CHAPTER 5

DISCUSSIONS

The thin layer chromatography of extracts from *Tacca integrifolia* (Table 4.12) showed the presence of essential oils, alkaloids, phenols, flavonoids, terpenoids and unsaturated conjugated compounds. The detection of these compounds was carried out using chemical reagents. The formation of blue color with anesaldehyde-sulphuric acid reagent indicated the presence of essential oils in hexane, petroleum ether, chloroform, methanol and water leaves extract and rhizome extract. The presence of alkaloids was detected using Dragendorff reagent with the formation of orange color in hexane, chloroform, methanol and water leaves extract, as well as in hexane, petroleum ether, chloroform, methanol, and water rhizome extract. Hexane was used to extract essential oil (Mindaryani et al., 2007), lipids, carotenoids and chlorophyll (Dai et al., 2010), phenol and flavonoids (Hossain et al., 2011). Whereas petroleum ether was used to extract steroids, alkaloids and saponin (Iwalewa et al., 2003).

The presence of terpenoids was carried out using vanillin sulphuric reagent with the formation of blue color in hexane (Table 4.02), chloroform (Table 4.04) and water leaves extract (Table 4.10), and in hexane (Table 4.06), petroleum ether (Table 4.07) and methanol rhizome extract (Table 4.09). The phenols and flavonoids were detected using vanillin sulphuric acid reagent and anesaldehyde sulphuric acid with the formation of red color in chloroform leaves extract (Table 4.04) and a green colour in water rhizome extract (Table 4.11) that identified as phenols and flavonoid respectively. The unsaturated compounds were detected using iodine vapors with the formation of brown color in petroleum ether, chloroform, and methanol leaves and rhizome extract.

Chloroform and methanol are polar solvent that has been used to extract polar compound of lipids (Dai et al., 2010), phenolic compound and flavonoids (Hossain et al., 2011), steroid, alkaloids and saponin (Iwalewa et al., 2003). In this study, the TLC of chloroform leaves extract showed the presence of the alkaloid, terpenoid, essential oil and phenol (Table 4.04) while in TLC of chloroform rhizome extract showed the presence of alkaloid, essential oil and unsaturated compounds (Table 4.08). The methanol which is more polar than chloroform has been reported to extract terpenoids (Iwalewa et al., 2003), phenols and flavonoids (Hossain et al., 2011), lower molecular weight of polyphenols, sugars, and organic acid (Dai et al., 2010). Similarly in this study the TLC of methanol leaves extract showed the presence of alkaloid, essential oil, unsaturated compounds (Table 4.05), while TLC of methanol rhizome extract showed the presence alkaloid, terpenoid, essential oil and unsaturated compounds (Table 4.09). It is also has been reported that water which is the most polar solvent was used to extract phenol and flavonoids (Alexandru et al., 2007). The results of the TLC of leaves water extract showed the presence of alkaloid, terpenoid and essential oil (Table 4.10) whereas in the water rhizomes extract showed the presence of alkaloid, essential oil and flavonoids compounds (Table 4.11). The active compounds identified in TLC of leaves and rhizomes extract was summarized as in Table 4.12.

The separations of the phytochemical compounds were carried out using High Performance Liquid Chromatography (HPLC) and Liquid Chromatography Mass Spectrometry tandem with mass spectrometry (LCMS/MS) in attempting to determine the phytochemical compounds presence in leaves and rhizome of *Tacca integrifolia*. The HPLC chromatogram profile chloroform of leaves (Figure 4.03) and rhizome extract (Figure 4.04) showed that there are peaks signals indicating the presence of chemical compounds. However, when it was detected with LCMS/MS, the chloroform leaves extract showed the presence of *p* hydroxybenzoic acid (Figure 4.15),

proanthocyanidin trimer (Figure 4.16), 1,3,5 tricaffeolquinic acid (Figure 4.17), and 2(3,4-Dihydroxyphenyl)-7-hydroxy-5-benzenepropanoic acid (Figure 4.18). Whereas, the LCMS/MS profile of chloroform rhizome extract showed the presence of triterpenoid saponin (Figure 4.38) and gypenosides (Figure 4.39).

