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CONTENT OF PHENOLIC COMPOUNDS OF *Pluchea indica* LEAVES EXTRACT FROM SOME ALTITUDE HABITATS

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Abstract

Potential of secondary metabolism (production, persistence and effectiveness) of an organism has diversity that is commonly caused by genetic or environmental factors. The diversity of secondary metabolites potential can be occur because of differences in population, life cycle, time of cultivation, soil, climate, and geographic condition. In the process of cultivation, to obtain effective secondary metabolite compounds, it must be supported by appropriate habitat. The aim of this study was to determine the content of phenolic compounds in *Pluchea indica* from some altitude habitats. This study was a descriptive research. *P.indica* leaves samples was obtained from Bangkalan for lowland (28.3-31,72 m asl), Dlundung - Trawas for middle-altitude land (727-937 m asl), and in Coban Talun – Batu for highland (1303-1322 m asl). Simplicia of *P.indica* leaves was macerated and extracted by dissolving in methanol, ethyl acetate, distilled water, and n–butanol. Total polyphenol (gallic acid/GAE) contents was determined using UV - VIS spectrophotometer. Result of the study showed that content of phenolic compounds (gallic acid) on *P. indica* in Bangkalan/lowland (0.441 mg/g) was higher than *P. indica* growing in Trawas/middle-altitude land (0.364 mg/g) and in Batu/highland (0.303 mg/g). Phenolic compound characteristic was mostly semi-polar which was indicated by the largest total phenol in ethyl acetate fraction (0.412mg/g bk).

Key words: phenolic compound, leaves extract, *Pluchea indica*, altitude habitat

INTRODUCTION

Pluchea indica, a perennial shrub or known as beluntas in Indonesia, grows in sub-tropical climate. *Pluchea indica* contains of some secondary metabolites such as alkaloids, flavonoids, phenols hydroquinone, tannins, and essential oils that can be used as a herbicide or insecticide (Andarwulan, 2010). Member of *Pluchea* with methanol extract has the effect of insecticides and with the chloroform extract can be used as anti-nematode and antibacterial agent. The other contents of the *Pluchea* are terpenes, benzenoid, phenylpropanoid, lignin, and steroid (Traithip, 2005). *Pluchea indica* has an antioxidant activity because it contains a number of phytochemical compounds such as lignans, terpene, phenylpropanoid, benzoid and alkane (Luger, 2000). Uchiyama *et. al.* (1989) isolated the glycoside terpene compound from *P. indica*, and Biswas *et . al* . (2007) isolated pure compounds from the extract of the roots of *Pluchea* and identified as antimicrobial compounds. Research on *P. indica* leaf as bioherbicide indicated that it contains coumarin phenol, benzoate acid, salicylic acid, and vanilic acid. Bioherbicide was treated in some weeds, those are: *Mimosa pudica, Ruellia tuberose, Celosia argentea*, and

Amaranthus spinosus. The result showed that beluntas was able to inhibit the growth of weeds. The greater the concentration of the extract given, the greater the growth inhibition (Yuliani, 2009).

Pluchea contains of secondary metabolites released into the environment, either in the form of compounds evaporated from leaves or decomposed in soil (Uchiyama *et. al.,* 1989). Research of Sharma *et. al.* (2011) showed that the leaf extract of *P. indica* have larvicidal activity on *Culex pipiens* mosquito. Research of Widyawati *et. al*. (2010) showed that *P. indica* leaf contains flavonoids, hydroquinone phenols, tannins, and sterols. Methanol extract of *P. indica* leaf has total phenols (gallic acid equivalents) amounted to 304.42 mg/100g dry weight and total flavonoids of 116.38 mg/100g dry weight.

