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SCREENING OF ANGIOTENSIN I-CONVERTING ENZYME INHIBITION OF THAI INDIGENOUS VEGETABLES

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Abstract: Nowadays, a trend which consuming foods preventing diseases becomes more popular in Thai people. Hypertension is one of chronic diseases in which can be prevented using this strategy. Thus, the researchers aim to investigate Thai indigenous vegetables which possess antihypertensive activity. In this article, angiotensin I-converting enzyme inhibition (ACEi) model coupling with HPLC analysis was used as a method for screening of vegetables possessed this activity. The vegetable extracts were obtained using sonication in ethanol. The ACEi activity was determined calculating from the present of hippuric acid. While 0.1 mg/ml captopril showed 100 percent ACEi activity, it was found that *Glochidion perakense* and *Litsea* sp. at the concentration 1 mg/ml showed high ACEi activity in which the values were 90.3 and 92.1 percent, respectively. To prevent hypertension, it is a possibility to include both vegetables into meal in combination with lifestyle modification.

Keywords: ACEi, HPLC, *Glochidion perakense*, *Litsea* sp.

บทคัดย่อ: ในปัจจุบันคนไทยหันมาใส่ใจสุขภาพโดยการรับประทานอาหารที่มีประโยชน์ต่อสุขภาพเพื่อป้องกันโรคภัยไข้เจ็บต่างๆ โรคความดันโลหิตสูงเป็นโรคเรื้อรังชนิดหนึ่งที่สามารถป้องกันได้โดยการกินอาหารที่ถูกต้อง ในงานวิจัยนี้ ผู้วิจัยจึงมีความสนใจที่จะทำการตรวจสอบหาผักพื้นบ้านที่มีแนวโน้มมีฤทธิ์ลดความดันโลหิต วิธีการวิจัยที่ใช้คือการดูการยับยั้งของตัวอย่างต่อเอนไซม์เปลี่ยนแปลง angiotensin I ร่วมกับการวิเคราะห์ปริมาณ hippuric acid ด้วยเทคนิค HPLC โดยทำการสกัดผักพื้นบ้านในเอทานอลด้วยวิธี sonication จากผลการวิจัยพบว่า มันปูและธัมมังที่ความเข้มข้น 1 mg/ml มีฤทธิ์ ACEi สูงใกล้เคียงกับยาลดความดันโลหิต captopril จึงมีความเป็นไปได้ที่ผักพื้นบ้านทั้งสองชนิดนี้จะมีสารที่มีฤทธิ์ลดความดันโลหิต การกินผักทั้งสองชนิดนี้จึงอาจมีส่วนช่วยในการรักษาระดับความดันโลหิตให้อยู่ในระดับปกติได้ ทั้งนี้การปรับวิถีทางการดำเนินชีวิตร่วมด้วยก็มีส่วนช่วยป้องกันโรคความดันโลหิตได้เช่นกัน

คำสำคัญ: ACEi, HPLC, *Glochidion perakense*, *Litsea* sp.

INTRODUCTION

Nowadays, a trend which consuming foods preventing diseases becomes more popular in Thai people. The World health Organization (WHO) also published a guideline which suggested the people to change their habits such as, lifestyles, food intakes in order to improve their health status. There are many scientific evidences showed that healthy foods can be used to prevent various kinds of chronic diseases, for an example, hypertension. Hypertension is occurred when blood pressure is 140/90 mmHg or higher in people under 60 years of age and it is considered to be a risk factor for cardiovascular diseases (Williams *et al.*, 2004; Mancia *et al.*, 2013). Various kinds of medicines can be used for treating hypertension but the side effects of taking those medicines in a period of time are taking in account, for the examples, renal and liver toxic, thus it is better to prevent this kind of symptom. One appropriate way is to find the food which possess antihypertensive activity and promote to the people to put it into their meals in combination with the lifestyle change (Appel *et al.*, 1998).

Plasma rennin angiotensin system is occurred in kidney in which used to balance the body fluid and blood pressure. Renin secretion is activated via sympathetic system. It has a role in conversion of angiotensinogen to angiotensin I. Since then angiotensin I is converted to angiotensin II using angiotensin I-converting enzyme (ACE) and angitensin II has the effect on increasing of blood pressure. Using *in vitro* angiotensin I-converting enzyme inhibition (ACEi) model is the most appreciated method for anti-hypertension screening. The experiment is fast and cheap. It is non-animal based model so it suit to trend in the promotion of restrict animal use for scientific experiment without necessary. In rennin-angiotensin system in human, inactivation of ACE is effective in lowering blood pressure or preventing the pathogenesis of hypertension. A number of assays have been developed using the substrate hippuryl-L-histydyl-L-leucine (HHL) which composition matches the terminal two amino acids of angiotensin I (Cheung et al, 1980). The protocol is based on the quantity of hippuric acid (HA) liberated from the substrate using reliable and quick HPLC technique. In this study, the screening of ACEi of Thai indigenous vegetables was investigated in order to gain the information about the possibility of these vegetables for antihypertensive activity.

