ISOLATION AND CHARACTERIZATION OF ANGIOTENSIN CONVERTING ENZYME (ACE) INHIBITORY COMPOUNDS DERIVED FROM MALAYSIAN MEDICINAL PLANTS

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ABSTRACT

The crude extract of rhizomes shows high percentage of ACE inhibition than leaves crude extracts. The methanol and water crude extracts give 51.36% and 42.53% of ACE inhibition respectively. Whereas in leaves crude extracts water and hexane give 45.59% and 36.31% of ACE inhibition respectively. These findings suggest that both of the leaves and rhizomes crude extract contain antihypertensive active compound for antihypertension treatment. However, further research is still in progress to evaluate its antihypertensive action by oral administration in Spontaneously Hypertensive Rats (SHR).

INTRODUCTION

Angiotensin Converting Enzyme inhibitors are considered one of the important drug beside β-receptor blockers and calcium-antagonists used in the treatment of high blood pressure (James 2003) as well as in regulation of cardiovascular function. The synthetic ACE inhibitors such as alacepril, benazepril, captopril, cilazipril, enalapril, fosinopril, lisinopril, moexipril, perindopril, quinapril, ramipril, tandolapril and zofenopril have been used extensively as antihypertensive drugs in the treatment of hypertension and heart failure. However, these antihypertensive drugs can cause adverse side effects including cough, taste disturbance and skin rashes (Messerli and Hypertens 1999). Many researchers have reported ACE inhibitors derived from plants (Anderson and Anderson,1997; Nyman et al 1998; Duncan et al,1999). Futhermore, naturally occurring compound of procyanidins and flavonoids isolated from plant such as Lespedezae capitata, Cistus clusii and Amelanchier ovalis have been found to have ACE-inhibiting activities (Elbl and Wagner 1991; Wagner and Elbl 1992). Besides that many natural ACE inhibitors peptides have been isolated from plants such as buckwheat (Li et al 2002), chickpea (Pedroche et al 2002; Yust et al 2003), garlic (Suetsuna 1998), spinach (Yang et al 2003, sunflower (Megias et al 2004) and Brassica oleracea (Ju-Eun Lee et al 2006).

In the search for new antihypertensive agents from medicinal plants inhibition of ACE will be one of the effective screening methods besides using animal model (Villar et al 1986). In this project the investigation of *Tacca integrifolia* which are potential to have ACE inhibitor compounds were carried out. There is no research have been reported on the ACE inhibitor compounds from *Tacca integrifolia.*

Tacca integrifolia, also known as white bat flower, is one of ten species that belong to the genus *Tacca*. It is distributed throughout Southeast Asia and is famous among native in

peninsular Malaysia. The genus *Tacca* is a rhizomatous or tuberous herbaceous plant and has attractive, entire leaves, with vertical growth habit, and bracts below the flowers. The bracts are beautifully veined with purple. The large and attractive leaves emerge from the top of the vertically growing rhizome. In the wild, *T. integrifolia* grows in the under story of rain forests in deep shade, but in a diversity of soil types. The species is most often found growing in accumulations of decayed organic matter. It has been used traditionally as preventing and treatment of high blood pressure and also haemorrhoids.

OBJECTIVES

1 To screen the bioactive chemical compounds for Converting Enzyme (ACE) inhibitors
2 To isolate, purify, identify and characterize the properties of the ACE inhibitors
3 To investigate the antihypertensive action of the purified ACE inhibitor compounds by oral administration in spontaneously hypertensive rats (SHR)

MATERIALS AND METHODS

Tacca integrifolia was collected at Field Study Centre (FSC), Gombak, Selangor. Leaves and rhizomes were cut into small pieces and dried in the oven. The sample was then grinded into powder form and extracted with hexane, petroleum ether, chloroform, methanol and water. Thin Layer Chromatography (TLC) was developed using chloroform and chloroform-ethanol as solvent system.