Similarly the HPLC profile of methanol leaves extract showed that there are chemical compounds in the extracts. However, further analysis with LCMS/MS had showed the presence of quinic acid (Figure 4.20), 3 caffeolquinic acid (Figure 4.21), *p* hydroxybenzoic acid (Figure 4.22), decaffeolquinic acid conjugate (Figure 4.23), isoflavone glycoside (Figure 4.24), and proanthocyanidin (Figure 4.25). The gypenosides was detected in the methanol rhizome extract showed in Figure 4.41.

The water leaves extract also showed the presence of chemical compounds in HPLC profile while the LCMS/MS profile showed the presence of quinic acid (Figure 4.27), protocatechuic acid (Figure 4.28), salicylic acid (Figure 4.29), phenolic acid conjugate (Figure 4.30), proanthocyanidin (Figure 4.31) and proanthocyanidin trimer (Figure 4.32). While water rhizome extract showed the presence of dicaffeolquinic acid conjugate (Figure 4.43), proanthocyanidin (Figure 4.44) and proanthocyanidin trimer (Figure 4.45) with LCMS/MS. The analysis of hexane leaves extract and petroleum ether leaves extract with LCMS/MS showed the presence of proanthocyanidin trimer as in Figure 4.11 and 4.13. The proanthocyanidin trimer (Figure 4.34) and proanthocyanidin trimer isomer (Figure 4.36) was detected in the hexane rhizome extract and in petroleum ether rhizome extract respectively with LCMS/MS. It has been reported from the previous research the presence of ochratoxin A, amino acids, n-triacontanol, castanogenin, betulinic acid, quercetin-3-α-arabinoside, and taccalin in rhizome of *Tacca integrifolia* (Kitjaroennirut et al., 2005). However, these compounds was unable to be detected could be due to the low amount of compounds presence. In this research the detection of proanthocyanidin,

proanthocyanidin trimer and isomer, phenolic acid conjugate, *p* hydroxybenzoic acid, protocatechuic acid, quinic acid, 2(3,4-dihydroxyphenyl)-7-hydroxy-5-benzene propanoic acid, 1,3,5-tricaffeoylquinic acid, 3 caffeoylquinic acid, dicaffeolquinic acid conjugate, gypenoside, triterpenoid saponin compounds with LCMS/MS in the leaves and rhizome of *Tacca integrifolia* was the first time to our best knowledge being carried out.

The formation of stable froth in saponin froth test in the rhizomes methanol extract indicated the presence of saponin and this was supported by detection of gypenoside as shown in LCMS/MS profile of methanol rhizome extract in Figure 4.41. The color changes in methanol leaves extract from green to dark green during tannin and phenol test indicating the presence of phenolic compounds of quinic acid (Figure 4.20), 3-caffeolquinic acid (Figure 4.21), *p*-hydrobenzoic acid (Figure 4.22) and dicaffeoquinic acid conjugate (Figure 4.23) that has been detected in LCMS/MS of methanol leaves extract.

Since the phenolic and flavonoid compounds has been detected presence in the leaves and rhizome extract of *Tacca integrifolia* with TLC and LCMS/MS, the total phenol and flavonoid contents were determined. Water leaves extract showed the highest phenolic contents 792.7mgGAE/g followed by chloroform extract 288.6mgGAE/g, petroleum ether extract 105.7mgGAE/g, methanol leaves extract 69.8mgGAE/g and hexane leaves extract 44.6mgGAE/g as in Table 4.18. Whereas water rhizome extract showed the highest phenolic contents 350.8mgGAE/g followed by hexane rhizome extract 130.3mgGAE/g, chloroform rhizome extract 84.9mgGAE/g, methanol rhizome extract 61.3mgGAE/g and petroleum ether rhizome extract 38.9mgGAE/g as in Table 4.19.

The high contents of phenolic leaves and rhizome water extraction of *Tacca integrifolia* could be due to the presence of phenolic acid conjugate, salicylic acid, protocatechuic acid and quinic acid which has been detected in LCMS/MS in the extracts. Similarly the high phenol contents in the chloroform leaves extract are due the presence of 2(3,4-dihydroxyphenyl)-7-hydroxy-5-benzenepropanoic acid, 1,3,5 caffeolquinic acid, *p*-hydrobenzoic acid whereas in methanol leaves extract it contains 3 caffeolquinic acid, quinic acid and dicaffeolquinic acid conjugate.

