Morphology of leaves and content of secondary metabolites asiaticoside in some accession of pegagan (*Centella asiatica* l. Urban) in North Sumatera

[1]Noverita Sprinse Vinolina, [2]Luthfi A.M. Siregar and [2]Justin A. Napitupulu

[1]Department of Agrotechnology, Sisingamangaraja XII University, Medan, Indonesia; Department of Agroecotechnology, University of Sumatera Utara, Medan 23111, Indonesia.
Coorresponding Author: noveritasitumorang@yahoo.com

Abstract. The objectives of this experiment was to observe morphology of leaves and content of asiaticoside in some accessions of *Centella asiatica* of lowland Sumatera Utara, Medan and Pantai Labu Deli Serdang and the highlands of Sumatera Utara, Berastagi, Kabanjahe and Samosir. The study also examines the link between altitude grow with the content of asiaticoside of *Centella asiatica*. Domestication research include: exploration and collection. This activity is carried out to collect *Centella asiatica* from different geographic locations with different altitudes. Later examination asiaticoside levels in the leaves of *Centella asiatica* by HPLC method and phytochemical test. Analysis of soil at all growing places were also conducted. The highest content of asiaticoside in accessions of *Centella asiatica* tested is as follows accession Deli Serdang (2.38%), Kabanjahe (1.43%), Medan (1.38%), Berastagi (1.38%), Samosir with shade (0.28%) and accession Samosir (0.24%). Altitude does not affect the content of asiaticoside *Centella asiatica* because in this study obtained the highest asiaticoside content found on lowland accessions namely (± 4 m asl), namely accession Pantai Labu Deli Serdang (2.38%).
Keywords: *Centella asiatica*, asiaticoside, altitude.

Introduction

Knowledge of medicinal plants in the archipelago comes from the inheritance of hereditary knowledge, and 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 the garden began to stray, neglected and cleaned. As a result of society in general are not familiar with medicinal plants and their use as medicines. Something similar is happening in our neighboring countries such as Japan, China, Taiwan, Hong Kong, Korea and other Eastern countries. But the last decade of the 20th century there is a global tendency to return to nature. The tendency of 'back to nature' (back to nature) in the field of medicine is a return to traditional medicinal plants. This trend is very strong in developed countries and have a big impact in developing countries. This is considering the complex nature of medicinal plants and organic. Thus, medicinal plants can be synchronized with the food, an ingredient that is consumed with the intention of reconstructing the damaged organ or system. Back to nature were widespread in the western world since about three decades ago, while in our country began to appear at the beginning of this millennium grouped mainly middle to high society (Januwati, Mariam and Yusron, 2005).

One plant that is widely known throughout the world as a medicine is *Centella asiatica* L. (Urban). *Centella asiatica* is still categorized as a wild plant that has not undergone domestication. *Centella asiatica* bioactive compounds are triterpenoid saponins and sapogenin with a framework that is asiaticoside and madekasosida ursane very interesting, as well as acid and acid madekasik Asiatic. Asiaticoside, asiatic acid and madekassosida which spur the production of collagen I, thankunsida, isothankunsida, brahmosida, brahmic acids, triterpene acids, meso-inositol, centellosa, carotenoids, salt K, Na, Ca, Fe, phosphorus, vellarin, tannins, mucilago, resin , pectin, sugar, B vitamins, fatty oil, calcium oxalate, amygdalin (Matsuda *et al*., 2001).

Materials and Methods

Location and schedule

The study was conducted by collecting material *Centella asiatica* from the lowlands of Medan (± 49 m asl N 03° 31 '49.3 "; E 098° 36' 24.2") climate type D1, Deli Serdang (± 4 m asl N 03° 38 '28.3', E 098° 54 '33.8 ") climate type D1, from the highlands among others Aek

Nauli village Samosir (± 1164 m asl N 02° 38' 31.3"; E 098° 43 '26.9 ") climate type E2, WDG (± 1400 m asl N 03° 11 '48.4 "; E 098° 30' 54.4") climate type E2 and Kabanjahe (± 1217 m asl N 03° 06 '32.3 "; E 098° 30 '33.3 ") climate type E2. The instrument used 12 XL 12-channel GPS Garmin, USA. Climate type is obtained from Meteorology, Climatology and Geophysics of North Sumatra. The experiment was conducted in May-August 2010.