ACE inhibitory activity was determined by a modification of the method of Cushman and Cheung, 1971. In each test tube 30 µl sample solution was added to 500 µl 7 mM HHL in 200 mM Borate buffer pH 8.3, 400 µl 2 M NaCl, 40 µl distilled water and 30 µl crude enzyme. After incubation at 37ºC for 30 minutes the reaction was terminated by adding 500 µl 1 N HCl. 3 ml ethyl acetate was added and vortexed for 5 seconds. The top layer was removed into crucible and air dried. Then 3 ml of distilled water was added and absorbance was read at 228 nm with spectrophotometer. The percentage of inhibition was calculated:

$$\text{Percentage of inhibition} = \frac{\text{Absorbance (control)} - \text{Absorbance (test)}}{\text{Absorbance (control)}} \times 100$$

RESULTS AND DISCUSSIONS

The result from Table 1 shows that the water crude extract from leaves of *Tacca integrifolia* have the highest percentage of ACE inhibition (45.59%) followed by hexane crude extract (36.31%). Whereas in rhizomes, methanol crude extract shows the highest percentage of inhibition (51.36%) followed by petroleum ether crude extract (42.99%) and water extract (42.53%).

The water and hexane leaves crude extract also shows high percentage of ACE inhibition compared with the other crude extracts as the concentrations increases (Table 2).

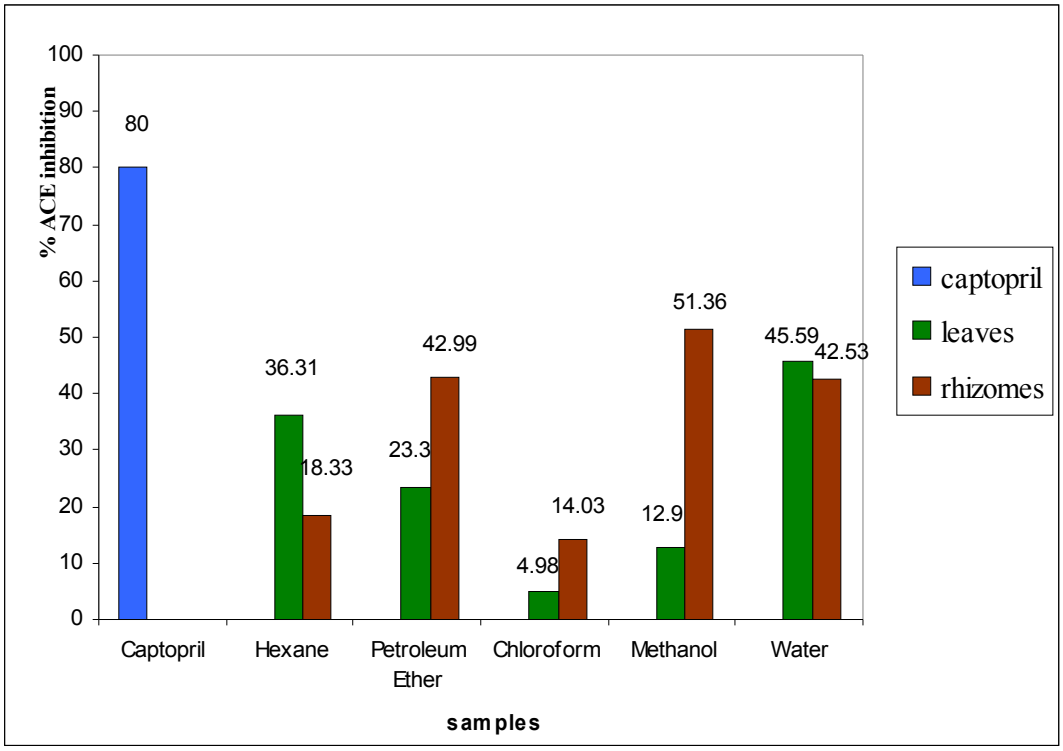

Figure 1. Percentage of ACE inhibition of leaves and rhizomes crude extracts from *Tacca integrifolia.*