The total flavonoids contents in leaves extract was highest in petroleum ether leaves extract 376.7mgQE/g followed by hexane leaves extract 266.9mgQE/g, chloroform leaves extract 242.5mgQE/g, methanol leaves extract 154.1mgQE/g and water leaves extract 89.5mgQE/g (Table 4.21). Whereas in rhizome extract it was highest in chloroform rhizome extract 193.4mgQE/g followed by hexane rhizome extract 179.7mgQE/g, petroleum ether rhizome extract 125.1mgQE/g, methanol rhizome extract 54.4mgQE/g and water rhizome extract 30.2mgQE/g (Table 4.22). The presence of proanthocyanidin trimer, proanthocyanidin and isoflavone glycoside in the leaves extract of hexane, petroleum ether, chloroform, methanol and water which has been detected by LCMS/MS contributed for its high flavonoids contents. Similarly, the high flavonoids in rhizome of hexane extract, petroleum ether and water are due to the presence of proanthocyanidin and proanthocyanidin trimer and isomer.

The anti-hypertension properties of the *Tacca integrifolia* extract were evaluated through angiotensin converting enzyme inhibition assay to evaluate its effectiveness in reducing high blood pressure. In this assay the captopril was used as standard for ACE inhibition with IC_{50} value of 58µg/ml. In the ACE inhibition assay of leaves extract of *Tacca integrifolia* as shown in Figure 4.49 showed that the ACE inhibition was highest in the water, hexane, petroleum ether, methanol and chloroform extract at 100µg/ml. Whereas in the rhizome extract (Figure 4.51) showed that methanol

rhizome extract gave highest ACE inhibition followed by petroleum ether extract, water extract, chloroform extract and hexane extract at 100µg/ml. As the ACE enzyme was inhibited it reduced the ACE activity and this would then eventually inhibit the formation of angiotensin II from angiotensin 1 that lead to reducing of 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 shown in the methanol rhizome extract could be due to the presence of gypenoside which has been detected by LCMS/MS and has been reported possesses antihypertensive properties by Circosta et al. (2005). While inhibition of ACE in water leaves and rhizome extract were due to the high phenols contents as phenolic acid conjugate, protocatechuic acid, quinic acid, flavonoid proanthocyanidin and proanthocyanidin trimer were found in water leaves extract while dicaffeolquinic acid conjugate, proanthocyanidin and proanthocyanidin trimer was presence in water rhizome extract as detected by LCMS/MS. Thus, it can be concluded that the presence of these compounds contributed to the ACE inhibition in the extracts. It has been reported by Lacaille-Dubois et al. (2001) that proanthocyanidin is potential in inhibiting ACE enzyme.

The ACE inhibition of the isolated compounds E3, E4 (hexane rhizome extract in Figure 4.56), B3, B4 (petroleum ether rhizome extract in Figure 4.54), A5, A7, A9 (hexane leaves extract in Figure 4.53), C3, C9 (chloroform leaves extract in Figure 4.55) and G1 (chloroform rhizome extract in Figure 4.58) showed more than 50% of ACE inhibition. The results of the thin layer chromatography showed that the isolated compounds of E3, E4, A5, A7 and A9 were detected as terpenoids (Table 4.06 and 4.02), C3 and G1 as alkaloid (Table 4.04 and 4.08), C9 as phenol (Table 4.04), B5

and B3 as unsaturated compounds (Table 4.03). Whereas labeled compounds of E1, G6, F1, G3 (detected as alkaloid) and A8 (detected as essential oil) showed less than 50% ACE inhibition. The inhibition of the isolated compounds suggested that the ACE inhibition was indeed due to the presence of phytochemicals compounds in the extract of *Tacca integrifolia* and reducing high blood pressure.

