Sains Malaysiana 45(3)(2016): 425–433

Antihypertension Activity of Water Extract of *Tacca integrifolia*

(Aktiviti Antihipertensi Ekstrak Air *Tacca integrifolia*)

FARAH LIYANA JAMALUDIN & JAMALUDIN MOHAMAD*

ABSTRACT

Tacca integrifolia *Ker-Gawl (Taccaceae) is locally known as 'Belimbing Tanah' . It has been traditionally used for the treatment of hypertension, hemorrhoids, heart failure and kidney disease. Analysis of leaf and rhizome extracts of* T. integrifolia *using LCMS/MS showed the presence of p-hydroxybenzoic acid, protocatechuic acid, quinic acid, gypenosides and 3-caffeolquinic acid. The total phenol contents are high in the leaves and rhizome water extracts at 792.75±0.21 and 350.83±0.15 mgGAE/g, respectively. Total flavonoid contents are also high in the leaf extract at 89.52±0.27 mgQE/g and rhizome extract at 30.22±0.37 mgQE/g. ACE inhibitory activity was highest in rhizome water extract. Sub-acute toxicity test showed that no mortality occurred in rats fed with these extracts under experimental conditions. Blood pressure of spontaneously hypertensive rats was reduced significantly (*$p<0.05$*) at 50 and 100 mg/kg of leaves and rhizome water extract, respectively. Liver function test indicated no significant difference in total protein, ALT and AST between control group fed with 100 mg/kg of both extracts. Similarly, renal function test indicated no significant difference in sodium and potassium levels as well as creatinine between control group fed with 100 mg/kg leaves and rhizome water extract. Thus, these results supported and provide scientific evidence for the traditional use of* T. integrifolia *as treatment for hypertension.*

Keywords: Angiotensin-converting enzyme; Antioxidant; hypertension; Tacca integrifolia

ABSTRAK

Tacca integrifolia *Ker-Gawl (Taccaceae) dikenali sebagai Belimbing Tanah. Tumbuhan ini digunakan secara tradisi untuk rawatan hipertensi, hemoroid, kegagalan jantung dan penyakit buah pinggang. Analisis ekstrak daun dan rizom* T. integrifolia *menggunakan LCMS/MS menunjukkan kehadiran asid p-hydroxybenzoik, asid protocatechuik, asid quinik, gypenosida dan asid 3-caffeolquinik. Jumlah kandungan fenol adalah tinggi dalam ekstrak air daun dan rizom iaitu masing-masing pada 792.75±0.21 dan 350.83±0.15 mgGAE/g. Jumlah kandungan flavonoid juga adalah tinggi iaitu pada 89.52±0.27 mgQE/g bagi ekstrak daun dan 30.22±0.37 mgQE/g pada ekstrak rizom. Aktiviti perencatan ACE didapati lebih tinggi dalam ekstrak air rizom berbanding ekstrak daun. Ujian ketoksikan akut menunjukkan tiada kematian berlaku pada haiwan diberi ekstrak dalam keadaan percubaan. Tekanan darah tikus hipertensif spontan didapati berkurangan dengan signifikan (*$p<0.05$*) dengan pemberian masing-masing pada 50 dan 100 mg/kg ekstrak air daun dan rizom. Ujian fungsi hati menunjukkan tiada perubahan signifikan dalam kandungan jumlah protein serta ALT dan AST antara kumpulan kawalan yang diberi makan kedua-dua ekstrak sebanyak 100 mg/kg. Begitu juga pada ujian fungsi renal tiada perbezaan signifikan diperhatikan pada aras natrium, kalium dan kreanitina bila dibandingkan antara kumpulan kawalan yang diberikan 100 mg/kg ekstrak air daun dan rizom. Dengan itu, keputusan ini memberikan sokongan saintifik terhadap kegunaan tradisi* T. integrifolia *sebagai rawatan hipertensi.*

Kata kunci: Antioksidan; enzim penukar-angiotensin; hipertensi; Tacca integrifolia

INTRODUCTION

Hypertension is a worldwide problem with epidemic proportions affecting 15-20% of all adult population (Wang et al. 2008). It is one of the major risk factors for coronary heart disease and development of cardiac hypertrophy to heart failure. It is also a high risk factor for brain ischemia, arteriosclerosis, stroke, myocardial infarction and end-stage renal disease (Sabbatini et al. 2000). The relationship between blood pressure and the risk of cardiovascular disease (CVD) is continuous and consistent with each increment of 20/10 mmHg doubling the risk of CVD (Erdogan et al. 2010). Drugs used to control blood pressure include calcium channel blockers, beta-blockers, angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor blockers (Podymow & August 2011). These drugs were used individually or in combination to optimize the mechanism of action in reducing the blood pressure. However, the unwanted side effects of these drugs cannot be disputed even though it was proven to reduce blood pressure effectively. Therefore, studies of active compounds from natural resources for the treatment of high blood pressure are important to be developed. Two-thirds of the world's plant species have been suggested to display