MATERIALS AND METHODS

1) *Materials*:

All fresh leaves materials including *Oenanthe javanica*, *Micromelum minutum*, *Cucurbita moschata*, *Eupatorium capillifolium*, *Ocimum gratissimum*, *Paederia linearis*, *Sesbania grandiflora*, *Careya sphaerica*, *Tamarindus indica*, *Solanum* sp., *Spondias pinnata*, *Anacardium occidentale*, *Barringtonia acutangula*, *Glochidion perakense*, *Litsea* sp., *Garcinia cowa*, *Ipomoea batatas*, *Antidesma ghaesembilla*, *Gnetum gnemon*, *Acacia pennata* subsp. *insuavis*, and *Clausena cambodiana* were purchased from Phatthalung province, Thailand. All plant materials were identified by Assoc. Prof. Thatree Phadungcharoen, Faculty of Pharmacy, Rangsit University. AR grade ethanol was purchased from Labscan (Thailand). HPLC grade acetonitrile, water, and formic acid were purchased from B&J (Korea). Hippuric acid (HA), N–hippuryl-L-histydyl-L-leucine (HHL), and angiotensin I-converting enzyme (ACE) from rabbit lung were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). HPLC system was from HPLC Agilent 1200 series coupling with an Agilent 1200 series photodiode-array detector (PDA).

2) *Methods*:

Preparations of plant extracts

The plants were washed and dried at 50 ^{o}C in hot air oven for 24 hours. Dried plant materials were grinded and the powders (1 g) were extracted three times ethanol (10 ml) using sonication for 60 min. Each extract was pooled and concentrated under reduced pressure and kept in cool and dry place.

ACEi assay

The total reaction volume was set at 70 µl according to previous report with some modifications (Wu *et al*., 2002). The assay was performed in 50 mM Tris buffer pH 8.3 containing 300 mM NaCl. The buffer was used to dilute enzyme and substrate while 5% ethanol in buffer was used to dilute samples. The reaction consisted of 50 µl of 2.17 mM HHL, 10 µl of 2 mU of ACE, and 10 µl of 1 mg/ml of plant extracts. The HHL and extract were mixed and incubated for 10 min at 37^{o}C in a water bath. ACE was also incubated at 37^{o}C for 10 min before the two solutions were combined and incubated at 37^{o}C with continuous agitation at 200 rpm for 30 min. The reaction was stopped by adding of 85 µl of 1 M HCl. Prior to HPLC analysis, all solution was filtered through 0.45 µm nylon filter. The filtered solution was used for the determination of HA resulting from ACE activity on the substrate.

Hippuric acid quantification (Pegg *et al*., 2007)

An Agilent 1200 Series HPLC system consisting of a quaternary pump with UV-Vis diode array detector and OpenLAB was used for the chromatography. The HPLC method was developed to separate and quantify free HA in the reaction. A reverse phased C18 column (Agilent 5 TC-C18(2), 150x4.6 mm, 5 µm) was used. The mobile phase comprised of 87.5% 0.05 M acetic acid in water and 12.5% acetonitrile. The injection volume was 10 µl; flow rate of 1 ml/min. HA was detected by monitoring the absorbance of the eluents at 228 nm. Standard HA samples were prepared freshly on a daily basis and used for calculation of the concentration of HA. The ACE inhibition ratio was calculated from the given formula;

$$\text{ACE inhibition ratio} = \frac{(\text{HA conc. in negative control} - \text{HA conc. in the sample}) \times 100}{\text{HA conc. in negative control}}$$

RESULTS AND DISCUSSION

Standard curve HA was prepared at range 0.05-0.5 mM. The linearity is 0.9998. Prior to analysis of the ACEi activity, the responsiveness assay system was calibrated with captopril, a positive control, and it showed ACEi activity of 100% at the concentration of 0.1 mg/ml (The final concentration of captopril is 0.014 mg/ml). Figure 1 shows representative chromatograms after injection of HA standard solution and assay mixtures. Only *G. perakense* and *Litsea* sp., at the final concentration of 0.14 mg/ml, showed high ACEi activity in which the values were 90.3 and 92.1, respectively. The other extracts showed no inhibitory activity with the percentage of ACEi between 1.8 - 23.5%. (Table 1). Previous reports were mentioned about high ACEi activity in *Solanum torvum* and *Apium graveolens* extract and also their pure compounds (Simaratanamongkol *et al.*, 2014a, b) in which the activity were closely to the plants from this study. Thus, *G. perakense* and *Litsea* sp. probably possessed some valuable active constituents which showed high ACEi activity and maybe explored in the next study.

