CHAPTER 4

RESULTS

4.1 Extraction of plant chemical compounds

The leaves and rhizome of *Tacca integrifolia* were separately extracted with hexane, petroleum ether, chloroform and methanol by using Soxhlet apparatus. Extraction process were started with low polarity of solvent, hexane, followed by medium polarity of solvent, petroleum ether and chloroform and finally with high polarity of solvent, methanol. The polar solvent extracted out the polar compound and the non-polar compound extracted by the non-polar solvent.

Water extractions were done by mixing 200g powder of grind leaves and rhizome with 500 ml distilled water respectively and the mixture were macerated approximately for 3 days. Extractions were filtered separately before evaporated to dryness using vacuum rotary evaporator at 40°C. Concentrated water extract were kept in air tight bottle and store in refrigerator until further use.

Table 4.00. Colour observation of leaves and rhizome extracts of *Tacca integrifolia*

Sample	Observations				
	Hexane	Petroleum ether	Chloroform	Methanol	Water
Leaves of *Tacca integrifolia*	Dark green	Dark green	Dark green	Green	Dark brown
Rhizome of *Tacca integrifolia*	Dark brown	Light brown	Light brown	Light brown	Brownish

4.2 Isolation and separation of chemical compounds

4.2.1 Thin Layer Chromatography (TLC)

Thin layer chromatography (TLC) was performed on a sheet of aluminum foil of size 20cm x 20cm and 10cm x 2cm, coated with a thin layer of silica gel(Silica gel 60 F_{254} sheet) while solvent systems used were chloroform, chloroform-ethanol; 9.7:0.3, and buthanol-acetic acid-water (60:15:25) with a slight modification. Solvent systems used for each extract were summarized as in Table 4.01.

Table 4.01. Solvent system used in Thin Layer Chromatography (TLC) of extracts from leaves and rhizomes of *Tacca integrifolia*

Plant Sample	Solvent of extraction	Solvent system for TLC.
Leaves extracts	Hexane Petroleum ether	Chloroform (Sherma, 2000)
	Chloroform Methanol	Chloroform: ethanol; 9.7:0.3 (Sherma, 2000)
	Water	Buthanol, acetic acid and water (60:15:25) (Beug et al., 1981)
Rhizome extracts	Hexane Petroleum ether	Chloroform (Sherma, 2000)
	Chloroform Methanol	Chloroform: ethanol; 9.7:0.3 (Sherma, 2000)
	Water	Buthanol, acetic acid and water (60:15:25) (Beug et al., 1981)

The presence of chemical compounds in the hexane extract from leaves of *Tacca integrifolia* (HLE) were observed under visible light and by using reagents including Dragendorff reagents, Vanillin-Sulphuric acid, Anesaldehyde-Sulphuric acid and iodine vapor. Chloroform was used as solvent system in TLC to separate 15 labeled compounds from hexane extract; HLE 1, HLE 2, HLE 3, HLE 4, HLE 5, HLE 6, HLE 7, HLE 8, HLE 9, HLE 10, HLE 11, HLE 12, HLE 13, HLE 14, and HLE 15. Table 4.02 showed 8 compounds were identified as essential oil, 4 compounds identified as alkaloid and 3 compounds identified as terpenoid.

Table 4.02. Thin Layer Chromatography of hexane extract from leaves of *Tacca integrifolia*

Labeled compound	R_f value (x 100)	Observations						Comment
		Colour under visible light	Colour under UV light	Reagents				
				Dragendorff reagent	Vanillin-sulphuric acid	Anesaldehyde-sulphuric acid	Iodine vapor	
HLE 1	7.4	-ve	-ve	Orange	-ve	-ve	-ve	Alkaloid
HLE 2	8.0	-ve	-ve	Orange	-ve	-ve	-ve	Alkaloid
HLE 3	10.3	-ve	-ve	Orange	-ve	-ve	-ve	Alkaloid
HLE 4	11.8	-ve	-ve	Orange	-ve	-ve	-ve	Alkaloid
HLE 5	16.4	-ve	-ve	-ve	Dark blue	-ve	-ve	Terpenoid
HLE 6	74.2	-ve	-ve	-ve	-ve	Blue	-ve	Essential oil
HLE 7	76.0	-ve	-ve	-ve	Dark blue	-ve	-ve	Terpenoid
HLE 8	77.9	-ve	-ve	-ve	-ve	Blue	-ve	Essential oil
HLE 9	88.1	-ve	-ve	-ve	Dark blue	-ve	-ve	Terpenoid
HLE 10	92.4	-ve	-ve	-ve	-ve	Blue	-ve	Essential oil
HLE 11	93.0	-ve	-ve	-ve	-ve	Blue	-ve	Essential oil
HLE 12	93.5	-ve	-ve	-ve	-ve	Blue	-ve	Essential oil
HLE 13	94.0	-ve	-ve	-ve	-ve	Blue	-ve	Essential oil
HLE 14	95.5	-ve	-ve	-ve	-ve	Blue	-ve	Essential oil
HLE 15	97.0	-ve	-ve	-ve	-ve	Blue	-ve	Essential oil

The presence of chemical compounds in the petroleum ether extract from leaves of *Tacca integrifolia* (PLE) were observed under visible light and by using reagents including Dragendorff reagents, Vanillin-Sulphuric acid, Anesaldehyde-Sulphuric acid and iodine vapor. Solvent systems used was chloroform to separate 8 labeled compounds named PLE 1, PLE 2, PLE 3, PLE 4, PLE 5, PLE 6, PLE 7 and PLE 8. Table 4.03 showed 5 labeled compounds were identified as essential oil and 3 compounds known as unsaturated compound with conjugated double chain.

Table 4.03. Thin Layer Chromatography of petroleum ether extract from leaves of *Tacca integrifolia*

Labeled compound	R_f value (x 100)	Observations						Comment
		Colour under visible light	Colour under UV light	Reagents				
				Dragendorff reagent	Vanillin-sulphuric acid	Anesaldehyde-sulphuric acid	Iodine vapor	
PLE 1	65.7	-ve	-ve	-ve	-ve	Blue	-ve	Essential oil
PLE 2	76.5	-ve	-ve	-ve	-ve	-ve	Brown	unsaturated double chain conjugated compound
PLE 3	84.1	-ve	-ve	-ve	-ve	-ve	Brown	unsaturated double chain conjugated compound
PLE 4	91.2	-ve	-ve	-ve	-ve	Blue	-ve	Essential oil
PLE 5	93.6	-ve	-ve	-ve	-ve	-ve	Brown	unsaturated double chain conjugated compound
PLE 6	94.2	-ve	-ve	-ve	-ve	Blue	-ve	Essential oil
PLE 7	94.8	-ve	-ve	-ve	-ve	Blue	-ve	Essential oil
PLE 8	98.1	-ve	-ve	-ve	-ve	Blue	-ve	Essential oil

Chemical compound in chloroform extract from leaves of *Tacca integrifolia* were separated using chloroform-ethanol (9.7:0.3) and colour presence were observed under visible light as well as by using Dragendorff reagents, Vanillin-Sulphuric acid, Anesaldehyde-Sulphuric acid and iodine vapor. 24 compounds were separated and labeled as CLE 1, CLE 2, CLE 3, CLE 4, CLE 5, CLE 6, CLE 7, CLE 8, CLE 9, CLE 10, CLE 11, CLE 12, CLE 13, CLE 14, CLE 15, CLE 16, CLE 17, CLE 18, CLE 19, CLE 20, CLE 21, CLE 22, CLE 23, and CLE 24. Table 4.04 showed 8 compounds identified as essential oil, 6 compounds identified as alkaloid, 4 compounds identified as phenol and 4 compounds identified as terpenoid. 2 unsaturated compounds with conjugated double chain were identified.

Table 4.04. Thin Layer Chromatography of chloroform extract from leaves of *Tacca integrifolia*

Labeled compound	R_f value (x 100)	Observations						Comment
		Colour under visible light	Colour under UV light	Reagents				
				Dragendorff reagent	Vanillin-sulphuric acid	Anesaldehyde-sulphuric acid	Iodine vapor	
CLE 1	9.4	-ve	-ve	Orange	-ve	-ve	-ve	Alkaloid
CLE 2	10.4	-ve	-ve	Orange	-ve	-ve	-ve	Alkaloid
CLE3	14.9	-ve	-ve	Orange	-ve	-ve	-ve	Alkaloid
CLE 4	29.7	-ve	-ve	-ve	Pink	-ve	-ve	Phenol
CLE 5	29.9	-ve	-ve	Orange	-ve	-ve	-ve	Alkaloid
CLE 6	39.0	-ve	-ve	Orange	-ve	-ve	-ve	Alkaloid
CLE 7	52.9	Green	-ve	-ve	Red	-ve	-ve	Phenol
CLE 8	63.6	Green	-ve	-ve	-ve	Blue	Brown	Essential oil
CLE 9	65.7	Green	-ve	-ve	Red	-ve	-ve	Phenol
CLE 10	71.2	-ve	-ve	-ve	Red	-ve	-ve	Phenol
CLE 11	74.0	Green	-ve	-ve	-ve	Blue	-ve	Essential oil
CLE 12	76.1	-ve	-ve	Orange	-ve	-ve	-ve	Alkaloid
CLE 13	79.2	Green	-ve	-ve	-ve	Blue	-ve	Essential oil
CLE 14	82.8	-ve	-ve	-ve	Purple	-ve	-ve	Terpenoid
CLE 15	84.5	-ve	-ve	-ve	Purple	-ve	-ve	Terpenoid
CLE 16	85.9	-ve	-ve	-ve	Purple	-ve	-ve	Terpenoid
CLE 17	86.3	-ve	-ve	-ve	Dark blue	-ve	Brown	Terpenoid
CLE 18	88.6	Green	-ve	-ve	-ve	Blue	-ve	Essential oil
CLE 19	92.9	Green	-ve	-ve	Green	-ve	Brown	unsaturated double chain conjugated

								compound
CLE 20	94.0	-ve	-ve	-ve	-ve	-ve	Brown	unsaturated double chain conjugated compound
CLE 21	95.5	-ve	-ve	-ve	-ve	Dark blue	Brown	Essential oil
CLE 22	96.3	-ve	-ve	-ve	-ve	Dark blue	-ve	Essential oil
CLE 23	97.0	-ve	-ve	-ve	-ve	Dark blue	Brown	Essential oil
CLE 24	97.4	-ve	-ve	-ve	-ve	Blue	-ve	Essential oil

The presence of chemical compounds in the methanol extract from leaves of *Tacca integrifolia* (MLE) were separated using chloroform-ethanol (9.7:0.3) and colour presence were observed under visible light and by using Dragendorff reagents, Vanillin-Sulphuric acid, Anesaldehyde-Sulphuric acid and iodine vapor. 10 compounds were separated and labeled as MLE 1, MLE 2, MLE 3, MLE 4, MLE 5, MLE 6, MLE 7, MLE 8, MLE 9, and MLE 10. Table 4.05 showed 5 compounds were identified as alkaloid, 2 compounds as essential oil, while 3 compounds known as unsaturated compound with conjugated double chain.

Table 4.05. Thin Layer Chromatography of methanol extract from leaves of *Tacca integrifolia*

Labeled compound	R_f value (x 100)	Observations						Comment
		Colour under visible light	Colour under UV light	Reagents				
				Dragendorff reagent	Vanillin-sulphuric acid	Anesaldehyde-sulphuric acid	Iodine vapor	
MLE 1	11.9	-ve	-ve	Orange	-ve	-ve	-ve	Alkaloid
MLE 2	14.9	-ve	-ve	Orange	-ve	-ve	-ve	Alkaloid
MLE 3	18.8	-ve	-ve	Orange	-ve	-ve	-ve	Alkaloid
MLE 4	36.0	-ve	-ve	Orange	-ve	-ve	-ve	Alkaloid
MLE 5	53.8	-ve	-ve	Orange	-ve	-ve	-ve	Alkaloid
MLE 6	85.0	-ve	-ve	-ve	-ve	-ve	Brown	unsaturated double chain conjugated compound
MLE 7	92.5	-ve	-ve	-ve	-ve	-ve	Brown	unsaturated double chain conjugated compound
MLE 8	94.6	-ve	-ve	-ve		Dark blue	Brown	Essential oil
MLE 9	95.5	-ve	-ve	-ve	Blue	Blue	-ve	Essential oil
MLE 10	97.1	-ve	-ve	-ve	-ve	-ve	Brown	unsaturated double chain conjugated compound

TLC of hexane rhizome extract was done using chloroform as solvent system and the presence of chemical compounds were observed under visible light and by using Dragendorff reagents, Vanillin-Sulphuric acid, Anesaldehyde-Sulphuric acid and iodine vapor. Separation of 9 labeled compounds were labeled as HRE 1, HRE 2, HRE 3, HRE 4, HRE 5, HRE 6, HRE 7, HRE 8 and HRE 9. Table 4.06 showed 5 compounds were identified as terpenoid, 3 compounds identified as essential oil and 1 compound was identified as alkaloid.

Table 4.06. Thin Layer Chromatography of hexane extract from rhizome of *Tacca integrifolia*

Labeled compound	R_f value (x 100)	Observations						Comment
		Colour under visible light	Colour under UV light	Reagents				
				Dragendorff reagent	Vanillin-sulphuric acid	Anesaldehyde -sulphuric acid	Iodine vapor	
HRE 1	15.5	-ve	-ve	Orange	-ve	-ve	-ve	Alkaloid
HRE 2	68.8	-ve	-ve	-ve	Dark purple	-ve	-ve	Terpenoid
HRE 3	69.9	-ve	-ve	-ve	Dark purple	-ve	-ve	Terpenoid
HRE 4	80.8	-ve	-ve	-ve	Dark blue	-ve	-ve	Terpenoid
HRE 5	81.4	-ve	-ve	-ve	Dark blue	-ve	-ve	Terpenoid
HRE 6	89.4	-ve	-ve	-ve	-ve	Blue	-ve	Essential oil
HRE 7	90.0	-ve	-ve	-ve	-ve	Blue	-ve	Essential oil
HRE 8	97.0	-ve	-ve	-ve	-ve	Blue	-ve	Essential oil
HRE 9	98.0	-ve	-ve	-ve	Dark blue	-ve	-ve	Terpenoid

TLC of petroleum ether extract from rhizome of *Tacca integrifolia* were done using chloroform as solvent system and separation of chemical compounds were observed under visible light and by using Dragendorff reagents, Vanillin-Sulphuric acid, Anesaldehyde-Sulphuric acid and iodine vapor. Separation of 18 compounds were labeled as PRE 1, PRE 2, PRE 3, PRE 4, PRE 5, PRE 6, PRE 7, PRE 8, PRE 9, PRE 10, PRE 11, PRE 12, PRE 13, PRE 14, PRE 15, PRE 16, PRE 17 and PRE 18. Table 4.07 showed 11 compounds were identified as essential oil, 4 compounds identified as alkaloid, 2 compounds were identified as terpenoid and 1 compound known as unsaturated compound with conjugated double chain.

Table 4.07. Thin Layer Chromatography of petroleum ether extract from rhizome of *Tacca integrifolia*

Labeled compound	R_f value (x 100)	Observations						Comment
		Colour under visible light	Colour under UV light	Reagents				
				Dragendorff reagent	Vanillin-sulphuric acid	Anesaldehyde-sulphuric acid	Iodine vapor	
PRE 1	18.8	-ve	-ve	Orange	-ve	-ve	-ve	Alkaloid
PRE 2	28.4	-ve	-ve	Orange	-ve	-ve	-ve	Alkaloid
PRE 3	53.6	-ve	-ve	-ve	-ve	Blue	-ve	Essential oil
PRE 4	63.4	-ve	-ve	-ve	Blue	-ve	Brown	Terpenoid
PRE 5	73.5	-ve	-ve	-ve	-ve	Blue	-ve	Essential oil
PRE 6	76.0	-ve	-ve	-ve	-ve	Blue	-ve	Essential oil
PRE 7	81.3	-ve	-ve	-ve	-ve	Blue	-ve	Essential oil
PRE 8	85.7	-ve	-ve	Orange	-ve	-ve	-ve	Alkaloid
PRE 9	88.3	-ve	-ve	-ve	-ve	Blue	-ve	Essential oil
PRE 10	92.0	-ve	-ve	-ve	-ve	Blue	Brown	Essential oil
PRE 11	92.6	-ve	-ve	-ve	-ve	Blue	-ve	Essential oil
PRE 12	94.0	-ve	-ve	-ve	-ve	Blue	-ve	Essential oil
PRE 13	94.2	-ve	-ve	-ve	-ve	Blue	-ve	Essential oil
PRE 14	94.3	-ve	-ve	-ve	-ve	Blue	-ve	Essential oil
PRE 15	95.6	-ve	-ve	Orange	-ve	-ve	-ve	Alkaloid
PRE 16	96.3	-ve	-ve	-ve	-ve	-ve	Brown	unsaturated double chain conjugated compound
PRE 17	96.5	-ve	-ve	-ve	Dark blue	-ve	-ve	Terpenoid
PRE 18	97.5	-ve	-ve	-ve	-ve	Blue	-ve	Essential oil

Separation of chemical compounds present in the chloroform extract from rhizome of *Tacca integrifolia* (CRE) were done using chloroform-ethanol (9.7:0.3) and colour presence were observed under visible light and by using Dragendorff reagents, Vanillin-Sulphuric acid, Anesaldehyde-Sulphuric acid and iodine vapor. 11 labeled compounds were separated from hexane extract; CRE 1, CRE 2, CRE 3, CRE 4, CRE 5, CRE 6, CRE 7, CRE 8, CRE 9, CRE 10 and CRE 11. Table 4.08 showed 7 compounds were identified as alkaloid, 2 compounds as essential oil and 2 compounds known as unsaturated compound with conjugated double chain.

Table 4.08. Thin Layer Chromatography of chloroform extract from rhizome of *Tacca integrifolia*

Labeled compound	R_f value (x 100)	Observations						Comment
		Colour under visible light	Colour under UV light	Reagents				
				Dragendorff reagent	Vanillin-sulphuric acid	Anesaldehyde-sulphuric acid	Iodine vapor	
CRE 1	5.9	-ve	-ve	Orange	-ve	-ve	-ve	Alkaloid
CRE 2	6.2	-ve	-ve	Orange	-ve	-ve	-ve	Alkaloid
CRE 3	7.6	-ve	-ve	Orange	-ve	-ve	-ve	Alkaloid
CRE 4	8.0	-ve	-ve	Orange	-ve	-ve	-ve	Alkaloid
CRE 5	8.4	-ve	-ve	Orange	-ve	-ve	-ve	Alkaloid
CRE 6	14.9	-ve	-ve	Orange	-ve	-ve	-ve	Alkaloid
CRE 7	65.7	-ve	-ve	-ve	-ve	-ve	Brown	unsaturated double chain conjugated compound
CRE 8	73.1	-ve	-ve	-ve	-ve	-ve	Brown	unsaturated double chain conjugated compound
CRE 9	74.6	-ve	-ve	Orange	-ve	-ve	-ve	Alkaloid
CRE 10	89.0	-ve	-ve	-ve	-ve	Dark blue	-ve	Essential oil
CRE 11	94.0	-ve	-ve	-ve	-ve	Dark blue	-ve	Essential oil

TLC of methanol extract from rhizome of *Tacca integrifolia* (MRE) was done using chloroform-ethanol (9.7:0.3) as solvent system and compounds separated were observed under visible light. Separation of 8 labeled compounds name MRE 1, MRE 2, MRE 3, MRE 4, MRE 5, MRE 6, MRE 7 and MRE 8 were tested using Dragendorff reagents, Vanillin-Sulphuric acid, Anesaldehyde-Sulphuric acid and iodine vapor. Table 4.09 showed 2 compounds were identified as alkaloid, 1 compound identified as essential oil, and 3 compounds known as unsaturated compound with conjugated double chain. MRE5 was detected to contain both unsaturated compound and terpenoid while essential oil, terpenoid and unsaturated compound were spotted at MRE8.

Table 4.09. Thin Layer Chromatography of methanol extract from rhizome of *Tacca integrifolia*

Labeled compound	R_f value (x 100)	Observations						Comment
		Colour under visible light	Colour under UV light	Reagents				
				Dragendorff reagent	Vanillin-sulphuric acid	Anesaldehyde-sulphuric acid	Iodine vapor	
MRE 1	7.5	-ve	-ve	Orange	-ve	-ve	-ve	Alkaloid
MRE 2	8.6	-ve	-ve	Orange	-ve	-ve	-ve	Alkaloid
MRE 3	34.0	-ve	-ve	-ve	-ve	-ve	Brown	unsaturated double chain conjugated compound
MRE 4	82.1	-ve	-ve	-ve	-ve	-ve	Brown	unsaturated double chain conjugated compound
MRE 5	91.0	-ve	-ve	-ve	Purple	-ve	Brown	Terpenoid and unsaturated double chain conjugated compound
MRE 6	92.0	-ve	-ve	-ve	-ve	-ve	Brown	unsaturated double chain conjugated compound
MRE 7	94.0	-ve	-ve	-ve	-ve	Dark blue	-ve	Essential oil
MRE 8	95.0	-ve	-ve	-ve	Blue	Dark blue	Brown	Essential oil, Terpenoid

								and unsaturated double chain conjugated compound

4.2.2 Column Chromatography (CC)

Maceration method was used to extract 200 g of grind leaves and rhizome of *Tacca integrifolia* with 500 ml of distilled water. Column chromatography (CC) of water extract were developed using buthanol-acetic acid-water (60:15:25) as solvent system with slight modifications. 20 fractions were collected with 2 ml each and were dried in fume cupboard before the dry weights were measured. TLC was developed for each fraction using buthanol-acetic acid-water (60:15:25) as solvent system to detect chemical compounds present.

The separation and detection of chemical compounds in the water extract from leaves of *Tacca integrifolia* (WLE) were observed under visible light and by using Dragendorff reagents, Vanillin-Sulphuric acid, Anesaldehyde-Sulphuric acid and iodine vapor. 7 compounds were separated from water extract from leaves of plant studied labeled WLE 1, WLE 2, WLE 3, WLE 4, WLE 5, WLE 6, and WLE 7. Table 4.10 showed 4 compounds were identified as alkaloid, 2 compounds were identified as terpenoid and 1 compound identified as essential oil.

Table 4.10. Thin Layer Chromatography of water extract from leaves of *Tacca integrifolia*

Labeled compound	R_f value (x 100)	Observations						Comment
		Colour under visible light	Colour under UV light	Reagents				
				Dragendorff reagent	Vanillin-sulphuric acid	Anesaldehyde-sulphuric acid	Iodine vapor	
WLE 1	21.6	-ve	-ve	Orange	-ve	-ve	-ve	Alkaloid
WLE 2	21.8	-ve	-ve	-ve	Dark blue	-ve	-ve	Terpenoid
WLE 3	21.9	-ve	-ve	Orange	-ve	-ve	-ve	Alkaloid
WLE 4	23.2	-ve	-ve	Orange	-ve	-ve	-ve	Alkaloid
WLE 5	24.4	-ve	-ve	-ve	Dark blue	-ve	-ve	Terpenoid
WLE 6	26.7	-ve	-ve	Orange	-ve	-ve	-ve	Alkaloid
WLE 7	35.5	-ve	-ve	-ve	-ve	Blue	-ve	Essential oil

Separation of chemical compounds present in the water extract from the rhizome of *Tacca integrifolia* (WRE) was done using the same solvent system as in TLC of water leaves extract. Each colour presence was observed under visible light and by using Dragendorff reagents, Vanillin-Sulphuric acid, Anesaldehyde-Sulphuric acid and iodine vapor. 11 compounds were separated and labeled as WRE 1, WRE 2, WRE 3, WRE 4, WRE 5, WRE 6, WRE 7, WRE 8, WRE 9, WRE 10, and WRE 11. Table 4.11 showed 8 compounds were identified as essential oil, 1 compound identified as alkaloid and 1 compound identified as flavonoid while both essential oil and alkaloid were also detected in WR11.

Table 4.11. Thin Layer Chromatography of water extract from the rhizome of *Tacca integrifolia*

Labeled compound	R_f value (x 100)	Observations						Comment
		Colour under visible light	Colour under UV light	Reagents				
				Dragendorff reagent	Vanillin-sulphuric acid	Anesaldehyde-sulphuric acid	Iodine vapor	
WRE 1	16.7	-ve	-ve	-ve	-ve	Green	-ve	Flavonoid
WRE 2	25.0	-ve	-ve	-ve	-ve	Dark blue	-ve	Essential oil
WRE 3	26.2	-ve	-ve	-ve	-ve	Blue	-ve	Essential oil
WRE 4	26.4	-ve	-ve	Orange	-ve	-ve	-ve	Alkaloid
WRE 5	30.0	-ve	-ve	-ve	-ve	Blue	-ve	Essential oil
WRE 6	37.3	-ve	-ve	-ve	-ve	Blue	-ve	Essential oil
WRE 7	38.5	-ve	-ve	-ve	-ve	Blue	-ve	Essential oil
WRE 8	47.5	-ve	-ve	-ve	-ve	Blue	-ve	Essential oil
WRE 9	47.8	-ve	-ve	-ve	-ve	Blue	-ve	Essential oil
WRE 10	50.0	-ve	-ve	Orange	-ve	Blue	-ve	Alkaloid Essential oil
WRE 11	52.9	-ve	-ve	-ve	-ve	Blue	-ve	Essential oil

Table 4.12. Summary of compounds identified from TLC of extracts from *Tacca integrifolia*

Extracts	Alkaloids	Flavonoids	Phenols	Terpenoids	Essential oils
Leaves hexane extract	⁄			⁄	⁄
Leaves petroleum ether extract					⁄
Leaves chloroform extract	⁄		⁄	⁄	⁄
Leaves methanol extract	⁄				⁄
Leaves water extract	⁄			⁄	⁄
Rhizome hexane extract	⁄			⁄	⁄
Rhizome petroleum ether extract	⁄			⁄	⁄
Rhizome chloroform extract	⁄				⁄
Rhizome methanol extract	⁄			⁄	⁄
Rhizome water extract	⁄	⁄			⁄

4.2.3 High Performance Liquid Chromatography (HPLC)

The extract of leaves and rhizome of *Tacca integrifolia* was analyzed using High Performance Liquid Chromatography (HPLC). Extract was filtered using 0.45µm Regenerated Cellulose (RC) membrane filter before 10µl from each extract were injected separately to C-18 column for 1 hour with flow rate of 1ml/min. Retention time obtain from each extracts were compared to standard reference of phenol and flavonoid. Gallic acid and tannic acid were used for standard phenol while quercetin for standard flavonoids.

i) HPLC profile of standard gallic acid

Gallic acid solution was prepared by dissolving 10mg of gallic acid in 1 ml of HPLC pure water. Gallic acid solution was filtered using 0.45µm Regenerated Cellulose (RC) membrane filter before was tested for solubility test with a mixture of water and acetic acid (97:3) as mobile phase A and methanol HPLC grade as mobile phase B. 10µl of gallic acid was injected to the C-18 column and was left for 10 minutes for HPLC analysis at 280nm wavelength.

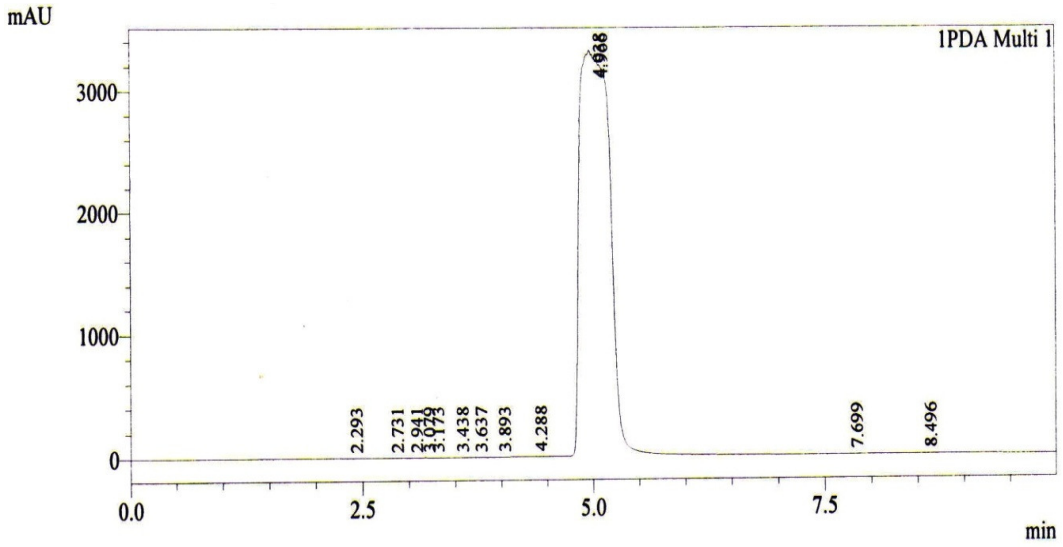

Figure 4.00. High Performance Liquid Chromatography (HPLC) chromatogram of standard gallic acid.

Retention time of standard gallic acid was detected at 4.966' at 280 nm wavelength.

ii) HPLC profile of standard tannic acid

Tannic acid is a type of polyphenols that also known as polymer of gallic acid molecules and glucose. Tannic acid solution was prepared by dissolving 10mg of tannic acid in 1 ml of pure water of HPLC before the solution was filtered using 0.45µm Regenerated Cellulose (RC) membrane filter and tested for solubility test. 10µl of tannic acid was injected to the C-18 column and was left for HPLC separation for 10 minutes.

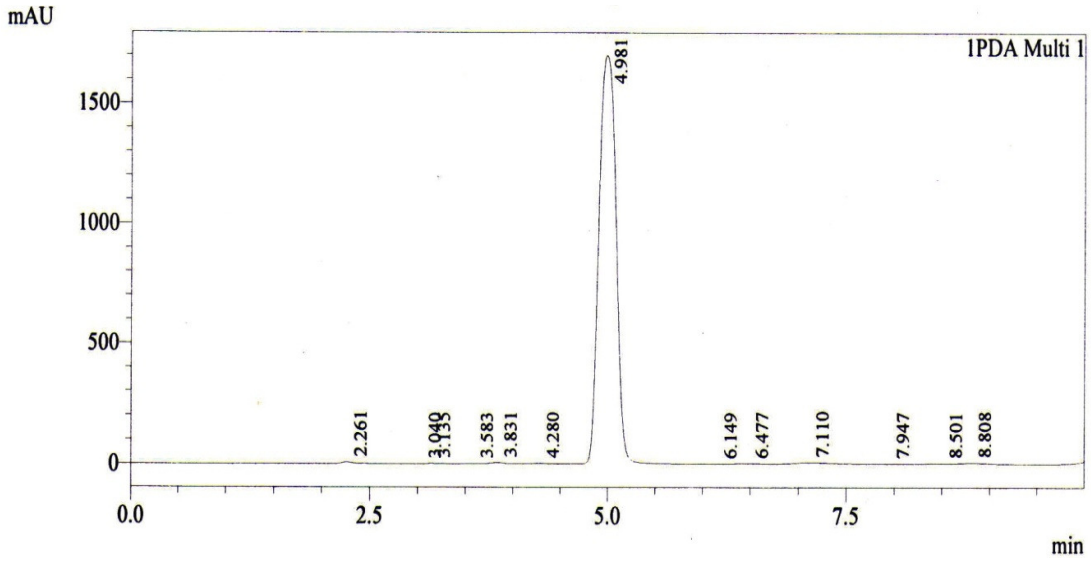

Figure 4.01. High Performance Liquid Chromatography (HPLC) chromatogram of standard tannic acid.

Retention time of standard tannic acid was detected at 4.981' at 280 nm wavelength.

iii) HPLC profile of standard flavonoid Quercetin

Quercetin known as a plant-derived flavonoid that widely distributed in fruits, vegetables, leaves and grains. Quercetin was used as reference standard in analysis of plant flavonoid in *Tacca integrifolia* via HPLC. Stock solution of quercetin was prepared by dissolving 10mg of quercetin in 1 ml of methanol HPLC grade before it was filtered and injected through HPLC C18 column.