medicinal values and traditional medicinal plants have thus become important resources for drug development (Krishnaiah et al. 2011; Tirapelli et al. 2010). They are potential sources of novel biologically active compounds; however, only a small percentage of these plants has been exploited for its bioactivity. Natural products derived from medicinal plants have been studied for its therapeutic agents in treating chronic disease such as cardiovascular disease, cancer, hypertension, diabetis and other oxidative stress-related ailments (Ahmad et al. 2013; Dwivedi & Dipti 2002; Rajandeep et al. 2011; Shankar Murthy & Kiran 2012; Sri Nurestri et al. 2008). Attention recently was focused on natural compounds for the treatment of hypertension as well as hypertension-related disease. The presence of natural compounds such as phenolics, diterpenoids and anthocyanins were reported to contribute to pharmacological effects on cardiovascular and hypertensive activities (Lena et al. 2009; Pechanova et al. 2008). About 80% of the world population rely on traditional medicine that is based on natural products for their primary health care needs. In Malaysia, medicinal plants have been used as daily supplements to reduce high blood pressure (Cicero et al. 2012; Marcia et al. 2009; Omar et al. 2013). In this study, the antihypertensive property of *Tacca integrifolia* was investigated. *T. integrifolia* is a herbaceous plant from the Taccaceae family of plants widely distributed in Southeast Asia (Zhang et al. 2006). *T. integrifolia*, also known as *Tacca cristata* and locally known as 'Belimbing Tanah' grows in deep shade of the rainforest. The genus *Tacca* is known to have long leaves and short stemmed. Asian *Tacca* is attractive and the entire leaves has vertical growth. The plant filiform resembles whiskers which hang below the flowers and may extend to 1 foot in length. The white bracts are beautifully veined with purple color and drift over white flowers thus *T. integrifolia* has been frequently used as an ornamental garden plant. Additionally, consumption of the plant as a daily supplement in maintaining normal blood pressure as well as reducing high blood pressure is a common practice. In Malay folk traditional medicine, it was also used to control diabetes. *Tacca* species have been used for the treatment of gastric ulcer, enteritis and hepatitis in China while the rhizomes have been used for improving sexual function and controlling blood pressure in Thai herbal medicine (Kitjaroennirut et al. 2005). The root broths of the plant were used for treatment of hypertension and hemorrhoids. For women health, the plant has been used in baths to remove the toxic substances from the body after child delivery. The sap from the leaves were used for skin diseases. In another application, the extracts from the tuber and roots were mixed with *Goniothalamus malayanus* (Mempisang) for treatment of kidney pain. Although it is useful in the treatment of hypertension and other diseases, limited scientific evidence exist for its antihypertensive activity. Thus, the aim of this study was to evaluate the effectiveness of *T. integrifolia* as a antihypertensive agent.

Materials and Methods

Plant materials

The leaves and rhizomes of *T. integrifolia* were collected from the Field Study Centre, University of Malaya, Gombak, Selangor. Professor Dr. Ong Hean Choi from Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur confirmed its taxonomic identification based on its ethnobotanical literature.

Extraction, separation, detection and determination of phytochemical compounds

The leaves and rhizomes of *T. integrifolia* were cleaned and dried in the oven at 30°C - 40°C for approximately 3 days. The dried plant materials were ground into powder form with grinding machine and stored in the glass bottle until further used. The water extract was prepared by macerated 20 g powder of the leaves and rhizome in 200 mL of distilled water at room temperature for 3 days. The phytochemical compounds were separated using TLC plate size 20×20 cm and developed in chloroform solvent. The presence of the phytochemical compounds were detected by spraying the TLC plate with chemical reagents. The Folin-ciocalteu, vanillin sulfuric acid and Dragendorffs' reagent were used to detect phenol, terpenoids and alkaloid compounds, respectively.

Determination of total phenol contents

The total phenolic contents of water extracts were determined with the Folin-Ciocalteu reagent using methods from Spanos and Wrosltad (1990). First, the Folin-Ciocalteu reagent was prepared by dissolving the Folin - Ciocalteu reagent in distilled water in 1: 10 ratio. 500 µL of the crude extracts was mixed with 5 mL of Folin-Ciocalteu reagent and 4 mL of 1 M of sodium carbonate (Na_2CO_3). The reaction mixture was incubated in a water bath at 45°C for 15 min. The absorbance of the sample was measured at 765 nm with a spectrophotometer. The total phenol content was determined using a standard curve prepared from 0, 50, 100, 150, 200, 250 and 500 mg/L of gallic acid in methanol solution (50:50/v:v). The results were expressed as mg of gallic acid equivalent per gram of dry sample. All of the aqueous extracts were tested in duplicate and mean value were calculated.

Determination of total flavonoids contents

The total flavonoid contents of the water extracts were determined using aluminium chloride colorimetric method with slightly modified as described by Liu et al. (2007). 10 mg/mL of standard concentration samples in methanol (1:1) were mixed with 0.3 mL 5% sodium nitrate and incubated for 5 min at 37°C. 0.3 mL of 10% aluminium chloride was added and incubated 6 min at 37°C. 2 mL of sodium hydroxide and 10 mL distilled water was added. Absorbance was measured at 510 nm using

a spectrophotometer. The total flavonoids content was determined using a standard curve prepared from 0, 50, 100, 150, 200, 250 and 500 mg/L of quercetin. The results from two replicates were expressed as mg of quarcetin equivalent per gram of dry sample.

ANGIOTENSIN CONVERTING ENZYME (ACE) INHIBITION ASSAY

ACE inhibition activity was carried out according to the Crushman and Cheung (1971) with slight modification (Choi et al. 2001). The water extract of the leaves and rhizome of *T. integrifolia* were prepared at the concentration of 6.25, 12.5, 25, 50 and 100 mg/mL. Briefly, 0.25 mL of 100 mM phosphate buffer pH8.3, 0.25 mL of 300 mM sodium chloride (NaCl), 0.25 mL of 5 mM HHL and 0.25 mL of samples were added in a test tube. Then, 0.25 mL ACE enzymes were added into the mixture and were incubated in a water bath at 37°C for 30 min. The reaction was terminated by adding 0.25 mL 1 N hydrochloric acid (HCl) and 2 mL of ethyl acetate was added to each test tube and vortex for 5 s. The top layer was removed and dried the crucible. Finally, 3 mL of distilled water was added and the absorbance was measured at 228 nm using a spectrophotometer. The captopril was used as a positive standard. The percentage of ACE inhibition of the standard and samples were determined using the formula:

