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THE DETERMINATION OF ASIATICOSIDE CONTENT AND SCREENING OF ACETYLCHOLINESTERASE INHIBITORY POTENCY OF GOTUCOLA (*Centella asiatica*) HARVESTED FROM DIFFERENT LOCATION

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Abstract

Gotu cola (C*entella asiatica*) with characterized coumpound was reported to have a potential anti dementia effect by modifying acetylcholinesterase activity (Ach) The activity of the gotucola to Ach may vary according to the place where gotucola is cultivated. This research was focused on the determination of asiaticoside content and screening of acetylcholinesterase inhibitory potency of gotu cola collected from different location namely Pacitan, Wonogari (a), Wonogiri (b), Solo, Wonosobo, Karanganyar and Bogor. Asiaticoside contents of gotucola was determined by HPLC method and inhibitory activity was determined by colorimetric method modified version of Ellman (Perry et al 2000). The result showed the three location of collected gotucola which have highest concentration of asiaticoside namely Wonogiri (b) , Solo, and Wonogiri (a) with range concentration per gram extract were obtained from 10.53 mg ,9,4 mg and 8.9 mg of respectively. In the point view of the inhibitory activity of extract on acetylcholinesterase, the three locations that have gotucola exctracts with hight potency as acetilcholinesterase inhibitor are wonogiri (a), Karanganyar and Pacitan with the percentase inhibitions are 62.16%, 59.5 %,and 56.8 % respectively. Based on asiaticoside content of different location from Wonogiri (a and b) and its inhibitory activity on acetylcholinesterase, inhibitory activity of extract on acetylcholinesterase may not correlate with amount of asiaticosid content.

Keywords: Acetylcholinesterase, Asiaticoside, *Centella asiatica*

INTRODUCTION

Centella asiatica (L.) Urb. (syn.:*Hydrocotyle asiatica* L.) (CA) is a small herbaceous perennial plant belong to Apiaceae (Umbelliferae) family. Its growing place spread throughout Indonesia, India, China, Australia, the South Pacific, Madagascar, and southern as well as middle Africa . *Centella asiatica* grows preferably in damp swampy areas, up to 700 meters above sea level (22).

Beside It is used as a foodstuff, CA is also used as drug ingredient to cure the disease symptom related to brain nerve disorder. Many researcher have reported the potencial activity of CA to improve learning and memory (10,23, 24) capability. Because of its activity against central nerve system , CA is used as a tonic to promote brain growth and improving memory (1) as well as improving general mental capability in children with learning difficulties and in people suffering from cognitive disorders (3, 10,12,19,25). In addition, CA also has pharmacological effects on Central Nerve system as rejuvenant, sedative and anxiolytic regiment, (13).

The Chemical compound of pegagan or gotukola are asiaticoside, thankuniside, isothankuniside, madecassoside, brahmoside, brahminoside, brahmic acid, madasiatic acid, meso-inositol, centellose, carotenoids, mineral salt such as salt of kalium, natrium, magnesium, kalsium, fe and, vellarine, as well as tanning substance.

Neuroprotective [1] effect of pegagan was suggested due to the asiaticoside content of the plant. Kumar *et al*. (2009) demonstrated the reversement of colchicines induced increase in acetylcholinesterase activity in mice following CA treatment. Alzheimer's disease in relation to brain damage is also showed the increment of Acetylcholinesterase activity. Controlling the severity of disease symptom which is identified as loss of memory capabilities need long term medical treatment and may have harmful effect on the human health overall. Thus it is necessary to provide a remedy by which the bioactive compounds used to control the disease symptom is being more safe and more effective.. Most of conventional drugs origins comes from medicinal plant and One belong to them is CA.

The pharmacological potency of CA, however, were reported to be different among remedies because of the variation in composition of bioactive compound. Randriamampionona et al 2007 reported the variation in the composition of India plant which is attributed to geographical condition. In addition, climate, seasonal , harvesting time and storage condition was suggested to have significant contribution to variation in chemical content of plant especially medicinal plant in India (18).

The aims of of this research was performing a comparative quantitative analysis of the active triterpenoids asiaticoside in *Centella asiatica* samples collected from different locations of java island and analyze the potential inhibitor activity of extract against acetylcholinesterase enzyme.