Contents of secondary metabolites in plants is formed as an attempt to defend themselves from its growing ecosystem. Hence the high and low contents of secondary metabolites in plants is influenced by the environment such as altitude, rain, and temperature (Vanhaelen et.al., 1991). The influence of environmental factors interact with genetic factors in the phenotypic expression of secondary metabolites, resulting in the production and excretion of secondary metabolites is influenced by temperature, light intensity, soil properties, microorganisms, and nutrient status (Olofsdotter, 2001). Thus, if *P. indica* that can be used as a biopesticide is cultivated, it must be adapted to its habitat so that the contents of secondary metabolites can be produced to the maximum.

Research of Pujiasmanto *et.al*. (2007) about the influence of habitat on the content of secondary metabolites showed that the content of androgapolid in Sambiloto plant in middle-altitude land (2.27 %) was higher than in lowland (1.73%) and highland (0.89%). Singh *et.al.*, (2003) *in* Junaedi (2006) reported that total phenolic compounds on *P. lanceolata* growing on cultivated land was more than in uncultivated. Similarly, quercetin compounds were found in cultivated land but not found on uncultivated land. Variations in the secondary metabolite were related to variations in climate and soil properties such as air and soil temperature, soil moisture, pH, C organic , and nutrient content.

Accordingly, the purpose of this study was to determine the content of phenolic compounds in *P. indica* found in a wide variety of habitats, those are in lowlands, middle-altitude land, and highlands. Genus of *Pluchea* generally contains phenolic compounds such as coumarin, flavonoids, and tannins, easy to grow in all places (home garden, roadside, drainage border, etc.), in lowland to highland (altitude up to 1500 meters above sea level) and easily cultivated, so the range was spread widely. Furthermore, *Pluchea* usually used as a medicinal plant or botanical pesticide (Ozgen, et.al., 2004) . *Pluchea indica* has the potential to be developed as a biopesticide. Information about the potential diversity of secondary metabolites is an important consideration as a biopesticide for plant cultivation.

RESEARCH METHOD

Sampling sites were selected by purposive sampling method in lowland < 50 m asl, 700-950 m asl for middle-altitude land, and > 1300 m asl for highland. They were Jl. Kapten Syafiri - Jl.Raya Ketengan (Bangkalan Madura) for lowland (28.3-31.72 m asl), Dlundung - Trawas (Mojokerto) for middle-altitude land (727-937 m asl) and Coban Talun - Bumiaji (Batu Malang) for highland (1303-1322 m asl). Implementation of the study included:

a). Preparation of phenolic standards: Gallic acid and preparation of plant material (simplicia). Leaf of plant used was powdered (simplicia) and the water content was determined.

b). Extraction and Fractionation process of *P. indica* leaf using the procedure of Dorman and Hiltunen (2004) : *P.indica* plant leaf powder (40 mesh size) was macerated with petroleum ether at room temperature for 24 hours. The dried residue was extracted with methanol using soxhlet extraction at a temperature of 65^{o}C for 3 hours. Methanol solvent was evaporated with a rotary

evaporator. The extract obtained was fractionated with ethyl acetate and distilled water. Next, distilled water phase was fractionated with n-butanol solvent. Solvents in fraction of ethyl acetate, n-butanol, and distilled water were evaporated with rotary evaporator, each extract and fractions were stored at 4°C until next analysis. Determination of total polyphenols (gallic acid equivalent/GAE) used UV - VIS spectrometer.

c). Parameter of this study were the contents of phenolic compounds in *P. indica* obtained at three locations based on altitude difference, with three replications of extraction. Data was analyzed using descriptive analysis.

RESULT AND DISCUSSION

The sampling method was conducted at three different areas showed in Figure 1. They were Bangkalan (average 28.7m asl), Trawas -Mojokerto (average 859.5 m asl) and Bumiaji - Batu (average 1312.2 m asl).