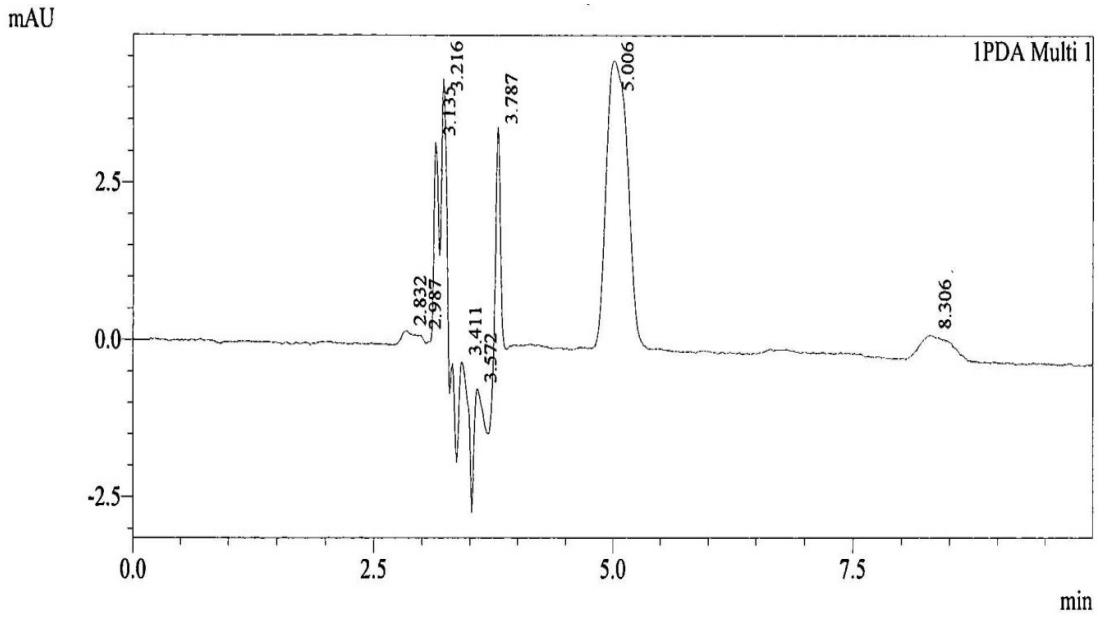

Figure 4.02. High Performance Liquid Chromatography (HPLC) chromatogram of standard flavonoid quercetin.

Retention time of quercetin was detected at 5.006' with 280 nm wavelength.

iv) HPLC profile of chloroform leaves extract of *Tacca integrifolia*

10µl chloroform extract from leaves of *Tacca integrifolia* was injected to injection valve and separated using C-18 column for 1 hour. However, only first 30 minutes of separation showed 2 peaks detected at 16.438 minutes and at 28.943 minutes with 280 nm wavelength.

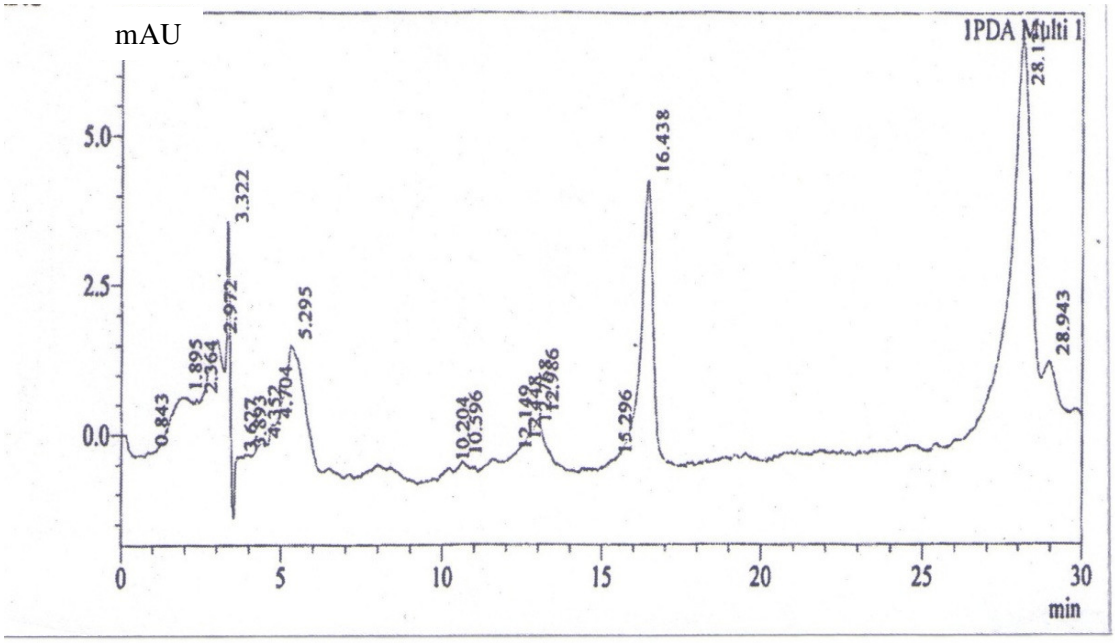

Figure 4.03.HPLCchromatogram of chloroform leaves extract from *Tacca integrifolia.*

Several peaks were detected indicated that there were many compounds presence in the extract. They were indicated that the presence of gallic acid, tannic acid and quercetin at the retention time of 4.704 and 5.295. The other peaks showed unknown compounds.

v) HPLC profile of chloroform rhizomes extract of *Tacca integrifolia*

10µl chloroform extract from rhizome of *Tacca integrifolia* was filtered using 0.45µm Regenerated Cellulose (RC) membrane filter before injected to injection valve and separated using C-18 column for 1 hour.

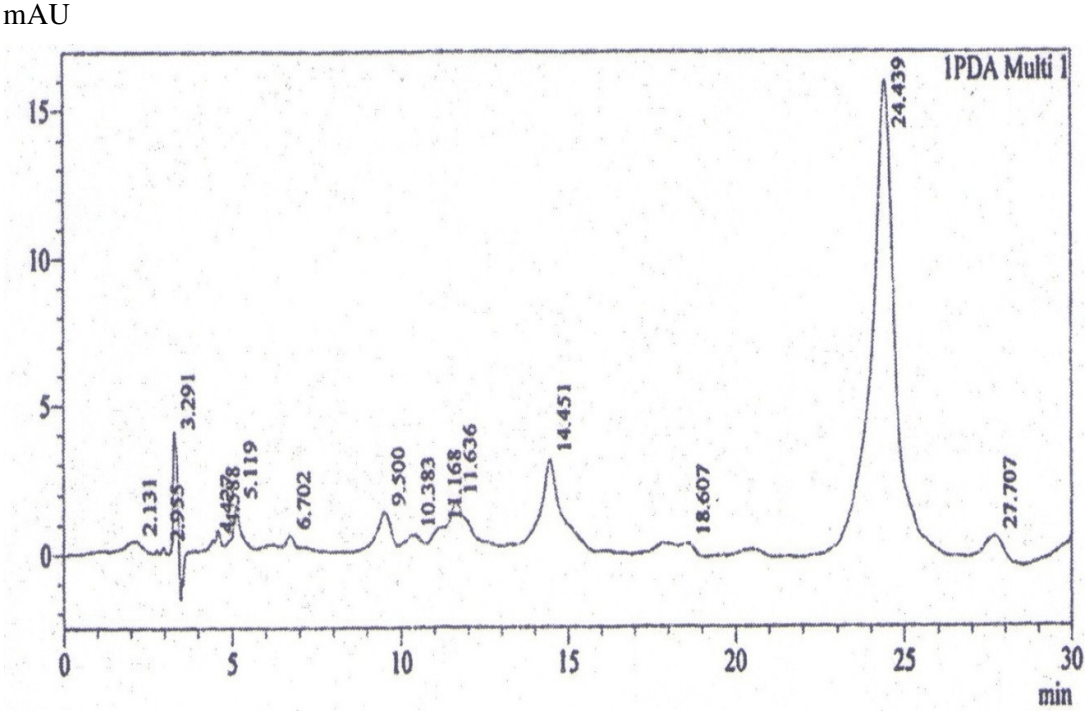

Figure 4.04. HPLC chromatogram of chloroform rhizome extract from *Tacca integrifolia.*

vi) HPLC profile of methanol leaves extract *Tacca integrifolia*

10µl methanol extract from leaves of *Tacca integrifolia* was filtered using 0.45µm Regenerated Cellulose (RC) membrane filter before injected to injection valve and separated using C-18 column for 1 hour.

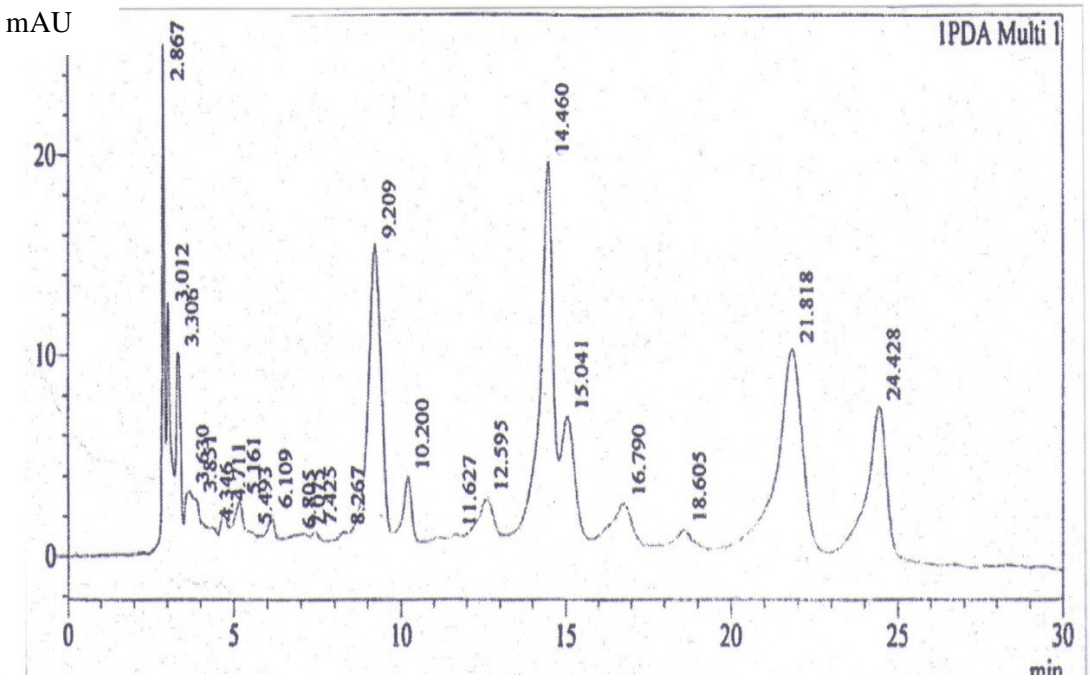

Figure 4.05.HPLC chromatogram of methanol leaves extract from *Tacca integrifolia.*

vii) HPLC profile of methanol rhizomes extracts *Tacca integrifolia*

10µl methanol extract from rhizome of *Tacca integrifolia* were filtered using 0.45µm Regenerated Cellulose (RC) membrane filter before injected to injection valve and separated using C-18 column for 1 hour..

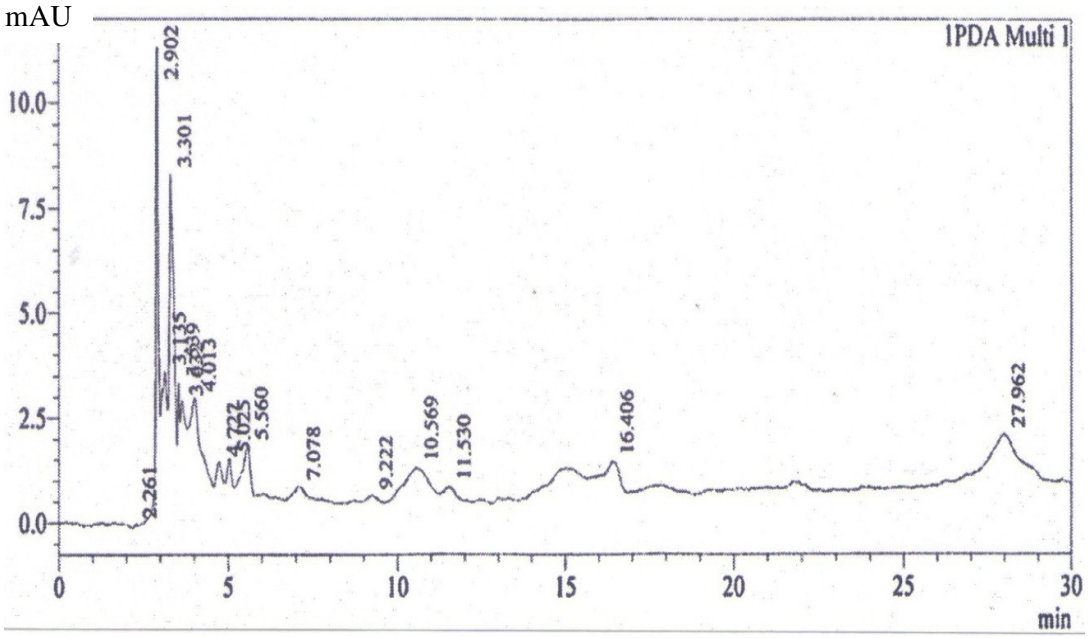

Figure 4.06.HPLC chromatogram of methanol rhizomes extract from *Tacca integrifolia.*

viii) HPLC profile of water leaves extract of *Tacca integrifolia*

10µl water extract from leaves of *Tacca integrifolia* was filtered using 0.45µm Regenerated Cellulose (RC) membrane filter, injected to injection valve, and separated using C-18 column for 1 hour.

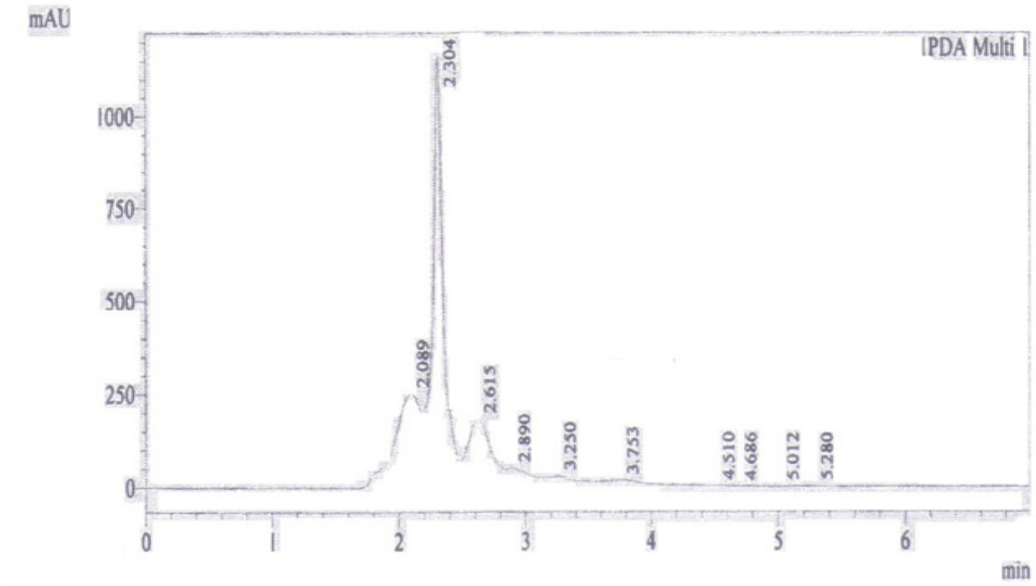

Figure 4.07.HPLC chromatograms of water leaves extract from *Tacca integrifolia.*

ix) HPLC profile of water rhizome extracts of *Tacca integrifolia*

10µl of water extract from rhizome of *Tacca integrifolia* was filtered using 0.45µm Regenerated Cellulose (RC) membrane filter and was injected to injection valve for separation using C-18 column. Wavelength was set at 228nm and analysis was run for 60 minutes.

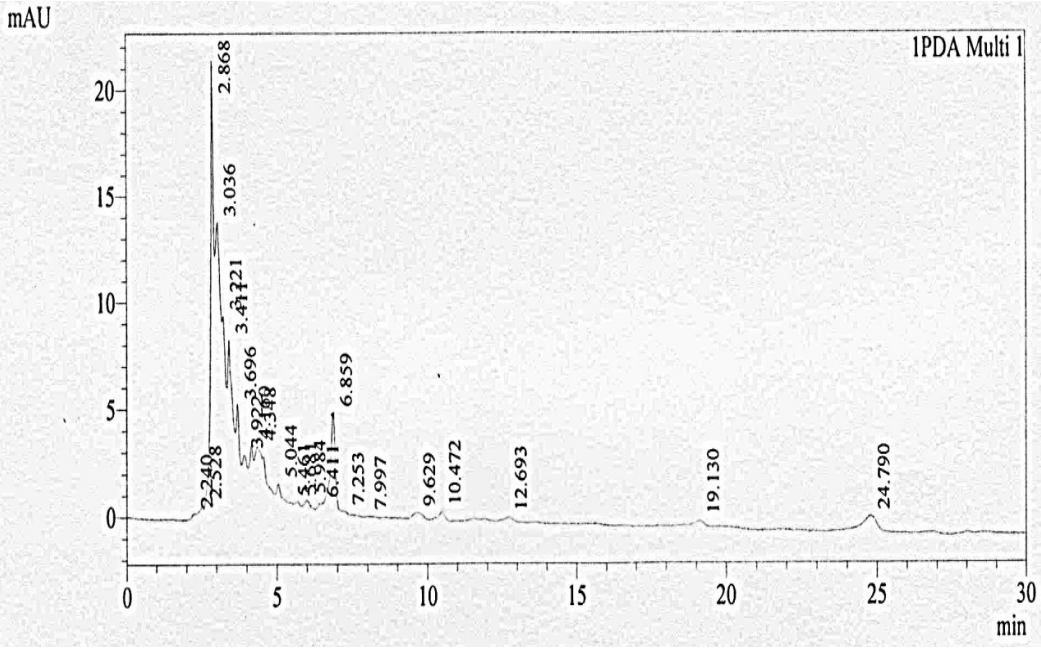

Figure 4.08.HPLC chromatogram of water rhizome extract from *Tacca integrifolia.*

x) HPLC profile of standard hippuric acid (HA)

Mobile phase A was prepared by dilution of 0.05% Trifluoroacetic acid (TFA) in water and mobile phase B was prepared by dilution of 0.05% TFA in acetonitrile. Mobile phase A and B were filtered before discarding bubbles using sonicator for 1 hour. 10µl of Hippuric acid with the concentration 62.5 µg/ml was injected into the C18 column of the HPLC system with flow rate of 1ml/min. HPLC was run for 20 minutes and wavelength was set at 228 nm. HPLC profile of standard hippuric acid was identified by comparing the HPLC chromatogram with the previous journal (Wu et al., 2002). One peak was detected at 4.115 minutes.

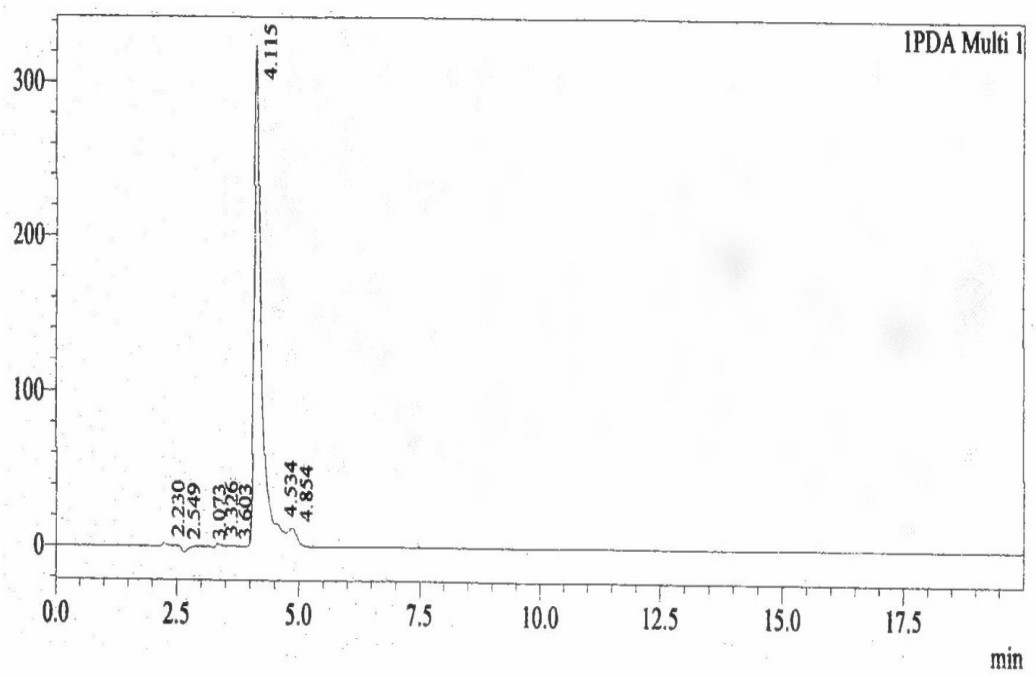

Figure 4.09.HPLC chromatogram of standard of Hippuric acid.

4.3 Determination of chemical compounds using Liquid Chromatography Mass Spectrometry combined with Mass Spectrometry (LCMS/MS)

Liquid Chromatography Mass Spectrometry combined with Mass Spectrometry (LCMS/MS) was used to determine the chemical compounds found in extracts of leaves and rhizome of *Tacca integrifolia.* The extracts have been fully screen with AB Sciex 3200QTrap LCMS/MS and fully scan with MS/MS data collection. All samples were appropriately diluted and filtered with 0.22µM nylon filter and injection volume for all samples is 20µL.Sample were run with gradient mode; 10% A to 90% B from 0.01 minute to 8.0 minute and were hold for 3 minutes and back to 10% A in 0.1 minute and re-equilibrated for 4 minutes. Pre-run equilibration time was 1.0 minute.

4.3.1 Liquid Chromatography Mass Spectrometry/Mass Spectrometry (LCMS/MS) for leaves extract of *Tacca integrifolia*

Table 4.13. Compounds detected in Liquid Chromatography Mass Spectrometry (LCMS/MS) of leaves extract from *Tacca integrifolia*

Extract	Compound detected	Reference Figure
Hexane	1) Proanthocyanidin trimer	Fig. 4.11
Petroleum Ether	1) Proanthocyanidin trimer	Fig. 4.13
Chloroform	1) *p* hydroxybenzoic acid	Fig. 4.15
	2) Proanthocyanidin trimer	Fig. 4.16
	3) 1,3,5 tricaffeolquinic acid	Fig. 4.17
	4) 2(3,4-Dihydroxyphenyl)-7-hydroxy-5-benzene propanoic acid	Fig. 4.18
Methanol	1) Quinic Acid	Fig. 4.20
	2) 3 caffeolquinic acid	Fig. 4.21
	3) *p* hydroxybenzoic acid	Fig. 4.22
	4) Dicaffeolquinic acid conjugate	Fig. 4.23
	5) Isoflavone glycoside	Fig. 4.24
	6) Proanthocyanidin	Fig. 4.25
Water	1) Quinic acid	Fig. 4.27
	2) Protocatechuic acid	Fig. 4.28
	3) salicylic acid	Fig. 4.29
	4) Phenolic acid conjugate	Fig. 4.30
	5) Proanthocyanidin	Fig. 4.31
	6) Proanthocyanidin trimer	Fig. 4.32

i) LCMS/MS profile of hexane leaves extracts of *Tacca integrifolia*

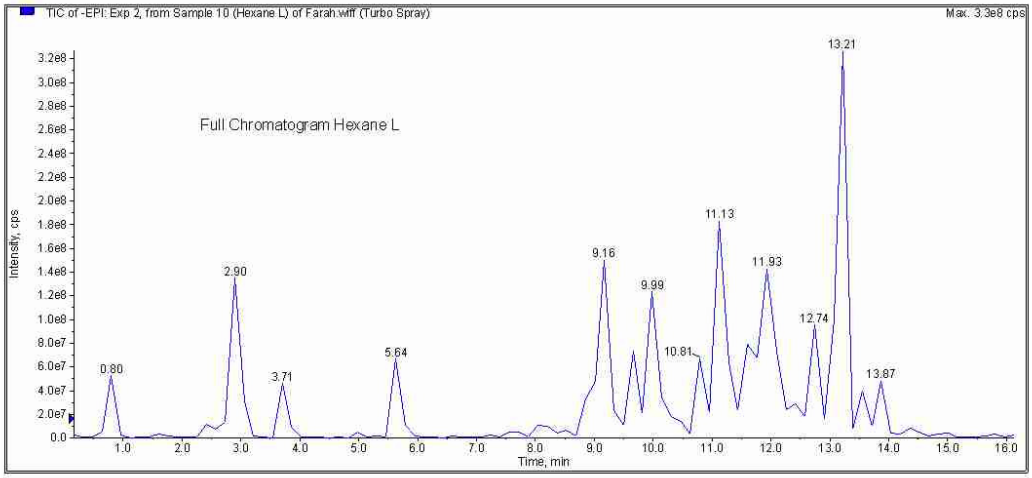

Figure 4.10. LCMS/MS chromatogram of hexane leaves extract from *Tacca integrifolia.*

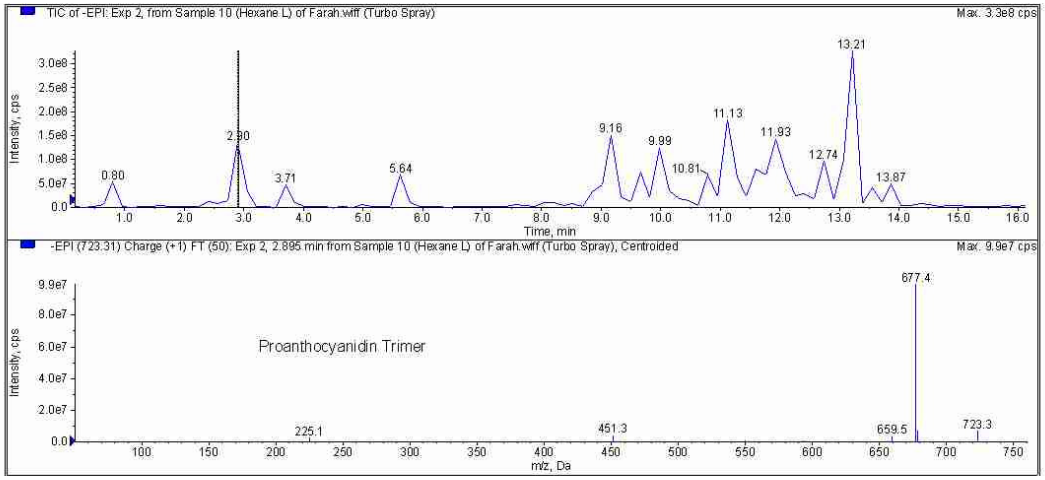

Figure 4.11. LCMS/MS chromatogram of proanthocyanidin trimer from hexane leaves extract of *Tacca integrifolia*

.

Full LCMS chromatogram of hexane leaves extract showed 12 chromatogram peaks separated at different time ranging from 0.80', 2.90', 3.71', 5.64', 9.16', 9.99', 10.81', 11.13', 11.93', 12.74', 13.21' and 13.87' as in Figure 4.10. At time of 2.90', proanthocyanidin trimer was detected as in Figure 4.11.

ii) LCMS/MS profile of petroleum ether leaves extracts of *Tacca integrifolia*

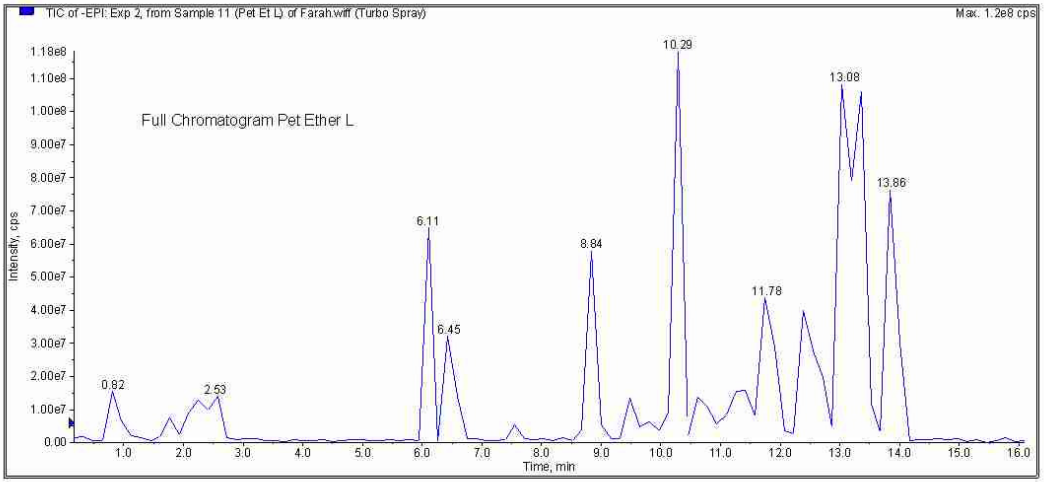

Figure 4.12. LCMS/MS chromatogram of petroleum ether leaves extract from *Tacca integrifolia.*

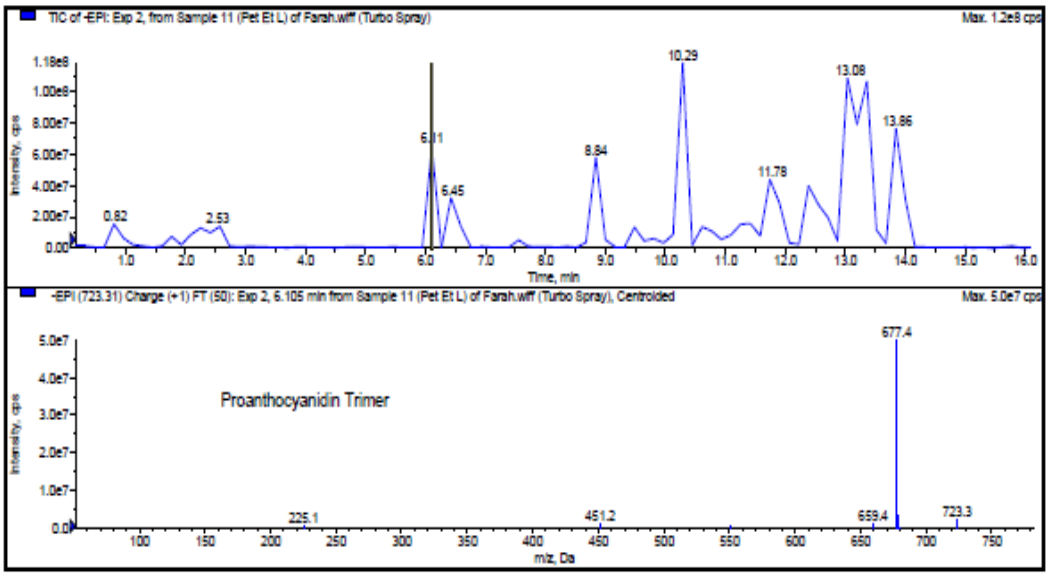

Figure 4.13. LCMS/MS chromatogram of proanthocyanidin trimer from petroleum ether leaves extract of *Tacca integrifolia.*

Full LCMS/MS chromatogram as in Figure 4.12 showed 9 peaks were separated at 0.82', 2.53', 6.11', 6.45', 8.84', 10.29', 11.78', 13.08' and 13.86'while Figure 4.13 showed the presence of proanthocyanidin trimer at 6.105'.