Methods

Observation of morphology, especially leaf, leaf area, leaf weight, leaf chlorophyll and asiaticoside content analysis in plant leaves. Soil analysis was also carried out from different places are growing pegagan. Soil analysis conducted at the Laboratory Institute for Agricultural Technology Assessment in North Sumatra. Domestication research include: exploration and collection.

Determination of Asiaticoside

Determination of asiaticoside in pegagan leaves obtained by HPLC method. Asiaticoside analysis conducted at the Laboratory of Research Institute for Medicinal and Aromatic Plants, Bogor.

Procedure

Centella asiatica leaf powder sample weight 2 g inserted into the measuring flask is added methanol solvent pro analysis until the volume 50 ml. Shaker for about 2 hours. Then filtered with filter paper Whatman 41, and filtered again with milliphore. Distillate extract injected into the HPLC instrument as much as 20 µL. This type of tool used Hitachi D-7000, Colom: C-18, detector: UV, eluent used was methanol: acetonitril: acetic acid = 70 : 30 : 0.6%, UV 254 absorbance, asiaticoside 1520 ppm standard.

Results and Discussion

Macroscopic characteristics of Pegagan

- Herb, annual, spreading.
- Trunk, not trunked.
- Leaves, single, arranged in a rosette root, from two to ten, kidney shape, base rounded, serrated edge, diameter of 1-9 cm, arranged in a rosette consisting of 20-10 leaves green, sometimes slightly hairy, pinnate, stem 10-40 cm, green.
- Flowers, plural, umbrella shape, axillary, ± 3 cm stems, leaves a protective two, ovate, long ± 4 mm , yellowish green, crown shape trumpet, long ± 1 ½ cm, ± 8 mm wide, light blue.
- Fruit, flattened, grooved two, ribbed, brownish purple.
- Roots, riding, round, white.

Morphology

Centella asiatica is still categorized as a wild plant that has not undergone domestication to crop cultivation, so we need some initial observations about the behavior of the plant itself grows. Observations on the behavior of gotu kola grows aims to provide further information for gotu kola cultivation system.

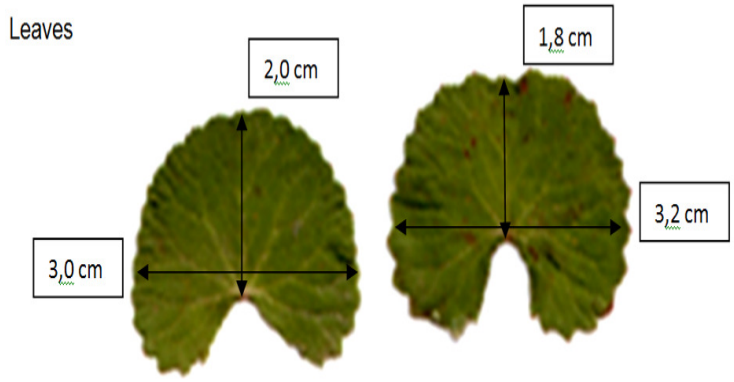

Figure 1. Samosir accession.

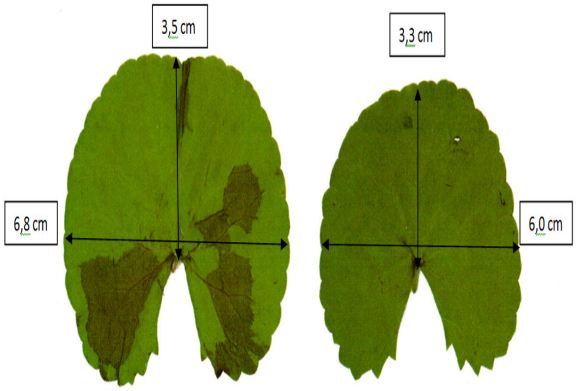

Figure 2. Samosir accession with shading.