Since the leaves and rhizome water extract showed high ACE inhibition and also due to its most commonly used as traditional medicine for hypertension treatment, its hypertension properties was further investigated in SHR rats. The sub-acute toxicity test of water extract from leaves and rhizome of *Tacca integrifolia* were carried out using female spontaneously hypertensive rats (SHR) prior to the treatment of the extract *in vivo* in SHR rats. The body weight of group control SHR and group SHR fed with water leaves extract at dose of 50mg/kg, 100mg/kg and 500mg/kg was increased significantly from 0 to 14 days followed by slightly reduced of body weight before it was increased back to 28 days (Table 4.44). Similarly, sub-acute toxicity test for water rhizome extract revealed that the body weight of SHR rats group fed with medium dose of 100mg/kg and high dose of 500mg/kg showed similarity as SHR control group. However, SHR group fed with low dose of 50mg/kg of water rhizome extract showed significantly increased of body weight from 0 day until 28 days (Table 4.45). There is no death or abnormalities in clinical sign were observed during the sub-acute toxicity test.

The toxicity properties of water extract from leaves and rhizome of *Tacca integrifolia* were further investigated for liver function and renal function test. In the liver function test the total protein, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) was investigated (Table 4.46) while in renal function test total sodium, potassium and creatinine was evaluated (Table 4.47). These tests were carried out in order to assess its side effect in SHR fed with water extract of leaves and rhizome

of *Tacca integrifolia* with the control group of SHR rats. The SHR groups at dose of 50mg/kg and 100mg/kg showed that the total protein is within normal range between 6.3 to 7.9 dL compared to control SHR group. However SHR group at dose of 500mg/kg showed high total protein.

The alanine aminotransferase (ALT) test showed that group fed with 500mg/kg of water leaves and rhizome extract give the high ALT activity compared to other group of rats which is in normal range ALT between 7 to 55 IU/L. The high activity of ALT is an indication of damage in 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 high in SHR group fed with 500mg/kg of water leaves and rhizome extract compared to other groups which is fall within normal range of AST between 10 to 34 IU/L. The high activity of AST indicates there is leaking in hepatocyte plasma membrane that caused by cytolysis or hepatocyte necrosis (Renner, 1995). The elevation of both AST and ALT also might be indicator for viral or non-viral, acute and chronic liver disease, drug induces and ischaemic liver injury as well (Renner, 1995).

An abnormal level of AST indicates liver problem 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 were commonly used to detect liver damage and the high level or lower level of ALT of its normal range give 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 dose of 50mg/kg and 100mg/kg of rat body weight.

The blood serums were further tested for renal function test to determine the amount of sodium, potassium and creatinine. The entire SHR group showed normal range of sodium amount between 135 – 145 mmol/L except for the SHR group fed with dose of 500mg/kg that showed high level of sodium. The high sodium intake will lead to development of hypertension. Thus, higher sodium balance also indicates high blood pressure. Similarly, potassium amount was in a normal range between 3.5 to 5.0 mmol/L with SHR rats fed 50mg/kg and 100kg/mg body weight. The SHR rats group fed with 500mg/kg showed high level of potassium. 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 are not working well, possibility of internal bleeding or blood pressure medications such as ACE inhibitor that might contain in water leaves extract and water rhizome extract while abnormal sodium levels may lead to sodium imbalance and disorders. The SHR group rats fed with 500mg/kg showed high level of creatinine compared with other group rats in normal range amount of creatinine of 0.8 to 1.2mg/dL. The high level of creatinine is an indication of kidney infection, urine blockage that will result in formation of kidney stone, dehydration and heart failure due to the high intake of extract of *Tacca integrifolia.* Since the high dose of 500mg/kg showed indication of side effect to the rats, therefore dose of 50mg/kg and 100mg/kg was used *in vivo* treatment of leaves and rhizomes in SHR rats.

The lethal dose concentration LC_{50} of the extract of *Tacca integrifolia* was also determined using Brine Shrimp Lethality Assay. The results showed that the water rhizome extract possess the highest (LC_{50} 22981 µg/ml) followed by chloroform leaves extract (LC_{50} 6921 µg/ml), methanol leaves extract (LC_{50} 3323 µg/ml), water leaves

extract (LC_{50} 4378 µg/ml), methanol rhizome (LC_{50} 6921 µg/ml), chloroform rhizome extract (LC_{50} 1182 µg/ml), petroleum ether leaves extract (LC_{50} with 975 µg/ml), petroleum ether rhizome extract (LC_{50} 282 µg/ml), hexane leaves extract (LC_{50} 135 µg/ml) and hexane rhizome extract (LC_{50} 100 µg/ml). Thus, the water rhizome extract, methanol rhizome extract, chloroform rhizome extract and water leaves extract showed low toxicity compared to hexane leaves and hexane rhizome extract. Therefore, it is safe to be used in SHR rats for *in vivo* treatment with leaves and rhizome extract of *Tacca integrifolia.*