iii) LCMS/MS profile of chloroform leaves extracts of *Tacca integrifolia*

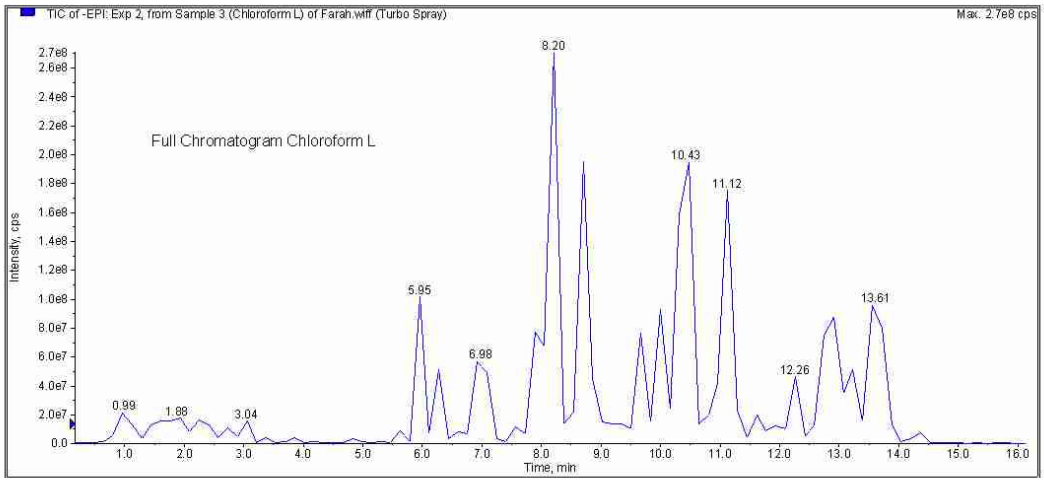

Figure 4.14. LCMS/MS chromatogram of chloroform leaves extract from *Tacca integrifolia.*

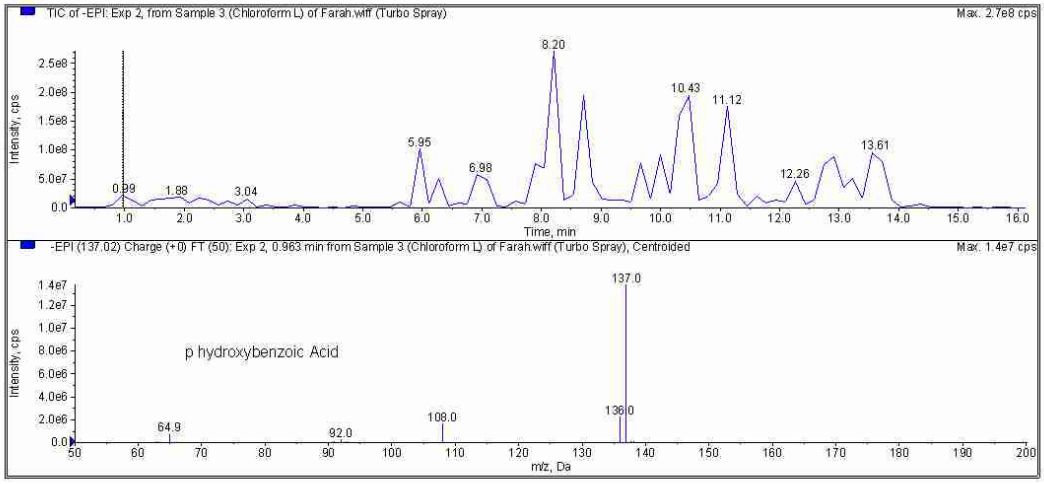

Figure 4.15. LCMS/MS chromatogram of *p*-hydroxybenzoic acid from chloroform leaves extract of *Tacca integrifolia.*

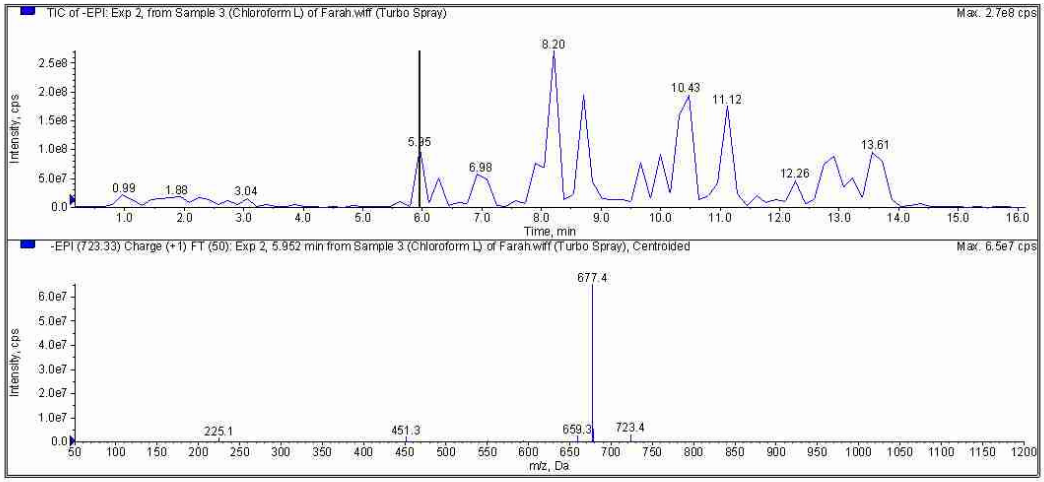

Figure 4.16. LCMS/MS chromatogram of proanthocyanidin trimer from chloroform leaves extract of *Tacca integrifolia.*

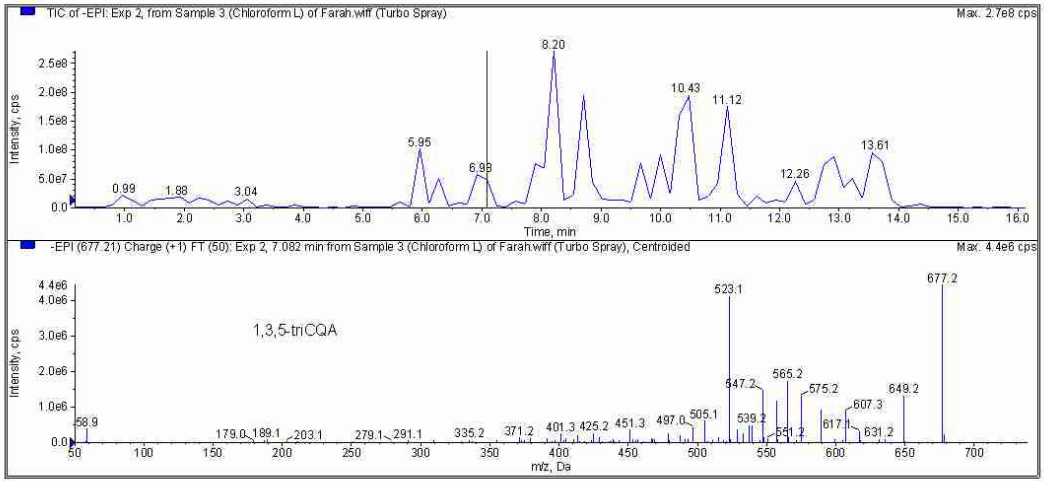

Figure 4.17. LCMS/MS chromatogram of 1,3,5-tricaffeolquinic acid from chloroform leaves extract of *Tacca integrifolia.*

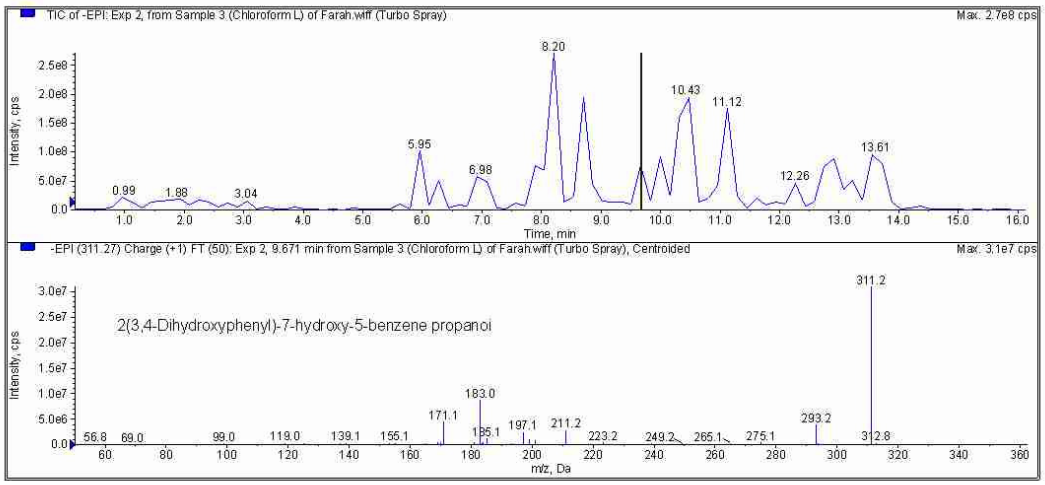

Figure 4.18. LCMS/MS chromatogram of 2(3,4-Dihydroxyphenyl)-7-hydroxy-5-benzene propanol)from chloroform leaves extract of *Tacca integrifolia.*

Full LCMS/MS chromatogram of chloroform leaves extract as in Figure 4.14 showed 10 peaks were separated at time of 0.99', 1.88', 3.04', 5.95', 6.98', 8.20', 10.43', 11.12', 12.26', and 13.71'. However, only four compounds were detected including *p* hydroxybenzoic acid that was detected at 0.963' (Figure 4.15), proanthocyanidin trimer was detected at 5.952' (Figure 4.16), 1,3,5-tricaffeolquinic acid was detected at 7.082' (Figure 4.17), and Figure 4.18 showed the detection of 2(3,4-Dihydroxyphenyl)-7-hydroxy-5-benzene propanol at 9.671'.

iv) LCMS/MS profile of methanol leaves extract of *Tacca integrifolia*

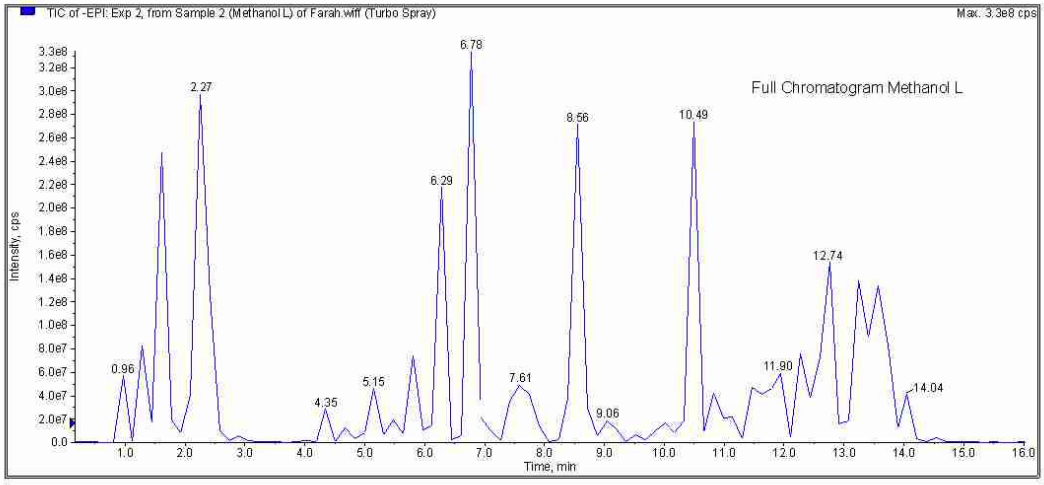

Figure 4.19. LCMS/MS chromatogram of methanol leaves extract from *Tacca integrifolia.*

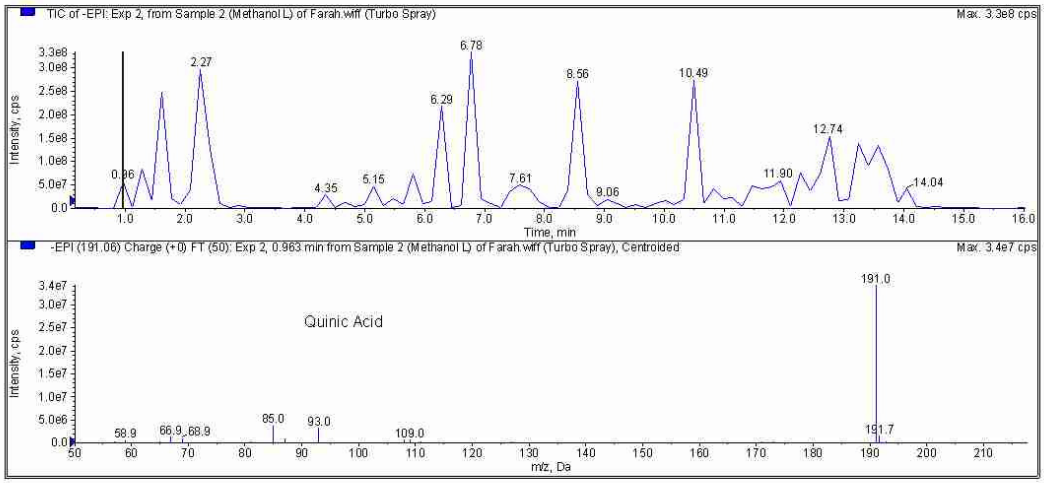

Figure 4.20. LCMS/MS chromatogram of quinic acid from methanol leaves extract of *Tacca integrifolia.*

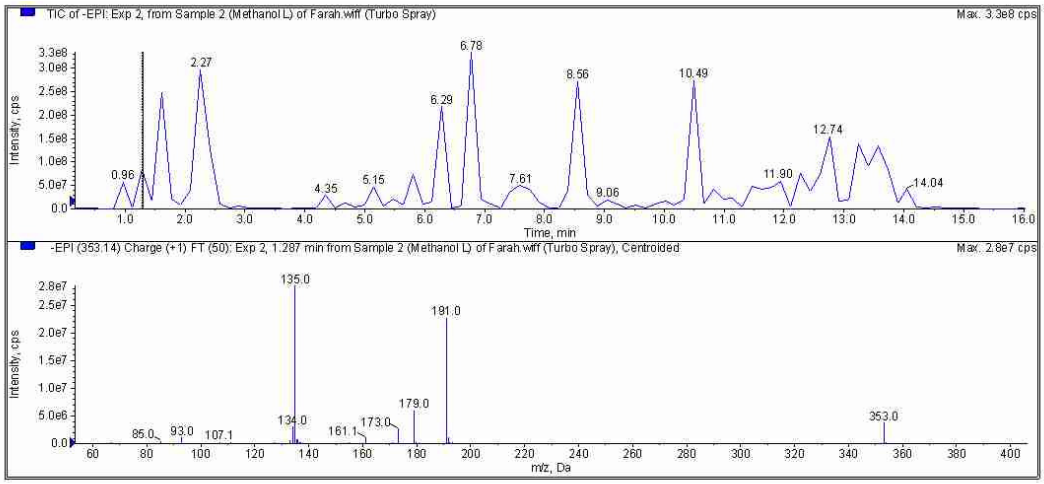

Figure 4.21. LCMS/MS chromatogram of 3-Caffeolquinic acid from methanol leaves extract of *Tacca integrifolia.*

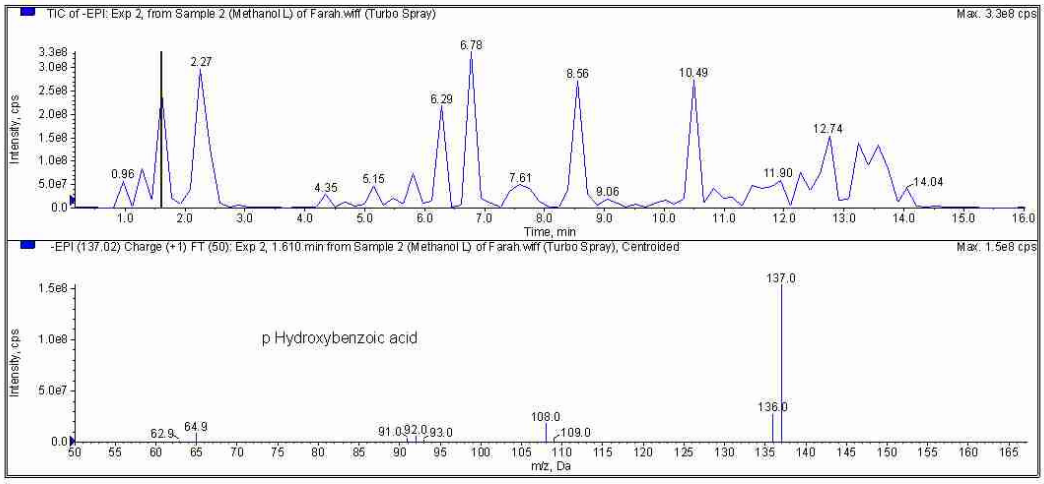

Figure 4.22. LCMS/MS chromatogram of *p* hydroxybenzoic acid from methanol leaves extract of *Tacca integrifolia.*

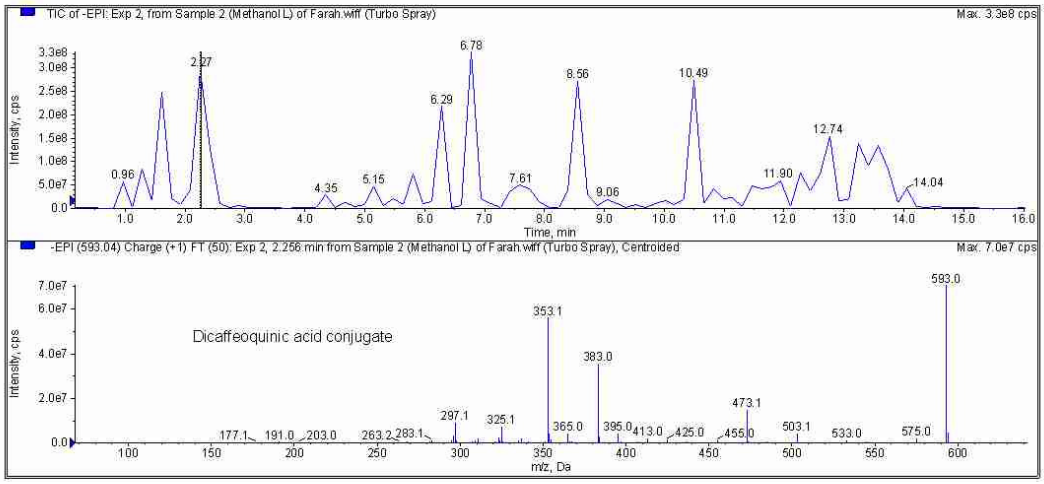

Figure 4.23. LCMS/MS chromatogram of dicaffeolquinic acid conjugate from methanol leaves extract of *Tacca integrifolia.*

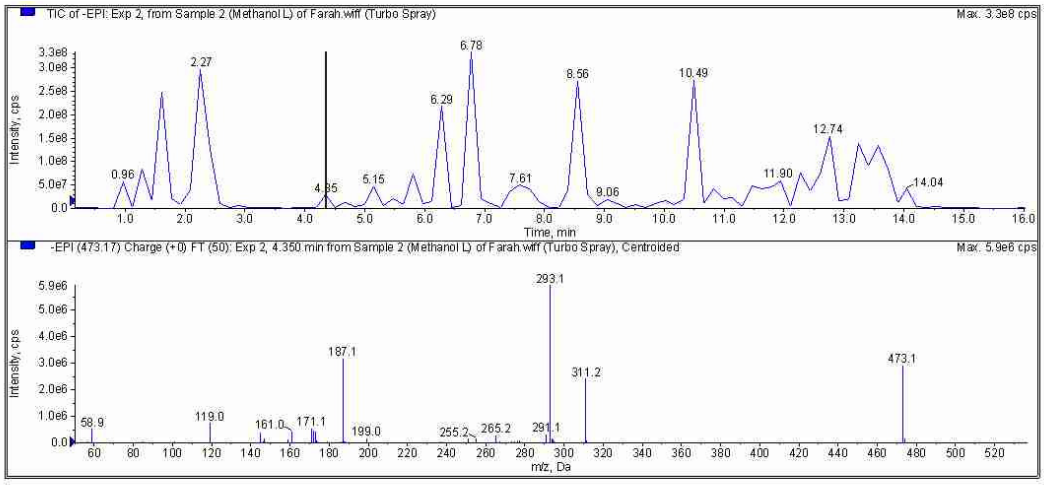

Figure 4.24. LCMS/MS chromatogram of isoflavone glycosides from methanol leaves extract of *Tacca integrifolia.*

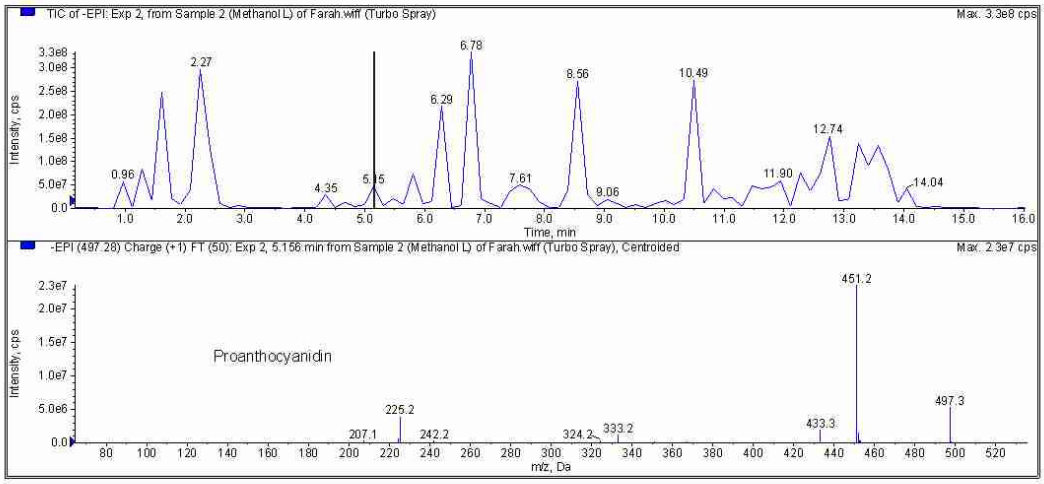

Figure 4.25. LCMS/MS chromatogram of proanthocyanidin from methanol leaves extract of *Tacca integrifolia.*

Full LCMS/MS of methanol leaves extract showed 13 peaks were separated at 0.96', 2.27', 4.35', 5.15', 6.29', 6.78', 7.61', 8.56', 9.06', 10.49', 11.90', 12.74' and 14.04' as in Figure 4.19. Six compounds were detected as quinic acid that was detected at 0.963' (Figure 4.20), 3-caffeolquinic acid detected at 1.287' as in Figure 4.21, *p* hydroxybenzoic acid was detected at 1.610 (Figure 4.22), dicaffeolquinic acid conjugate at 2.256' (Figure 4.23), isoflavone glycosides at 4.350' (Figure 4.24'), and proanthocyanidin at 5.156' (Figure 4.25).

v) LCMS/MS profile of water leaves extract of *Tacca integrifolia*

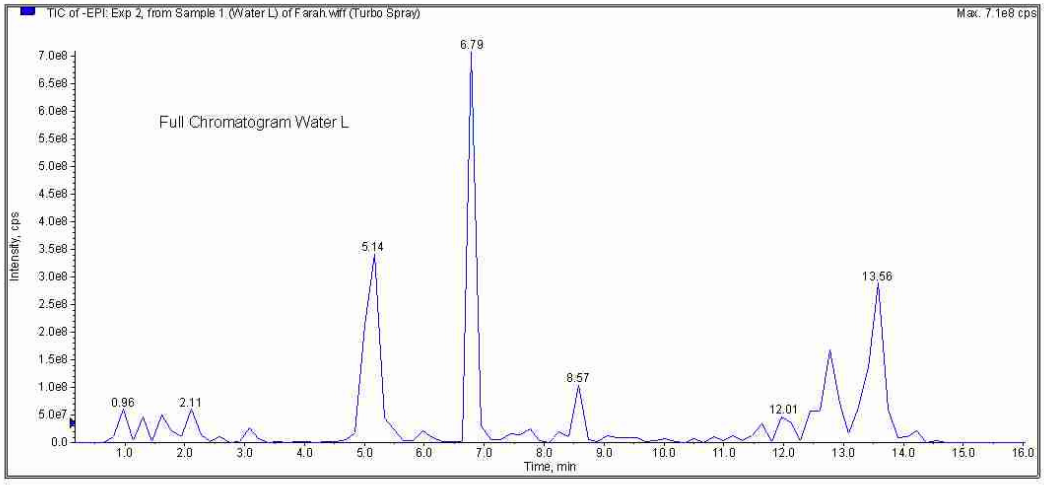

Figure 4.26. LCMS/MS chromatogram of water leaves extract from *Tacca integrifolia.*

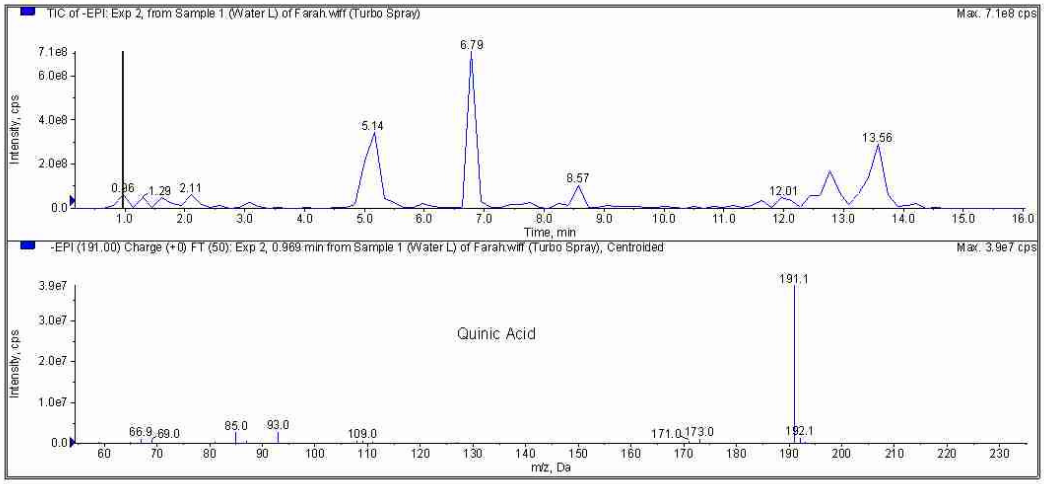

Figure 4.27. LCMS/MS chromatogram of quinic acid from water leaves extract of *Tacca integrifolia.*

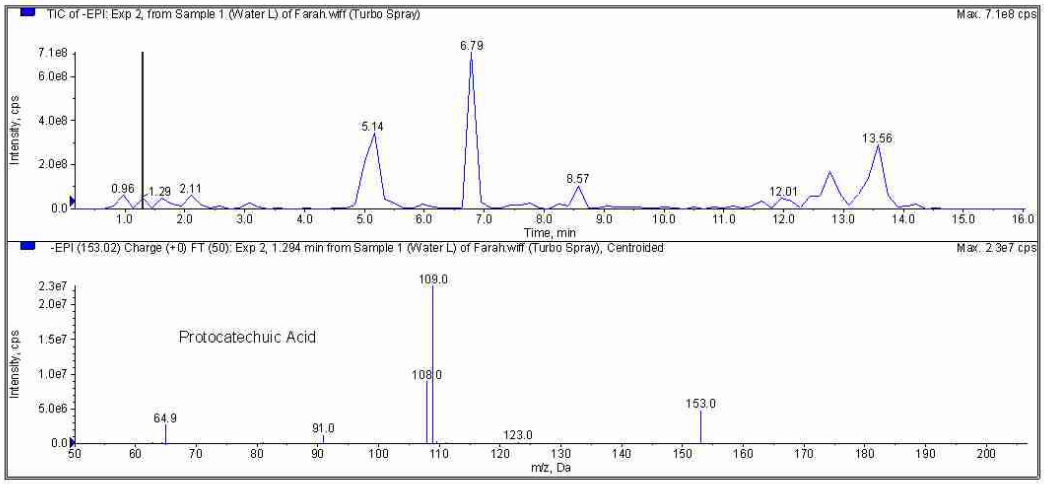

Figure 4.28. LCMS/MS chromatogram of protocatechuic acid from water leaves extract of *Tacca integrifolia.*

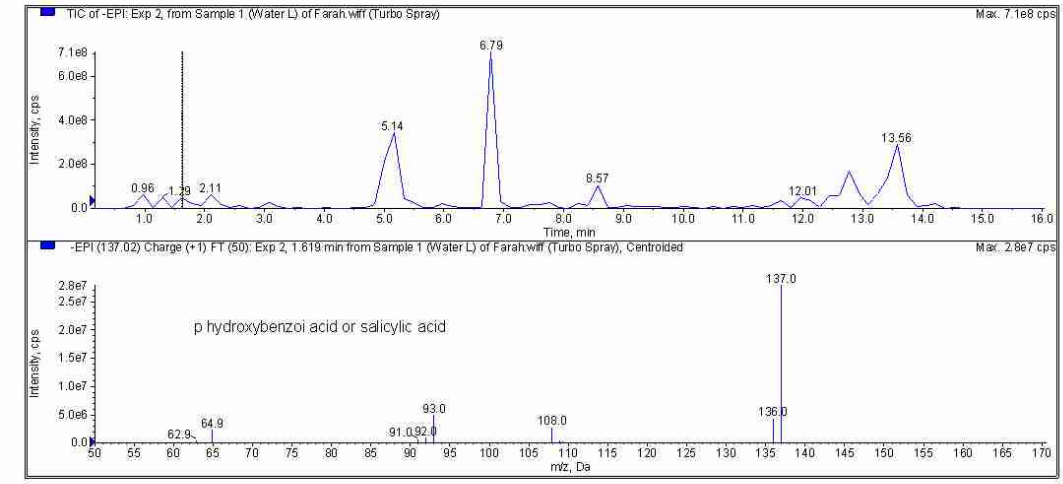

Figure 4.29. LCMS/MS chromatogram of salicylic acid from water leaves extract of *Tacca integrifolia.*

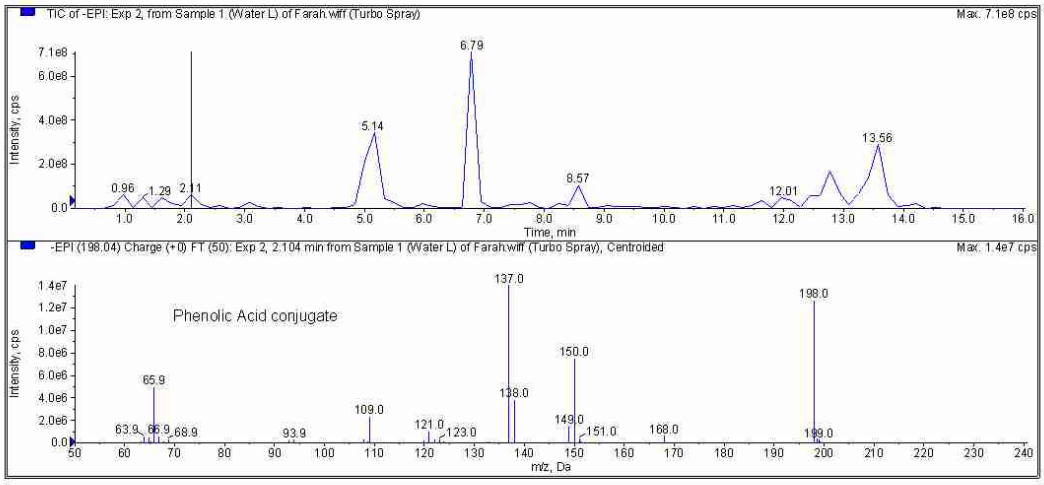

Figure 4.30. LCMS/MS chromatogram of phenolic acid conjugate from water leaves extract of *Tacca integrifolia.*

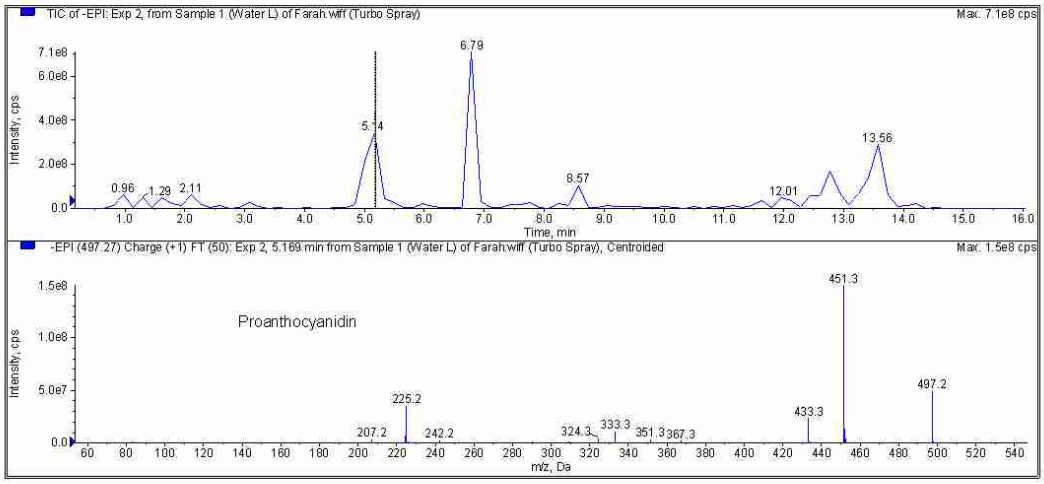

Figure 4.31. LCMS/MS chromatogram of proanthocyanidin from water leaves extract from *Tacca integrifolia.*

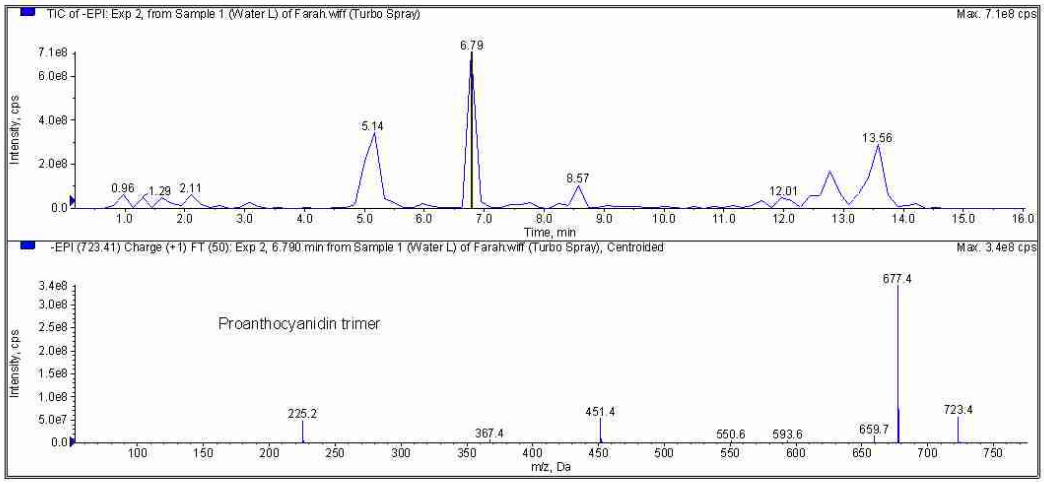

Figure 4.32. LCMS/MS chromatogram of proanthocyanidin trimer from water leaves extract from *Tacca integrifolia.*

Full LCMS/MS analysis of water leaves extract showed 7 peaks were separated at 0.96', 2.11', 5.14', 6.79', 8.57', 12.01', and 13.56' as in Figure 4.26. Figure 4.27 showed the detection of quinic acid at 0.969', while protocatechuic acid was detected at 1.294' (Figure 4.28), *p* hydroxybenzoic acid at 1.619' (Figure 4.29), phenolic acid conjugate at 2.104' (Figure 4.30), proanthocyanidin at 5.169' (Figure 4.31), and proanthocyanidin trimer was detected at 6.790' as in Figure 4.32.

