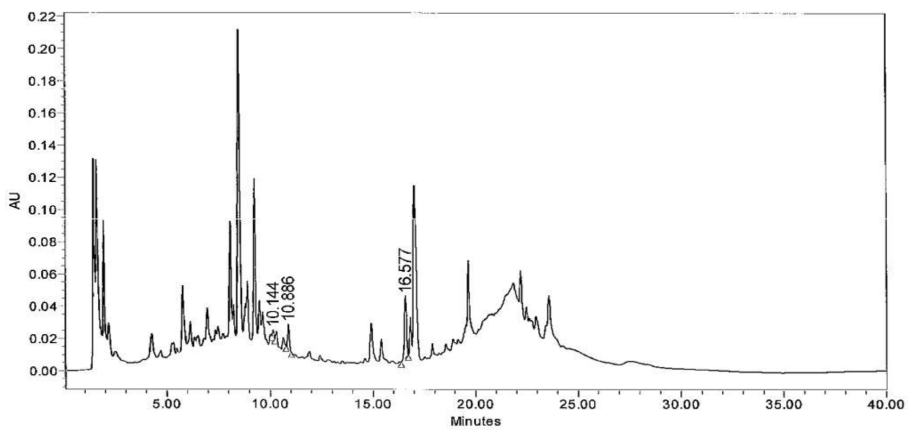


	RT	Area	% Area	Height
1	16.665	4787634	100.00	534601

Appendix 3. chromatograms Standard of Asiatic Acid



Appendix 4. Leaf Sample chromatograms of Deli SerdangAccession
Note: asiaticoside (53.603 g / ml), madecassoside (23.082 g / ml), asiatic acid (31.027 g / ml)



Appendix 5. Petiole Sample chromatograms of Deli SerdangAccession
Note: asiaticoside (46.489 g / ml), madecassoside (9.637 ug / ml), asiatic acid (57.185 g / ml)

Result of Compost analysis in early studies

No	Analysis Type	Compost	Criteria	Method
1	C-Organic (%)	44,53	Very High	(Walkley and Black)
2	N-Total (%)	0,72	High	K
3	P-Bray I (ppm)	857,25	Very High	S
4	K-dd (cmol(+) kg^{-1})	9,43	Very High	AAS
5	Ca-dd(cmol(+) kg^{-1})	21,92	Very High	AAS
6	Mg-dd(cmol(+) kg^{-1})	20,51	Very High	AAS
7	pH (H_2O)	6,11	Very High	pHMeter
8	C/N-Ratio	61,85	Very High	