The anti-hypertension treatment of water leaves extract and water rhizome extract were carried out *in vivo* with the doses of 50mg/kg and 100mg/kg body weight. The mean body weight in all groups were increased significantly within group at $p<0.05$. The captopril is used as standard and the results showed that body weight of SHR group fed with dose of 50mg/kg captopril and 100mg/kg was significantly increased (Table 4.48).

The mean systolic blood pressure of SHR showed that the systolic blood pressures were significantly reduced from day 0 to 28 days. The mean systolic blood pressure of SHR fed with water leaves and rhizome extract were also significant compared to the mean systolic blood pressure of control normal Sprague Dawley rat and control SHR. However, there is no significant difference between group fed with 50mg/kg of captopril and with 50mg/kg of water rhizome extract and significantly difference in group fed with 100mg/kg of captopril with group fed with 50mg/kg of water leaves extract (Table 4.49). The decreasing of the systolic blood pressure could be due to the presence of proanthocyanidin and proanthocyanidin trimer in the leaves and rhizome extract that has been detected in LCMS/MS of *Tacca integrifolia.* It has been shown that the proanthocyanidin and proanthocyanidin trimer possesses vasodilatory effects in SHR leading to decreasing of blood pressure (Bagchi et al., 2005). There are

also researches have reported that purified proanthocyanidin inhibited ACE activity *in vitro* (Eriz et al., 2011).

The liver function test of SHR in Table 4.50 showed that the amount of total protein was in a normal range of protein between 6.3 to 7.9g/dL in all SHR groups. Similarly, the ALT activity was in normal range indicating that there no side effect to the liver at dose of 50mg/kg and 100mg/kg of body weight. The AST activity was also in normal range of 10 to 34 IU/L. The mean difference of total protein level of SHR were significant to control normal SD rat and control SHR except for SHR group treated with 50mg/kg of water leaves extract. Whereas, ALT level were significant when compared to control normal SD rat except in SHR treated with 50mg/kg of Captopril. However, all SHR treated with water extract showed no significant difference compared to control SHR. As in AST level, all SHR showed significant compared to control normal SD and control SHR except for SHR treated with 100mg/kg of water rhizome extract.

Renal function test of SHR in Table 4.51 showed the significance difference in the amount of sodium when compared with SHR treated with water leaves and rhizome extract, control SHR and SHR treated with 50mg/kg of Captopril. Significance difference also found when compared control SHR to SHR treated with 50mg/kg water rhizome extract and SHR treated with 50mg/kg and 100mg/kg of Captopril. The mean difference were significance in the amount of potassium except when compared control normal SD rat to SHR treated with 100mg/kg of water leaves extract and when compared control SHR with SHR treated with 50mg/kg and 100mg/kg water extract. The mean difference in creatinine level were significant only when compared SHR group treated with 100mg/kg of Captopril, water leaves and water rhizome extract separately to control normal SD rat, and also were not significant when compare control

SHR with SHR treated with 50mg/kg and 100mg/kg water leaves and 100mg/kg water rhizomes extract of *Tacca integrifolia* as well as 100mg/kg Captopril.

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 rhizomes extract reduced the blood pressure without negative effect on kidney. As the blood pressure decreased, renin activity has increased (Yen et al., 2010). The liver and renal function test on sub-acute toxicity test and SHR anti-hypertension treatment showed that the leaves water and rhizome extract of Tacca integrifolia indeed is safe to be used for hypertension treatment.