4.3.2 Liquid Chromatography Mass Spectrometry/Mass Spectrometry (LCMS/MS) for rhizomes extracts of *Tacca integrifolia*

Table 4.14. Compounds detected in Liquid Chromatography Mass Spectrometry/ Mass Spectrometry (LCMS/MS) of rhizomes extract from *Tacca integrifolia*

Extract	Compound detected	Reference Figure
Hexane	1) Proanthocyanidin trimer	Fig. 4.34
Petroleum Ether	1) Proanthocyanidin trimer isomer	Fig. 4.36
Chloroform	1) Triterpenoid saponin 2) Gypenosides	Fig. 4.38 Fig. 4.39
Methanol	1) Gypenoside	Fig. 4.41
Water	1) Dicaffeolquinic acid conjugate 2) Proanthocyanidin 3) Proanthocyanidin trimer	Fig. 4.43 Fig. 4.44 Fig. 4.45

i) LCMS/MS chromatogram of hexane rhizome extract of *Tacca integrifolia*

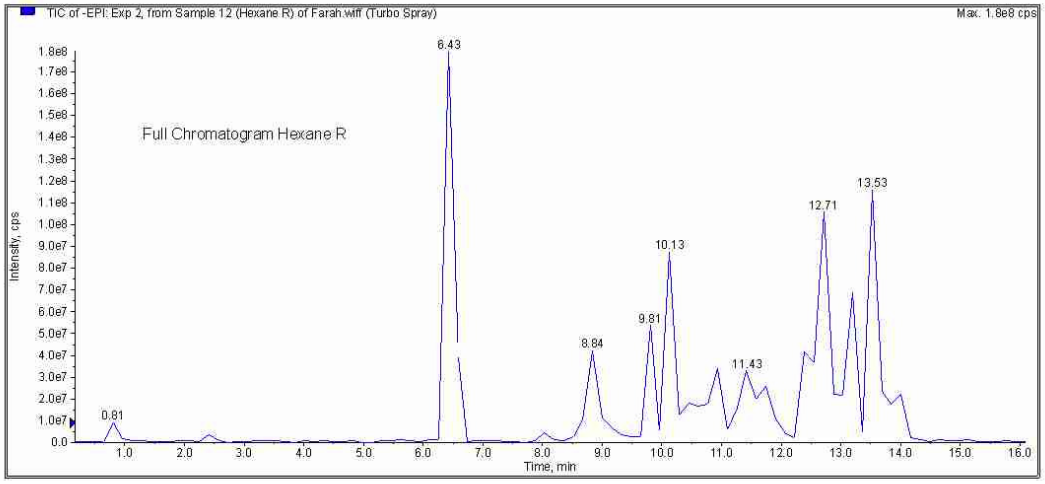

Figure 4.33. LCMS/MS chromatogram of hexane rhizome extract from *Tacca integrifolia.*

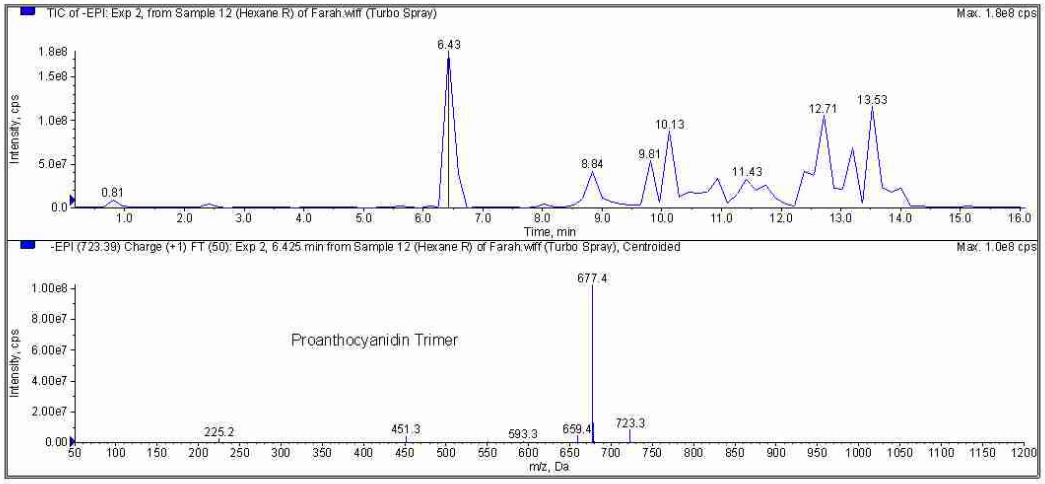

Figure 4.34. LCMS/MS chromatogram of proanthocyanidin trimer from hexane rhizome extract of *Tacca integrifolia.*

Full LCMS/MS chromatogram of hexane rhizome extract showed 8 peaks were separated at 0.91', 6.43', 8.84', 9.81', 10.13', 11.43', 12.71', and 13.53' as in Figure 4.33. However, only proanthocyanidin trimer was detected at 6.425' as in Figure 4.34.

ii) LCMS/MS profile of petroleum ether rhizome extract from of *Tacca integrifolia*

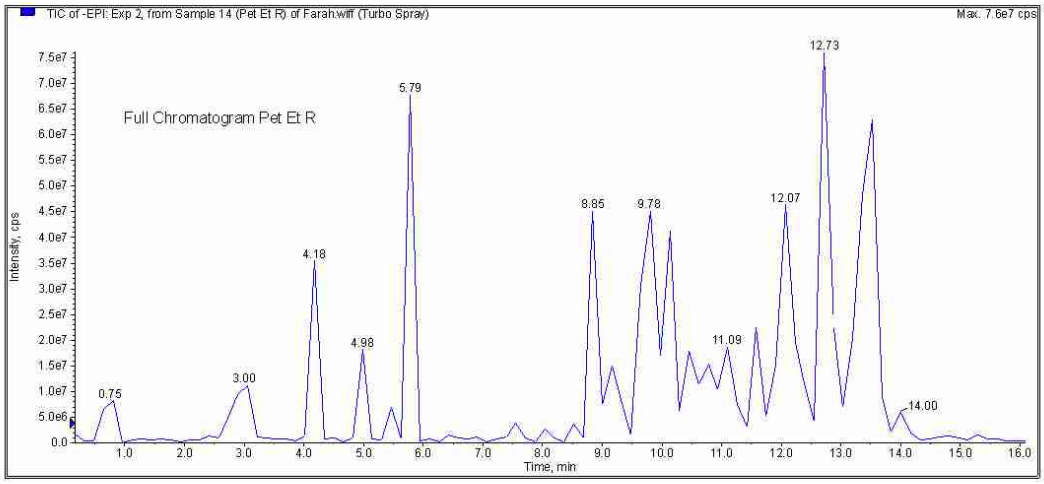

Figure 4.35. LCMS/MS chromatogram of petroleum ether rhizome extract from *Tacca integrifolia.*

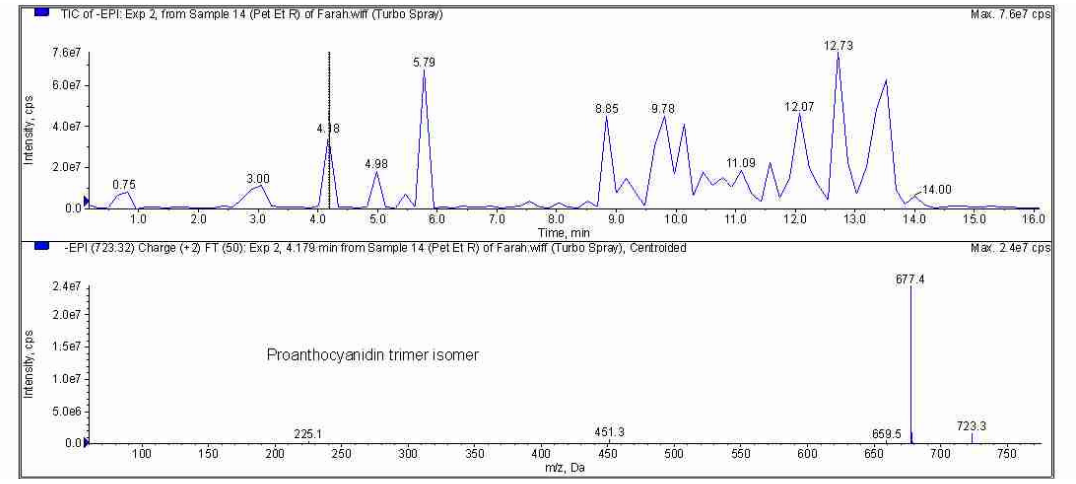

Figure 4.36. LCMS/MS chromatogram of proanthocyanidin trimer isomer from petroleum ether rhizome extract of *Tacca integrifolia.*

Full LCMS/MS chromatogram of petroleum ether rhizome extract as in Figure 4.35 showed the separation of 11 peaks at 0.75', 3.00', 4.18', 4.98', 5.70', 8.85', 9.78', 11.09', 12.07', 12.73', and 14.00'. Proanthocyanidin trimer isomer was detected at 4.179' as in Figure 4.36.

iii) LCMS/MS profile of chloroform rhizome extract of *Tacca integrifolia*

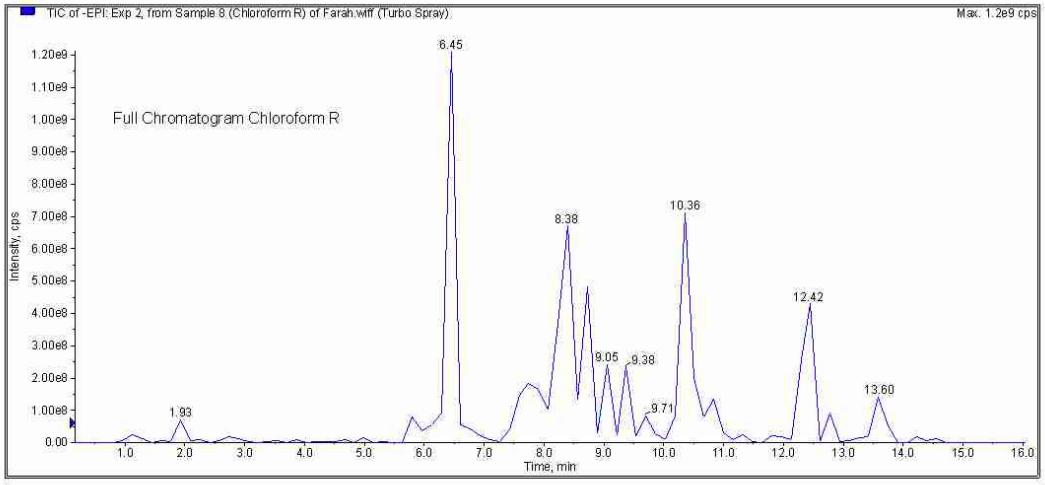

Figure 4.37. LCMS/MS chromatogram of chloroform rhizome extract from *Tacca integrifolia.*

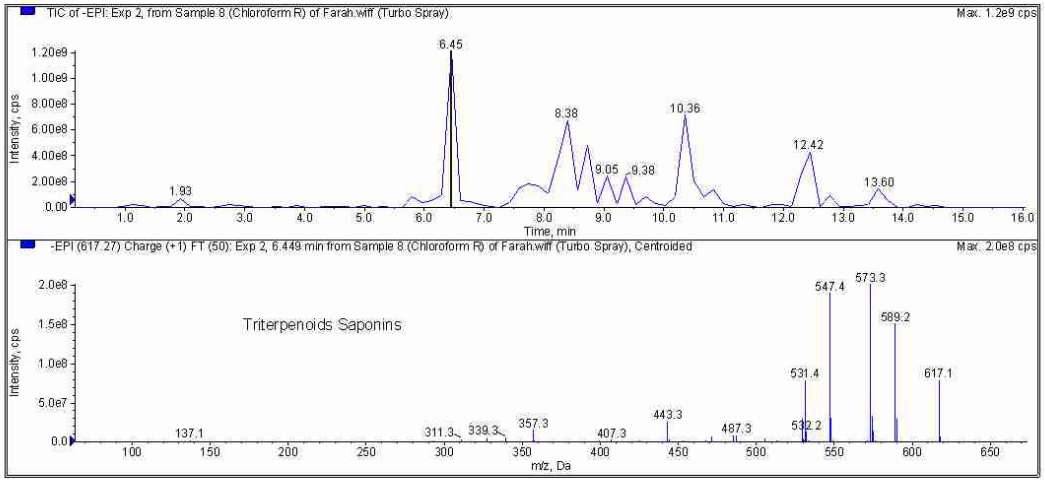

Figure 4.38. LCMS/MS chromatogram of triterpenoids saponins from chloroform rhizome extract of *Tacca integrifolia.*

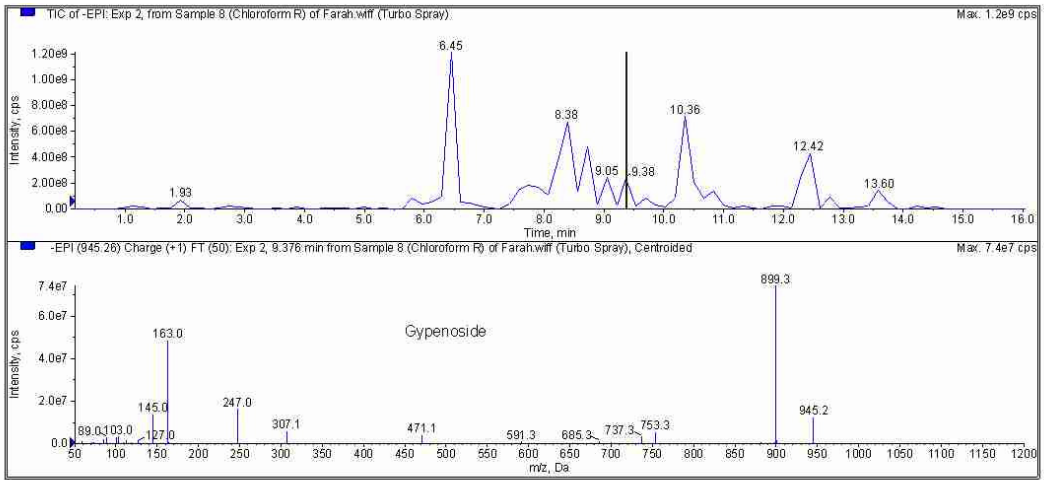

Figure 4.39. LCMS/MS chromatogram of gypenoside from chloroform rhizome extract of *Tacca integrifolia.*

Full LCMS/MS chromatogram of chloroform rhizome extract showed 9 peaks were separated at 1.93', 6.45', 8.38', 9.05', 9.38', 9.71', 10.36', 12.42', and 13.60' as in Figure 4.37. However, only 2compounds were detected as in Figure 4.38 and Figure 4.39 that showed the detection of triterpenoid saponin and gypenoside at 6.449' and 9.376' respectively.

iv) LCMS/MS profile of methanol rhizome extract of *Tacca integrifolia*

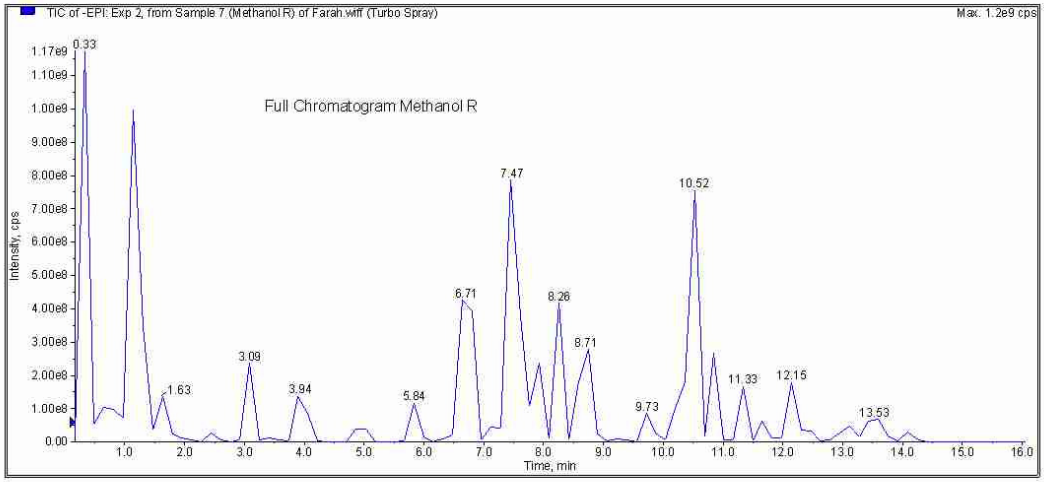

Figure 4.40. LCMS/MS chromatogram of methanol rhizome extract from *Tacca integrifolia.*

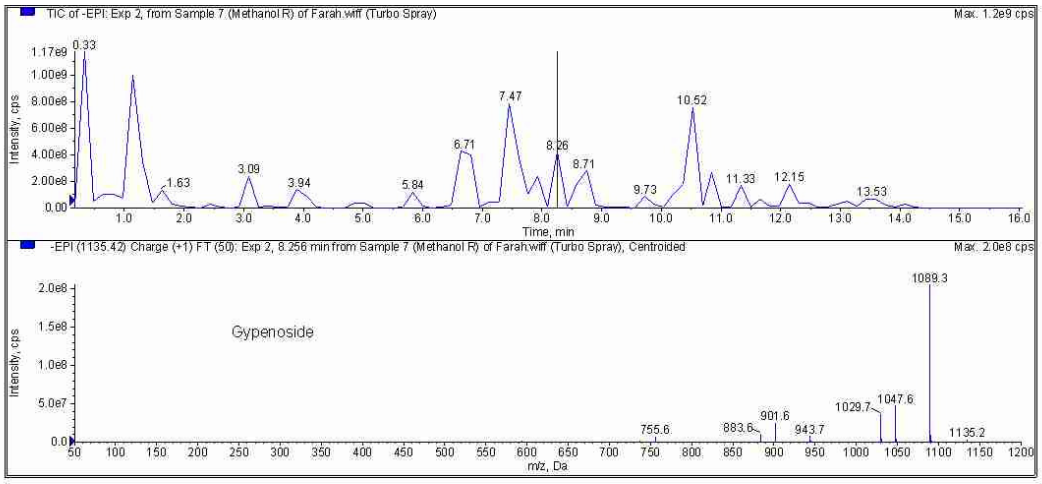

Figure 4.41. LCMS/MS chromatogram of gypenoside from methanol rhizomes extract from *Tacca integrifolia.*

Full LCMS/MS chromatogram of methanol rhizome extract in Figure 4.40 showed 14 peaks were separated at 0.33', 1.63', 3.09', 3.94', 5.84', 6.71', 7.47', 8.26', 8.71', 9.73', 10.52', 11.33', 12.15', and 13.53' and Figure 4.41 showed the detection of gypenoside at 8.256'

v) LCMS/MS profile of water rhizome extract of *Tacca integrifolia*

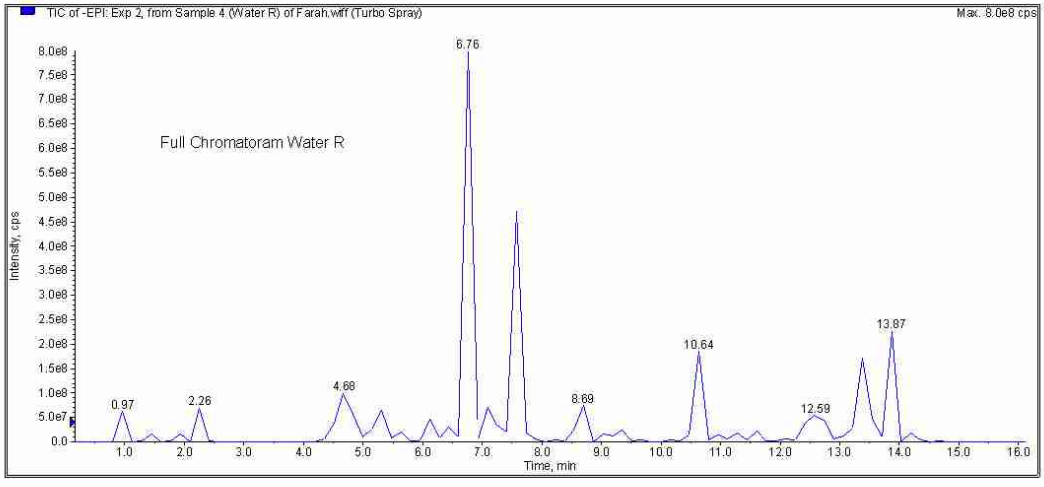

Figure 4.42. LCMS/MS chromatogram of water rhizome extract from *Tacca integrifolia.*

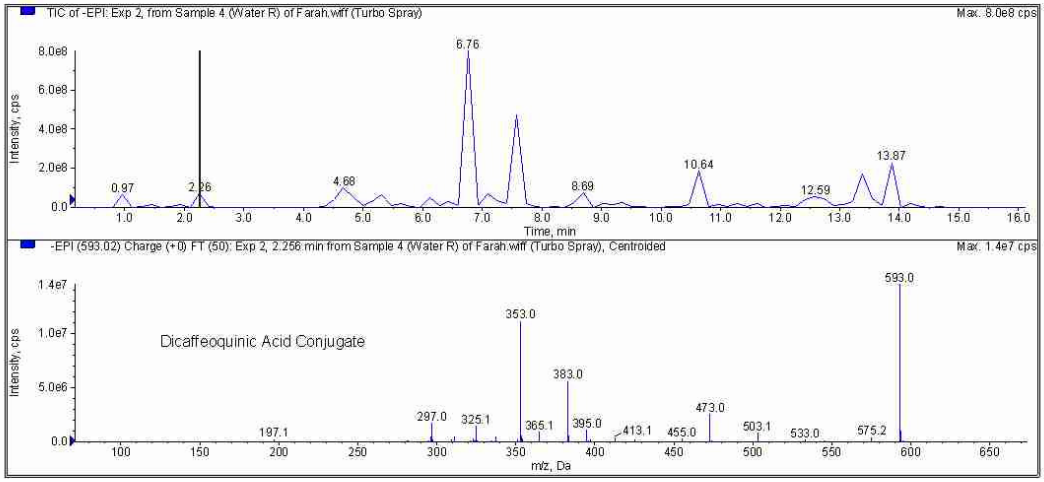

Figure 4.43. LCMS/MS chromatogram of dicaffeolquinic acid conjugate from water rhizome extract of *Tacca integrifolia.*

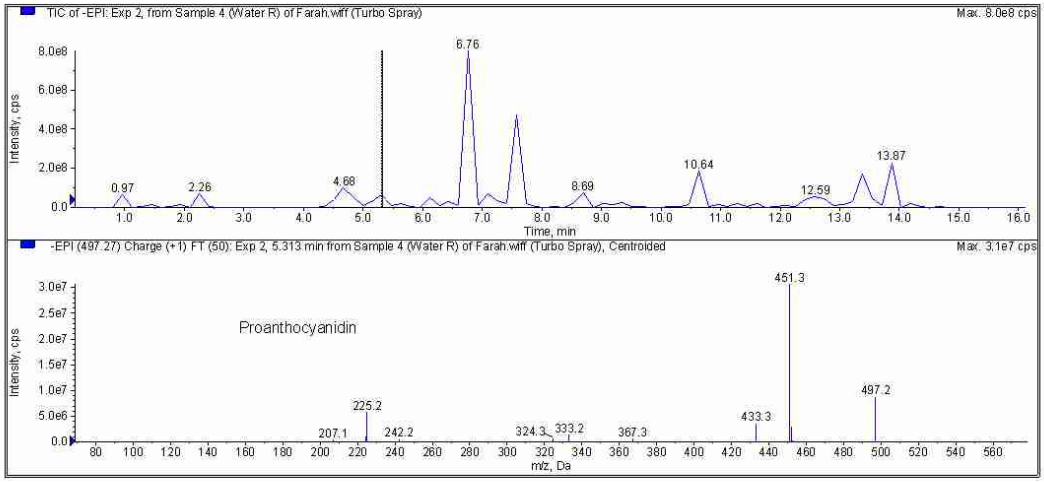

Figure 4.44. LCMS/MS chromatogram of proanthocyanidin from water rhizome extract of *Tacca integrifolia.*

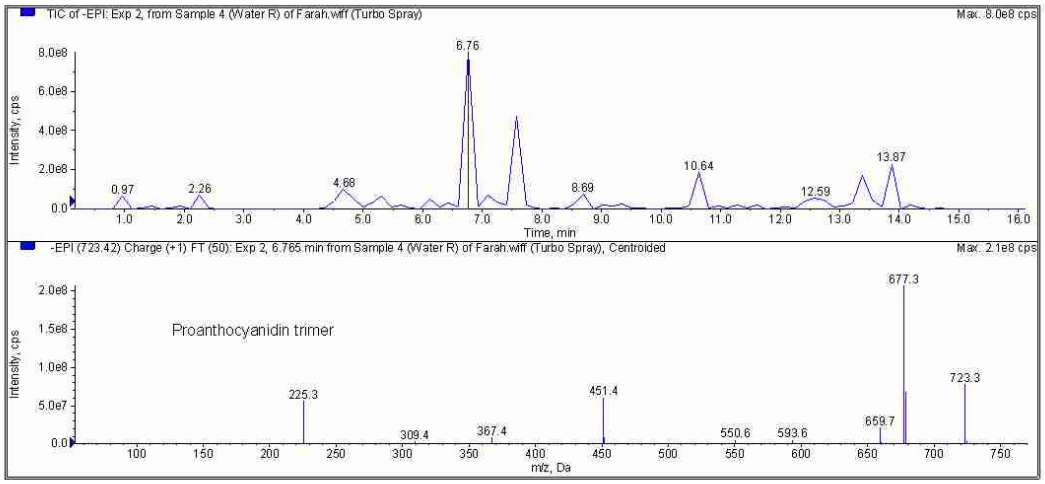

Figure 4.45. LCMS/MS chromatogram of proanthocyanidin trimer from water rhizome extract from *Tacca integrifolia.*

Full LCMS/MS chromatogram of water rhizome extract showed the separation of 8 peaks at 0.97', 2.26', 4.68', 6.76', 8.69', 10.64', 12.59', and 13.87' as in Figure 4.42. Dicaffeolquinic acid, proanthocyanidin and proanthocyanidin trimer were detected at 2.256', 5.313' and 6.765' as in Figure 4.43, Figure 4.44 and Figure 4.45.

4.4 Phytochemical detection of chemical compounds

4.4.1 Saponin froth test

200 g grinded sample of leaves and rhizome of *Tacca integrifolia* were extracted separately with 400 ml methanol and were left overnight in environmental shaker at room temperature. Extractions were filtered and 1 ml of extract was transferred into a small tube containing 5 ml distilled water. The mixture were shaken well for 30 seconds and allowed to stand at room temperature. After 30 minutes, a formation of a stable froth was observed and it indicates the presence of saponin.

Table 4.15. Saponin froth test

Sample	Froth
Leaves methanol extract	+ve
Rhizome methanol extract	+ve

4.4.2 Tannin and phenolic compounds

200 g grinded sample of leaves and rhizome of *Tacca integrifolia* were extracted separately with 400 ml methanol and left overnight in environmental shaker at room temperature. 2 ml of filtered sample were transferred into test tube separately. Each test tube was added with 6 drops of 1 % $FeCl_3$. Changes in colour for each extract were observed.