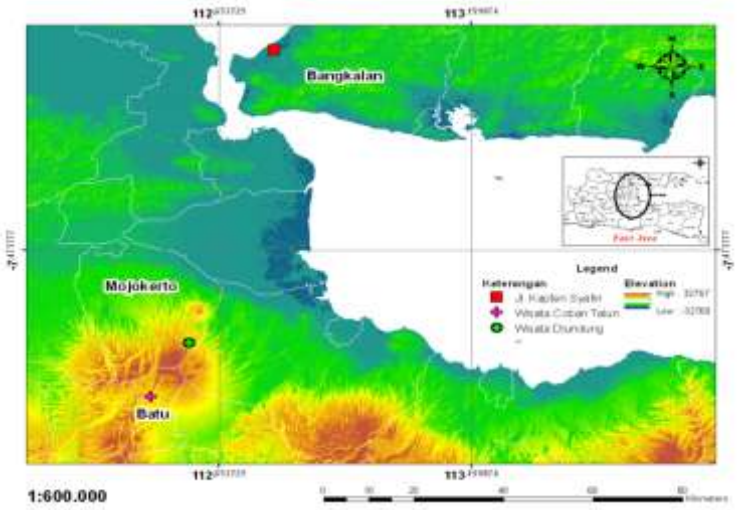

Figure 1.Location map of three sampling sites in East Java

The sampling site in Bangkalan was terrain and roadside, which had lots of shrubs and herbaceous plants. In Trawas Mojokerto, sampling site was an area near waterfall, roadside with lots of trees. In Bumiaji Batu, sampling site took place in a terrain with lots of shrubs and herbs.

Based on the ecological characters, *P. indica* could grow in altitude from 28.7 meters to1312.2 meters asl, temperature from 27.57°C to 38.63°C, humidity from 59% to 83.5%, atmospheric oxygen level from 17.35% to 21.25% , and light intensity from 153.21 to 808.580 lux . Based on the chemical analysis of soil, *P. indica* could live in the slightly low soil pH (middle-altitude land: 6.26) to neutral (lowland/highland were each 7.05 and 6.08), low organic C (lowland/1.18%), medium (middle-altitude land/2.7%) and high (highland/3.31%), low N nutrient (lowland/0.127%) to medium (middle-altitude land/0.272% and highland/0.335%). Based on soil physical properties, *P. indica* can be grown on sandy clay-loam soil with soil porosity from 46.25 to 70.08 (% vol), and soil water level from 0.19 to 0.46 cm3.cm^{-3}. Highlands have higher level of organic materials, N nutrients, and water level than the lowlands and middle-altitude land.

Analysis by UV-VIS spectrometer showed that the content of phenolic compounds in *Pluchea indica* growing in various altitude habitat, as in Table 1 below.

Table 1. The content of phenolic compounds of *P. indica* in Madura

	The content of phenolic compounds in habitat				
Kind of plant	Madura				
	Methanol	Et.acetate	Butanol	Water Fr.	average
Pluchea indica 1	0.419	0.466	0.449	0.388	0.431
Pluchea indica 2	0.420	0.473	0.512	0.406	0.453
Pluchea indica 3	0.424	0.487	0.492	0.353	0.439
average	0.421	0.475	0.484	0.382	**0.441**

Table 1 showed that the content of phenolic compounds in *P. indica* in lowland/Madura was 0.441 mg/g dry weight, while for Pluchea grown in Trawas Mojokerto, content of phenolic compounds were fewer, as 0.364 mg/g dry weight, showed in table 2.

Table 2. The content of phenolic compounds of *P. indica* in Trawas

	The content of phenolic compounds in habitat				
Kind of plant	Trawas				
	Methanol	Et.acetate	Butanol	Water Fr.	average
Pluchea indica 1	0.360	0.433	0.347	0.330	0.368
Pluchea indica 2	0.359	0.366	0.353	0.349	0.357
Pluchea indica 3	0.377	0.400	0.367	0.325	0.367
average	0.365	0.400	0.356	0.335	**0.364**

Pluchea indica grown in the highland (Batu) have the lowest phenol contents, equal to 0303 mg/g dry weight as showed in Table 3.