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

ANIMAL STUDY
SUB-ACUTE TOXICITY TEST OF THE WATER EXTRACT FROM THE LEAVES AND RHIZOME OF THE *TACCA INTEGRIFOLIA* ON SPONTANEOUSLY HYPERTENSIVE RATS (SHR)

The toxicity test of the water extract from the leaves and rhizome of *Tacca integrifolia* in rats was studied according to Organization for Economic Coorporation and Development (OECD) Test Guideline 423 with animal care and Use Committee (ACUC). The animal ethic number ISB/10/11/2008/JM(R) was given by Animal House, University of Malaya, Kuala Lumpur, Malaysia to conduct and characterize the potential toxic effects of water extract of the *Tacca integrifolia*. In all cases, 500 mg/kg oral dose of *Tacca integrifolia* extract was found to be safe as no mortality was observed during the study. On the basis of this study, the doses of 50 and 100 mg/kg were selected for *Tacca integrifolia* treatment in SHR.

SPONTANEOUSLY HYPERTENSIVE RATS (SHR) TREATMENT OF HYPETENSION WITH THE WATER EXTRACT FROM THE LEAVES AND RHIZOME OF *TACCA INTEGRIFOLIA*

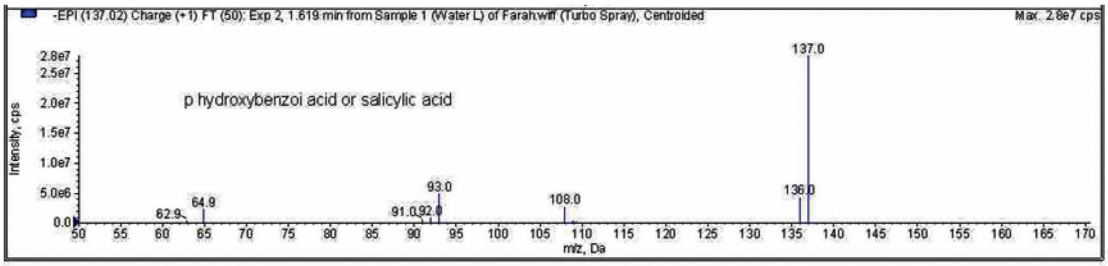
In this study, 56 male SHR with body weight of 180-190 g were acclimatized for 2 weeks before the experiments. It was divided into 8 groups of 6 rats. Group 1: Normal control, Group 2: Control SHR, Group 3: Standardization captopril (50 mg/kg), Group 4: Standard captopril (100 mg/kg), Group 5: SHR + leaves, water extract (50 mg/kg), Group 6: SHR + leaves, water extract (100 mg/kg), Group 7: SHR + rhizome water extract (50 mg/kg) and Group 8: SHR + rhizomes water extract (100 mg/kg). The blood pressures were measured every 10 days using the tail cuff method with the PowerLab instrument in conscious, lightly restrained rat (Ibrahim et al. 2005). The SHR rats were fasted approximately 12 h before being sacrificed with diethyl ether and the blood serums were taken for liver function test using ALT and AST kit purchased from Sigma-Aldrich. The renal function test for serum sodium and potassium was determined according to Akhigbe et al. (2008).

STATISTICAL ANALYSIS

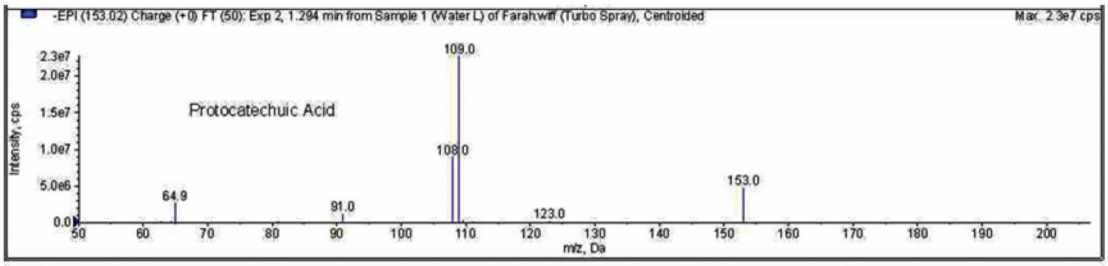
All statistical analysis, ANOVA was conducted using SPSS 10.0 (SPSS Inc., Illinois, USA) computer program.