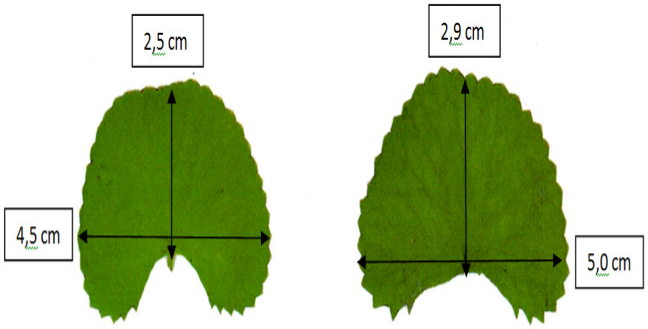

Figure 3. Medan accession.

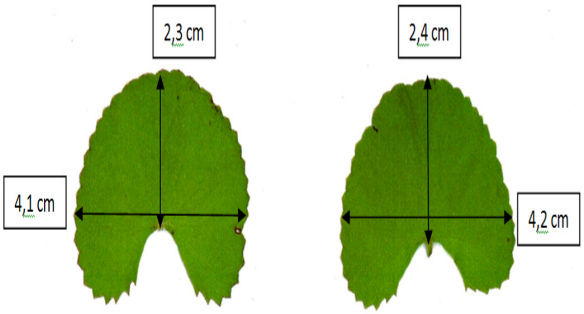

Figure 4. Pantai Labu accession.

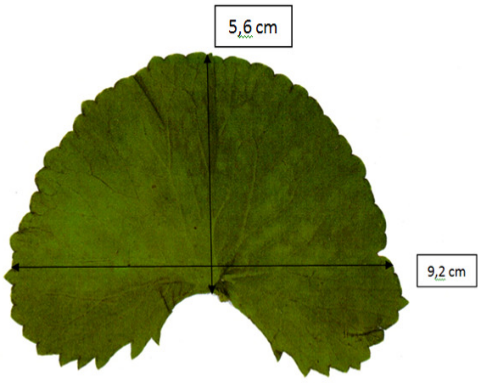

Figure 5. Berastagi accession.

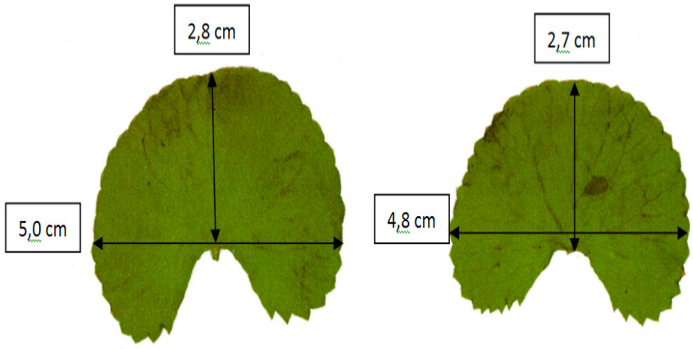

Figure 6. Kabanjahe accession.

Content of Asiaticoside

The results of analysis asiaticoside content can be seen in Table 1.

Table 1. Asiaticoside concentration of *Centella asiatica* leaf.

Accession	Asiaticoside concentration (%)
Medan	1.38
Pantai Labu	2.37
Samosir	0.24
Samosir with Shading	0.28
Berastagi	1.38
Kabanjahe	1.43

Table 2. Soil Analysis Results at Various Locations

No	Analysis	Medan	Pantai Labu	Samosir	Berastagi	Kabanjahe	Method
1	C-Organik (%)	1,61	1,09	1,03	**5,94**	**5,07**	Spectrop
2	N-Total (%)	0,15	0,14	0,14	**0,26**	**0,24**	Kjeldahl
3	P-Bray I (ppm)	15,60	**31,30**	9,97	3,03	14,25	Spectrop
4	K-dd (cmol(+) kg^{-1})	0,51	0,81	2,34	**2,48**	0,74	AAS
5	Ca-dd(cmol(+) kg^{-1})	3,12	4,25	2,64	**7,84**	3,10	AAS
6	Mg-dd(cmol(+) kg^{-1})	2,25	2,24	1,82	**3,86**	2,09	AAS
7	S (ppm)	97,50	42,50	114,75	168,81	**192,50**	Spectrop
8	pH (H_2O)	5,92	6,35	6,20	**6,55**	5,97	Elktromt
9	C/N-Rasio	10,73	7,79	7,36	**22,85**	**21,13**	Kalkulasi

