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RESEARCH ARTICLE

CENTELLOSIDE CONTENT OF PEGAGAN (*Centella asiatica*) DELI SERDANG.

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Abstract

Conservation efforts *Centella asiatica* and utilization of medicinal plants has been existing but very limited. *Centella asiatica* is not only harvested from the nature but also cultivated to obtain uniform quality. Saponins compound such as asiaticoside, madecassoside, asiatic acid and some chemical content has already known. The study was conducted in lowland and highland of North Sumatera. The materials is pegagan accession from Deli Serdang. Tools used are waters alliance type of HPLC system, auto sampler 2695, photodiode array detector 996, coulombsorbosphere C18 5 μ , 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 Pegagan affect the content of centelloside. Centelloside pattern is influenced by growing medium condition and phosphorus content.

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Introduction:-

Generally public does not recognize medicinal plants and their use as drugs. 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 a 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 Yusron, 2005).

Bioactive compound of *Centella asiatica* is triterpenoid saponin and sapogenin with ursane structure, namely asiaticoside and madecassoside as 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, antispasmodic, 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).

Some chemical compound of *Centella asiatica* that already known, among other are saponins compound such as asiaticoside, asiatic acid and madecassoside acid that stimulate the production of collagen I, thankunside, isothankunside, brahmioside, brahmikacid, madecassicacid, triterpene acid, meso-inositol, centellosa, carotenoids, K salt, Na, Ca, Fe, phosphorus, vellarin, tannins, mucilago, resin, pectin, sugar, B vitamins, fatty oils, calcium oxalate, amigdaline (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 (Ghulamahdi, 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 domestic plants, 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 necessary to 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 scale development of pegagan. Pegagan with high yield potency and high content of bioactive compounds is needed to produce qualified pegagan product.

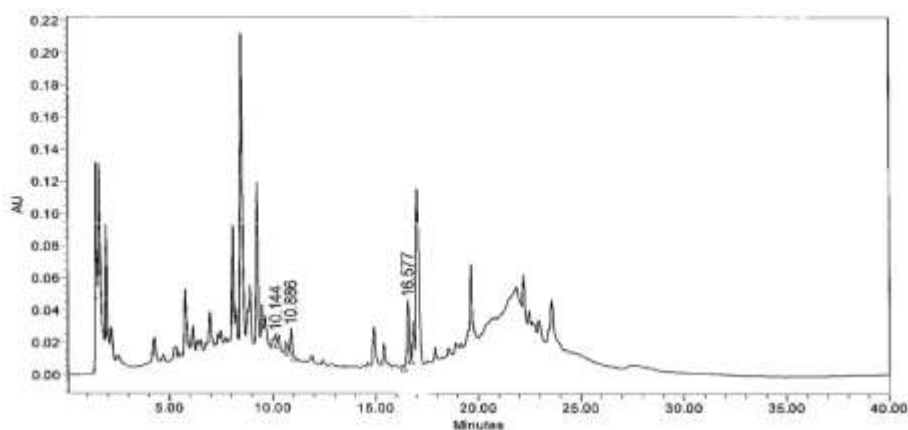
Research's Objective:-

The research's objective is to determine the content of centelloside (asiaticoside, madecassoside, and asiatic acid) in lowland pegagan, Deli Serdang.

Appendix:-

Appendix 1:- Leaf Sample chromatograms of Deli Serdang Accession

Note: asiaticoside (53.603 $\mu\text{g/ml}$), madecassoside (23.082 $\mu\text{g/ml}$), asiatic acid (31.027 $\mu\text{g/ml}$)



Result of Compost analysis in early studies

No	Analysis Type of Compost	Criteria	Method
1	C-Organic (%)	Very High	(Walkley and Black)
2	N-Total (%)	High	K
3	P-Bray I (ppm)	Very High	S
4	K-dd ($\text{cmol}(+) \text{ kg}^{-1}$)	Very High	AAS
5	Ca-dd ($\text{cmol}(+) \text{ kg}^{-1}$)	Very High	AAS
6	Mg-dd ($\text{cmol}(+) \text{ kg}^{-1}$)	Very High	AAS
7	pH (H_2O)	Very High	pHMeter
8	C/N-Ratio	Very High	

Research Methods:-

The study was conducted in *lowland of North Sumatera*. The materials is pegagan accession, Deli Serdang. Tools used are waters alliance type of HPLC system, auto sampler 2695, photodiode array detector 996, coulomb adsorbosphere C₁₈ 5 μ , 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 μ m.
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 μ m 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 μ L.

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 compound is 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 Pegagan Harvested from Nature

Sample	Asiaticoside	Madecassoside	Asiatic Acid
	(μg/ml)		
Deli Serdang Accession	40	120	325

Table 3:-Pegagan Samples from Deli Serdang

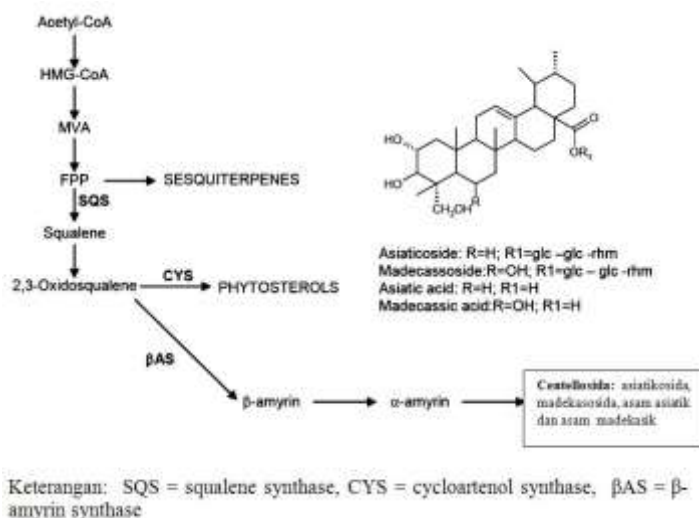
Sample	Asiaticoside	Madecassoside	Asiatic acid
Leaves	51	20	30
Petiole	42	10	55
Root	101	26	69

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.

Noverita, Siregar and Napitupulu (2012) and Noverita (2016) 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.



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 pattern is influenced by growing medium condition, phosphorus content.

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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