Table 4.16. Colour changes in tannin and phenolic compound test

Sample	Colour changes
Leaves methanol extract	Green → dark green
Rhizome methanol extract	Light yellow → yellow

4.5 Determination of Total Phenol Contents

Total phenol content in hexane, petroleum ether, chloroform, methanol and water extracts from leaves and rhizome of *Tacca integrifolia* were determined using standard curve of Gallic acid as positive reference standard. The total phenol content of each extracts were measured by using equation obtain from the standard curve as showed in Figure 4.46 while Table 4.18 and 4.19 showed the total phenolic contents of hexane, petroleum ether, chloroform, methanol and water extracts from the leaves and rhizomes of *Tacca integrifolia.*

i) Gallic acid as positive reference standard

Table 4.17. Absorbance of Gallic acid

Concentration of Gallic acid (µg/ml)	Absorbance 765 nm			Mean ± S.D.
	1	2	3	
50	0.251	0.249	0.252	0.251 ± 0.002
100	0.521	0.524	0.498	0.514 ± 0.014
150	0.714	0.712	0.715	0.714 ± 0.002
200	0.939	1.002	0.981	0.974 ± 0.032
250	1.113	1.115	1.115	1.114 ± 0.001

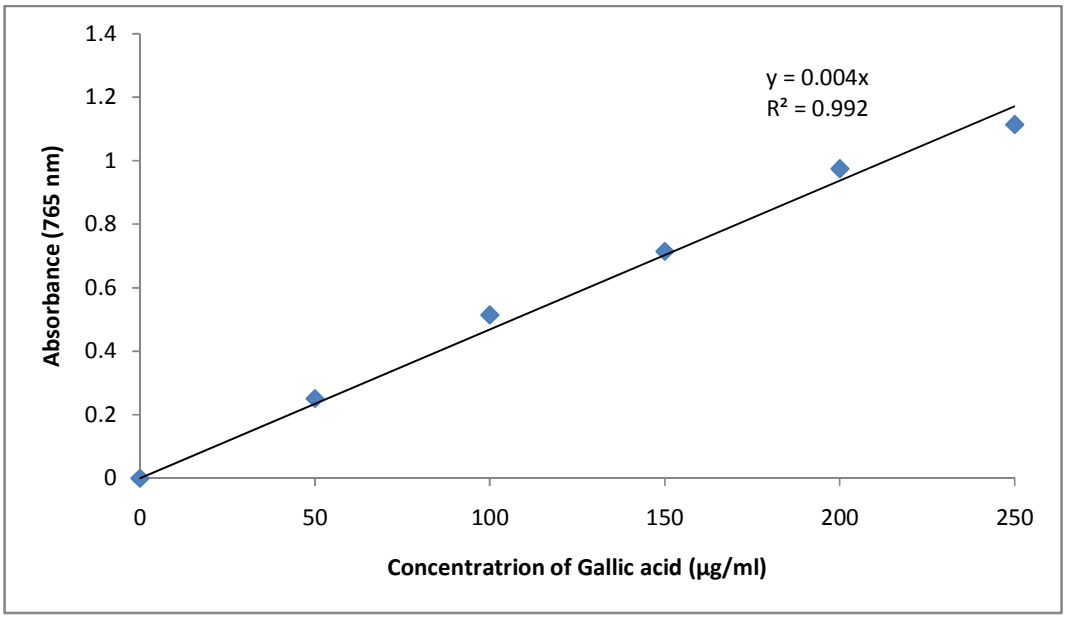

Figure 4.46. Standard curve of Gallic acid

ii) Total phenolic content of leaves extracts of *Tacca integrifolia*

Table 4.18. Total phenolic content from leaves extract of *Tacca integrifolia*

Sample (2500µg/ml)	Absorbance 765 nm			Mean ± SD	Total phenolic content (mgGAE/g)
	1	2	3		
Hexane extract	0.083	0.080	0.081	0.081 ± 0.002	44.6
Petroleum ether extract	0.192	0.194	0.190	0.192 ± 0.002	105.7
Chloroform extract	0.526	0.528	0.525	0.526 ± 0.002	288.6
Methanol extract	0.127	0.128	0.125	0.127 ± 0.002	69.8
Water extract	1.446	1.449	1.443	1.446 ± 0.003	792.7

At the concentration of (2500µg/ml), leaves water extract of *Tacca integrifolia* showed the highest concentration of phenolic compound (792.7mgGAE/g) followed by leaves chloroform extract (288.6mgGAE/g), leaves petroleum extract (105.7 mgGAE/g), leaves methanol extract (69.8 mgGAE/g) and leaves hexane extract (44.6 mgGAE/g).

iii) Total phenolic content from rhizome extract of *Tacca integrifolia*

Table 4.19. Total phenolic content from rhizome extract of *Tacca integrifolia*

Sample (2500µg/ml)	Absorbance 765 nm			Mean ± SD	Total phenolic content (mgGAE/g)
	1	2	3		
Hexane extract	0.239	0.237	0.236	0.237 ± 0.002	130.3
Petroleum ether extract	0.073	0.069	0.071	0.071 ± 0.002	38.9
Chloroform extract	0.153	0.157	0.158	0.156 ± 0.003	84.9
Methanol extract	0.106	0.118	0.119	0.114 ± 0.007	61.3
Water extract	0.642	0.641	0.639	0.641 ± 0.002	350.8

At the concentration of (2500µg/ml), rhizome water extract of *Tacca integrifolia* showed the highest concentration of phenolic compound (350.8 mgGAE/g) followed by rhizome hexane extract (130.3 mgGAE/g), rhizome chloroform extract (84.9 mgGAE/g), rhizome methanol extract (61.3 mgGAE/g) and rhizome petroleum ether extract (38.9 mgGAE/g).

4.6 Determination of Total flavonoid contents

i. Quercetin as Positive Reference Standard

A stock solution of Quercetin was prepared by dissolving 100mg of quercetin into 1ml of methanol. The stock solution with concentration of 100 mg/ml was diluted into 5 different concentration at 500 µg/ml, 1000 µg/ml, 1500 µg/ml, 2000 µg/ml, and 2500 µg/ml. 1 ml diluted standard quercetin from each concentration were mixed with 0.3ml 5% sodium nitrate ($NaNO_3$) and were incubated for 5 minutes in water bath at 37°C. 0.3 ml 10% aluminum chloride ($AlCl_2$) was added to the mixture and were left for incubation in water bath for 6 minutes at 38°C followed by addition of 2ml 1 M sodium hydroxide (NaOH) and 10 ml distilled water. Table 4.20 showed the absorbance reading at 510 nm and Figure 4.47 showed the standard curve of Quercetin as positive reference standard.

Table 4.20. Absorbance of Quercetin

Concentration of Quercetin (µg/ml)	Absorbance 510 nm			Mean ± S.D.
	1	2	3	
500	0.064	0.065	0.065	0.065 ± 0.001
1000	0.110	0.110	0.130	0.117 ± 0.01
1500	0.174	0.176	0.176	0.175 ± 0.001
2000	0.236	0.237	0.231	0.235 ± 0.003
2500	0.276	0.274	0.275	0.275 ± 0.001

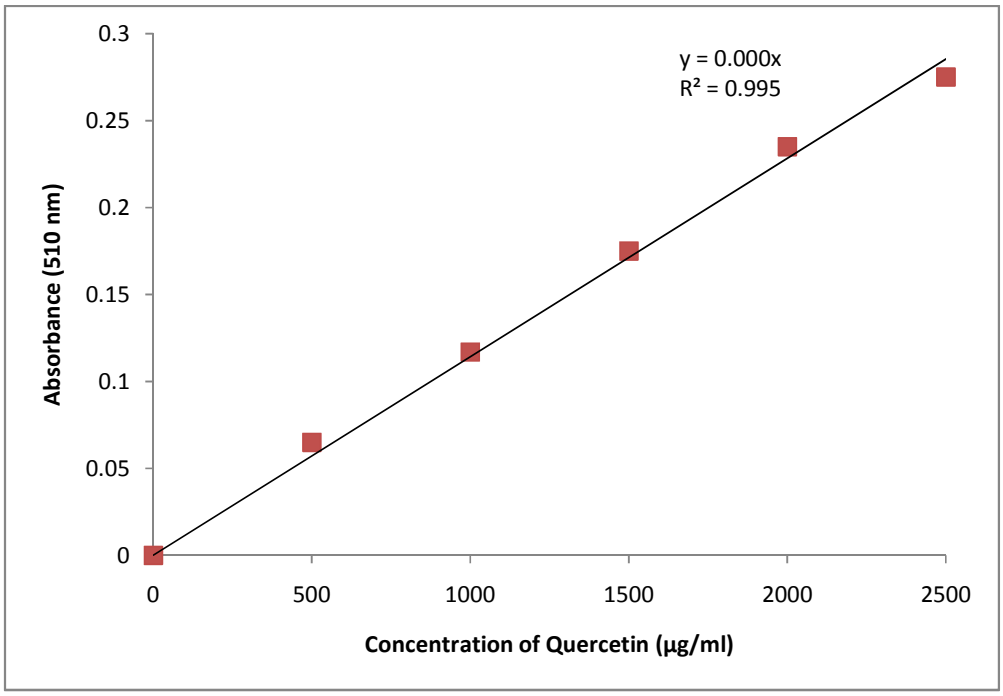

Figure 4.47. Standard curve of Quercetin

ii. Determination of Total Flavonoid content from leaves extract of *Tacca integrifolia*

Sample of extract from leaves of *Tacca integrifolia* were prepared in 2500µg/ml concentration. 1 ml from each samples were mixed with 0.3ml 5% sodium nitrate ($NaNo_3$) and were incubated for 5 minutes in water bath at 37°C. Test were continued by adding 0.3 ml 10% aluminum chloride ($AlCl_2$) to the mixture and incubation was followed for 6 minutes in water bath at 38°C. The mixture were then added with 2ml 1 M sodium hydroxide (NaOH) and 10 ml distilled water before absorbance reading at 510 nm.

Table 4.21. Total flavonoid content from leaves extract of *Tacca integrifolia*

Sample (2500µg/ml)	Absorbance 765 nm				Total flavonoid content (mgQE/g)
	1	2	3	Mean ± SD	
Hexane extract	0.36	0.41	0.38	0.38 ± 0.025	266.9
Petroleum ether extract	0.53	0.53	0.53	0.53 ± 0.002	376.7
Chloroform extract	0.49	0.49	0.49	0.49 ± 0.001	242.5
Methanol extract	0.22	0.22	0.22	0.22 ± 0.002	154.1
Water extract	0.12	0.12	0.12	0.12 ± 0.002	89.5

Leaves petroleum ether extract showed the highest concentration of total flavonoid content (376.7mgQE/g) followed by leaves hexane extract (266.9mgQE/g), leaves chloroform extract (242.5mgQE/g), leaves methanol extract (154.1mgQE/g) and leaves water extract (89.5mgQE/g).

iii. Determination of Total Flavonoid content from rhizomes extract of *Tacca integrifolia*

Extract from rhizome of *Tacca integrifolia* were prepared at 2500µg/ml. Experiment was initially started by adding 1 ml sample with 0.3ml 5% sodium nitrate ($NaNo_3$). The mixture were left for 5 minutes in water bath at 37°C followed by addition of 0.3 ml 10% aluminum chloride ($AlCl_2$). The mixtures again were left incubated in water bath for 6 minutes at 38°C. 2ml 1 M sodium hydroxide (NaOH) and 10 ml distilled water was added to the mixture before absorbance was read at 510 nm.

Table 4.22. Total flavonoid content from rhizome extract of *Tacca integrifolia*

Sample (2500µg/ml)	Absorbance 765 nm				Total flavonoid content (mgQE/g)
	1	2	3	Mean ± SD	
Hexane extract	0.255	0.256	0.255	0.255 ± 0.001	179.7
Petroleum ether extract	0.175	0.176	0.179	0.177 ± 0.002	125.1
Chloroform extract	0.273	0.272	0.276	0.274 ± 0.002	193.4
Methanol extract	0.075	0.081	0.075	0.077 ± 0.003	54.4
Water extract	0.043	0.041	0.044	0.043 ± 0.002	30.2

Rhizome chloroform extract showed the highest total flavonoid content which is 193.4mgQE/g followed by rhizome hexane extract (179.7mgQE/g), rhizome petroleum ether extract (125.1mgQE/g), rhizome methanol extract (54.4mgQE/g) and rhizome water extract (30.2mgQE/g).

Table 4.23.Summary of total phenol and total flavonoid content from leaves and rhizome extracts of *Tacca integrifolia*

Sample (2500µg/ml)	Total Phenol Content (mgGE/g)	Total Flavonoid Content (mgQE/g)
Hexane leaves extract	44.6	266.9
Hexane rhizome extract	130.3	179.74
Petroleum ether leaves extract	105.7	376.68
Petroleum ether rhizome extract	38.9	125.09
Chloroform leaves extract	288.6	343.463
Chloroform rhizome extract	84.9	193.4
Methanol leaves extract	69.8	154.06
Methanol rhizome extract	61.3	54.42
Water leaves extract	792.7	89.52
Water rhizome extract	350.8	30.153

4.7 Angiotensin Converting Enzyme (ACE) Bioassay

4.7.1 Determination of Angiotensin Converting Enzyme (ACE) inhibition by Captopril as positive reference standard

Captopril was used as positive reference standard in ACE bioassay as it acts as ACE inhibitor to inhibit the hydrolysis of Hippuryl-L-Histidyl-L-Leucine (HHL) to form Hippuric acid and Histidyl-L-Leucine (HL). Five difference concentration of Captopril were prepared and tested at 100, 50, 25, 12.5 and 6.25µg/ml as in Table 4.24.

Table 4.24. ACE inhibition and activity of standard of Captopril

Captopril (µg/ml)	Absorbance 228 nm			Mean ± S.D	Percentage of ACE inhibition	ACE activity (U)
	1	2	3			
6.25	0.341	0.349	0.345	0.345 ± 0.004	21.95	170
12.5	0.308	0.305	0.309	0.307 ± 0.002	30.54	151
25	0.275	0.279	0.276	0.277 ± 0.001	37.33	137
50	0.232	0.234	0.233	0.233 ± 0.002	47.29	115
100	0.159	0.158	0.156	0.158 ± 0.002	64.25	78

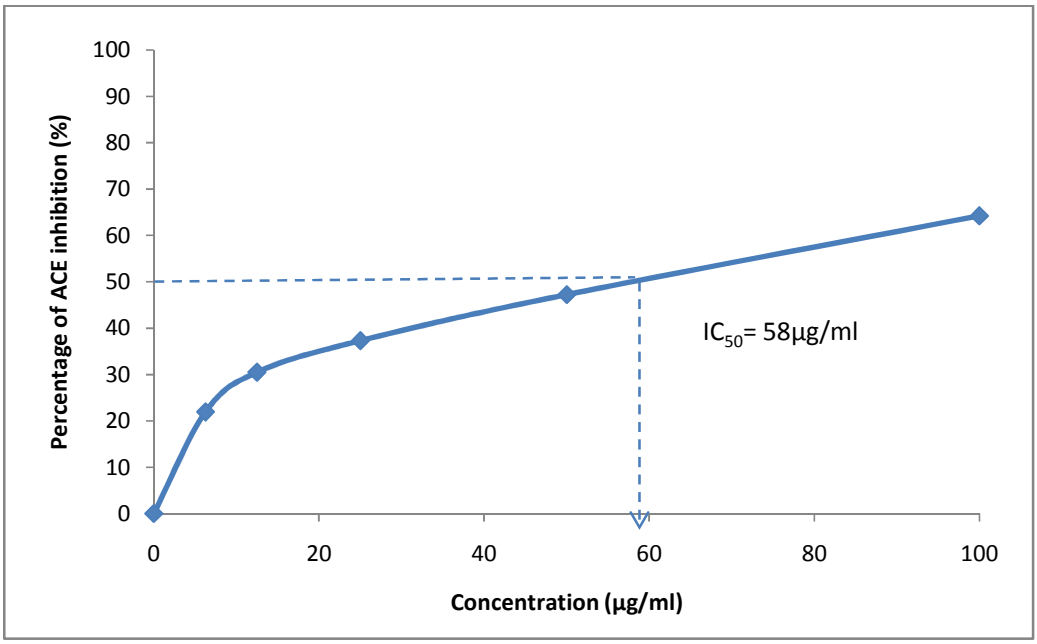

Figure 4.48. ACE inhibition of Captopril.

ACE inhibitory assay in this study was based on the hydrolysis of Hippuryl-L-Histidyl-L-Leucine (HHL) as subtract, by ACE to form Hippuric acid (HA) and Histidyl-L-Leucine (HL). The extent of the Hippuric acid form was directly related to the ACE activity. Therefore, ACE activity was determined spectrophotometrically at 228 nm. Figure 4.48 showed the IC_{50} of Captopril was 58µg/ml.

4.7.2 Determination of ACE inhibition of leaves extracts of *Tacca integrifolia*

The extracts from leaves were prepared in five different concentrations at 6.25µg/ml, 12.5µg/ml, 25µg/ml, 50µg/ml and 100µg/ml. The percentage of angiotensin converting enzyme (ACE) inhibition and angiotensin converting enzyme (ACE) activity of hexane, petroleum ether, chloroform, methanol and water extracts from the leaves of *Tacca integrifolia* were determined as in Table 4.25 to Table 4.29.

Table 4.25.ACE inhibition and activity of hexane leaves extract of *Tacca integrifolia*

Sample (µg/ml)	Absorbance 228 nm			Mean ± S.D	Percentage of ACE inhibition	ACE activity (U)
	1	2	3			
6.25	0.390	0.388	0.388	0.389 ± 0.001	11.99	191.8
12.5	0.343	0.352	0.349	0.348 ± 0.005	21.27	171.6
25	0.311	0.313	0.312	0.312 ± 0.001	29.41	153.8
50	0.280	0.282	0.284	0.282 ± 0.002	36.20	139.1
100	0.261	0.257	0.258	0.259 ± 0.002	41.40	127.7

ACE activity of leaves hexane extract showed that ACE activity reduced while the concentrations increased, thus the ACE inhibition increased together with the concentration. ACE activity was lowest at 100µg/ml (127.7U) and highest at 6.25µg/ml (191.8U) while percentage of ACE inhibition was highest at 100µg/ml (41.40%) and lowest at 6.25µg/ml (11.99%).

Table 4.26. ACE inhibition and activity of petroleum ether leaves extract of *Tacca integrifolia*

Sample (µg/ml)	Absorbance 228 nm			Mean ± S.D	Percentage of ACE inhibition	ACE activity (U)
	1	2	3			
6.25	0.409	0.413	0.408	0.410 ± 0.003	7.24	202.2
12.5	0.381	0.383	0.382	0.382 ± 0.001	13.57	188.4
25	0.360	0.356	0.353	0.356 ± 0.004	19.46	175.5
50	0.340	0.338	0.337	0.338 ± 0.002	23.53	166.7
100	0.336	0.334	0.336	0.335 ± 0.001	24.21	165.2

ACE activity of leaves petroleum ether extract showed that ACE activities reduced while the ACE inhibition increased when concentration of sample increased. ACE activity was lowest at 100µg/ml (165.2U) and highest at 6.25µg/ml (202.2U) while percentage of ACE inhibition was highest at 100µg/ml (24.21%) and lowest at 6.25µg/ml (7.24%).

Table 4.27.ACE inhibition and activity of chloroform leaves extract of *Tacca integrifolia*

Sample (µg/ml)	Absorbance 228 nm			Mean ± S.D	Percentage of ACE inhibition	ACE activity (U)
	1	2	3			
6.25	0.423	0.425	0.418	0.422 ± 0.004	4.52	208.1
12.5	0.389	0.386	0.388	0.388 ± 0.002	12.22	191.3
25	0.362	0.364	0.365	0.364 ± 0.002	17.65	179.5
50	0.354	0.358	0.358	0.357 ± 0.002	19.23	176
100	0.356	0.358	0.356	0.357 ± 0.001	19.23	176

Table 4.27 showed the percentage of ACE inhibition and ACE activity of leaves chloroform extract in five different concentrations. Percentage of ACE inhibition increased when concentration increased while ACE activities reduced when concentration of sample increased. ACE activity was lowest at 100µg/ml (176U) and highest at 6.25µg/ml (208.1U) while percentage of ACE inhibition was highest at 100µg/ml (19.23%) and lowest at 6.25µg/ml (4.52%).

Table 4.28. ACE inhibition and activity of methanol leaves extract of *Tacca integrifolia*

Sample (µg/ml)	Absorbance 228 nm			Mean ± S.D	Percentage of ACE inhibition	ACE activity (U)
	1	2	3			
6.25	0.421	0.420	0.419	0.420 ± 0.001	4.98	207.1
12.5	0.401	0.404	0.403	0.403 ± 0.002	8.82	198.7
25	0.380	0.386	0.382	0.383 ± 0.003	13.35	188.9
50	0.366	0.364	0.368	0.366 ± 0.002	17.19	180.5
100	0.347	0.353	0.349	0.350 ± 0.003	20.81	172.6

Table 4.28 showed the percentage of ACE inhibition and ACE activity of leaves methanol extract. At 100µg/ml percentage of ACE inhibition was highest (20.81%) while ACE activity was lowest (172.6U).

Table 4.29. ACE inhibition and activity of water leaves extract of *Tacca integrifolia*

Sample (µg/ml)	Absorbance 228 nm			Mean ± S.D	Percentage of ACE inhibition	ACE activity (U)
	1	2	3			
6.25	0.430	0.434	0.435	0.433 ± 0.003	2.04	213.5
12.5	0.393	0.391	0.392	0.392 ± 0.001	11.31	193.3
25	0.352	0.354	0.357	0.354 ± 0.003	19.91	174.6
50	0.298	0.303	0.301	0.301 ± 0.003	31.90	148.4
100	0.245	0.244	0.241	0.243 ± 0.002	45.02	119.8

Table 4.29 showed the percentage of ACE inhibition and ACE activity of leaves water extract. At 100µg/ml percentage of ACE inhibition was highest (45.02%) while ACE activity was lowest (119.8U).

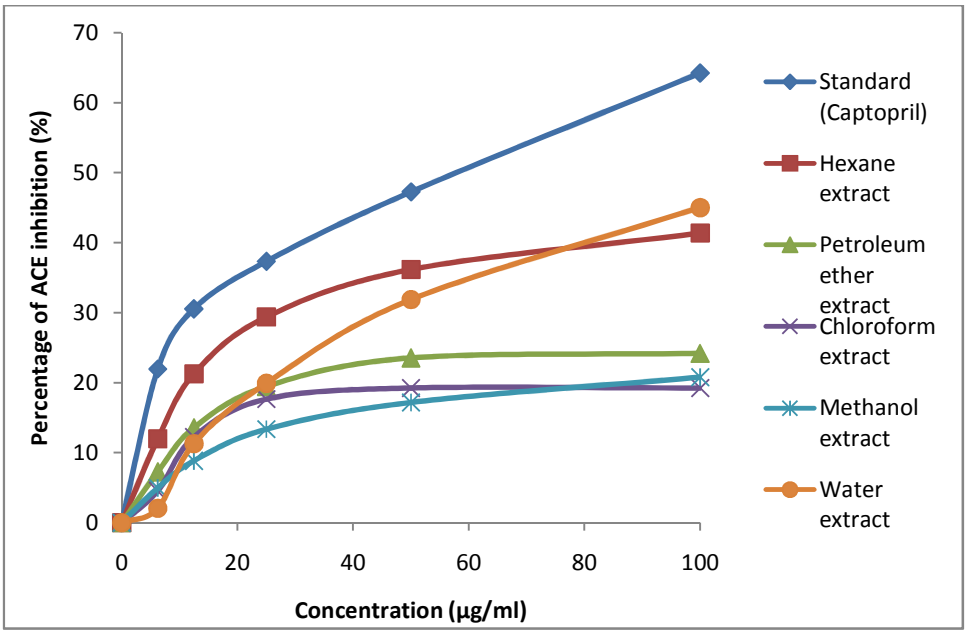

Figure 4.49. ACE inhibitions of leaves extracts from *Tacca integrifolia*

Figure 4.49 showed the percentage of ACE inhibition of 5 extract from leaves of *Tacca integrifolia* compared to the captopril as positive reference standard. All extracts showed dose dependent manner in ACE inhibition as increasing in sample concentration has increased the percentage of ACE inhibition significantly at $p<0.05$.

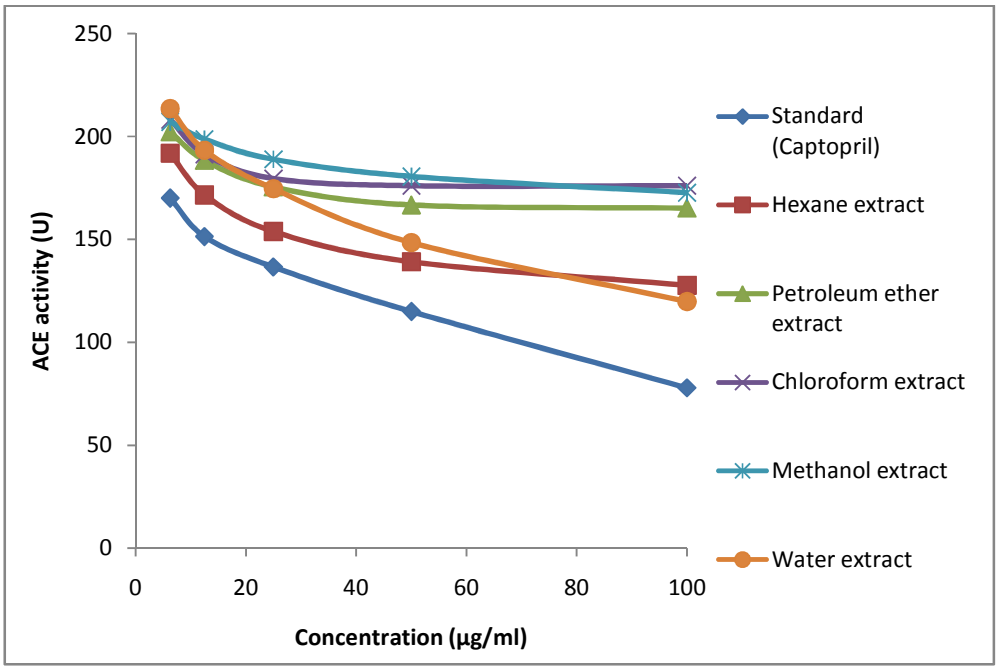

Figure 4.50. ACE activity of leaves extract from *Tacca integrifolia*

Figure 4.50 showed the ACE activity of five extracts from leaves of *Tacca integrifolia* compared to the ACE activity of Captopril as positive reference standard. All extracts showed dose dependent manner when increasing of sample concentration has reduced the ACE activity.

4.7.3 Determination of Angiotensin Converting Enzyme (ACE) inhibition of rhizome extract of *Tacca integrifolia*

The ACE inhibition and ACE activity of hexane, petroleum ether, chloroform, methanol and water extracts from the rhizome of *Tacca integrifolia* were determined (Table 4.30 to Table 4.34). The percentage of ACE inhibition showed the dose dependent manner to concentration of sample. Increasing of sample concentration showed the increasing of ACE inhibition while ACE activity was reduced while sample concentration increased.

Table 4.30.ACE inhibition and activity of hexane rhizome extract of *Tacca integrifolia*

Sample (µg/ml)	Absorbance 228 nm			Mean ± S.D	Percentage of ACE inhibition	ACE activity (U)
	1	2	3			
6.25	0.382	0.389	0.387	0.386 ± 0.004	12.67	190.3
12.5	0.376	0.375	0.379	0.377 ± 0.002	14.71	185.9
25	0.369	0.365	0.366	0.367 ± 0.002	16.97	181
50	0.359	0.366	0.361	0.362 ± 0.004	18.10	178.5
100	0.322	0.289	0.321	0.311 ± 0.019	29.63	153.4

Table 4.30 showed the percentage of ACE inhibition and ACE activity of rhizome hexane extract. At 100µg/ml percentage of ACE inhibition was highest at 29.63% while ACE activity was lowest at 153.35U.

Table 4.31.ACE inhibition and activity of petroleum ether rhizome extracts of *Tacca integrifolia*

Sample (µg/ml)	Absorbance 228 nm			Mean ± S.D	Percentage of ACE inhibition	ACE activity (U)
	1	2	3			
6.25	0.256	0.251	0.254	0.254 ± 0.003	42.53	125.25
12.5	0.253	0.251	0.252	0.252 ± 0.001	42.99	124.26
25	0.222	0.242	0.233	0.232 ± 0.01	47.51	114.4
50	0.219	0.226	0.225	0.223 ± 0.004	49.55	109.96
100	0.219	0.217	0.217	0.218 ± 0.001	50.68	107.49

Table 4.31 showed the percentage of ACE inhibition and ACE activity of rhizome petroleum ether extract. At 100µg/ml percentage of ACE inhibition was highest at 50.68% while ACE activity was lowest at 107.49U.

Table 4.32. ACE inhibition and activity of chloroform rhizome extract of *Tacca integrifolia*

Sample (µg/ml)	Absorbance 228 nm			Mean ± S.D	Percentage of ACE inhibition	ACE activity (U)
	1	2	3			
6.25	0.414	0.411	0.415	0.413 ± 0.002	6.56	203.6
12.5	0.384	0.386	0.387	0.380 ± 0.002	12.67	190.3
25	0.335	0.323	0.334	0.331 ± 0.007	25.11	163.2
50	0.290	0.310	0.312	0.304 ± 0.012	31.22	149.9
100	0.275	0.278	0.272	0.275 ± 0.003	37.78	135.6

Table 4.32 showed the percentage of ACE inhibition and ACE activity of rhizome chloroform extract. At 100µg/ml percentage of ACE inhibition was highest at 37.78% while ACE activity was lowest at 135.6U.

Table 4.33. ACE inhibition and activity of methanol rhizome extract of *Tacca integrifolia*

Sample (µg/ml)	Absorbance 228 nm			Mean ± S.D	Percentage of ACE inhibition	ACE activity (U)
	1	2	3			
6.25	0.238	0.244	0.241	0.241 ± 0.003	45.48	118.8
12.5	0.219	0.221	0.222	0.221 ± 0.002	50.0	108.97
25	0.215	0.219	0.216	0.217 ± 0.002	50.9	107
50	0.215	0.214	0.216	0.215 ± 0.001	51.36	106
100	0.204	0.205	0.206	0.205 ± 0.001	53.62	101.1

Table 4.33 showed the percentage of ACE inhibition and ACE activity of rhizome methanol extract. At 100µg/ml percentage of ACE inhibition was highest (53.62%) while ACE activity was lowest (101.1U).

Table 4.34. ACE inhibition and activity of water rhizome extract of *Tacca integrifolia*

Sample (µg/ml)	Absorbance 228 nm			Mean ± S.D	Percentage of ACE inhibition	ACE activity (U)
	1	2	3			
6.25	0.298	0.296	0.295	0.296 ± 0.002	33.03	146
12.5	0.249	0.258	0.256	0.254 ± 0.005	42.53	125.2
25	0.242	0.249	0.246	0.246 ± 0.004	44.34	121.3
50	0.239	0.241	0.243	0.241 ± 0.002	45.48	118.8
100	0.219	0.226	0.225	0.223 ± 0.004	49.55	110

Table 4.34 showed the percentage of ACE inhibition and ACE activity of rhizome water extract. At 100µg/ml percentage of ACE inhibition was highest at 49.55% while ACE activity was lowest at 110U.

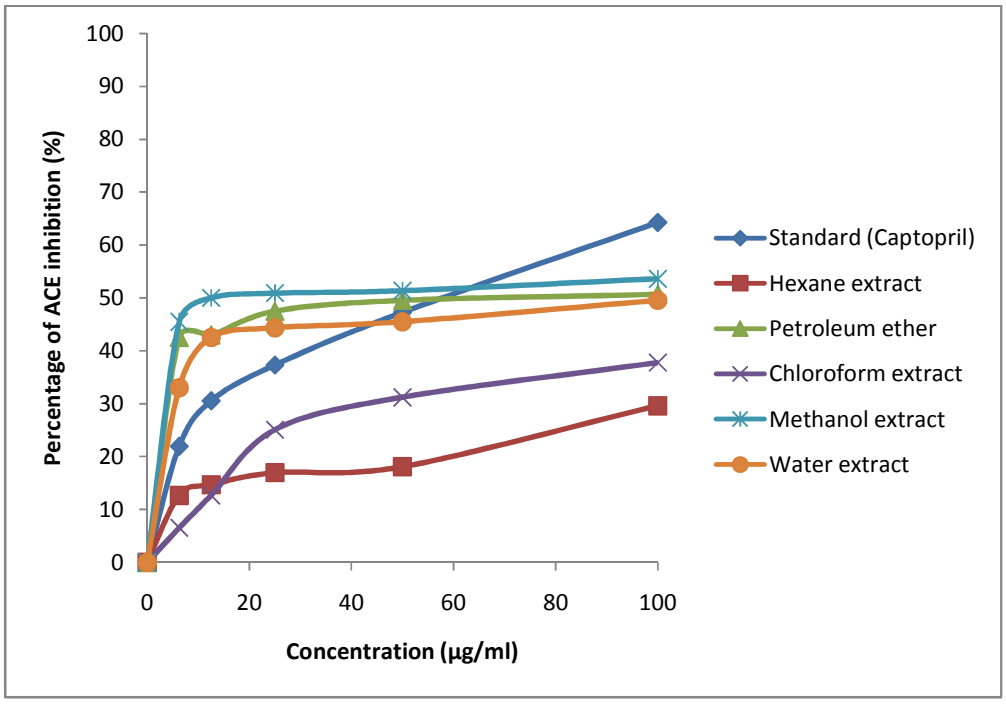

Figure 4.51. ACE inhibition of rhizome extracts from *Tacca integrifolia*

Figure 4.51 showed the percentage of ACE inhibition of extract from rhizome of *Tacca integrifolia* compared with Captopril as reference standard. All extracts showed the increasing of percentage of ACE inhibition significantly at $p<0.05$ when sample concentration increased.