DISCUSSION

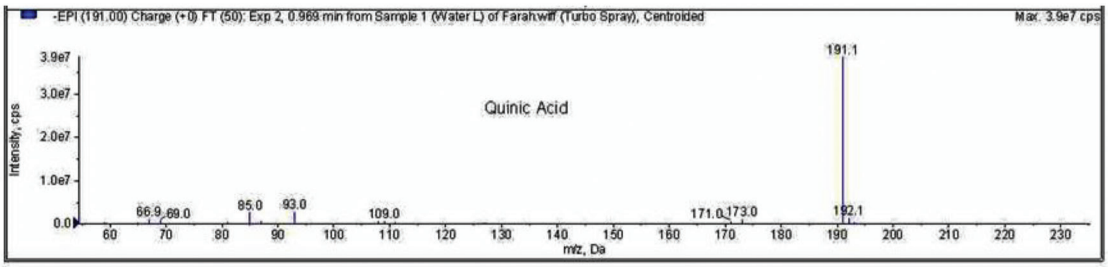
The identification and determination of the phytochemical compounds that has been separated with thin layer chromatography in the leaves and rhizomes of *Tacco integrifolia* were carried out using liquid chromatography mass spectrometry tandem with mass spectrometry (LCMS/MS). The chromatogram profile of LCMS/MS of leaves and rhizome water extract showed the presence of *p*-hydroxybenzoic acid, protocatechuic acid, quinic acid, gypenosides and 3-caffeolquinic acid (Figure 1(a)-1(f)). Kitjaroennirut et al. (2005) has reported the presence of ochratoxin A, amino acids, n-triacontanol, castanogenin, betulinic acid, quercetin-3-α-arabinoside and taccalin in rhizome of *Tacca integrifolia*. The results in Table 1 shows that leaves water extract contained the highest phenolic contents at 792.75±0.21 mgGAE/g followed by rhizome extract at 350.83±0.15 mgGAE/g. The high contents of phenols in leaves and rhizome, water extract of *Tacca integrifolia* could be due to the presence of *p* hydroxybenzoic acid, protocatechuic acid, 3-caffeolquinic acid and quinic acid, which has been detected in LCMS/MS in the extracts. The total flavonoids contents (Table 1) in leave extract were highest at 89.52±0.27 mgQE/g than rhizome extract at 30.22±0.37 mgQE/g. The presence of proanthocyanin and isoflavone glycoside in the leave extract which has been detected by LCMS/MS contributed to its high flavonoids contents. The anti-hypertension activity of *Tacca integrifolia* crude extract were evaluated through angiotensin converting enzyme inhibition assay to determine its effectiveness in reducing high blood pressure. In this assay the captopril was used as standard for ACE inhibition with IC_{50} values of 58 µg/mL. In the ACE inhibition assay of leave extract of *Tacca integrifolia* as shown in Figure 2 shows that the ACE inhibition was higher in the rhizome extract than the leaf extracts. As the ACE enzyme was inhibited, it reduced the ACE activity and this


-EPI (137.02) Charge (+1) FT (50): Exp 2, 1.619 min from Sample 1 (Water L) of Farah.wiff (Turbo Spray), Centroided
Max. 2.8e7 cps
p hydroxybenzoi acid or salicylic acid
137.0
136.0
93.0
108.0
64.9
62.9
91.0
92.0
Intensity, cps
m/z, Da

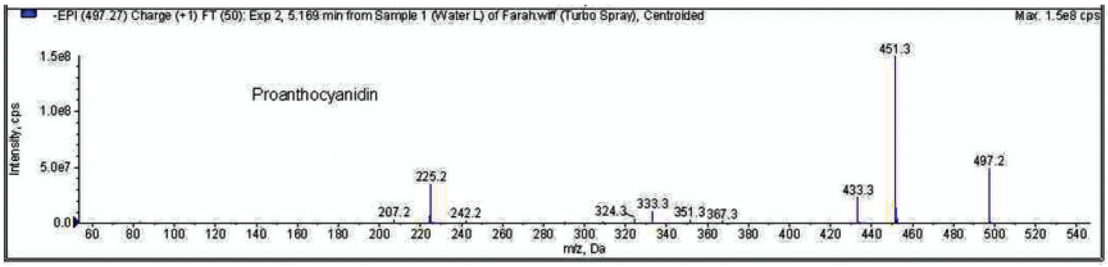
(a)


-EPI (153.02) Charge (+0) FT (50): Exp 2, 1.294 min from Sample 1 (Water L) of Farah.wiff (Turbo Spray), Centroided
Max. 2.3e7 cps
Protocatechuic Acid
109.0
108.0
153.0
64.9
91.0
123.0
Intensity, cps
m/z, Da

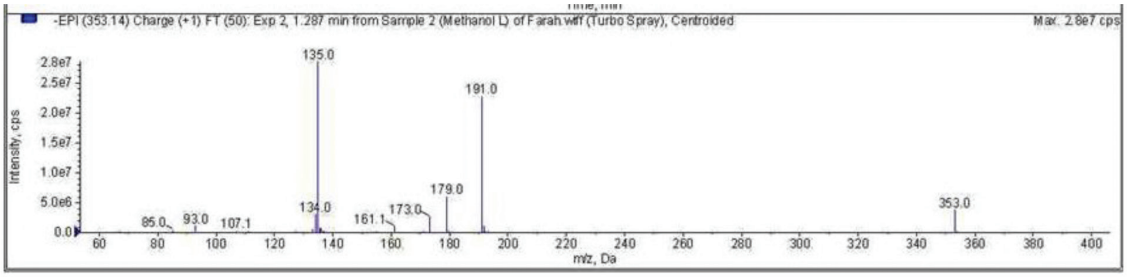
(b)


-EPI (191.00) Charge (+0) FT (50): Exp 2, 0.969 min from Sample 1 (Water L) of Farah.wiff (Turbo Spray), Centroided
Max. 3.9e7 cps
Quinic Acid
191.1
192.1
66.9
69.0
85.0
93.0
109.0
171.0
173.0
Intensity, cps
m/z, Da

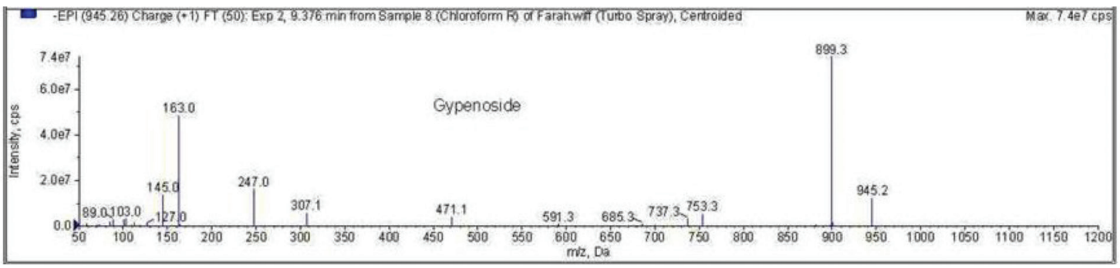
(c)