Table 3. The content of phenolic compounds of *P. indica* in Batu

	The content of phenolic compounds in habitat				
Kind of plant	Batu				
	Methanol	Et.acetate	Butanol	Water Fr.	average
Pluchea indica 1	0.296	0.369	0.229	0.321	0.304
Pluchea indica 2	0.290	0.382	0.168	0.327	0.292
Pluchea indica 3	0.307	0.335	0.259	0.352	0.313
average	0.298	0.362	0.219	0.333	**0.303**

The results of the overall data from the content of phenolic compounds in the leaf of *P. indica* in varied habitats were shown in Table 4 and the content of phenolic compounds in the various fractions was shown in Figure 2 below.

Table 4. Data of phenol contents in beluntas (*P. indica*)

Altitude	Methanol extract	Et.Acetate fraction	Butanol fraction	Water fraction	Average phenol mg/ml
Madura	0.421	0.475	0.485	0.382	**0.441**
Trawas	0.365	0.400	0.356	0.335	**0.364**
Batu	0.298	0.362	0.219	0.333	**0.303**
	0.361	0.412	0.353	0.350	0.369

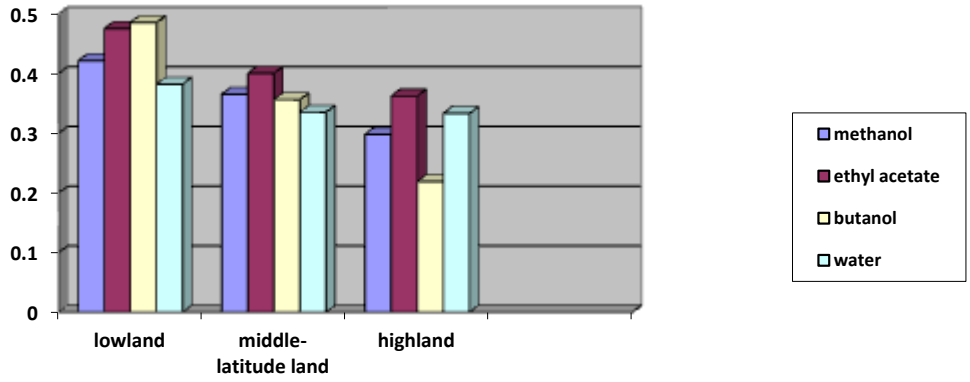

Figure 2. The content of phenolic compounds in Beluntas fractions grown in three different altitude habitat.

The results showed that the average content of phenolic compounds (gallic acid) of *P.indica* in Madura (lowland) was higher than the middle-altitude land (Trawas) and highland (Batu). For plant extract, ethyl acetate fraction showed higher content of phenolic compounds than methanol, butanol fraction and water fraction. This suggested that the content of semi-polar component in *P. indica* plants (ethyl acetate fraction) were higher compared to polar components (butanol fraction) and highly polar components (water fraction).

Total high content of secondary metabolites (phenolic compounds) in Madura was supported by *P. indica* plant growth data. It showed that *P. indica* leaf area in Madura were 18.182 cm, 10.173 cm in Trawas, and 3.57 cm in Batu. With a high rate of photosynthesis ability, *P. indica* in Madura was able to supply more C nutrients for the need of secondary metabolites synthesis. As explained by Sharafzadeh and Kourosh (2011), secondary metabolite was established through special primary metabolism pathway, and its existence as a response to the stimulus, so that the factors affecting primary metabolism also affect the presence of secondary metabolites.

The existence of organic material in the soil was also support secondary metabolites synthesis in plants. Diversity of secondary metabolites in plants due to interactions between plants and its changing environment produced a variety of compounds to defend themselves against abiotic and biotic environment. The compounds were the result of plant metabolic adaptation to the environment. Secondary metabolites played several important functions in plants, including structural role in a variety of supporting and protective tissues, and involvement in defense strategy (herbivores and pathogens).

It was expected that through cultivation on suitable habitat, carbon supply will be obtained for plant growth and development, which means secondary metabolites were also obtained effectively and can be used as a biopesticide.