Value of medicinal plants are located in the content of active ingredient or secondary metabolites and the presence of secondary metabolites in plants is highly dependent on particular environmental factors that affect enzymatic processes such as soil type, nutrients, rainfall, temperature and light. Besides, part of the plants used as raw materials or medicinal drugs for a variety of different plant species (Herlina, 2010). Which contained the highest content of asiaticoside on Pantai Labu accession (2.37%) and the lowest was found in Samosir accession (0.24%). When compared with data obtained from previous studies of various sites of origin, Pantai Labu accession has a fairly high level of asiaticoside. Some previous research data, Aziz *et al*. (2006), contained the highest content of terpenoids in the leaves, which contain asiaticoside (0.79 ± 0.03% and 1.15 ± 0.10%) of dry mass. From the data obtained can be seen that the content of asiaticoside on Deli Serdang, Medan and Berastagi Kabanjahe investigated asiaticoside leaves contain a high enough compared to accessions from Malaysia.

Munif, *et al*. (2007) declared the results of research studies the diversity of pegagan (*Centella asiatica* L (Urban.) based on morphological and agronomic characters through field trials. The results showed that the real accession affects all growth variables. This means that there are accessions have different growth diversity. From the analysis found that 8 of accession has asiaticoside levels above the average, the accession of Bengkulu, Malaysia, Ciwidey, Smukren, Boyolali, Karanganyar, Cilember, and Smugrim (0.72, 0.80, 0.77, 0.67, 0, 91; 0.68, 0.77 and 0.81%). When compared with the content of asiaticoside Deli Serdang accession (2.37%), Kabanjahe accession (1.43%), Medan accession (1.38%) and Berastagi accession (1.38%) was much higher. According to Munif, *et al*. (2007), in the highlands of *Centella asiatica* plant production is lower, but the content asiaticoside higher than the lowlands.

Table 3. Correlation Between Variables

	Asiaticoside
Asiaticoside	1
Accession	-0.17762ns
C-organic	0.239594ns
N-total	0.22582ns
P	**0.681052****
K-dd	-0.67661ns
Ca-dd	0.392049ns
Mg-dd	0.343718ns
S	-0.2755ns
pH	0.142419ns
C/N	0.266246ns
Weight of leaf	-0.0391ns
Leaf area	-0.06719ns
Total of chlorophyll	-0.46062ns

Based on survey results that we get that content on lowland asiaticoside higher than *Centella asiatica* growing in the highlands. In this study, obtained there is a link to the content of phosphorus element asiaticoside *Centella asiatica*. Results of chemical analysis of soil, P content in soil of Pantai Labu 31.30 ppm (very high), Medan 15.60 ppm (medium), Kabanjahe 14.25 ppm (medium), Samosir 9.97 ppm (medium), and Berastagi 3 , 03 ppm (low). P content of soil greatly affect the levels of asiaticoside. High P levels in the Land Deli Serdang cause levels of asiaticoside of Deli Serdang is also high compared to other accessions. According to Kim *et al*. (2005), have found several genes related to triterpene saponin biosynthesis in the lane on Centella like β-amryn synthase (CabAS), cycloartenol synthase (CaCYS), squalene synthase (CaSQS) and farnesyl diphosphate synthase. Kim *et al*. (2010) states, Farnesyl diphosphate synthase (FPS) plays an important role in organ development in plants. Farnesyl diphosphate synthase identified as a key regulatory enzyme in the biosynthesis of triterpene. Biosynthesis is thought to take place in the leaves where

asiaticoside content increases with time. During leaf development there CabAS m RNA levels increased to peak at 2 to 3 weeks after 4 weeks while there is a decrease (Mangas *et al*., 2009).

Phosphorus is an essential part of various sugar phosphates play a role in the reactions in the dark phase of photosynthesis, respiration, and various other metabolic processes. Phosphorus is also a part of nucleotides (RNA and DNA) and phospholipide membrane constituent (Lakitan, 2008). In addition, phosphorus acts as a constituent metabolites and complex compounds, activators, cofactors or constituent enzymes, and plays a role in physiological processes (Soepardi, 1983).