-EPI (497.27) Charge (+1) FT (50): Exp 2, 5.169 min from Sample 1 (Water L) of Farah.wiff (Turbo Spray), Centroided
Max. 1.5e8 cps
Proanthocyanidin
451.3
497.2
433.3
225.2
207.2
242.2
324.3
333.3
351.3
367.3
Intensity, cps
m/z, Da

(d)


-EPI (353.14) Charge (+1) FT (50): Exp 2, 1.287 min from Sample 2 (Methanol L) of Farah.wiff (Turbo Spray), Centroided
Max. 2.8e7 cps
135.0
191.0
179.0
353.0
134.0
173.0
161.1
85.0
93.0
107.1
Intensity, cps
m/z, Da

(e)



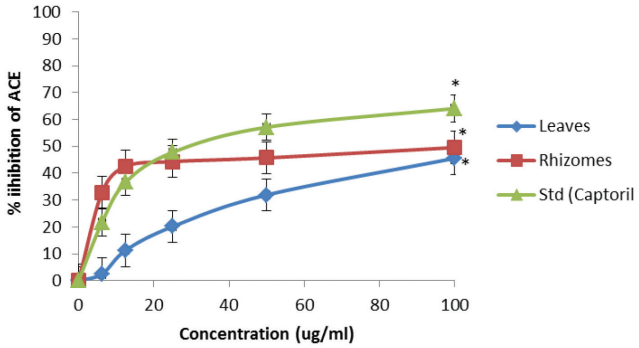
(f)

FIGURE 1. The LCMS/MS chromatogram profile: a) p-hydroybenzoic acid, b) protocatechuic acid, c) quinic acid, d) proanthocyanidin, e) 3-caffeolquinic acid and f) gypenosides of leaves and rhizomes water extract of *Tacca integrifolia*

TABLE 1. The total phenols and flavonoids content from leaves and rhizome extracts of *Tacca integrifolia*

Sample	Total phenol content (mgGAE/g)	Total flavonoid content (mgQE/g)
Water leaves extract	792.75±0.21*	89.52±0.27*
Water rhizome extract	350.83±0.15*	30.22±0.37*

Values are expressed as mean ± S.D., n = 3. *Significant at $p \leq 0.05$



Values are expressed as mean ± S.D., $n = 3$. *Significant at $p \leq 0.05$

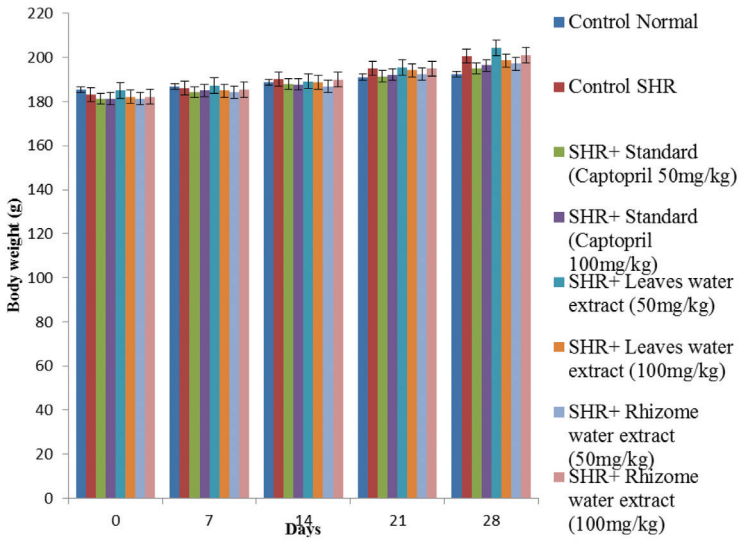
FIGURE 2. The ACE inhibitions of extracts from *Tacca integrifolia*

would then eventually inhibit the formation of angiotensin II from angiotensin I that lead to reduce the blood pressure. This will prevent the formation of angiotensin II and stimulated the synthesis and releasing of aldosterone from adrenal cortex that lead in increasing sodium, water retention and blood pressure (Lacaille-Dubois et al. 2001). Thus, the inhibition of angiotensin II will reduce high blood pressure. The highest ACE inhibition in the rhizome extract could be due to the presence of gypenoside which has been detected by LCMS/MS. It has been reported to possess antihypertensive properties (Circosta et al. 2005). Meanwhile inhibition of ACE in water, could be due to the high phenol contents in the leave extracts. The LCMS/MS of water leaves extract has shown that it contains phenol compounds of *p* hydroxybenzoic acid, protocatechuic acid, quinic acid and flavonoid proanthocyanidin. Thus, it can be concluded that the presence of these compounds contributed to the ACE inhibition in the extracts. Lacaille-Dubois et al. (2001) have reported that proanthocyanidin is potential in inhibiting the ACE enzyme. Since the leaves and rhizome, water extract was most commonly used as traditional medicine for hypertension treatment, its hypertension properties were further investigated in SHR rats. The sub-acute toxicity test of water crude extract from leaves and rhizome of *Tacca integrifolia* was carried out using female spontaneously hypertensive rats (SHR) prior to the treatment of the extract *in vivo* in SHR rats. There are no death or abnormalities in clinical sign were observed during the sub-acute toxicity test. There were no significant abnormalities in body weight, liver and kidney of the rats. This indicated that consumption of water crude extract at high dose of 500 mg/kg has no negative effect on SHR rat. The anti-hypertension treatment of leaves and rhizome water extract were carried out *in vivo* with the doses of 50 and 100 mg/kg body weight. The mean body weight in all groups was increased significantly within group and between groups at $p<0.05$. The captopril is used as standard and the results showed that group fed with a dose

of 20 mg/kg captopril and was significantly increased body weight when compared with a group fed with 100 mg/kg of captopril (Figure 3).