MATERIALS AND METHODE

Materials

Glassware, HPLC, acetylcholinesterase reagent kit purchased from sigma. Centella asiatica were collected from Central Java and Bogor, alcohol 30 %, grinder, 40 mesh strainer, asiaticoside standard.

Methods

Samples of pegagan were collected from Bogor, wonogiri (a dan b), Pacitan, Solo, Karang Anyar (Tawangmangu), and Wonosobo. All sample were dried using oven at 40.^{o} C, mashed into particle size of no 40 mesh strainer. The powder was extracted by methanoll absolute. The extract then evaporated by rotavapore. Aciaticoside content of extract was determined by HPLC using Rafamantanana *et al*. 2009 methode while acetylcholinesterase activity was determined according to method described by Ellman, et al 1961.

RESULT AND DISCUSSION

The value of water and ash content of Centella asiatica simplicia collected from Central Java are depicted in table 1. The value of water content of all simplicia are less than 10 %. This value fulfill the standard requirement written in Indonesia materia medica , which is standardirized the water value being less than 10 %. The ash value of simplicia is vary among the samples. The highest ash content was found in sample collected from Pacitan (19.3 %) and Wonogiri a (19.4 %). The ash value is depicted in table 1.

Table 1. The value of Water , Ash of Centella Simplisia collected from different location in Central Java (%)

Location of Sample collection	Water value	Ash value	Yield extract (%)
Pacitan	7.40	19.32	19.0
wonogiri a	8.42	19.41	19.11
Wonogiri b	6.82	14.89	23.20
Solo	6.94	12.37	23.08
Wonosobo	7.69	13.09	18.91
Karanganyar	8.76	12.53	20.37
Bogor	9.27	11.29	18.07

The yield value of extracts were also varies. The lowest value of yield was found in extract of CA collected from Bogor (18.70). Determination of asiaticoside content of CA indicated that asiaticoside content which was obtained from CA from Wonogiri (b) has highesht value. It is followed by CA from Solo, and Wonogiri (a) with concentration per

gram extract range from 10.53 mg ,9,4 mg and 8.9 mg respectively. Variation in asiaticoside content demonstrated in this study may represent of the geography, and cultivation as reported by Randriamampionona et al 2007 and Kurniawan et al 2005 . Randriamampionona et al 2007 reported the natural variability in active triterpenoid content of centella which was detected in *Centella asiatica* samples collected from different locations in Madagascar (21). It is also reported that plants from Madadagasgar contained the higher level of asiaticoside. compare to asiaticoside of India .

Table 2. The asiaticoside content of pegagan simplicia from different location

Location	Asiaticoside Content (mg/g)
Pacitan	4.2407
Wonogiri (a)	8.9176
Wonogiri (b)	10.5333
Solo	9.3718
Wonosobo	5.7230
Karanganyar	5.5753
Bogor	4.5458

Geographical condition , climate, seasonal , harvesting time and storage condition may contribute to variation in chemical content which was produced by plant especially medicinal plan (18).

The distinction of altitude where CA is growing may determine the composition of bio-active compound as reported by Das and Mallick (1991)(10). Centella which was growing at high altitude produce more asiaticoside than CA growing at lower altitude. Plants collected from 609 m above sea level contained 0.11 % of asiaticoside per leaf d.m.,whereas those from a lower altitude (*ca*. 5 m above sea level) contained almost half of this value. The result of their study is consistent with the finding of our study which also showed that CA collected from Bogor have lowest asiaticoside and yield value than CA from other place. *Bogor city that is known as rainy city* located on the *altitude* of ±190 m above sea level, The altitude of Bogor is lower than of Solo where is located *on* ± 1000 meter Wonogiri on ± 832 metres, Karanganyar on, ± 292, Pacitan *on* ± 600 meters. Wonosobo altitude is about 275 to 2,100 meters above the sea level. Furthermore high level of rainfall in Bogor may lead the intensity of light necessary for synthesis of metabolite to be lowered. This circumstance may have effect which is similar with shading effect on metabolite production Kurniawan et al 2005 reported the influence of the light intensity on yield and composition of triterpenoid content of CA. The shading cause a decrease in yield and triterpenoid content of centella (15). Light is essential for synthesis of metabolite thus lowering light intensity may lead to decreasing in asiaticoside content..