Research conducted Sutardi (2008), P2O5 fertilizer treatment significantly affected the parent root weight, weight of wet and dry biomass and production asiaticoside. Production of wet and dry weight biomass and production of the highest asiaticoside in harvest 4 months at dosages of 108 kg P2O5/ha are 694.01 and 185 g, the same is obtained for the highest asiaticoside content production reached 1.50%.

Conclusions

The content of the highest asiaticoside in accessions of *Centella asiatica* tested in succession is as follows accessions of Pantai Labu Deli Serdang (2.38%), Kabanjahe (1.43%), Medan (1.38%), Berastagi (1.38%), Samosir with shade (0.28%) and Samosir accessions without shade (0.24%). In the lowlands pegagan Deli Serdang and Medan, which contain high asiaticoside. In this study, obtained the highest asiaticoside content found on lowland accessions (± 4 m asl), Pantai Labu accession (2.38%). Asiaticoside of *Centella asiatica* Samosir has the lowest content. *Centella asiatica* grown in the highlands do not affect the content of *Centella asiatica* asiaticoside be higher. Phosphorus affects asiaticoside production, there are higher levels of asiaticoside on a high phosphorus conditions.

Acknowledgements

The authors are thankful to University of Sumatera Utara and Laboratory of Research Institute for Medicinal and Aromatic Plants, Bogor.

References

Andria Afrida. 2009. Pengaruh Pemupukan Fosfor Terhadap Pertumbuhan dan Produksi Tanaman Pegagan (*Centella asiatica* (L.) Urban) di Dataran Tinggi. Institut Pertanian Bogor. Bogor.

Aziz, Z.A, M.R. Davey, J.B.Power, P. Anthony, R.M.Smith and K.C.Lowe. 2007. Production of asiatikosida and madekasosida in Centella asitica in vitro and in vivo. Plant Sciences Division, School of Biosciences, University of Nottingham,UK. Biologia Plantarum 51(1): 34-42.

Depkes RI. 1979. Materia Medika Indonesia. Jilid III. Jakarta: Ditjen POM.

Duke.J, 2005, Phytochemical and Etnobotanical Databases, Maryland, Beltsuille Agricultural Researah Center.

Harborne, J.B, 1987, Metode Fitokimia, *Penuntun Cara Modern Menganalisis Tumbuhan*, diterjemahkan oleh Dr. Kosasih Patmawinata dan Dr. Iwang Soediro, ITB, Bandung.

Harborne, J.B., 1995. Metode Fitokimia MMI jilid VI. Penerbit ITB, Bandung.

Havlin, J.L., J.D. Beaton, S.L. Tisdale, and W.L. Nelson. 2005. Soil Fertility and Fertilizers: An Introduction to Nutrient Management. Pearson Prentice Hall. New Jersey.

Hernandez, Liliana-Vazquez, Mercedes Bonfill, Elisabeth Moyano, Rosa M. Cusido, Arturo Navarro-Ocana, Javier Palazon, 2010. Conversion of α-amryn into Centellosides by Plant Cell Cultures of *Centella asiatica*. Biotechnol Lett 32: 315 – 319.

James, Jacinda T. and Ian A. Dubery. 2009. Pentacyclic Triterpenoids from the Medicinal Herb, *Centella asiatica* (L.) Urban. *Review.* Molecules 14, 3922-3941

Januwati, Mariam dan M. Yusron. 2005. Budidaya Tanaman Pegagan. Balai penelitian dan Pengembangan Pertanian. Balai Penelitian Tanaman Obat dan Aromatika.

Kim, O.T., M.Y. Kim, M.H.Hong (2004). Stimulation of asiaticoside in the whole plant cultures of *Centella asiatica (L)* Urban by elicitors, Physiology and Biochemistry 23: 339-344.

Kim OT, Kim SH, Ohyama K, Muranaka T, Choi YE, Lee HY, Kim MY, Hwang B. 2010. Upregulation of phytosterol and triterpene biosynthesis in *Centella asiatica* hairy roots overexpressed ginseng farnesyl diphosphate synthase. Plant Cell Rep. 29(4):403-11.

Lakitan, B. 2008. Dasar-dasar Fisiologi Tumbuhan. Raja Grafindo Persada. Jakarta.