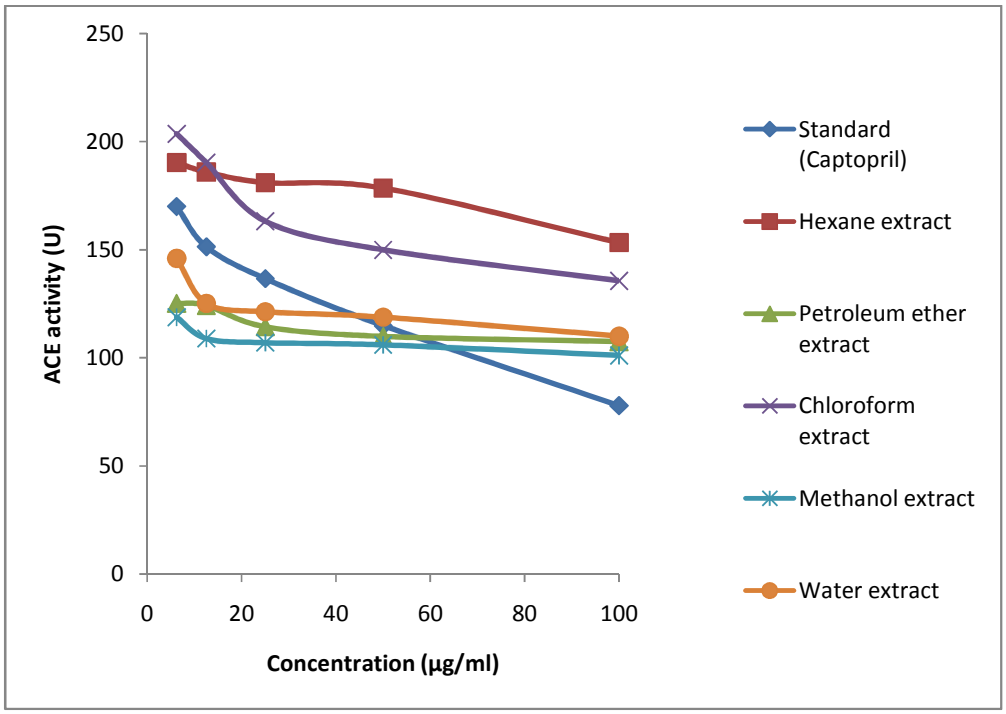

Figure 4.52. ACE activity of rhizome extract from *Tacca integrifolia*

Figure 4.52 showed the ACE activity of five extracts from rhizome of *Tacca integrifolia.* All extracts showed decreasing of ACE activity when sample concentration increased.

4.7.4 Determination of ACE inhibition of compounds isolated from extracts of *Tacca integrifolia*

Thin Layer Chromatography (TLC) of extracts of *Tacca integrifolia* has been developed using TLC plate size 20 x 20 cm. Each spotted colour presences were scrap separately before dissolved with distilled water in 1.5 ml vial. The mixtures were centrifuge using micro centrifuge and were kept for further use in ACE inhibitory activity assay. Table 4.35 to Table 4.42 showed the ACE inhibition and activity from the isolated chemical compounds from extract of *Tacca integrifolia* while Figure 4.53 to Figure 4.58 illustrated the histogram of the ACE inhibition of the isolated chemical compounds from extracts of *Tacca integrifolia.*

Table 4.35. ACE inhibitions and activity of the chemical compounds isolated from leaves hexane extract of *Tacca integrifolia*

Isolated compound (1 mg/ml)	Absorbance 228 nm			Mean ± S.D	Percentage of ACE inhibition	ACE activity (U)
	1	2	3			
A1	0.560	0.565	0.561	0.562 ± 0.003	No inhibition	277.12
A2	0.988	0.985	0.986	0.986 ± 0.002	No inhibition	486.19
A3	0.655	0.648	0.654	0.652 ± 0.004	No inhibition	321.5
A4	0.567	0.568	0.572	0.569 ± 0.003	No inhibition	280.57
A5	0.144	0.147	0.138	0.143 ± 0.005	67.65	70.51
A6	0.697	0.708	0.706	0.704 ± 0.006	No inhibition	347.14
A7	0.156	0.152	0.159	0.156 ± 0.004	64.71	76.92
A8	0.302	0.298	0.303	0.301 ± 0.003	31.9	148.42
A9	0.184	0.180	0.183	0.182 ± 0.002	58.82	89.74

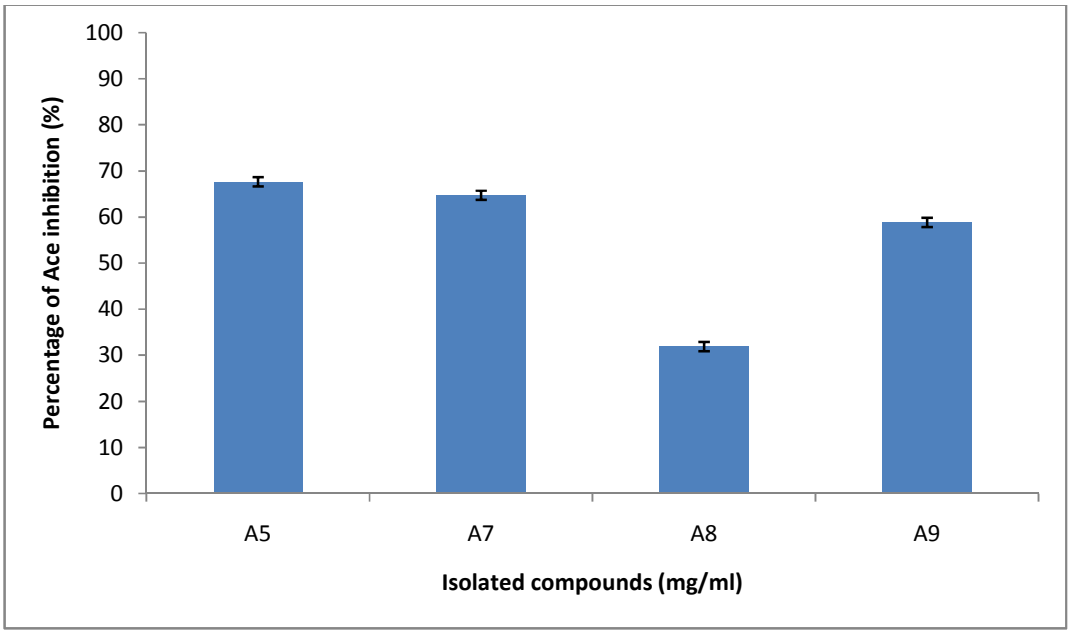

Figure 4.53. Histogram of percentage of ACE inhibition of chemical compounds isolated from hexane leaves extract of *Tacca integrifolia*

Table 4.36. ACE inhibitions and activity of the chemical compounds isolated from leaves petroleum ether extract of *Tacca integrifolia*

Isolated compound (1 mg/ml)	Absorbance 228 nm			Mean ± S.D	Percentage of ACE inhibition	ACE activity (U)
	1	2	3			
B1	0.618	0.614	0.616	0.616 ± 0.002	No inhibition	303.75
B2	0.835	0.833	0.836	0.835 ± 0.002	No inhibition	411.74
B3	0.162	0.171	0.168	0.167 ± 0.005	62.22	82.35
B4	0.789	0.794	0.792	0.792 ± 0.003	No inhibition	390.53
B5	0.142	0.144	0.138	0.141 ± 0.003	68.10	69.53
B6	0.588	0.592	0.586	0.589 ± 0.003	No inhibition	290.43
B7	0.615	0.614	0.617	0.615 ± 0.002	No inhibition	303.25
B8	0.388	0.394	0.392	0.391 ± 0.003	11.54	192.80
B9	0.708	0.709	0.711	0.709 ± 0.002	No inhibition	349.61

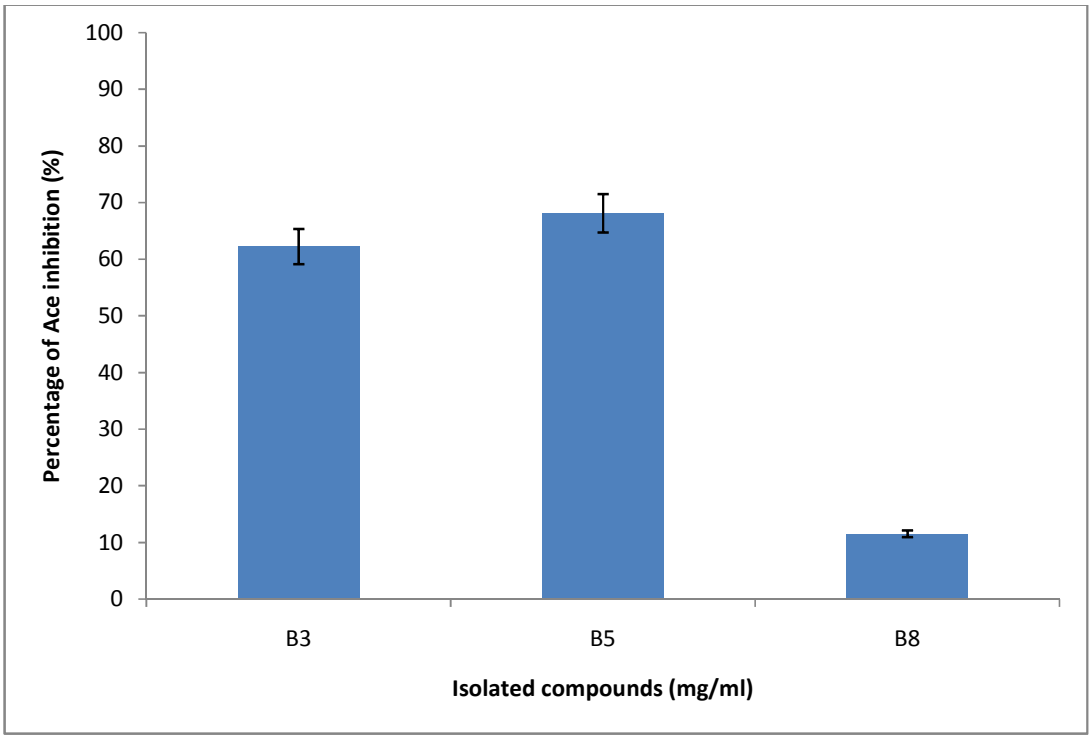

Figure 4.54. Histogram of percentage of ACE inhibition of chemical compounds isolated from petroleum ether leaves extract of *Tacca integrifolia*

Table 4.37. ACE inhibitions and activity of the chemical compounds isolated from leaves chloroform extract of *Tacca integrifolia*

Isolated compound (1 mg/ml)	Absorbance 228 nm			Mean ± S.D	Percentage of ACE inhibition	ACE activity (U)
	1	2	3			
C1	0.763	0.768	0.764	0.765 ± 0.003	No inhibition	377.22
C2	0.885	0.886	0.883	0.885 ± 0.002	No inhibition	436.39
C3	0.151	0.154	0.156	0.154 ± 0.003	65.16	75.94
C4	0.613	0.612	0.611	0.612 ± 0.001	No inhibition	301.78
C5	1.058	1.061	1.057	1.059 ± 0.002	No inhibition	522.19
C6	0.785	0.784	0.786	0.785 ± 0.001	No inhibition	387.08
C7	1.061	1.062	1.055	1.059 ± 0.004	No inhibition	522.19
C8	0.664	0.669	0.667	0.667 ± 0.003	No inhibition	328.90
C9	0.148	0.146	0.142	0.145 ± 0.003	67.19	71.50
C10	0.456	0.452	0.451	0.453 ± 0.003	No inhibition	223.37

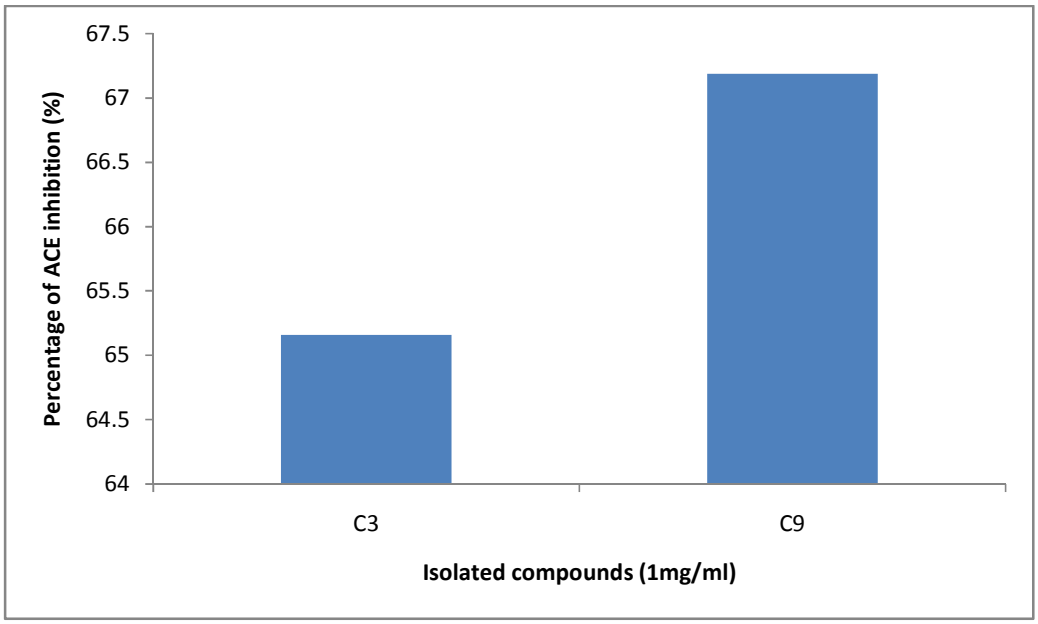

Figure 4.55. Histogram of percentage of ACE inhibition of chemical compounds isolated from chloroform leaves extract of *Tacca integrifolia*

Table 4.38. ACE inhibitions and activity of the chemical compounds isolated from leaves methanol extract of *Tacca integrifolia*

Isolated compound (1 mg/ml)	Absorbance 228 nm			Mean ± S.D	Percentage of ACE inhibition	ACE activity (U)
	1	2	3			
D1	1.124	1.132	1.128	1.128 ± 0.004	No inhibition	556.21
D2	0.641	0.649	0.644	0.645 ± 0.004	No inhibition	318.05
D3	0.475	0.479	0.482	0.479 ± 0.004	No inhibition	236.19
D4	0.995	0.991	0.994	0.993 ± 0.002	No inhibition	489.4
D5	1.172	1.171	1.176	1.173 ± 0.003	No inhibition	578.4
D6	1.195	1.191	1.194	1.193 ± 0.002	No inhibition	588.26
D7	1.146	1.145	1.151	1.147 ± 0.003	No inhibition	565.58
D8	1.086	1.089	1.091	1.089 ± 0.003	No inhibition	536.98
D9	0.657	0.661	0.654	0.657 ± 0.004	No inhibition	323.96

Table 4.39. ACE inhibitions and activity of the chemical compounds isolated from rhizome hexane extract of *Tacca integrifolia*

Isolated compound (1 mg/ml)	Absorbance 228 nm			Mean ± S.D	Percentage of ACE inhibition	ACE activity (U)
	1	2	3			
E1	0.223	0.227	0.221	0.224 ± 0.003	49.32	110.45
E2	0.445	0.448	0.442	0.445 ± 0.003	No inhibition	219.43
E3	0.108	0.109	0.105	0.107 ± 0.002	75.79	52.76
E4	0.122	0.121	0.125	0.123 ± 0.002	72.17	60.65
E5	0.756	0.755	0.752	0.754 ± 0.002	No inhibition	371.79
E6	0.846	0.848	0.851	0.848 ± 0.003	No inhibition	418.15
E7	0.657	0.653	0.658	0.656 ± 0.003	No inhibition	323.47

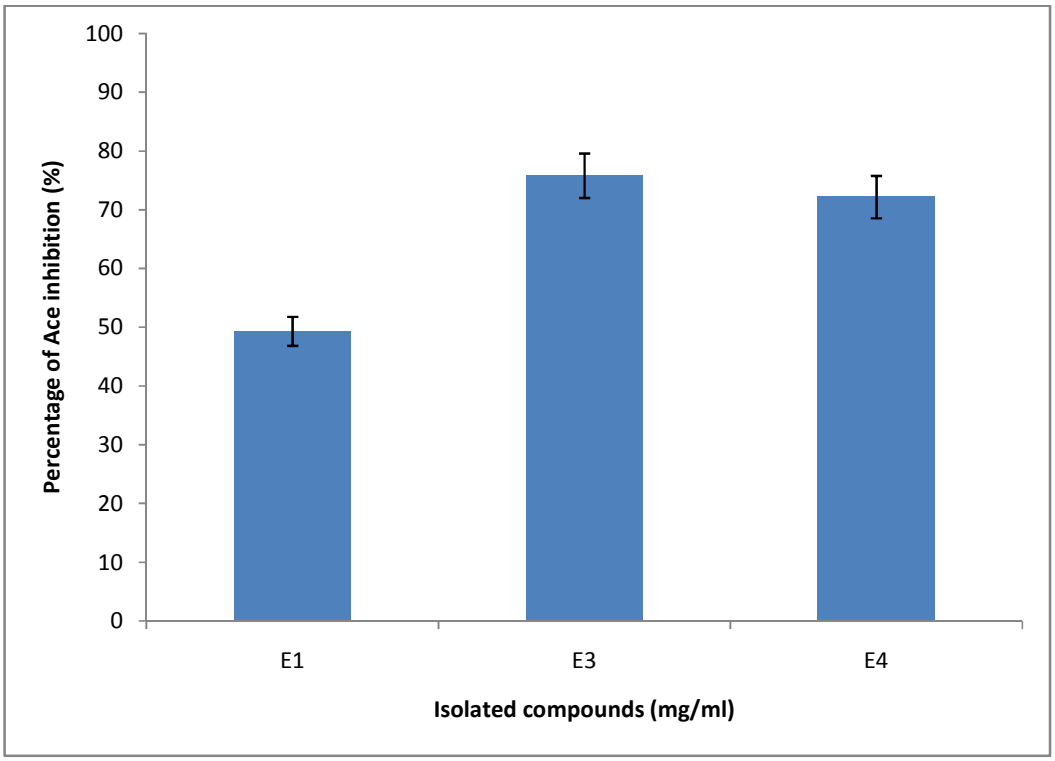

Figure 4.56. Histogram of percentage of ACE inhibition of chemical compounds isolated from hexane rhizome extract of *Tacca integrifolia*

Table 4.40. ACE inhibitions and activity of the chemical compounds isolated from rhizome petroleum ether extract of *Tacca integrifolia*

Isolated compound (1 mg/ml)	Absorbance 228 nm			Mean ± S.D	Percentage of ACE inhibition	ACE activity (U)
	1	2	3			
F1	0.266	0.269	0.264	0.266 ± 0.003	39.82	131.16
F2	0.557	0.562	0.563	0.561 ± 0.003	No inhibition	276.63
F3	0.592	0.587	0.593	0.591 ± 0.003	No inhibition	291.42
F4	0.494	0.497	0.498	0.496 ± 0.002	No inhibition	244.58
F5	0.604	0.605	0.606	0.605 ± 0.001	No inhibition	298.32
F6	0.461	0.458	0.459	0.459 ± 0.002	No inhibition	226.33
F7	0.699	0.696	0.695	0.697 ± 0.002	No inhibition	343.69

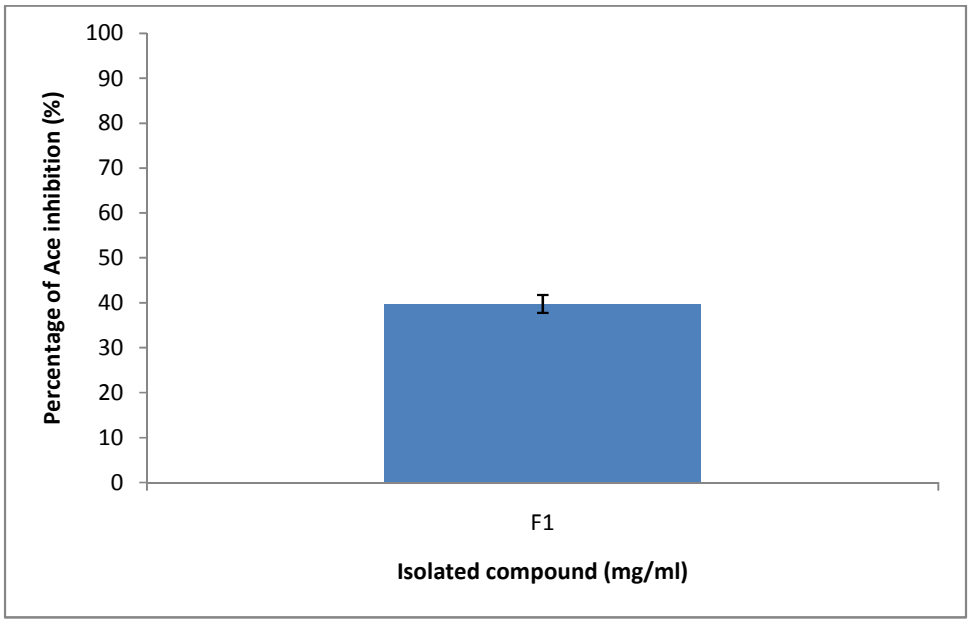

Figure 4.57. Histogram of percentage ACE inhibition of chemical compounds isolated from petroleum ether rhizome extract of *Tacca integrifolia*

Table 4.41. ACE inhibitions and activity of the chemical compounds isolated from rhizome chloroform extract of *Tacca integrifolia*

Isolated compound (1 mg/ml)	Absorbance 228 nm			Mean ± S.D	Percentage of ACE inhibition	ACE activity (U)
	1	2	3			
G1	0.159	0.154	0.158	0.157 ± 0.003	64.48	77.42
G2	0.546	0.548	0.545	0.546 ± 0.002	No inhibition	269.23
G3	0.367	0.371	0.365	0.368 ± 0.003	16.74	181.46
G4	0.676	0.678	0.682	0.679 ± 0.003	No inhibition	334.81
G5	0.728	0.724	0.725	0.726 ± 0.002	No inhibition	357.99
G6	0.229	0.231	0.228	0.229 ± 0.002	48.19	112.92
G7	0.720	0.719	0.715	0.718 ± 0.003	No inhibition	354
G8	1.213	1.211	1.216	1.213 ± 0.003	No inhibition	598.13
G9	1.159	1.161	1.162	1.161 ± 0.002	No inhibition	572.49
G10	0.636	0.638	0.635	0.636 ± 0.002	No inhibition	313.61

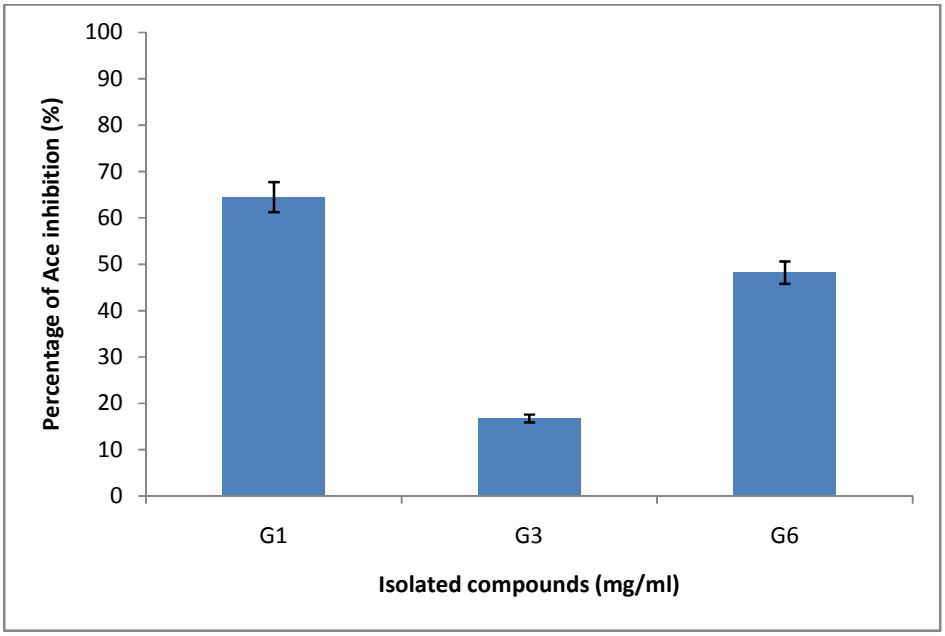

Figure 4.58. Histogram of percentage of ACE inhibition of chemical compounds isolated from chloroform rhizome extract of *Tacca integrifolia*

Table 4.42. ACE inhibitions and activity of the chemical compounds isolated from rhizome methanol extract of *Tacca integrifolia* using TLC

Isolated compound (1 mg/ml)	Absorbance 228 nm			Mean ± S.D	Percentage of ACE inhibition	ACE activity (U)
	1	2	3			
H1	0.485	0.480	0.482	0.482 ± 0.003	No inhibition	237.67
H2	0.506	0.504	0.502	0.504 ± 0.002	No inhibition	248.52
H3	0.605	0.604	0.598	0.602 ± 0.004	No inhibition	296.84
H4	0.485	0.483	0.482	0.583 ± 0.002	No inhibition	238.17
H5	0.715	0.713	0.712	0.713 ± 0.002	No inhibition	351.58
H6	0.589	0.588	0.585	0.587 ± 0.002	No inhibition	289.45
H7	0.646	0.645	0.649	0.647 ± 0.002	No inhibition	319.03

4.7.5 Standard curve of Hippuric acid

Standard curve of Hippuric acid (HA) was used to determine the ACE activity of the extracts of plant studied. Figure 4.59 illustrated the standard curve of HA while Table 4.44 showed the absorbance of Hippuric acid at 228 nm.

Table 4.43. Absorbance of Hippuric acid (HA)

Concentration of HA (µg/ml)	Absorbance 228 nm			Mean ± S.D
	1	2	3	
1.9	0.116	0.118	0.113	0.116 ± 0.003
3.8	0.328	0.332	0.331	0.330 ± 0.002
7.5	0.462	0.464	0.461	0.462 ± 0.002
15	0.989	0.992	0.987	0.989 ± 0.003
30	2.044	2.046	2.045	2.045 ± 0.001

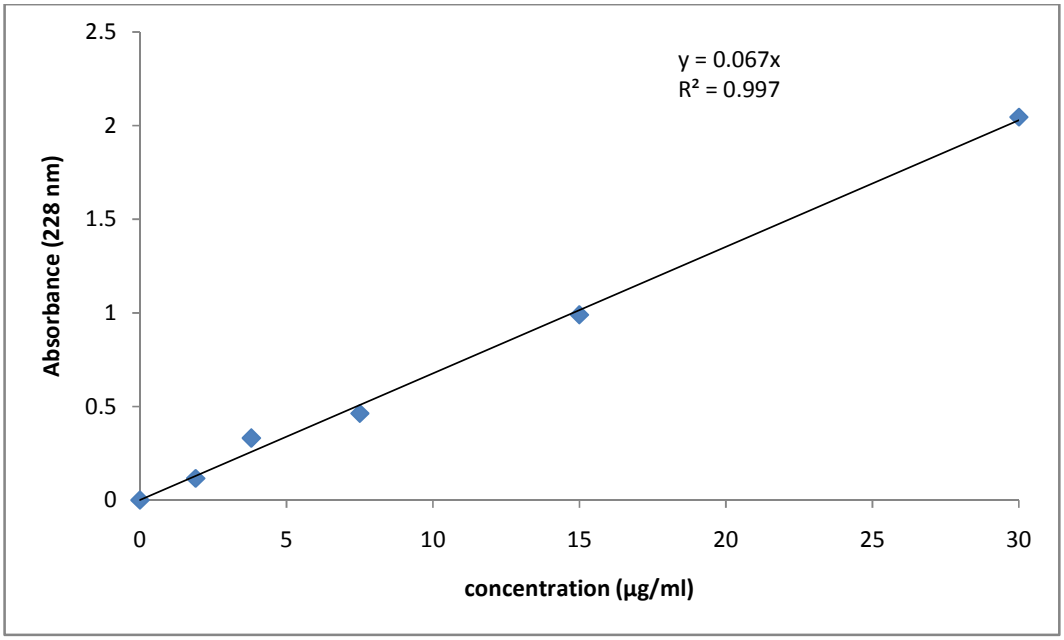

Figure 4.59. Standard curve of Hippuric acid (HA)

4.8 Animal Study

4.8.1 Sub-acute Toxicity Test of water extracts from leaves and rhizome of *Tacca integrifolia* on SHR

Toxicity properties of water extract from leaves and rhizome of *Tacca integrifolia* were analyzed using Sub-acute toxicity test method. The water extracts were administered to the respective groups of female spontaneously hypertensive rats (SHR) accordingly at doses 50mg/kg, 100mg/kg and 500mg/kg via oral gavage for consecutively 28 days. Body weights of each SHR were measured before experiment started, on days 7, 14, 21 and 28 days. SHR were sacrificed at the end of experiment to obtain their blood serums for liver function test and renal function test purposes.

Table 4.44. Body weight measurement of SHR on sub-acute toxicity test of water leaves extract of *Tacca integrifolia*

Animal group	Mean Body weight (g)				
	Day 0	Day 7	Day 14	Day 21	Day 28
Control SHR	171 ± 2	179 ± 10.1	187 ± 6.4	162 ± 8	173 ± 0.6
SHR + Water leaves extract (50mg/kg)	142 ± 1.2	150 ± 2.1	162 ± 2.1	152 ± 5.9	154 ± 1
SHR + Water leaves extract (100mg/kg)	150 ± 4.7	160 ± 2.9	167 ± 2.6	155 ± 9.6	165 ± 7.4
SHR + Water leaves extract (500mg/kg)	150 ± 5.9	152 ± 3.2	162 ± 2.5	147 ± 4.6	159 ± 2.1

Values are expressed as mean ± S.D., n = 3.

Body weight measurements of SHR on sub-acute toxicity test of leaves water extract were statistically analyzed using two-way ANOVA, followed by Tukey test using SPSS 14.0 software. Results were presented as mean ± S.D. From two-way ANOVA analysis showed significant value when $p<0.05$. Post-hoc comparison using Tukey indicated that the means body weight were significant within group and between groups ($p<0.05$).

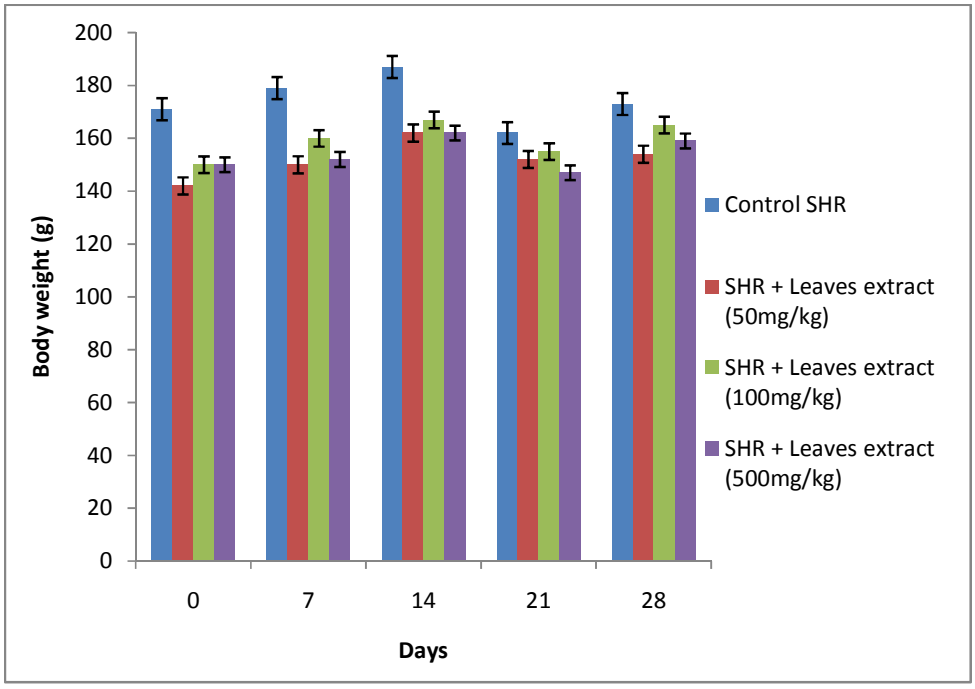

Figure 4.60. Histogram of body weight of SHR sub-acute toxicity test of water leaves extract of *Tacca integrifolia*

Table 4.45. Body weight measurement of SHR on Sub-acute toxicity test of water rhizomes extract of *Tacca integrifolia*

Animal group	Mean Body weight (g)				
	Day 0	Day 7	Day 14	Day 21	Day 28
Control SHR	171 ± 2	179 ± 10.1	187 ± 6.4	162 ± 8	173 ± 0.6
SHR + Rhizome extract (50mg/kg)	144 ± 3.6	155 ± 2.9	161 ± 2.1	165 ± 6.7	171 ± 8
SHR + Rhizome extract (100mg/kg)	151 ± 2.6	161 ± 4	167 ± 4.7	163 ± 4.4	172 ± 4.6
SHR + Rhizome extract (500mg/kg)	145 ± 1	155 ± 2.5	165 ± 1	157 ± 1.7	168 ± 1

Values are expressed as mean ± S.D., n = 3.