Our Data which were obtained in determination of asiaticoside content by HPLC, showed the Three higest content of asiaticoside to be found in CA collected from Wonogiri (b) , Solo, and Wonogiri (a). The concentration of asiaticacoside per gram extracts were range from 10.53 mg ,9,4 mg and 8.9 mg of respectively.

Table 3. The Inhibitory acetilcholinesterase activity of pegagan simplicia collected from different location

Origin of simplisia	Prosen Inhibition of acetilcholinesterase
Pacitan	56.7568
wonogiri a	62.1622
Wonogiri b	27.0270
Solo	5.4054
Wonosobo	43.2432
Karanganyar	59.4595
Bogor	-21.6216

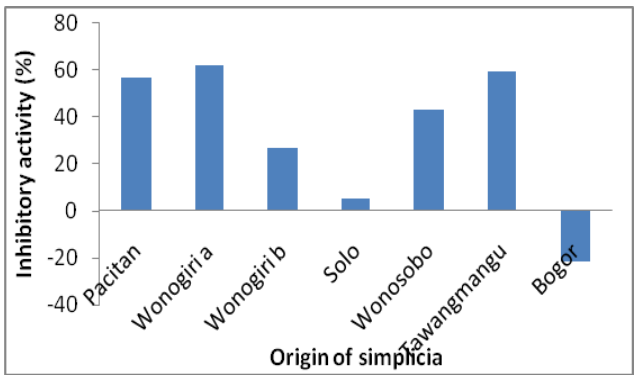

FIG 1. Inhibitory activity acetilcholinesterase vs origin of simplicia.

For pegagan sample we sceened the asiaticoside content using content using HPLC method, the result of the asiaticoside content is depicted in Table 4.

Pannizi et al (1993) reported the influence of Geographical and climate condition on variability in the composition of CA content between glycoside and aglycone ratio ..

It wa also reported that Plants from India , Sri Lanka and Madagascar are distinct in presence of glycosides, such as isothankuniside, brahmoside, centelloside and madecassoside (6-7; 8,9). Variation has also been observed in the constituents of essential oil extracted from the herb in the samples from Japan , Malaysia and Sri Lanka (4; 11). This variability may lead to difference in the pharmacological potency of CA Remedy including its activity to inihibit acetylcholynesterase enzyme .

Determination of the inhibitory activity of extract on enzyme resulted the data which is depicted in table 3..The three locations where highest potency of CA as acetylcholinesterase inhibitor were identified coming from wonogiri (a), Karanganyar and Pacitan. The inhibitory activity are 62.16%, 59.5 %,and 56.8 % respectively.. Based on the result of chemical analysis on the value of asiaticoside content of centella and their potency as inhibitor of asetylcholinesterase activity, it was clearly showed that the value of asiaticoside content is not in line with their activity on asetylcholinesterase. This is indicated by asiaticoside content of centella asiatica extract collected from wonogiri b which has highest content of asiaticoside,. However, incase of its potency to inhibit acetycholinesterase is lower than that of the centella collected from wonogiri a. Asiaticoside content of centella collected from wonogiri a is less than that of wonogir b. This data is also found in CA collected from Solo, Surprisingly asiaticoside content of CA collected from Bogor demonstrated the activator effect on asetilcholinesterase activity.

Variation in activity of the the extract which was not parallel with the asiaticoside content indicated the possibility of synergistic or antagonistic effect among component constitute the content of chemical complexity. Component of plant which are not active them self can act to improve the stability, solubility, bioavaibility or half life of the active compound (16) . This is demonstrated by ascorbic acid in a citrus extract was more bio-available than ascorbic acid alone.Vinson JA. Bose PB. 1988.

Geography difference may affect the composition of chemical content of CA which was produced collected from those of 5 location. It will in turn give a high contribution to availability of active compound to modify the level of their activity as reported by many researchers.

CONCLUSIONS

1. Asiaticoside content is varies among the sample collected from different location The highest Asiaticoside content was obtained from sample collected from Wonogiri, followed by Solo and Karang Anyar.
2. Centella asiatica has activity as inhibitor and activator.
3. Highest activity as inhibitor is found in the sample collected from Wonogiri a and the lowest activity is found in the sample from Solo as inhibitor however ,It was found in sample obtained from Bogor.
4. Asiaticoside value was not in line with inhibitory activity againts acetylcholinesterase.

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