Mangas, Susana, Elisabeth Moyano, Lidia Osuna, Rosa M. Cusido, Mercedes Bonfill, Javier Palazo. Triterpenoid saponin content and the expression level of some related genes in calli of *Centella asiatica* Lett (2008) 30:1853-1859.

Mangas S. , Moyano E. [2], Hernandez-Vazquez L. [1] and Bonfill M. *Centella asiatica* (L) Urban: An updated approach Terpenoids, 2009: ISBN: 978-81-308-0332-6 Editors: Javier Palazón and Rosa M. Cusidó [1]Laboratorio de Fisiología Vegetal, Facultad de Farmacia, Universidad de Barcelona, 08028 Barcelona, Spain; [2]Departament de Ciencies Experimentals.

Marschner, H. 1985. Mineral Nutrition in Higher Plants. Academic Press. London.

Matsuda H, Morikawa T, Ueda H, *et al*. Medicinal foodstuffs. XXVII. Saponin constituents of gotu kola (2): structures of new ursane- and oleanane-type triterpene oligoglycosides, centellasaponins B, C, and D, from *Centella asiatica* cultivated in Sri Lanka[J].Chem Pharm Bull (Tokyo), 2001, 49(10): 1368-1371.

Munif Ghulamahdi , Sandra Arifin Aziz dan Nurliani Bermawie. 2007. Evaluasi Karakter Morfologi Fisiologi dan Genetik Pegagan Mendukung Standarisasi Mutu Pegagan. Lab Balai Besar dan Pengembangan Pasca Panen, Lab PSPT IPB, Lab Pusat Studi Biofarmaka IPB Lab Tanah IPB.

Natalini, Nova K., Edy D. Jauhari Kusumah dan Putri Karina Lailani. 2009. Analisis Fitokimia dan Penampilan Polapita Protein Tanaman Pegagan (*Centella asiatica*) Hasil Konservasi In Vitro. Bul. Littro 20(1) : 11 -20.

Norton. 1999. The Significance of Tanins in Tropical Animal Production. In Tanin Livestock and Human Nutrition. Proc. Of an International Workshop. Australia.

Noverita Sprinse Vinolina. 2006. Pengaruh Pemberian Dosis Kompos dan Konsentrasi Pupuk Organik Cair Lengkap Super ACI terhadap Pertumbuhan Tanaman Pegagan (*Centella asiatica* L.).Jurnal Penelitian Bidang Ilmu Pertanian 4 (3): 141-150.

Noverita Sprinse Vinolina. 2010. Kandungan Metabolit Sekunder pada Tanaman Pegagan (*Centella asiatica* L.). Akademia 14 (1) : 57-62.

Nurliani Bermawie, Susi Purwiyanti dan Mardiana. 2008. Keragaan Sifat Morfologi, Hasil dan Mutu Plasmanutfah Pegagan (*Centella asiatica* (L.) Urban.) Balai Penelitian Tanaman Obat dan Aromatik. Bul. Littro. Vol. XIX (1), 1 – 17.

Sutardi, 2008. Kajian Waktu Panen dan Pemupukan Fosfor terhadap Pertumbuhan dan Produksi Asiatikosida Tanaman Pegagan *(Centella Asiatica* L. Urban*)* Di Dataran Tinggi. Tesis. Sekolah Pascasarjana, Institut Pertanian Bogor.

Usaman, H. Dan R.P.S. Akbar. 1995. Pengantar Statistik. Bumi Angkasa. Jakarta.

Vickery, Margaret L. and Brian Vickery, 1981, Secondary Plant Metabolism, University Park Press, Baltimore. Tim Kimia Organik. 2007. Penuntun Praktikum Kimia Organik II. Padang : FMIPA UNP.

Wijayanti, K. 2007. Keragaan Tiga Nomor Harapan Pegagan (*Centella asiatica* L.(Urban)) Berdasarkan Pertumbuhan, Produktivitas, dan Mutu Simplisia pada Perlakuan Naungan (Skripsi). Fakultas Pertanian. IPB.

Zainol N.A., S.C. Voo, M.R. Sarmidi, dan R.A. Aziz. 2008. Profiling Of *Centella asiatica* (L.) Urban Extract. The Malaysian Journal of Analytical Sciences, Vol 12(2): 322 -327