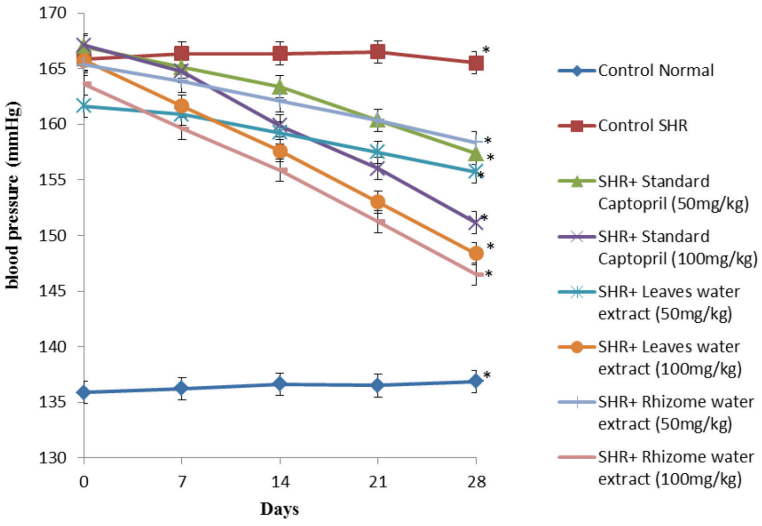
The mean systolic blood pressure of SHR (Figure 4) showed that the systolic blood pressure were significantly reduced from day 0 to 28 days at a dose of 100 mg/kg of leaves and rhizome extract between control SHR and treated SHR rats. However, there is no significant difference between group fed with 20 mg/kg of captopril and with 50 mg/kg of water rhizome extract. The group fed with 100 mg/kg of captopril and the group fed with 50 mg/kg of a leave extract shows significant differences in reducing blood pressure. The decreasing of the systolic blood pressure could be due to the presence of phytochemical compounds in the leaves and rhizome extract that has been detected in LCMS/MS of Tacca integrifolia (Figure 1). It has been shown that the proanthocyanidin and proanthocyanidin trimer possesses vasodilatory effects in SHR leading to decreasing of blood pressure (Bagchi et al. 2000). Researches also reported that purified proanthocyanidin inhibited ACE activity *in vitro* (Eriz et al. 2011).

The liver function test (Table 2) of SHR showed that the amount of total protein was in a normal range of protein between 6.3 to 7.9 g/dL in all SHR groups. The alanine aminotransferase (ALT) test showed that group fed with 50 and 100 mg/kg of leaves and rhizome water extract



Values are expressed as mean ± S.D., $n = 8$

FIGURE 3. The histogram of the mean body weight of spontaneously hypertensive rats (SHR)



Values are expressed as mean ± S.D., n = 8. * Significant $p \leq 0.05$ compared with control

FIGURE 4. The mean systolic blood pressure (mmHg) measurement of SHR

TABLE 2. The liver function and renal function test of blood serum collected from spontaneously hypertensive rats (SHR)

Group of SHR	Liver function test			Renal function test		
	Mean total protein (g/L)	Mean ALT (IU/L)	Mean AST (IU/L)	Mean N+ (mmol/L)	Mean K+ (mmol/L)	Mean creatinine (umol/L)
Control						
Normal (SD)	6.41 ± 0.18	46.56 ± 1.82	14.61 ± 0.27	140.13 ± 2.05	5.12 ± 0.28	0.83 ± 0.02
Control						
SHR	6.72 ± 0.07	53.51 ± 0.35	16.86 ± 0.05	136.11 ± 0.74	4.53 ± 0.01	0.83 ± 0.01
SHR + Standard Captopril (50 mg/kg)	5.81 ± 0.12	46.12 ± 1.12	16.95 ± 0.26	142.14 ± 1.25	4.22 ± 0.10	0.85 ± 0.01
SHR + Standard Captopril (100 mg/kg)	5.71 ± 0.13	75.13 ± 0.83	17.72 ± 0.08	153.14 ± 1.31	5.53 ± 0.09	0.94 ± 0.02
SHR + Leaves water extract (50 mg/kg)	6.86 ± 0.04	53.31 ± 0.43	20.22 ± 0.08	138.32 ± 0.28	4.44 ± 0.24	0.86 ± 0.02
SHR + Leaves water extract (100 mg/kg)	7.05 ± 0.06	52.21 ± 0.47	15.37 ± 0.05	136.62 ± 0.33	4.81 ± 0.19	0.87± 0.02
SHR + Rhizome water extract (50 mg/kg)	6.88 ± 0.09	52.28 ± 0.79	18.62 ± 0.18	139.12 ± 1.69	4.45 ± 0.26	0.84 ± 0.01
SHR + Rhizome water extract (100 mg/kg)	7.11 ± 0.08	54.32 ±1.09	16.79 ± 0.08	137.13 ± 1.32	4.68 ± 0.18	0.87 ± 0.02

Values are expressed as mean ± S.D., $n = 6$. No significant different ($p \geq 0.05$) compared with control

gives the normal ALT activity compared to other group of rats, which is in the normal range ALT between 7 to 55 IU/L (Table 2). The high activity of ALT is an indication of damage in the liver and it has been used in the prediction of cardiovascular disease (Saely et al. 2008). The high level of ALT in blood serum could be due to the inflammation of the liver and the damage, liver cell will result in releasing ALT into the blood vessel. The ALT value has been also reported to associate with metabolic syndrome, such as high triglycerides and high blood pressure traits, but not coronary artery disease (Saely et al. 2008).

Similarly, aspartate aminotransferase (AST) level was higher in SHR group fed with 100 mg/kg of leaves and rhizome water extract compared to other groups which falls within normal range of AST between 10 to 34 IU/L (Table 2). The high activity of AST indicates there is leaking in the hepatocyte plasma membrane that caused by cytolysis or hepatocyte necrosis (Renner 1995). The elevation of both AST and ALT also might be indicated for viral or non-viral, acute and chronic liver disease, drug induces and ischemic liver injury as well (Renner 1995).