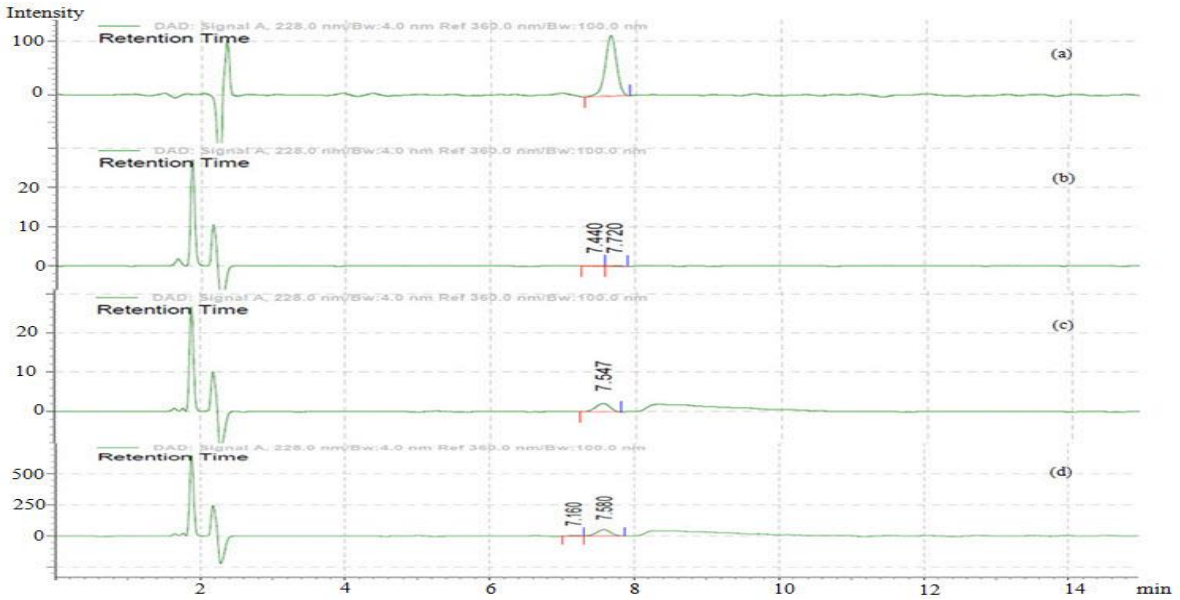


Fig. 1 Representative chromatograms using a reversed phase C18 column for HA determination (a) HA standard with R_T = 7.5, (b) a mixture of HHL, ACE, and 0.1 mg/ml captopril (positive control), (c) a mixture of HHL, ACE, and *G. perakense* leaves extract, (d) a mixture of HHL and ACE (negative control).

Table 1 ACE inhibition ratio of the plant extracts (n=3)

Samples	ACE inhibitory ratio (%)
O. javanica extract	20.4 ± 0.7
M. minutum extract	15.1 ± 0.5
C. moschata extract	3.8 ± 0.1
E. capillifolium extract	16.7 ± 0.5
O. gratissimum extract	16.4 ± 1.1
P. linearis extract	1.8 ± 0.1
S. grandiflora extract	10.0 ± 0.5
C. sphaerica extract	20.5 ± 1.0
T. indica extract	11.7 ± 0.5
Solanum sp. extract	15.0 ± 0.5
S. pinnata extract	23.5 ± 0.8
A. occidentale extract	9.2 ± 0.5
B. acutangula extract	11.8 ± 0.3
G. perakense extract	90.3 ± 4.7
Litsea sp. extract	92.1 ± 3.8
G. cowa extract	2.1 ± 0.1
I. batatas extract	2.5 ± 0.2
A. ghaesembilla extract	18.9 ± 0.6
G. gnemon extract	6.3 ± 0.2
A. pennata subsp. *insuavis* extract	20.1 ± 1.1
C. cambodiana extract	5.8 ± 0.1
Positive control (captopril)	100.0 ± 0.0
Negative control (ACE + HHL)	0.0

CONCLUSION

Considering using ACEi as a model for preliminary anti-hypertension screening of vegetables, *G. perakense* and *Litsea* sp. showed high ACEi activity. Thus, these vegetables may be useful for people who seek for an alternative way in preventing hypertension. Both vegetables are locally consumed freshly with Thai chili paste and also in combination with many types of food in Thailand. Provide the people such information along with lifestyle modification campaign; maintenance of health in a population will be achieved.

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