Body weight measurements of SHR on sub-acute toxicity test of rhizome water extract were statistically analyzed using two-way ANOVA, followed by Tukey test using SPSS 14.0 software. Results were presented as mean ± S.D. From two-way ANOVA analysis showed significant value when $p<0.05$. Post-hoc comparison using Tukey indicated that the means body weight were significant within group and between groups ($p<0.05$).

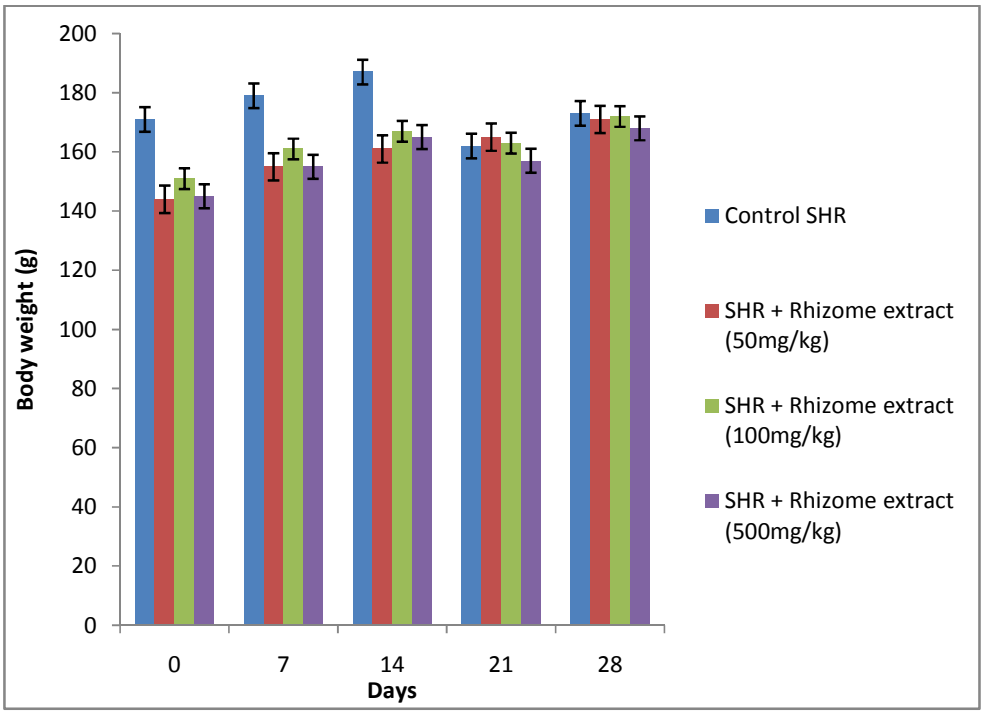

Figure 4.61. Histogram of body weight of SHR sub-acute toxicity test of water rhizome extract of *Tacca integrifolia*

Table 4.46. Liver function test of spontaneously hypertensive rats (SHR) undergoing sub-acute toxicity test

SHR group	Mean Total Protein (g/dL)	Mean ALT (IU/L)	Mean AST (IU/L)
Control SHR	6.7 ± 0.2	53.67 ± 0.6	16.87 ± 0.2
SHR + Leaves water extract (50mg/kg)	6.9 ± 0.1	52.3 ± 0.6	20.27 ± 0.2
SHR + Leaves water extract (100mg/kg)	7.0 ± 0.2	51.3 ± 0.6	15.37 ± 1.1
SHR + Leaves water extract (500mg/kg)	8.8 ± 0.1	68.3 ± 0.6	36.6 ± 1.1
SHR + Rhizome water extract (50mg/kg)	6.8 ± 0.1	53.3 ± 0.6	19.3 ± 1.3
SHR + Rhizome water extract (100mg/kg)	7.1 ± 0.1	52.3 ± 0.6	16.9 ± 0.2
SHR + Rhizome water extract (500mg/kg)	8.9 ± 0.3	68.7 ± 0.6	35.7 ± 0.4

Values are expressed as mean ± S.D., n = 3.

Liver function test of SHR undergoing sub-acute toxicity test of leaves and rhizome water extract were statistically analyzed using one-way ANOVA, followed by Tukey test using SPSS 14.0 software. Results were presented as mean ± S.D. From one-way ANOVA analysis showed significant value when $p<0.05$. Post-hoc comparison using Tukey indicated that the means total protein, AST and ALT were not significant compared to control SHR group.

Table 4.47. Renal function test of spontaneously hypertensive rats (SHR) undergoing sub-acute toxicity test

SHR group	Mean N^+ (mmol/L)	Mean K^+ (mmol/L)	Mean Creatinine (mg/dL)
Control SHR	137.7 ± 0.6	4.7 ± 0.2	0.84 ± 0.04
SHR + Leaves water extract (50mg/kg)	138.7 ± 1.5	4.3 ± 0.2	0.84 ± 0.02
SHR + Leaves water extract (100mg/kg)	137.3 ± 1.2	4.8 ± 0.7	0.87 ± 0.03
SHR + Leaves water extract (500mg/kg)	147.3 ± 1.5	5.4 ± 0.3	1.5 ± 0.1
SHR + Rhizome water extract (50mg/kg)	134 ± 2.6	4.5 ± 0.32	0.84 ± 0.03
SHR + Rhizome water extract (100mg/kg)	137.3 ± 1.53	4.7 ± 0.17	0.87 ± 0.02
SHR + Rhizome water extract (500mg/kg)	149.0 ± 2.5	5.3 ± 0.4	1.57 ± 0.15

Values are expressed as mean ± S.D., n = 3.

Renal function test of SHR on sub-acute toxicity test of leaves and rhizome water extract were statistically analyzed using one-way ANOVA, followed by Tukey test using SPSS 14.0 software. Results were presented as mean ± S.D. From one-way ANOVA analysis showed significant value when $p<0.05$. Post-hoc comparison using Tukey indicated that the means sodium, the means potassium and the means creatinine were not significant compared to control SHR group ($p>0.05$).

No deaths or abnormalities in clinical signs were observed during the 28 days experiment. LD_{50} or lethal dose with 50% mortality was determined as greater than 500mg/kg under the experimental conditions.

4.8.2 Anti-hypertension treatment of water extract from leaves and rhizome of *Tacca integrifolia*

42 Spontaneously Hypertensive Rats (SHR) age between 6 weeks to 7 weeks were group into 7 groups with 6 rats in each group and all SHR were acclimatized for two weeks before experiment started. Group A were orally feed with normal saline as SHR control group (NS), Group B with low dose of Captopril (50mg/kg), Group C with high dose of Captopril (100mg/kg), both as positive reference standard, Group D were fed with low dose of water leaves extract (50mg/kg), Group E fed with high dose of water leaves extract(100mg/kg), Group F fed with low dose of water rhizome extract (50mg/kg)and Group G fed with high dose of water rhizome extract (100mg/kg). Systolic blood pressure and body weight of 42 SHR were measured on day 0, 7, 14, 21 and day 28. Water and food were given *ad libitum* and all 42 SHR were fasting before experiment started and at the end of experiment. SHR were sacrificed using neck dislocation method to collect blood serum for liver and renal function test purposes.

Table 4.48. Mean body weight of Spontaneously Hypertensive Rats (SHR)

Animal Group	Mean body weight (g)				
	Day 0	Day 7	Day 14	Day 21	Day 28
Control Normal (SD)	185 ± 1.5	187 ± 1.3	189 ± 1.7	191 ± 1.7	193 ± 4.0
Control SHR	183 ± 1.6	186 ± 1.6	190 ± 1.6	195 ± 1.2	201 ± 2.8
SHR + Standard Captopril (50mg/kg)	182 ± 2.1	184 ± 2.1	188 ± 2.4	192 ± 3.0	195 ± 2.9
SHR + Standard Captopril (100mg/kg)	181 ± 1.5	185 ± 1.3	188 ± 1.7	192 ± 1.8	196 ± 1.3
SHR + Leaves water extract (50mg/kg)	185 ± 1.2	187 ± 1.1	189 ± 1.0	196 ± 0.5	204 ± 1.7
SHR + Leaves water extract (100mg/kg)	182 ± 1.1	185 ± 1.1	189 ± 1.7	194 ± 1.2	199 ± 0.9
SHR + Rhizome water extract (50mg/kg)	181 ± 1.5	184 ± 1.4	187 ± 0.8	192 ± 1.9	197 ± 1.4
SHR + Rhizome water extract (100mg/kg)	182 ± 1.8	185 ± 1.7	190 ± 1.7	195 ± 2.2	201 ± 2.8

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

Body weight measurement of SHR on treatment using leaves and rhizome water extract were statistically analyzed using two-way ANOVA, followed by Tukey test using SPSS 14.0 software. Results were presented as mean ± S.D. From two-way ANOVA analysis showed significant value when $p<0.05$.

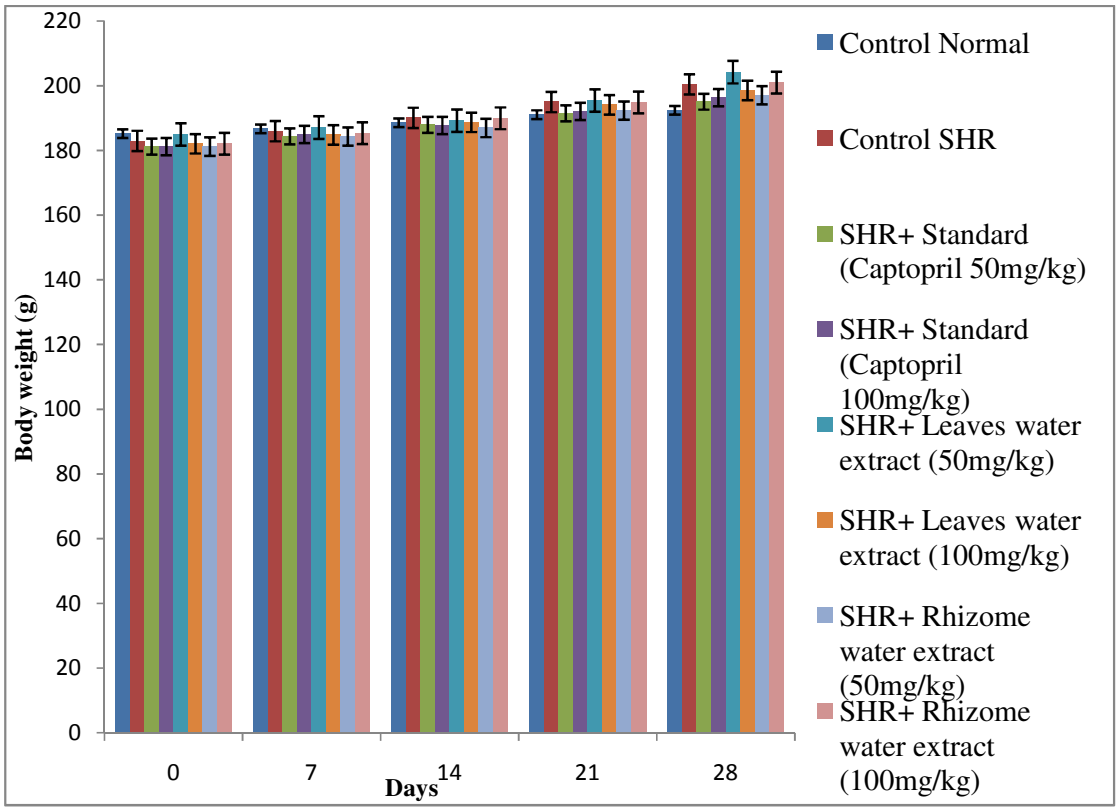

Figure 4.62. Histogram of mean body weight of Spontaneously Hypertensive Rats (SHR)

Table 4.49. Mean Systolic blood pressure of Spontaneously Hypertensive Rats (SHR)

Group	Mean Systolic Blood pressure (mmHg)				
	Day 0	Day 7	Day 14	Day 21	Day 28
Control Normal (SD)	136 ± 2.3	136 ± 2.2	137 ± 2.0	137 ± 1.1	137 ± 1.4
Control SHR	166 ± 2.3	166 ± 1.9	166 ± 1.5	167 ± 1.3	166 ± 0.9
SHR + Standard Captopril (50mg/kg)	167 ± 1.1	165 ± 1.2	163 ± 1.2	160 ±1.6	157 ± 1.4
SHR + Standard Captopril (100mg/kg)	167 ± 1.5	165 ± 1.4	160 ± 2.2	156 ± 1.3	151 ± 1.8
SHR + Leaves water extract (50mg/kg)	162 ± 3.8	161 ± 4.7	159 ± 5	158 ± 4.8	156 ± 4.5
SHR + Leaves water extract (100mg/kg)	166 ± 3.5	162 ± 3.1	158 ± 3.2	153 ± 3.1	148 ± 2.9
SHR + Rhizome water extract (50mg/kg)	165 ± 2.9	164 ± 2.9	162 ± 2.7	160 ± 3.1	158 ± 3.5
SHR + Rhizome water extract (100mg/kg)	164 ± 3.3	160 ± 3.6	156 ± 3.4	151 ± 4.1	147 ± 3.2

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

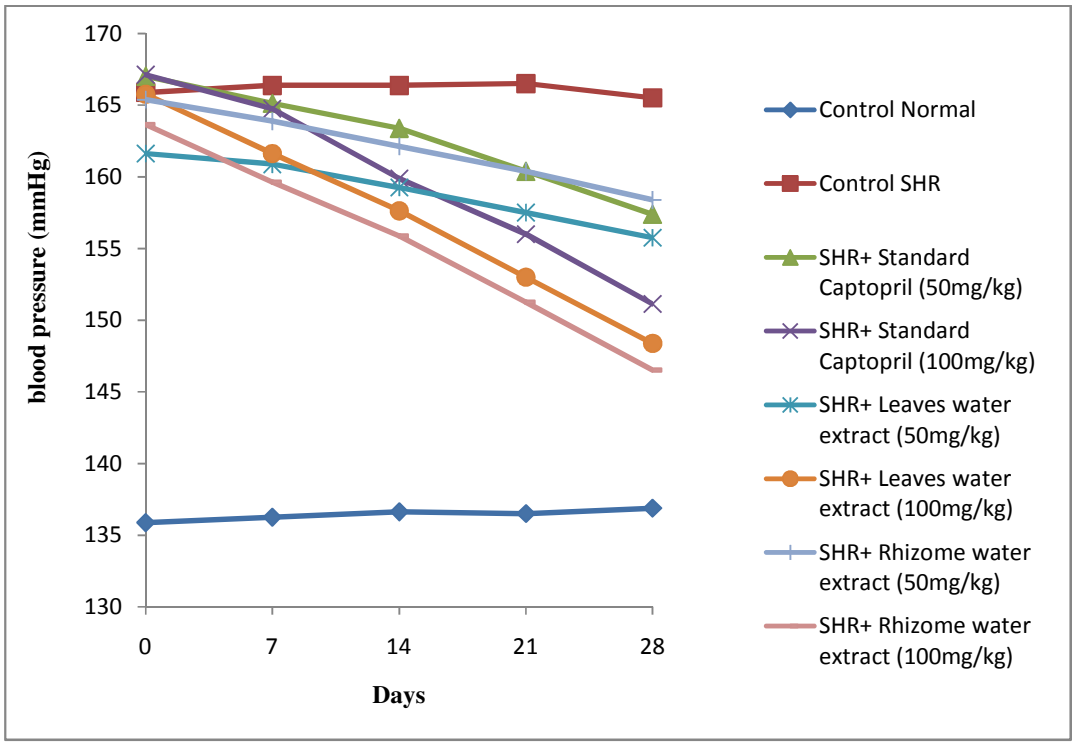

Figure 4.63. Graph of mean systolic blood pressure (mmHg) measurement of SHR

Table 4.50. Liver function test of blood serum collected from Spontaneously Hypertensive Rats (SHR)

Group of SHR	Mean Total Protein (g/L)	Mean ALT (IU/L)	Mean AST (IU/L)
Control Normal (SD)	6.4 ± 0.18	46.56 ± 1.82	14.6 ± 0.27
Control SHR	6.7 ± 0.07	53.5 ± 0.35	16.86 ± 0.05
SHR + Standard Captopril (50mg/kg)	5.8 ± 0.12	46 ± 1.12	16.95 ± 0.26
SHR + Standard Captopril (100mg/kg)	5.7 ± 0.13	45 ± 0.83	17.7 ± 0.08
SHR + Leaves water extract (50mg/kg)	6.86 ± 0.04	53.3 ± 0.43	20.22 ± 0.08
SHR + Leaves water extract (100mg/kg)	7.05 ± 0.06	52.2 ± 0.47	15.37 ± 0.05
SHR + Rhizome water extract (50mg/kg)	6.88 ± 0.09	52.28 ± 0.79	18.6 ± 0.18
SHR + Rhizome water extract (100mg/kg)	7.11 ± 0.08	54.3 ± 1.09	16.79 ± 0.08

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

Liver function test were analyzed using one-way ANOVA followed by Tukey test using SPSS 14.0. Post-hoc comparison using Tukey has indicated that the means difference of Total protein and AST were not significant compared to control group, however mean difference in ALT was significance at $p>0.05$.

Table 4.51. Renal function test of blood serum collected from Spontaneously Hypertensive Rats (SHR)

Group of SHR	Mean N+ (mmol/L)	Mean K+ (mmol/L)	Mean Creatinine (umol/L)
Control Normal (SD)	140.1 ± 2.05	5.1 ± 0.28	0.83 ± 0.02
Control SHR	136 ± 0.74	4.53 ± 0.01	0.83 ± 0.01
SHR + Standard Captopril (50mg/kg)	142 ± 1.25	4.2 ± 0.10	0.85 ± 0.01
SHR + Standard Captopril (100mg/kg)	153 ± 1.31	5.5 ± 0.09	0.94 ± 0.02
SHR + Leaves water extract (50mg/kg)	138.3 ± 0.28	4.4 ± 0.24	0.86 ± 0.02
SHR + Leaves water extract (100mg/kg)	136.6 ± 0.33	4.8 ± 0.19	0.87 ± 0.02
SHR + Rhizome water extract (50mg/kg)	139 ± 1.69	4.45 ± 0.26	0.84 ± 0.01
SHR + Rhizome water extract (100mg/kg)	137 ± 1.3	4.68 ± 0.18	0.87 ± 0.02

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

Renal function test were analyzed using one-way ANOVA followed by Tukey test using SPSS 14.0. Post-hoc comparison using Tukey has indicated that the means difference of

sodium test were significance except for group fed with 100mg/kg of water leaves and water rhizome extract while mean difference significance in potassium test except for group fed with 20mg/kg of captopril 20mg/kg and 100mg/kg water leaves extract. Mean difference in creatinine level were significance compared to control group except for group fed with 100mg/kg of water rhizome extract.

4.9 Antioxidants

4.9.1 DPPH radical scavenging activity

DPPH radical scavenging assay was used to determine the ability of hexane, petroleum ether, chloroform, methanol and water extracts from the leaves and rhizome of *Tacca integrifolia* to scavenge the free radical activity. Percentage inhibitions of DPPH radical were determined using ELISA with absorbance reading at 517 nm. IC_{50} value is the concentration which the extracts inhibit 50% of DPPH radical was obtained from the graph.

i) Ascorbic acid as positive reference standard

In the DPPH radical scavenging assay, ascorbic acid was used as positive reference standard. Table 4.52 showed the scavenging ability of the ascorbic acid on DPPH radicals. At 500ug/ml, the percentage inhibition of ascorbic acid against DPPH radicals was 91.45% and its IC_{50} value was determined at 5.5ug/ml.

Table 4.52. DPPH radical scavenging activity of Ascorbic acid

Concentration of Ascorbic acid (µg/ml)	Absorbance 517nm				Percentage of DPPH inhibition
	1	2	3	Mean ± S.D	
Control	0.735	0.790	0.791	0.772 ± 0.032	-
2.5	0.59	0.571	0.576	0.579 ± 0.01	25
5.0	0.42	0.488	0.492	0.467 ± 0.04	39.55
10.0	0.101	0.092	0.091	0.095 ± 0.006	87.74
12.5	0.065	0.064	0.064	0.064 ± 0.001	91.71
37.5	0.063	0.063	0.063	0.063 ± 0	91.84
125	0.066	0.065	0.065	0.007 ± 0.001	91.58
250	0.066	0.066	0.065	0.066 ± 0.006	91.45
500	0.066	0.066	0.065	0.066 ± 0.006	91.45

ii) DPPH radical scavenging activity of leaves extracts of *Tacca integrifolia*

The DPPH radical scavenging activity of hexane, petroleum ether, chloroform, methanol and water extracts from the leaves of *Tacca integrifolia* showed that methanol extract possess the highest DPPH inhibition at 500µg/ml (93.65%) followed by chloroform extract with 74.18%, water leaves extract with 53.07%, hexane leaves extract with 43.83% and petroleum ether leaves extract possess only 1.3% at the same concentration. Table 4.53 to Table 4.57 showed the percentage of DPPH radical scavenging activity for each extract in eight different concentrations while Figure 4.64 illustrated the graph of inhibition for leaves extract. IC_{50} values for chloroform, methanol and water extract were determined as 350µg/ml, 88µg/ml and 480µg/ml.

Table 4.53. DPPH radical scavenging activity of leaves hexane extract from *Tacca integrifolia*

Concentration of sample (µg/ml)	Absorbance 517nm				Percentage of DPPH inhibition
	1	2	3	Mean ± S.D	
2.5	0.771	0.805	0.810	0.795 ± 0.02	No inhibition
5.0	0.848	0.840	0.836	0.841 ± 0.006	No inhibition
10.0	1.021	1.025	1.028	1.025 ± 0.004	No inhibition
12.5	0.807	0.810	0.813	0.81 ± 0.003	No inhibition
37.5	0.817	0.819	0.831	0.822 ± 0.008	No inhibition
125	0.698	0.705	0.703	0.702 ± 0.004	9.07
250	0.504	0.507	0.509	0.507 ± 0.003	34.37
500	0.436	0.438	0.427	0.434 ± 0.006	43.83

Table 4.54. DPPH radical scavenging activity of leaves petroleum ether extract from *Tacca integrifolia*

Concentration of sample (µg/ml)	Absorbance 517nm				Percentage of DPPH inhibition
	1	2	3	Mean ± S.D	
2.5	0.851	0.878	0.892	0.874 ± 0.02	No inhibition
5.0	0.840	0.822	0.829	0.83 ± 0.009	No inhibition
10.0	0.832	0.832	0.824	0.829 ± 0.005	No inhibition
12.5	0.834	0.829	0.836	0.833 ± 0.004	No inhibition
37.5	0.841	0.844	0.851	0.845 ± 0.005	No inhibition
125	0.832	0.847	0.856	0.845 ± 0.012	No inhibition
250	0.810	0.813	0.810	0.811 ± 0.002	No inhibition
500	0.766	0.763	0.757	0.762 ± 0.005	1.3

Table 4.55. DPPH radical scavenging activity of leaves chloroform extract of *Tacca integrifolia*

Concentration of sample (µg/ml)	Absorbance 517nm				Percentage of DPPH inhibition
	1	2	3	Mean ± S.D	
2.5	0.786	0.855	0.849	0.83 ± 0.038	No inhibition
5.0	0.934	1.033	0.968	0.978 ± 0.05	No inhibition
10.0	0.861	0.942	0.879	0.894 ± 0.043	No inhibition
12.5	0.824	0.830	0.829	0.828 ± 0.003	No inhibition
37.5	0.811	0.836	0.830	0.826 ± 0.013	No inhibition
125	0.687	0.684	0.697	0.689 ± 0.007	10.71
250	0.518	0.517	0.522	0.519 ± 0.003	32.77
500	0.193	0.200	0.205	0.199 ± 0.006	74.18

Table 4.56. DPPH radical scavenging activity of leaves methanol extract from *Tacca integrifolia*

Concentration of sample (µg/ml)	Absorbance 517nm				Percentage of DPPH inhibition
	1	2	3	Mean ± S.D	
2.5	0.852	0.875	0.898	0.875 ± 0.023	No inhibition
5.0	0.779	0.819	1.227	0.942 ± 0.248	No inhibition
10.0	0.892	0.900	0.956	0.916 ± 0.035	No inhibition
12.5	0.870	0.984	0.882	0.912 ± 0.063	No inhibition
37.5	0.712	0.725	0.736	0.724 ± 0.012	6.17
125	0.196	0.192	0.191	0.193 ± 0.003	75
250	0.051	0.048	0.049	0.049 ± 0.002	93.61
500	0.048	0.049	0.050	0.049 ± 0.001	93.65

Table 4.57. DPPH radical scavenging activity of leaves water extract from *Tacca integrifolia*

Concentration of sample (µg/ml)	Absorbance 517nm				Percentage of DPPH inhibition
	1	2	3	Mean ± S.D	
2.5	0.931	0.905	0.910	0.915 ± 0.014	No inhibition
5.0	0.898	0.901	0.910	0.903 ± 0.006	No inhibition
10.0	0.927	0.942	0.924	0.931 ± 0.01	No inhibition
12.5	0.915	0.941	0.944	0.93 ± 0.016	No inhibition
37.5	0.917	0.924	0.939	0.927 ± 0.011	No inhibition
125	0.867	0.869	0.846	0.861 ± 0.013	No inhibition
250	0.708	0.701	0.706	0.705 ± 0.004	8.68
500	0.370	0.357	0.360	0.362 ± 0.007	53.07

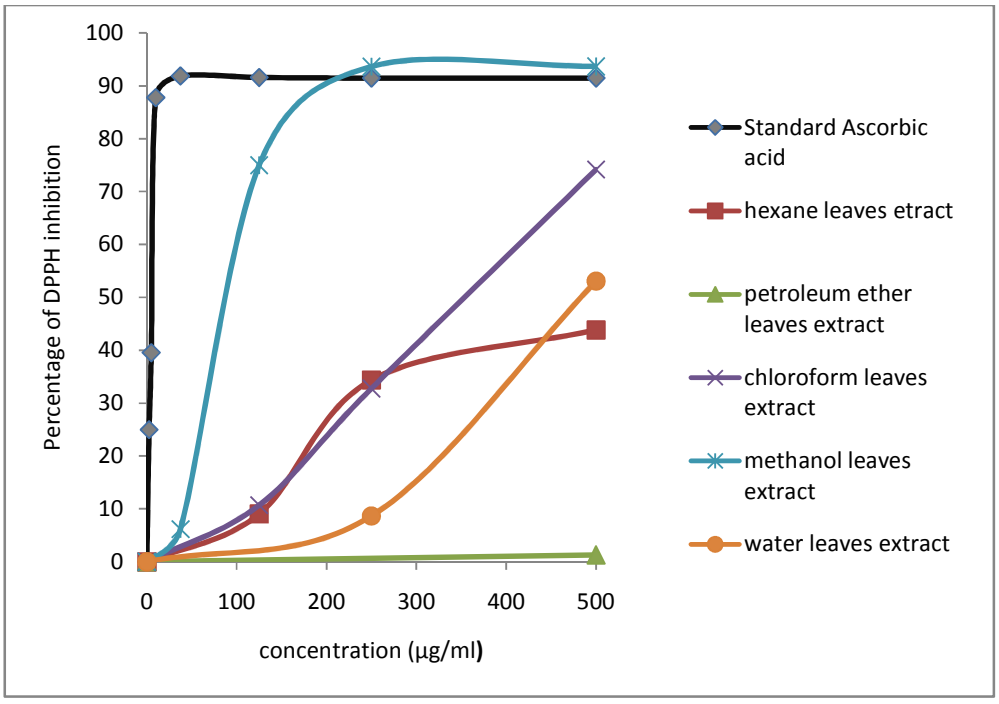

Figure 4.64. DPPH inhibition of leaves extracts from *Tacca integrifolia*

iii) DPPH radical scavenging activity from rhizome extracts of *Tacca integrifolia*

DPPH scavenging effect of hexane, petroleum ether, chloroform, methanol and water extracts from the rhizome of *Tacca integrifolia* were showed in Table 4.58 to 4.62. Methanol extract possess 41.71% at concentration of 500µg/ml while water extract and chloroform extract showed 18.96% and 5.66% respectively. No DPPH inhibition showed in hexane and petroleum ether.

Table 4.58. DPPH radical scavenging activity of rhizome hexane extract from *Tacca integrifolia*

Concentration of sample (µg/ml)	Absorbance 517nm				Percentage of DPPH inhibition
	1	2	3	Mean ± S.D	
2.5	0.913	0.935	0.940	0.929 ± 0.014	No inhibition
5.0	0.929	0.936	0.919	0.928 ± 0.009	No inhibition
10.0	0.917	0.930	0.966	0.938 ± 0.003	No inhibition
12.5	0.890	0.945	0.976	0.937 ± 0.044	No inhibition
37.5	0.902	0.928	0.942	0.924 ± 0.02	No inhibition
125	0.903	0.923	0.957	0.928 ± 0.027	No inhibition
250	0.889	0.917	0.935	0.914 ± 0.023	No inhibition
500	0.854	0.849	0.85	0.851 ± 0.003	No inhibition

Table 4.59. DPPH radical scavenging activity of rhizome petroleum ether extract of *Tacca integrifolia*

Concentration of sample (µg/ml)	Absorbance 517nm				Percentage of DPPH inhibition
	1	2	3	Mean ± S.D	
2.5	0.884	0.923	0.929	0.912 ± 0.024	No inhibition
5.0	1.337	1.236	1.194	1.256 ± 0.074	No inhibition
10.0	0.971	0.985	1.032	0.996 ± 0.032	No inhibition
12.5	0.954	0.949	0.957	0.953 ± 0.004	No inhibition
37.5	0.941	0.946	0.950	0.946 ± 0.005	No inhibition
125	1.259	1.264	0.837	1.12 ± 0.245	No inhibition
250	0.906	0.949	0.945	0.933 ± 0.024	No inhibition
500	1.124	1.160	1.191	1.16 ± 0.034	No inhibition

Table 4.60. DPPH radical scavenging activity of rhizome chloroform extract from *Tacca integrifolia*

Concentration of sample (µg/ml)	Absorbance 517nm				Percentage of DPPH inhibition
	1	2	3	Mean ± S.D	
2.5	1.050	0.998	0.942	0.997 ± 0.054	No inhibition
5.0	0.915	0.946	0.921	0.927 ± 0.016	No inhibition
10.0	0.948	0.976	0.947	0.957 ± 0.016	No inhibition
12.5	0.943	0.975	0.964	0.961 ± 0.002	No inhibition
37.5	0.918	0.950	1.150	1.006 ± 0.126	No inhibition
125	0.873	0.888	0.876	0.879 ± 0.008	No inhibition
250	0.816	0.825	0.805	0.815 ± 0.01	No inhibition
500	0.724	0.732	0.729	0.728 ± 0.004	5.66

Table 4.61. DPPH radical scavenging activity of rhizome methanol extract from *Tacca integrifolia*

Concentration of sample (µg/ml)	Absorbance 517nm				Percentage of DPPH inhibition
	1	2	3	Mean ± S.D	
2.5	0.722	0.906	0.829	0.819 ± 0.092	No inhibition
5.0	0.893	0.890	0.889	0.890 ± 0.002	No inhibition
10.0	0.898	0.907	0.905	0.900 ± 0.005	No inhibition
12.5	0.895	0.893	0.885	0.891 ± 0.005	No inhibition
37.5	0.873	0.866	0.874	0.871 ± 0.004	No inhibition
125	0.770	0.768	0.768	0.769 ± 0.001	0.43
250	0.655	0.654	0.652	0.654 ± 0.002	15.33
500	0.456	0.446	0.448	0.450 ± 0.005	41.71

Table 4.62. DPPH radical scavenging activity of rhizome water extract from *Tacca integrifolia*

Concentration of sample (µg/ml)	Absorbance 517nm				Percentage of DPPH inhibition
	1	2	3	Mean ± S.D	
2.5	1.614	1.346	1.613	1.524 ± 0.154	No inhibition
5.0	0.908	0.892	0.879	0.893 ± 0.015	No inhibition
10.0	0.902	0.904	0.904	0.903 ± 0.001	No inhibition
12.5	0.887	0.898	0.878	0.890 ± 0.01	No inhibition
37.5	0.897	0.905	0.896	0.899 ± 0.005	No inhibition
125	0.903	0.906	0.904	0.904 ± 0.002	No inhibition
250	0.796	0.786	0.786	0.789 ± 0.006	No inhibition
500	0.663	0.659	0.555	0.626 ± 0.06	18.96

4.9.2 Ferric Reducing Power Assay (FRAP)

Antioxidant studies of extract from leaves and rhizome of *Tacca integrifolia* was continued using ferric reducing power assay. This assay was carrying out in triplicates and absorbances reading were taken at 700 nm using spectrophotometer.

i) Butylated Hydroxyanisole (BHA) as positive reference standard.