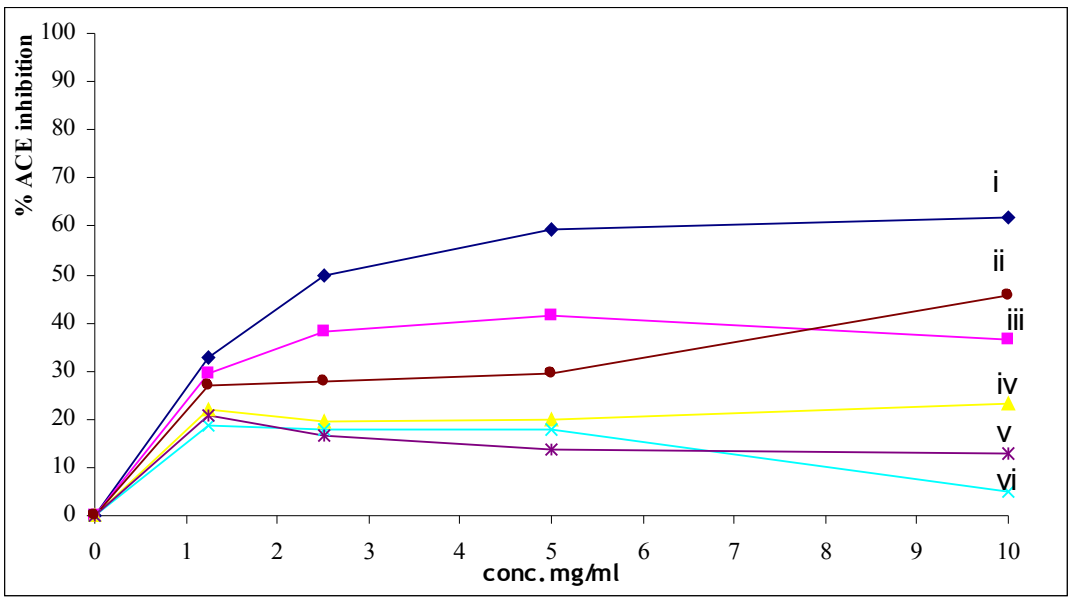

Figure 2. Percentage of ACE inhibition of leaves crude extract from *Tacca integrifolia*
i) Captopril (std), ii) Water extract, iii) Hexane extract, iv) Petroleum Ether extract
v) Methanol extract, vi) Chloroform extract

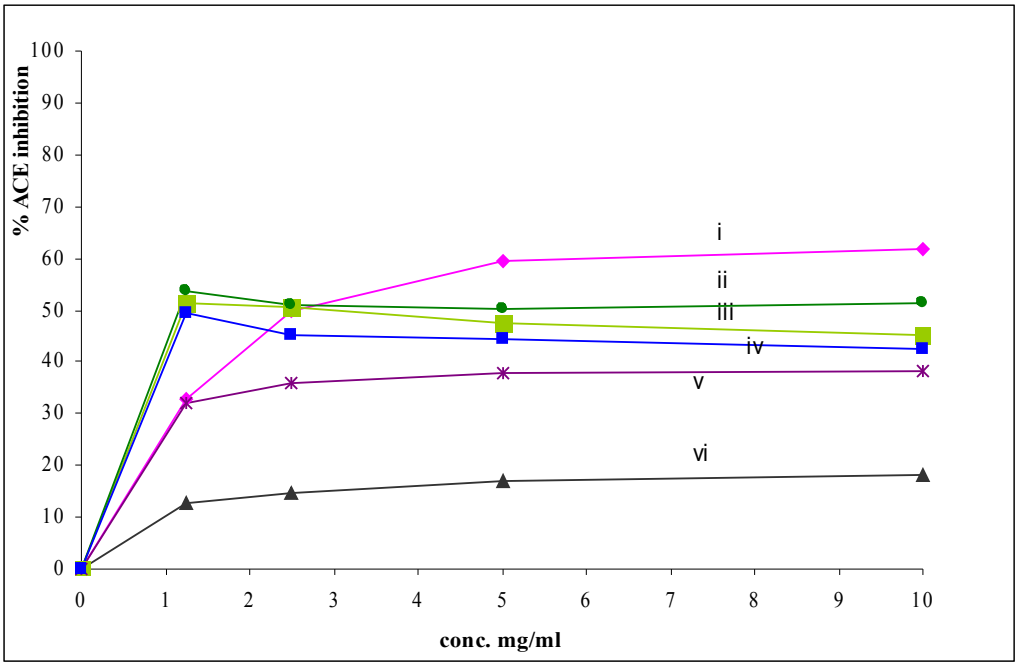

Figure 3. Percentage of ACE inhibition of rhizome crude extract from *Tacca integrifolia*
i) Captopril (std), ii) Methanol extract, iii) Petroleum Ether extract, iv) Water extract
v) Chloroform extract, vi) Hexane extract

Similarly in rhizome crude extract of methanol, petroleum ether and water also shows high percentage of ACE inhibition with the increased of crude extract concentration (Table 3).

This suggests that the crude extract contained ACE inhibitors which could be used as antihypertension agents. However further analysis need to be carried out to evaluate the effectiveness of the ACE inhibitors as antihypertension agents.

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