The antioxidant properties of leaves and rhizome extract were evaluated with DPPH radical scavenging assay, Ferric Reducing Power Assay (FRAP) and Metal Chelating Assay. In this assay the IC_{50} of standard ascorbic acid was 5.5µg/ml. The methanol leaves (IC_{50} 88µg/ml) extract showed the highest percentage of DPPH inhibition followed by chloroform (IC_{50} 350µg/ml), water (IC_{50} 480µg/ml), hexane and petroleum ether extract at 500ug/ml (Figure 4.64). However, the rhizome extract showed very low DPPH inhibition. The high inhibition of leaves methanol and water extract is due to the presence of proanthocyanidin and proanthocyanidin trimer which has been detected in the extract with LCMS/MS. Proanthocyanidin was known as antioxidant vitamins and has the ability in protection against free radicals and oxidative stress by donating its hydrogen to scavenging free radicals activity while singlet oxygen quenchers will predict their antioxidant activity (Bagchi et al., 2000). The chloroform and methanol contain *p* hydroxybenzoic acid which has been detected in LCMS/MS possess inhibition of low-density lipoprotein (LDL) oxidation and slowing down the process of atherosclerosis (Elzaawely et al., 2007). Similarly, the presence of

protocatechuic acid in water leaves extract was known to possess scavenges free radicals and protection against oxidative damage (Tseng et al., 1996). Whereas the quinic acid presence in water and methanol leaves extract might be responsible in radical scavenging DPPH as ortho or a para-diphenolic group was also effective in free radical scavengers by an electron transfer (Xu et al., 2008).

In the ferric reducing power assay the leaves chloroform extract of *Tacca integrifolia* showed highest ferric reducing power followed by hexane, methanol and water extract. Whereas, chloroform rhizomes extract showed the highest reducing activity followed by methanol, hexane, petroleum ether and water rhizome extract. The reducing power was determined by the reduction of ferric cyanide $[Fe(CN)_6]^{3-}$ to ferro cyanide $[Fe(CN)_6]^{4-}$ via electron donation (Rohman et al., 2012). The addition of Fe^{3+} ions to form $(Fe^{3+})_4$ $[Fe2+(CN-)_6]_3$, a complex of blue colour were determined by absorbance at 700nm (Rohman et al., 2010). Increasing of absorbance indicated the increasing of reducing activity.

In the metal chelating assay of leaves crude extract showed that the hexane extract (IC_{50} 1.92mg/ml) possess the highest metal chelating activity followed by chloroform extract (IC_{50} 1.98mg/ml), methanol (IC_{50} 2.4mg/ml), water (IC_{50} 4.1mg/ml) and petroleum ether extract. While in rhizome crude extract, the water rhizome extract showed highest metal chelating activity (IC_{50} 3.2mg/ml) followed by methanol (IC_{50} 3.6mg/ml), chloroform rhizome extract (IC_{50} 4.7mg/ml), petroleum ether rhizome extract (IC_{50} 5mg/ml) and hexane extract. The IC_{50} of standard EDTA was evaluated in metal chelating assay was determined at 1.7mg/ml at concentration of 5mg/ml. The higher metal chelating activity was indicated by the smaller IC_{50} value (Rohman et al., 2010). Iron ferrous (Fe^{2+}) is an unstable form of iron that was responsible in the formation of ROS that cause lipid peroxidation, nucleic acid or protein damage whereas

ferric (Fe^{3+}) is an inactive but more stable iron (Chvatalova et al., 2008). The complex formation of ferrozine with Fe^{2+} can be disturbed by chelating agents via inhibition of heavy metal. They stabilized the oxidized form of metal iron by forming σ-bonds with the metal.

The presence of compounds that were detected in extract from leaves and rhizome with LCMS/MS of *Tacca integrifolia* showed the antihypertension and antioxidant activities. Plant polyphenols were reported to involve in the reactions of free radicals via scavenge them or chelating the transition of metal ions (Chvatalove et al., 2008) while the presence of proanthocyanidin, protocatechuic acid, quinic acid, *p* hydroxybenzoic acid and gypenoside in *Tacca integrifolia* were responsible for the antioxidant properties against free radical of DPPH. The phenolics compounds donate it's hydrogen atom to free radicals when it interfere in the oxidation of lipids and other molecules will resulting the formation of stable substance phenoxy radical intermediates that act as terminators of propagation route by reacting with other free radicals (Dai et al., 2010). It scavenged free radical as it has phenolic hydroxyl group that prone to donate an electron or hydrogen atom to a free radical and it has extended conjugated aromatic system that able to delocalize an unpaired electron (Dai et al., 2010).