In Ferric reducing power assay, a synthetic antioxidant, butylated hydroxyanisole (BHA) was used as positive reference standard. Table 4.63 showed the absorbance of reducing power assay of BHA at 700 nm.

Table 4.63. Reducing power of butylated hydroxyanisole (BHA)

Concentration (µg/ml)	Absorbance (700nm)			Mean ± S.D
	1	2	3	
62.5	0.800	0.803	0.744	0.782 ± 0.03
125	1.205	1.190	1.138	1.178 ± 0.04
250	1.787	1.860	1.565	1.737 ± 0.15
500	3.135	3.215	3.215	3.188 ± 0.05
1000	3.215	3.913	3.913	3.680 ± 0.4

ii) Reducing power of leaves extract of *Tacca integrifolia*

Ferric reducing power of hexane, petroleum ether, chloroform, methanol and water extracts from the leaves of *Tacca integrifolia* were determined at five concentration at 62.5 µg/ml, 125 µg/ml, 250 µg/ml, 500 µg/ml and 1000 µg/ml. Table 4.64 to Table 4.68 showed the absorbance reading taken at 700 nm and Figure 4.65 illustrated the curve of reducing power of extract from the leaves of *Tacca integrifolia*.

Table 4.64. Reducing power of hexane leaves extract from *Tacca integrifolia*

Concentration (µg/ml)	Absorbance (700nm)			Mean ± S.D
	1	2	3	
62.5	0.455	0.480	0.490	0.475 ± 0.18
125	0.507	0.512	0.512	0.510 ± 0.003
250	0.564	0.541	0.544	0.550 ± 0.013
500	0.588	0.553	0.656	0.599 ± 0.052
1000	0.806	0.769	1.281	0.952 ± 0.29

Table 4.65. Reducing power of petroleum ether leaves extract from *Tacca integrifolia*

Concentration (µg/ml)	Absorbance (700nm)			Mean ± S.D
	1	2	3	
62.5	0.514	0.557	0.526	0.532 ± 0.02
125	0.570	0.505	0.515	0.530 ± 0.035
250	0.593	0.568	0.588	0.583 ± 0.013
500	0.549	0.498	0.511	0.519 ± 0.027
1000	0.557	0.580	0.547	0.561 ± 0.017

Table 4.66. Reducing power of chloroform leaves extract from *Tacca integrifolia*

Concentration (µg/ml)	Absorbance (700nm)			Mean ± S.D
	1	2	3	
62.5	0.504	0.503	0.539	0.515 ± 0.02
125	0.548	0.551	0.530	0.543 ± 0.01
250	0.675	0.693	0.645	0.671 ± 0.02
500	0.563	0.597	0.521	0.560 ± 0.04
1000	0.960	0.988	1.050	0.999 ± 0.05

Table 4.67. Reducing power of methanol leaves extract from *Tacca integrifolia*

Concentration (µg/ml)	Absorbance (700nm)			Mean ± S.D
	1	2	3	
62.5	1.023	0.935	1.170	1.043 ± 0.12
125	0.860	0.756	0.744	0.787 ± 0.06
250	0.535	0.533	0.532	0.533 ± 0.002
500	0.603	0.613	0.627	0.614 ± 0.01
1000	0.819	0.775	0.806	0.8 ± 0.02

Table 4.68. Reducing power of water leaves extract from *Tacca integrifolia*

Concentration (µg/ml)	Absorbance (700nm)			Mean ± S.D
	1	2	3	
62.5	0.490	0.553	0.468	0.503 ± 0.04
125	0.481	0.481	0.488	0.483 ± 0.004
250	0.534	0.528	0.579	0.547 ± 0.03
500	0.586	0.610	0.654	0.617 ± 0.03
1000	0.715	0.726	0.741	0.727 ± 0.01

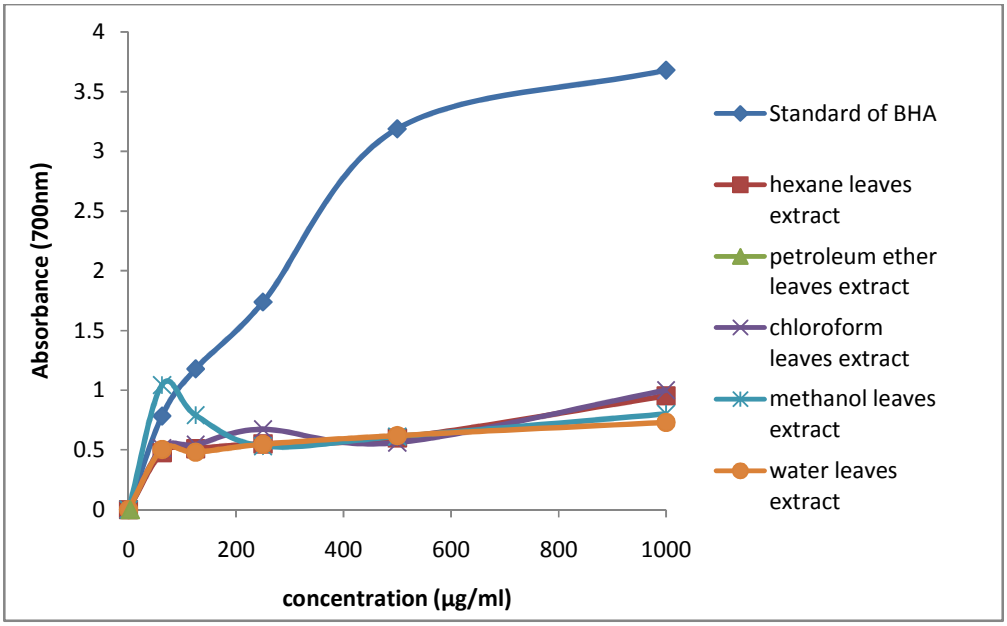

Figure 4.65. Ferric Reducing Power Assay of leaves extract from *Tacca integrifolia*

Analysis of ferric reducing power assay using Two-Way ANOVA has showed the mean difference is significance between all extract with standard of BHA at $p<0.05$. However, the mean difference between hexane leaves extract to petroleum ether leaves extract, to chloroform leaves extract and to water leaves extract, and chloroform leaves extract to methanol leaves extract are not significant at $p<0.05$. Comparison using Tukey test also has showed non-significant when petroleum ether leaves extract and chloroform leaves extract were compared to water leaves extract. The mean difference was significance within groups in difference concentration when $p<0.05$ accept for sample with concentration 62.5µg/ml and 125µg/ml.

iii) Reducing power of rhizome extract of *Tacca integrifolia*

Determination of reducing power of hexane, petroleum ether, chloroform, methanol and water extracts from the rhizome of *Tacca integrifolia* were done at various concentration at 62.5, 125, 250, 500 and 1000 µg/ml. Table 4.69 to Table 4.73 showed the absorbance reading taken at 700 nm.

Table 4.69. Reducing power of hexane rhizome extract from *Tacca integrifolia*

Concentration (µg/ml)	Absorbance (700nm)			Mean ± S.D
	1	2	3	
62.5	0.708	0.726	0.745	0.726 ± 0.02
125	0.761	0.765	0.711	0.746 ± 0.03
250	0.819	0.817	0.803	0.813 ± 0.01
500	0.833	0.824	0.890	0.849 ± 0.04
1000	0.957	1.023	1.008	0.996 ± 0.03

Table 4.70. Reducing power of petroleum ether rhizome extract from *Tacca integrifolia*

Concentration (µg/ml)	Absorbance (700nm)			Mean ± S.D
	1	2	3	
62.5	0.810	0.896	0.736	0.814 ± 0.08
125	0.744	0.791	0.761	0.765 ± 0.02
250	0.841	0.828	0.906	0.858 ± 0.04
500	0.885	0.805	0.840	0.843 ± 0.04
1000	0.959	0.963	1.036	0.986 ± 0.04

Table 4.71. Reducing power of chloroform rhizome extract from *Tacca integrifolia*

Concentration (µg/ml)	Absorbance (700nm)			Mean ± S.D
	1	2	3	
62.5	0.882	0.879	0.872	0.878 ± 0.01
125	0.936	0.899	0.848	0.894 ± 0.04
250	1.035	1.134	0.939	1.036 ± 0.1
500	1.012	1.129	0.991	1.044 ± 0.07
1000	1.183	1.170	1.157	1.17 ± 0.013

Table 4.72. Reducing power of methanol rhizome extract from *Tacca integrifolia*

Concentration (µg/ml)	Absorbance (700nm)			Mean ± S.D
	1	2	3	
62.5	0.834	0.839	0.962	0.878 ± 0.07
125	1.011	1.119	0.920	1.017 ± 0.1
250	1.032	1.185	1.307	1.175 ± 0.14
500	0.975	0.957	0.913	0.948 ± 0.03
1000	1.176	1.065	1.170	1.137 ± 0.06

Table 4.73. Reducing power of water rhizome extract from *Tacca integrifolia*

Concentration (µg/ml)	Absorbance (700nm)			Mean ± S.D
	1	2	3	
62.5	0.729	0.783	0.728	0.747 ± 0.03
125	0.729	0.710	0.752	0.730 ± 0.02
250	0.862	0.872	0.841	0.858 ± 0.02
500	0.839	0.818	0.880	0.846 ± 0.03
1000	0.865	0.963	0.878	0.902 ± 0.05

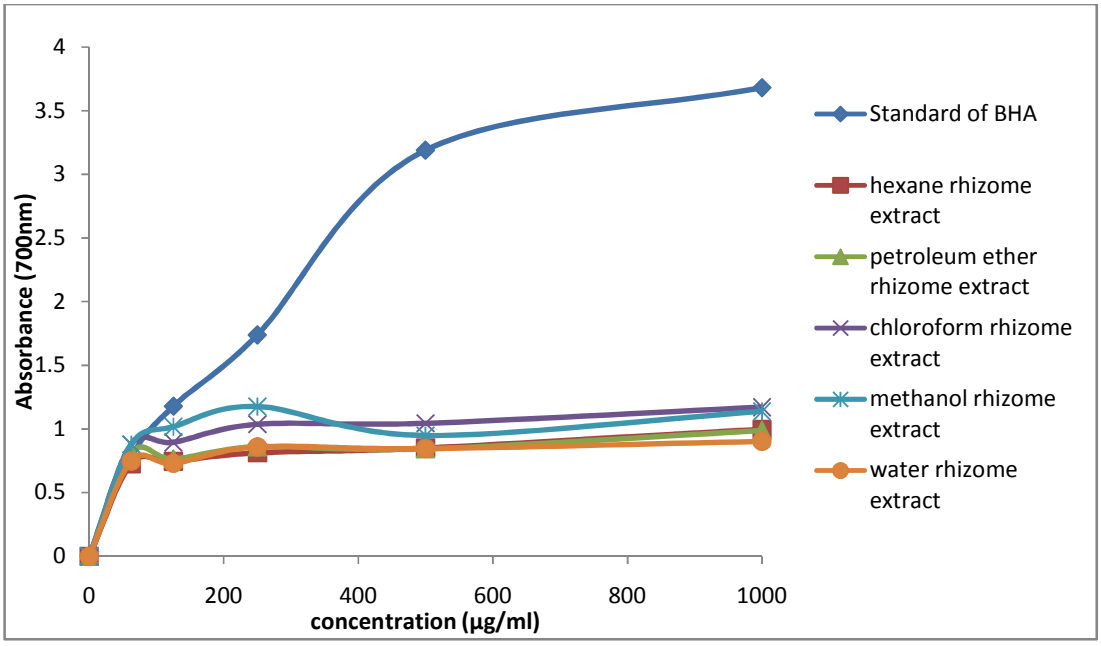

Figure 4.66. Graph of Ferric Reducing Power Assay of rhizome extract from *Tacca Integrifolia*

Analysis of ferric reducing power assay using Two-Way ANOVA has showed the mean difference is significance between all extract with standard of BHA at $p<0.05$. However, the mean difference between hexane rhizome extract to petroleum ether rhizome extract, and to water rhizome extract, and between chloroform rhizome extract to methanol rhizome extract are not significant at $p<0.05$. Comparison using Tukey test also has showed non-significant in the mean difference of concentration 62.5µg/ml and 125µg/ml while other had showed significance at $p<0.05$.

4.9.3 Metal Chelating Power Assay

The antioxidant activity of hexane, petroleum ether, chloroform, methanol and water from the leaves and rhizome of *Tacca integrifolia* were determined using metal chelating assay that was based on the chelating effects of Fr^{2+} ions by ferrozine reagent. Assay was carried out in triplicates and the absorbance reading was read using ELISA.

i) Ethylenediaminetetraacetic acid (EDTA) as standard

Ethylenediaminetetraacetic acid (EDTA) was used as positive reference standard in Metal Chelating activity. Five different concentration of EDTA were prepared at 1mg/ml, 2mg/ml, 3mg/ml, 4mg/ml and 5mg/ml and was added with ferrozine and $FeCl_2$ followed by absorbance reading at 562nm ELISA. Reading was carried out in triplicate to obtain mean absorbance, thus determined the percentage of inhibition.

Table 4.74. Metal Chelating activities of EDTA

EDTA concentration (mg/ml)	Absorbance 562 nm			Mean ± SD	Percentage of Metal Chelating Inhibition
	1	2	3		
Control	1.141	1.116	1.158	1.138 ± 0.02	-
1	0.884	0.896	0.897	0.892 ± 0.007	21.59
2	0.451	0.444	0.446	0.447 ± 0.004	60.72
3	0.302	0.306	0.299	0.302 ± 0.004	73.64
4	0.271	0.268	0.267	0.269 ± 0.002	76.39
5	0.190	0.190	0.191	0.190 ± 0.001	83.28

ii) Metal chelating activity of leaves extracts of *Tacca integrifolia*

Metal Chelating Activity of extracts from leaves was done using five different concentrations for each sample. Absorbance was read in triplicates at 562nm using ELISA. Mean absorbance were calculated and percentage of inhibition were determined as in Table 4.75 to Table 4.79 while graph percentage of inhibition against concentration was plotted as in Figure 4.67.

Table 4.75. Metal Chelating activities of hexane leaves extracts from *Tacca integrifolia*

Sample concentration (mg/ml)	Absorbance 562 nm			Mean ± SD	Percentage of Metal Chelating Inhibition
	1	2	3		
1	0.993	0.957	1.011	0.987 ± 0.027	13.27
2	0.570	0.491	0.570	0.544 ± 0.046	52.23
3	0.500	0.407	0.511	0.473 ± 0.057	58.46
4	0.342	0.347	0.363	0.351 ± 0.01	69.19
5	0.324	0.306	0.300	0.310 ± 0.012	72.76

Table 4.76. Metal Chelating activities of petroleum ether leaves extracts from *Tacca integrifolia*

Sample concentration (mg/ml)	Absorbance 562 nm			Mean ± SD	Percentage of Metal Chelating Inhibition
	1	2	3		
1	1.121	1.125	1.122	1.123 ± 0.002	1.35
2	1.136	1.148	1.054	1.110 ± 0.05	2.23
3	0.946	0.983	0.845	0.925 ± 0.07	18.75
4	0.854	0.861	0.859	0.858 ± 0.004	24.6
5	0.699	0.702	0.703	0.701 ± 0.002	38.37

Table 4.77. Metal Chelating activities of chloroform leaves extracts from *Tacca integrifolia*

Sample concentration (mg/ml)	Absorbance 562 nm			Mean ± SD	Percentage of metal chelating inhibition
	1	2	3		
1	1.123	1.097	1.086	1.102 ± 0.019	3.16
2	0.523	0.780	0.351	0.551 ± 0.22	51.55
3	0.463	0.382	0.390	0.411 ± 0.044	63.83
4	0.260	0.261	0.161	0.227 ± 0.06	80.02
5	0.055	0.073	0.115	0.081 ± 0.03	92.88

Table 4.78. Metal Chelating activities of methanol leaves extracts of *Tacca integrifolia*

Sample concentration (mg/ml)	Absorbance 562 nm			Mean ± SD	Percentage of metal chelating inhibition
	1	2	3		
1	0.872	0.868	0.866	0.869 ± 0.003	23.67
2	0.661	0.662	0.661	0.661 ± 0.001	41.89
3	0.441	0.444	0.442	0.442 ± 0.002	61.13
4	0.386	0.388	0.387	0.387 ± 0.001	65.99
5	0.334	0.332	0.335	0.334 ± 0.002	70.68

Table 4.79. Metal Chelating activities of water leaves extracts from *Tacca integrifolia*

Sample concentration (mg/ml)	Absorbance 562 nm			Mean ± SD	Percentage of metal chelating inhibition
	1	2	3		
1	0.681	0.685	0.682	0.683 ± 0.002	40.01
2	0.640	0.642	0.638	0.640 ± 0.002	43.76
3	0.610	0.603	0.605	0.606 ± 0.004	46.75
4	0.583	0.570	0.583	0.579 ± 0.008	49.15
5	0.473	0.473	0.477	0.474 ± 0.002	58.32

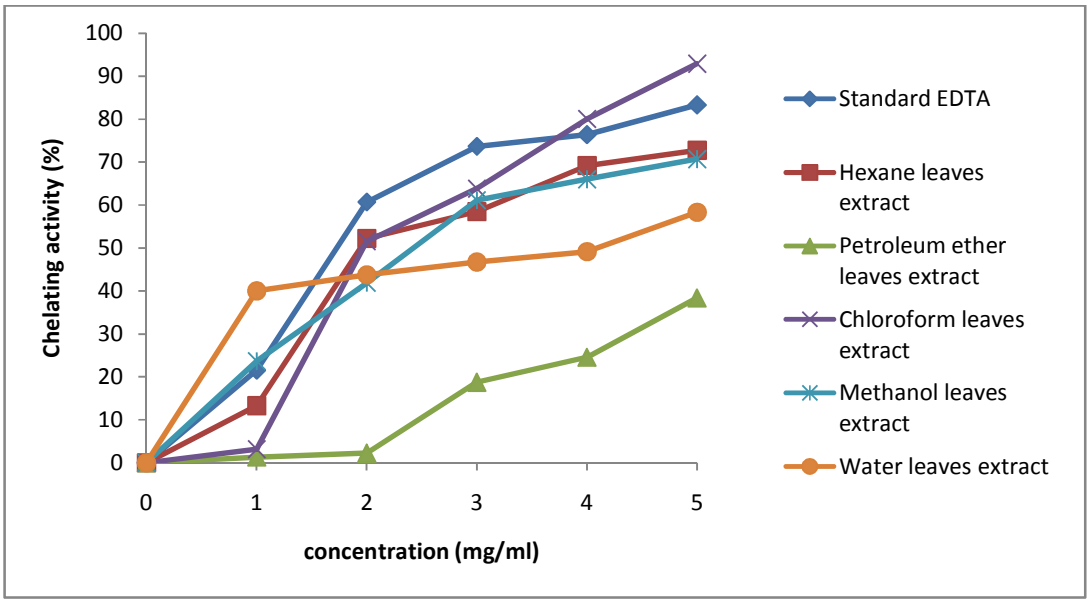

Figure 4.67. Metal Chelating activities of leaves extract from *Tacca integrifolia*

Metal chelating activity of extract from leaves of *Tacca integrifolia* showed that IC_{50} of hexane extract, chloroform extract, methanol extract and water extract was determined at 1.92mg/ml, 1.98mg/ml, 2.4mg/ml and 4.1mg/ml respectively. IC_{50} obtained was higher compared to IC_{50} of standard of EDTA (1.7mg/ml).

iii) Metal chelating activity of rhizome extracts of *Tacca integrifolia*

Metal Chelating Activity of rhizome extracts was determined in five different concentrations at 1mg/ml, 2mg/ml, 3mg/ml, 4mg/ml and 5mg/ml. Absorbance 562nm was read using ELISA and reading was carried out in triplicate. Mean absorbance were calculated and percentage of inhibition were determine as in Table 4.80 to Table 4.84.

Table 4.80. Metal Chelating activities of hexane rhizome extracts from *Tacca integrifolia*

Sample concentration (mg/ml)	Absorbance 562 nm			Mean ± SD	Percentage of metal chelating inhibition
	1	2	3		
1	1.065	1.066	1.064	1.065 ± 0.001	6.41
2	0.762	0.761	0.762	0.762 ± 0.001	33.07
3	0.703	0.701	0.702	0.702 ± 0.001	38.31
4	0.587	0.599	0.603	0.596 ± 0.008	47.6
5	0.578	0.576	0.579	0.578 ± 0.002	49.24

Table 4.81. Metal Chelating activities of petroleum ether rhizome extracts from *Tacca integrifolia*

Sample concentration (mg/ml)	Absorbance 562 nm			Mean ± SD	Percentage of metal chelating inhibition
	1	2	3		
1	1.031	1.032	1.031	1.03 ± 0.001	9.37
2	0.899	0.896	0.898	0.898 ± 0.002	21.12
3	0.864	0.866	0.865	0.865 ± 0.001	23.99
4	0.807	0.803	0.804	0.805 ± 0.002	29.29
5	0.566	0.564	0.568	0.566 ± 0.002	50.26

Table 4.82. Metal Chelating activities of chloroform rhizome extracts from *Tacca integrifolia*

Sample concentration (mg/ml)	Absorbance 562 nm			Mean ± SD	Percentage of metal chelating inhibition
	1	2	3		
1	0.708	0.708	0.709	0.708 ± 0.001	37.76
2	0.632	0.633	0.634	0.633 ± 0.001	44.38
3	0.621	0.619	0.621	0.620 ± 0.001	45.49
4	0.606	0.607	0.606	0.606 ± 0.001	46.72
5	0.561	0.56	0.559	0.560 ± 0.001	50.79

Table 4.83. Metal Chelating activities of methanol rhizome extracts from *Tacca integrifolia*

Sample concentration (mg/ml)	Absorbance 562 nm			Mean ± SD	Percentage of metal chelating inhibition
	1	2	3		
1	1.103	1.102	1.103	1.103 ± 0.001	31
2	0.968	0.888	0.970	0.942 ± 0.05	17.22
3	0.751	0.741	0.757	0.750 ± 0.008	34.12
4	0.476	0.451	0.452	0.460 ± 0.01	59.61
5	0.433	0.460	0.471	0.455 ± 0.02	60.05

Table 4.84. Metal Chelating activities of water rhizome extracts from *Tacca integrifolia*

Sample concentration (mg/ml)	Absorbance 562 nm			Mean ± SD	Percentage of metal chelating inhibition
	1	2	3		
1	1.117	1.091	1.092	1.1 ± 0.01	3.34
2	0.982	0.838	0.841	0.887 ± 0.08	22.06
3	0.611	0.612	0.612	0.612 ± 0.001	46.25
4	0.422	0.446	0.443	0.437 ± 0.01	61.6
5	0.372	0.373	0.372	0.372 ± 0.001	67.28

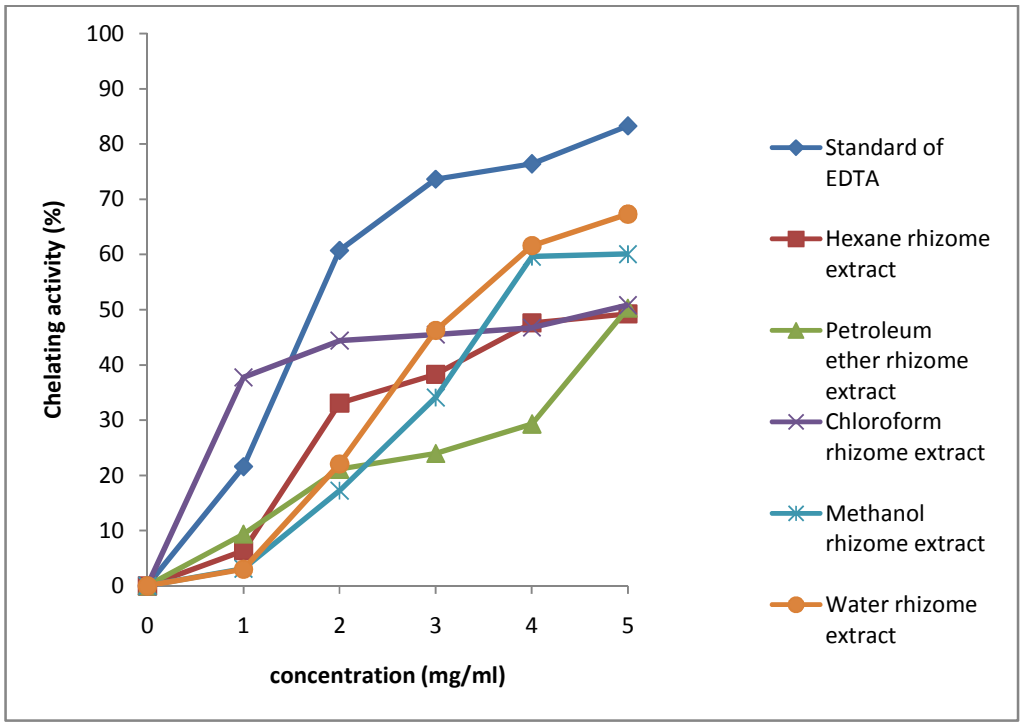

Figure 4.68. Metal Chelating activities of rhizome extracts from *Tacca integrifolia*

Metal chelating activity of extract from rhizome of *Tacca integrifolia* showed that IC_{50} of petroleum ether extract, chloroform extract, methanol extract and water extract were determined at 5mg/ml, 4.7mg/ml, 3.6mg/ml and 3.2 mg/ml respectively.

4.10 Brine Shrimp Lethality Assay (BSLA)

LC_{50} are the value of lethal concentration of the sample that can cause lethality to the subject exposed. The higher LC_{50} value meant the toxicity of the extracts was lower and vice versa. The highest LC_{50} value was the water extracts from rhizome of *Tacca integrifolia* which was 22981µg/ml, while hexane extract from rhizome of *Tacca integrifolia* gives the lowest L_{C50} value with 100µg/ml. This meant that 100 µg/ml was needed to inhibit the 50% population of the brine shrimp. Table 4.85and Table 4.86 showed the number of dead shrimp that exposed to the extracts from leaves and rhizome of *Tacca integrifolia*.

Table 4.85. Number of dead shrimp in BSLA of leaves extract from *Tacca integrifolia*

Sample (µg/ml)	Total number of shrimp					Number of dead shrimp				
	HLE	PLE	CLE	MLE	WLE	HLE	PLE	CLE	MLE	WLE
10	10	10	10	10	10	3	3	2	1	1
100	10	10	10	10	10	3	4	3	2	1
1000	10	10	10	10	10	8	5	4	4	4

Table 4.86. Number of dead shrimp in BSLA of rhizomes extract from *Tacca integrifolia*

Sample (µg/ml)	Total number of shrimp					Number of dead shrimp				
	HLE	PLE	CLE	MLE	WLE	HLE	PLE	CLE	MLE	WLE
10	10	10	10	10	10	4	3	2	2	1
100	10	10	10	10	10	5	4	3	3	2
1000	10	10	10	10	10	6	6	5	4	3

4.10.1 BSLA of extract from leaves of *Tacca integrifolia*

BSLA analyses of leaves extract were present as in Table 4.89 to Table 4.93. Chloroform extract from leaves showed the highest LC_{50} value (6921µg/ml), followed by water extract with 4378µg/ml, methanol extract (3323µg/ml), petroleum ether extract (975µg/ml) and hexane extract showed the lowest LC_{50} value which is 135µg/ml.

Table 4.87. Probit analysis of hexane extract from leaves of *Tacca integrifolia*

Sample (µg/ml)	Log10 (conc. Sample)	Total number of shrimp	Number of dead	Percentage mortality	LC_{50} (µg/ml)	95% confidence
10	1	10	3	30	135	8.35-12645.69
100	2	10	3	30		
1000	3	10	8	80		

Table 4.88. Probit analysis of petroleum ether extract from leaves of *Tacca integrifolia*

Sample (µg/ml)	Log10 (conc. Sample)	Total number of shrimp	Number of dead	Percentage mortality	LC_{50} (µg/ml)	95% confidence
10	1	10	3	30	975	0-infinity
100	2	10	4	40		
1000	3	10	5	50		

Table 4.89. Probit analysis of chloroform extract from leaves of *Tacca integrifolia*

Sample (µg/ml)	Log10 (conc. Sample)	Total number of shrimp	Number of dead	Percentage mortality	LC_{50} (µg/ml)	95% confidence
10	1	10	2	20	6921	156.89-infinity
100	2	10	3	30		
1000	3	10	4	40		

Table 4.90. Probit analysis of methanol extract from leaves of *Tacca integrifolia*

Sample (µg/ml)	Log10 (conc. Sample)	Total number of shrimp	Number of dead	Percentage mortality	LC_{50} (µg/ml)	95% confidence
10	1	10	1	10	3323	299.79-infinity
100	2	10	2	20		
1000	3	10	4	40		

Table 4.91. Probit analysis of water extract from leaves of *Tacca integrifolia*

Sample (µg/ml)	Log10 (conc. Sample)	Total number of shrimp	Number of dead	Percentage mortality	LC_{50} (µg/ml)	95% confidence
10	1	10	1	10	4378	400.1-infinity
100	2	10	1	10		
1000	3	10	4	40		

4.10.2 BSLA of extract from rhizome of *Tacca integrifolia*

BSLA analyses of rhizome extract were present as in Table 4.94 to Table 4.98. Water extract from rhizome showed the highest LC_{50} value (22981µg/ml), followed by methanol extract with 6921µg/ml, chloroform extract (1182µg/ml), petroleum ether extract (282µg/ml) and hexane extract showed the lowest LC_{50} value which is 100µg/ml.

Table 4.92. Probit analysis of hexane extract from rhizome of *Tacca integrifolia*

Sample (µg/ml)	Log10 (conc. Sample)	Total number of shrimp	Number of dead	Percentage mortality	LC_{50} (µg/ml)	95% confidence
10	1	10	4	40	100	0-infinity
100	2	10	5	50		
1000	3	10	6	60		

Table 4.93. Probit analysis of petroleum ether extract from rhizome of *Tacca integrifolia*

Sample (µg/ml)	Log10 (conc. Sample)	Total number of shrimp	Number of dead	Percentage mortality	LC_{50} (µg/ml)	95% confidence
10	1	10	3	30	282	0-infinity
100	2	10	4	40		
1000	3	10	6	60		

Table 4.94. Probit analysis of chloroform extract from rhizome of *Tacca integrifolia*

Sample (µg/ml)	Log10 (conc. Sample)	Total number of shrimp	Number of dead	Percentage mortality	LC_{50} (µg/ml)	95% confidence
10	1	10	2	20	1182	86.21-infinity
100	2	10	3	30		
1000	3	10	5	50		

Table 4.95. Probit analysis of methanol extract from rhizome of *Tacca integrifolia*

Sample (µg/ml)	Log10 (conc. Sample)	Total number of shrimp	Number of dead	Percentage mortality	LC_{50} (µg/ml)	95% confidence
10	1	10	2	20	6921	156.89-infinity
100	2	10	3	30		
1000	3	10	4	40		

Table 4.96. Probit analysis of water extract from rhizome of *Tacca integrifolia*

Sample (µg/ml)	Log10 (conc. Sample)	Total number of shrimp	Number of dead	Percentage mortality	LC_{50} (µg/ml)	95% confidence
10	1	10	1	10	22981	484.96-infinity
100	2	10	2	20		
1000	3	10	3	30		