An abnormal level of AST indicates liver problems such as hepatitis, virus infection, acute and chronic hepatitis, the blockage of bile ducts, cirrhosis, cancer, alcohol and heart attack. However, abnormalities of AST level might also because of different factor as the test is not specifically to detect liver damage but also in the brain and kidney. The liver function test was commonly used to detect liver damage and higher level or lower level of ALT of its normal range gives indication of abnormal in liver function or liver damage. In this study, all of the SHR groups showed normal activity of ALT and AST at a dose of 50 and 100 mg/kg of rat body weight.

The blood serums were further tested in renal function test to determine the amount of sodium, potassium and creatinine. All of the SHR group showed normal range of sodium amount between 135-145 mmol/L. The high sodium intake will lead to the development of hypertension. Thus, higher sodium balance also indicates high blood pressure. Similarly, potassium amount was in a normal range of 3.5 to 5.0 mmol/L with SHR rats fed 50 and 100 kg/mg body weight. The potassium is important for normal heart and nervous system function. Thus, a high level of potassium in blood serum may lead to a serious fatal heart rhythm problem. Furthermore, higher level of potassium indicates the kidneys or adrenal glands not working well, the possibility of internal bleeding or blood pressure medications such as an ACE inhibitor that might contain in water, leaves and rhizome water extract while abnormal sodium levels may lead to sodium imbalance and disorders. The SHR group rats fed with 50 mg/kg showed normal level of creatinine compared with other group rats in the normal range amount of creatinine of 0.8 to 1.2 mg/dL. The high level of creatinine is an indication of kidney infection, urine blockage that will result in the formation of kidney stone, dehydration and heart failure due to the high intake of crude extract of *Tacca integrifolia*. The determination of sodium, potassium and creatinine level in renal function test can predict the abnormalities in kidney functions and kidney disease related to hypertension. However, the results of the renal function test showed that the administration of leaves and rhizome extract reduced the blood pressure without negative effect on kidney. Thus, the leaves and rhizome water extract of *Tacca integrifolia* indeed are safe to be used at 50 and 100 mg/kg for hypertension treatment.

Conclusion

The water extracts of leaves and rhizomes contained *p*-hydroxybenzoic acid, protocatechuic acid, quinic acid, proanthocyanin, gypenosides and 3-caffeolquinic acid as detected with LCMS/MS. The total phenol contents were higher in water crude extract from leaves than in the rhizomes. While total flavonoids content was higher in the leaf extract than in the rhizome extract of *Tacca integrifolia*. The water crude extract from both leaves and rhizome of *Tacca integrifolia* resulted in significant lowering of blood pressure in spontaneously-induced hypertensive rats during the 28 day experiment. The crude water extract of the leaves and rhizome showed no toxic effect on spontaneously hypertensive rats (SHR). Furthermore, renal and liver function tests showed no toxic effects in both organs. Further research on its chemical properties as well as its medicinal value needs to be investigated in order to explain its antihypertensive mechanism of action. The findings provide scientific evidence to support the traditional use of *T. integriloia* for the treatment of high blood pressure. Thus, it will contribute to the pharmaceutical industry and human health.

ACKNOWLEDGEMENTS

The authors thank the Malaysian government research grant (MOSTI - ScienceFund RMK-9; No: 12-02-03-2064 (8422064) and University of Malaya research grant No: PS156/2008B.

REFERENCES

Ahmad Ajaz, Khan Rao, Alkharfy & Khalid. 2013. Effects of selected bioactive natural products on the vascular endothelium. *Cardiovascular Pharmacology* 62(2): 111-121.

Akhigbe, R.E., Ige, S.F., Afolabi, A.O., Oyeyipo, P.I., Ajao, F.O. & Ajayi, F.A. 2008. Water balance and serum levels of some electrolytes in oral contraceptive-treated female wistar rats. *Journal Medicinal Science* 8: 591-594.

Bagchi, D., Bagchi, M., Stohs, S.J., Das, D.K., Ray, S.D., Kuszynski, C.A., Joshi, S.S. & Pruess, H.G. 2000. Free radicals and grape seed proanthocyanidin extract: Importance in human health and disease prevention. *Toxicology* 148: 187-197.

Choi, S.H., Cho, H.Y., Yang, H.C., RA, K.S. & Suh, H.J. 2001. Angiotensin I-converting enzyme inhibitor from *Grifola frondosa*. *Food Research International* 34: 177-182.

Cicero, A.F., De Sando, V., Izzo, R., Vasta, A., Trimarco, A. & Borghi, C. 2012. Effect of a combined nutraceutical containing Orthosiphon stamineus effect on blood pressure and metabolic syndrome components in hypertensive

dyslipidaemic patients: A randomized clinical trial. *Complementary Therapies in Clinical Practice* 18(3): 190-194.

Circosta, C., Pasquale, R.D. & Occhiuto, F. 2005. Cardiovascular effects of the aqueous extract of *Gynostemma pentaphyllum* Makino. *Phytomedicine* 12: 638-643.

Cushman, D.W. & Cheung, H.S. 1971. Spectrophotometric assay and properties of the angiotensin-converting enzyme of rabbit lung. *Biochemical Pharmacology* 20(7): 1637-1648.

Dwivedi. S. & Dipti. 2002. Useful medicinal plants in cardiovascular ailments. *Natural Product Radiance* 1(5): 22-26.

Erdogan, D., Gonul, E., Icli, A., Yucel, H., Arslan, A., Akcay, S. & Ozaydin, M. 2010. Effects of normal blood pressure, prehypertension, and hypertension on autonomic nervous system function. *International Journal of Cardiology* 151(1): 50-53.