The protocatechuic acid can protect against oxidative damage induced by tert-butylhydroperoxide in a primary culture of rat hepatocytes as well as it potent to scavenge free radicals (Tseng et al., 1996). It is widely distributed in fruits, vegetables and nuts and was responsible in reducing carcinogenic action of diethylnitrosamine in the liver, 4-nitroquinoline 1-oxide in the oral cavity, azoxymethane in the colon, *N*-methyl-*N*-nitrosourea in glandular stomach tissue and *N*-butyl-*N*-(4-hydroxybutyl) nitrosamine in the bladder (Tseng et al., 1996). As quinic acid fight against free radicals activity (Hung et al., 2006), *p* hydroxybenzoic acid inhibits oxidation of low-density lipoprotein to slow down the process of atherosclerosis as well as reduce the risk of

cancer and other disease (Elzaawely et al., 2007). Hydroxybenzoic acid also was previously reported to protect ferrous ions from autoxidation but it has no ability in reducing ferric ions (Chvatalove et al., 2008). While gypenoside, and triterpenoid saponin that was detected in LCMS/MS of chloroform and methanol rhizome extract lower blood pressure via direct release of endothelium-derived nitric oxide, leading to vasorelaxation (Tanner et al., 1999). Similarly, proanthocyanidin that has a structural unit of phenolic flavan-3-ol possess antioxidant activity It is also known to have vasodilatory effects and cardioprotective properties that will lead to lowering high blood pressure (Bagchi et al., 2000). The presence of dicaffeoylquinic acid that has been reported to be an effective free radicals scavengers as well as an efficient metal ion chelator, was responsible in inhibition of LDL oxidation and can be used in prevention of cardiovascular disease including atherosclerotic (Hung et al., 2006). The antioxidant treatment using dicaffeoylquinic acid might as well be useful in reducing high blood pressure.

The contribution of ROS production in organs such as kidney and center of the brain that involves in blood pressure regulation has been widely known (Harrison et al., 2007). Nicotinamide adenine dinucleotide phosphate (NADPH) produced ROS in two different ways. NADPH produces ROS directly and it is also stimulated other ROS-generating enzymes and make it as predominant vascular source of ROS (Harrison et al., 2007). The hypertension known to be associated with oxidative stress as NADPH oxidases, uncoupled nitric oxide synthase, xanthine oxidase and mitochondrial sources increased their ROS production thus lead to hypertension as well as other vascular disease (Harrison et al., 2007). The vascular growth, vascular inflammation as well as impaired endothelium dependency has reported to associated with oxidative stress that known to contribute in the pathogenesis of essential hypertension or in arterial damage associated with essential hypertension (Yen et al., 2010). The hypertension caused by

oxidative stress started with the excessive production of ROS by NADPH in blood vessel and reaction of ROS with thiol group that activated the matrix metalloproteinase (MMP), a group of zinc-endopeptidase that mediate the vascular remodeling and vascular dysfunction (Castro et al., 2009). The activation of angiotensin II also contribute in the alterations of vascular wall in terms of function and structure that caused endothelial dysfunction, increased contractility, vascular hypertrophy and increased deposition of extracellular matrix proteins as well (Castro et al., 2009). Previous research also has reported that MMP-2 activation by ROS were important in pathophysiological cardiovascular conditions (Castro et al., 2009). Therefore, antioxidant therapy is an alternative way to prevent alterations of function and structure of vascular that will help in lowering high blood pressure (Castro et al., 2009). Inhibition of MMP-2 activity can be suggested as alternative approach in antioxidant treatment.

The oxidative stress in kidney occurred as NADPH oxidase was richly found in the vasculature, interstitium, juxtaglomerular apparatus and the distal nephron that stimulates the production of superoxide anion thus help transduced the signal of angiotensin II to oxidative stress (Agarwal et al., 2004). Therefore, antioxidant treatment focus on renal function might be an alternative approach to prevent hypertension associated with renal disease. In conclusion, there are relation between oxidative stress and hypertension and this will provide fundamental knowledge regarding natural antioxidant that has additional medicinal value in lowering high blood pressure. This relation has brings new approach in hypertension treatment as well as oxidative stress related disease. The results of this research has shown that the water of leaves and rhizomes extracts of *Tacca integrifolia* possesses antihypertension and antioxidant activity that provide scientific evidence to claiming of the traditional usage in treatment of high blood pressure.