CONCLUSION AND SUGGESTION

Contents of phenolic compounds (GAE) of *P. indica* in Madura (lowlands) was higher (0.441mg/g dry weight) than *P. indica* grown in Trawas/middle-altitude land (0.364 m/g dry weight) and Batu/highland (0.303 mg/g dry weight). Most of phenolic compounds obtained were semi-polar showed by the largest total phenol of ethyl acetate fraction (0.412 mg/g dry

weight).

REFERENCES

Andarwulan, N. 2010. Flavonoid content and antioxidant activity of vegetables from Indonesia. *Food Chemistry*, 121:1231- 1235.

Biswas R, Dutta PK, Achari B, Bandyopadhyay D, Mishra M, Pramanik KC, Chatterjee TK. 2007. Isolation of pure compound R/J/3 from Pluchea indica (L.) Less. and its anti-amoebic activities against Entamoeba histolytica. *Phytomedicine* (Jena) 14: 534-537.

Dorman,H.J.D.and Hiltunen,R.2004. Fe(III) reductive and free radical-scavenging properties of summer savory (Satureja hortensis L.) extract and subfractions. *Food Chemistry*. 88;193-199.

Junaedi, Ahmad, M.Ahmad Chozin, Kwang Ho Kim. 2006. Perkembangan terkini Kajian Alelopati. *Hayati* 13(2): 79-84.

Luger P. 2000. The Crystal structure of hop-17(21) en β Asetat of Pluchea pteropoda Hemsl from Vietnam.*Crystal Res Technology* 35(3) :355-362

Olofsdotter, M. 2001. Rice-a step toward use allelopathy. *Agron J*. 93:3-8.

Ozgen, U. , Ahmet Mavi, Zeynep Terzi, Maksut Coskun, Ali Yildirim. 2004. Antioxidant activities and Total Phenolic Compound amount of Some Asteraceae Species.*Turkish J.Pharm,Sci*.1(3): 203-216.

Pujiasmanto, Bambang, Jody Moenandir, Syamsulbahri, Kuswanto.2007. Study on the morphology and agroecology of creat (*Andrographis paniculata* Ness.) in various habitat. *Biodiversitas*. 8(4):326-329

Sharma,Surendra Kr. and Naveen Goyal. 2011. Biological studi of the Plants from Genus Pluchea.*Annals of Biological Research* 2(3): 25-34

Sharafzadeh,Shahram and Kourosh Ordookhani.2011. Influence of Carbon Dioxide Enrichment on Accumulation of Secondary Metabolites in Plants. Australian *Journal of Basic and Applied Sciences* 5 (11): 1681-1686

Traithip.A.2005. Phytochemistry and antioxidant activity of Pluchea indica. Mahidol University

Uchiyama T, Miyase T, Ueno A, Usmanghani K (1989) Terpenic Glycosides from Pluchea-Indica. *Phytochemistry* (Oxford) 28: 3369-72.

Vanhaelen,M.J.Lejoly,Mhanocq and L.Molle.1991.Climate and geographical aspect of medicinal plant constituents.*The Medicinal Plant Industry*.2 (1): 59-76.

Widyawati, Paini Sri,C.Hanny Wijaya,Peni S.H.,Dondin S. 2010. Pengaruh Ekstraksi dan Fraksinasi Terhadap Kemampuan Menangkap Radikal bebas DPPH (1,1-Difenil-2-Pikrilhidrazil) Ekstrak dan Fraksi Daun Beluntas (Pluchea indica).Proseding Seminar rekayasa Kimia dan Proses.Jurusan Tehnik Kimia Fakultas Teknik.Univ.Diponegoro Semarang.

Yuliani, Rahayu, Y.S, Mitarlis, dan Ratnasari, E. 2009. Potensi senyawa Alelokemi Daun Pluchea indica (L.) Less dan *Mikoriza Vesikular Arbuskular* Sebagai Model Mekanisme Pengendalian Gulma Terpadu Secara Hayati.*Berkala Penelitian Hayati*.