Eriz, G., Sanhueza, V., Roeckel, M., & Fernandez, K. 2011. Inhibition of the angiotensin-converting enzyme by grape seed and skin proanthocyanidins extracted from *Vitis vinifera* L.Cv. Pais. LWT. *Food Science and Technology* 44: 860-865.

Ibrahim, M.A., Kanzaki, T., Yamagata, S.I., Satoh, N. & Ueda, S. 2005. Effect of diabetes on aortic nitric oxide synthesis in spontaneously hypertensive rats; does captopril modulate this effect? *Life Sciences* 77: 1003 - 1014.

Kitjaroennirut, N., Jansakul, C. & Sawangchote, P. 2005. Cardiovascular effects of *Tacca integrifolia* Ker-Gawl. extract in rats. *Journal Science Technology* 27: 281-289.

Krishnaiah, D., Sarbatly, R. & Nithyanandam, R. 2011. A review of the antioxidant potential of medicinal plant species. *Food and Bioproducts Processing* 89: 217-233.

Lacaille-Dubois, M.A., Franck, U. & Wagner, H. 2001. Search for potential angiotensin converting enzyme (ACE)-inhibitors from plants. *Phytomedicine* 8: 47-52.

Liu, X., Dong, M., Chen, X., Jiang, M., Lv, X. & Yan, G. 2007. Antioxidant activity and phenolics of endophytic *Xylaria* sp. from *Ginkgo biloba*. *Food Chemistry* 105: 548-554.

OECD 2001. OECD Guidelines for Testing of Chemical No. 423. Acute oral toxicity - Acute toxic class method adopted.

Omar Saad Saleh Abrika, Mun Fei Yam, Mohd. Zaini Asmawi, Amirin Sadikun, Hamady Dieng & Elssanousi Ali Hussain. 2013. Effects of extracts and fractions of *Gynura procumbens* on rat atrial contraction. *Journal of Acupuncture Meridian Studies* 6(4): 199-207.

Pechanova, O., Rezzani, R., Babal, P., Bernatova, I. & Andriantsitohaina, R. 2006. Beneficial effects of provinols™ : Cardiovascular system and kidney. *Physiology Resource* 55(Suppl. 1): S17-S30.

Pinto, M.D.S., Kwon, Y.I., Apostolidis, E., Lajolo, F.M., Genovese, M.I. & Shetty, K. 2009. Potential of *Ginkgo biloba* L. leaves in the management of hyperglycemia and hypertension using *in vitro* models. *Bioresource Technology* 100: 6599-6609.

Podymow, T. & August, P. 2011. Antihypertensive drugs in pregnancy. *Seminars in Nephrology* 31(1): 70-85.

Rajandeep Kaur, Karan Kapoor, & Harpreet Kaur. 2011. Plants as a source of anticancer agents. *Journal of Natural Product Plant Resource* 1(1): 119-124.

Ranilla, L.G., Apostolidis, E., Genovese, M.I., Lajolo, F.M. & Shetty, K. 2009. Evaluation of indigenous grains from the Peruvian Andean Region for antidiabetes and antihypertension potential using *in vitro* methods. *Journal of Medicinal Food* 12(4): 704-713.

Renner, E.L. 1995. Liver funcion tests. *Bailliere's Clinical Gastroenterology* 9(4): 661-677.

Sabbatini, M., Strocchi, P., Vitaioli, L. & Amenta, F. 2000. The hippocampus in spontaneously hypertensive rats: A quantitative microanatomical study. *Neuroscience* 100: 251-258.

Saely, C.H., Vonbank, A., Rein, P., Woess, M., Beer, S., Aczel, S., Jankovic, V., Boehnel, C., Risch, L., & Drexel, H. 2008. Alanine aminotransferase and gamma-glutamyl transferase are associated with the metabolic syndrome but not with angiographically determined coronary atherosclerosis. *Clinica Chimica Acta* 397: 82-86.

Shankar Murthy, K. & Kiran, B.R. 2012. Medicinal plants usage in cardiovascular diseases - A Review. *International Journal of Advanced Scientific and Technical Research* 2(6): 264-280.

Spanos, G.A. & Wrosltad, R.E. 1990. Influence of processing and storage on the phenolic composition of Thompson seedless grape juice. *Journal of Agricultural & Food Chemistry* 38: 1565-1571.

Sri Nurestri, A.B., Norhanom, A.W., Hashim, Y., Sim, K.S., Hong, S.L., Lee, G.S. & Syarifah, N.S.A.R. 2008. Cytotoxic activity of *Pereskia bleo* (Cactaceae) against selected human cell lines. *International Journal of Cancer Research* 4: 20-27.

Tirapelli, C.R., Ambrosio, S.R., Oliveira, A.M.D. & Tostes, R.C. 2010. Hypotensive action of naturally occurring diterpenes: A therapeutic promise for the treatment of hypertension. *Fitoterapia* 81: 690-702.

Wang, J., Hu, J., Cui, X., Bai, Y., Du, Y., Miyaguchi & Lin, B. 2008. Purification and identification of a ACE inhibitory peptide from oyster proteins hydrolysate and the antihypertensive effect of hydrolysate in spontaneously hypertensive rats. *Food Chemistry* 111: 302-308.

Zhang, L., Li, Q.J. & Li, D.Z. 2006. Genetic diversity of *Tacca integrifolia* (Taccaceae) in the Brahmaputra valley, Tibet. *Biodiversity Science* 14: 65-72.

Institute of Biological Sciences
Faculty of Science
University of Malaya
50603 Kuala Lumpur
Malaysia

*Corresponding author; email: jamal@um.edu.my

Received: 16 October 2014
Accepted: 2 September 2015