4.0 RESULT

4.1 Extraction

This study was conducted to evaluate the bioactivity and chemical investigations of *Ervatamia coronaria* (roots, stems and leaves) and *Tinospora crispa* (stems). The plant samples were prepared by solvent extraction method using four different solvents namely petroleum ether, chloroform, methanol and water consecutively. The percentage yield of each crude extract obtained from 30 g dried plant immersed in 300 ml solvent was tabulated below (Table 4.1, Table 4.2, Table 4.3, Table 4.4):

Table 4.1: Percentage yield of crude extracts of *Ervatamia coronaria* (roots)

Type of crude extracts	Weight of crude extracts (g)	Yield of crude extracts (%)
Petroleum ether	0.20	0.66
Chloroform	0.30	1.00
Methanol	0.40	1.33
Water	0.99	3.30

Table 4.2: Percentage yield of crude extracts of *Ervatamia coronaria* (stems)

Type of crude extracts	Weight of crude extracts (g)	Yield of crude extracts (%)
Petroleum ether	0.10	0.33
Chloroform	0.17	0.57
Methanol	0.99	3.30
Water	1.16	3.87

Table 4.3: Percentage yield of crude extracts of *Ervatamia coronaria* (leaves)

Type of crude extracts	Weight of crude extracts (g)	Yield of crude extracts (%)
Petroleum ether	0.42	1.40
Chloroform	0.61	2.03
Methanol	1.47	4.90
Water	1.85	6.17

Table 4.4: Percentage yield of crude extracts of *Tinospora crispa* (stems)

Type of crude extracts	Weight of crude extracts (g)	Yield of crude extracts (%)
Petroleum ether	0.20	0.67
Chloroform	0.37	1.23
Methanol	1.53	5.10
Water	2.23	7.43

In comparison with the other extracts, petroleum ether crude extract exhibited the lowest weight of crude extracts (g) as well as percentage yield of crude extract (%) in both plants. While chloroform crude extract exhibited slightly higher than petroleum ether crude extract followed by methanol crude extract. Water crude extract exhibited the highest weight of crude extracts (g) and percentage yield of crude extracts (%).

4.2 Thin Layer Chromatography (TLC)

4.2.1 Thin Layer Chromatography (TLC) of *Ervatamia coronaria* (roots)

According to Table 4.5, 15 compounds were detected in *Ervatamia coronaria* (roots) petroleum ether extract using dichloromethane solvent system. Thin Layer Chromatography (TLC) revealed the presence of alkaloid, terpenoid, flavonoid and conjugated bond due to colour appeared when sprayed with dragendorff reagent, vanillin-sulphuric acid reagent, anisaldehyde-sulphuric acid reagent and iodine vapour. Two compounds could not be detected using the reagents.

Besides, 23 compounds were detected in *Ervatamia coronaria* (roots) chloroform extract using 9:1 (chloroform:methanol) solvent system (Table 4.6). Thin Layer Chromatography (TLC) revealed the presence of alkaloid, terpenoid, flavonoid, saponin and conjugated bond due to colour appeared when sprayed with dragendorff reagent, vanillin-sulphuric acid reagent, anisaldehyde-sulphuric acid reagent and iodine vapour. Four compounds could not be detected using the reagents.

In addition, 16 compounds were detected in *Ervatamia coronaria* (roots) methanol extract using 9:1 (chloroform:methanol) solvent system (Table 4.7). Thin Layer Chromatography (TLC) revealed the presence of alkaloid, terpenoid, phenol, flavonoid, saponin and conjugated bond due to colour appeared when sprayed with dragendorff reagent, vanillin-sulphuric acid reagent, anisaldehyde-sulphuric acid reagent and iodine vapour. Two compounds could not be detected using the reagents.

Only 5 compounds were detected in *Ervatamia coronaria* (roots) crude water extract using 7:3 (acetone: chloroform) solvent system (Table 4.8). Thin Layer Chromatography (TLC) revealed the presence of alkaloid and conjugated bond due to colour appeared when sprayed with dragendorff reagent and iodine vapour. Three compounds could not be detected using the reagents.

Table 4.5: TLC Analysis of *Ervatamia coronaria* (roots) petroleum ether crude extract in dichloromethane

Label Compounds	Rf x100	Colour		Dragendorff	Vanillin-sulphuric acid	Anisaldehyde-sulphuric acid	Iodine Vapour	Comment
		Visible light	UV-light					
Ae-1	4	-	-	-	Pp+	-		Terpenoid
Ae-2	7	-	-	Or++	-	Pp+	Br++	Alkaloid/ Flavonoid/ Comjugated bond
Ae-3	11	Y+	G++	Or+++	Bb+	-	Br++	Alkaloid/ Terpenoid/ Conjugated bond
Ae-4	15	-	-	Or++	-	-		Alkaloid
Ae-5	20	-	Or+	-	B++	-		Terpenoid
Ae-6	21	-	-	-	-	Pp+		Flavonoid
Ae-7	33	-	-	-	-	-	Br+	Conjugated bond
Ae-8	36	-	*G++	-	Pp++	Pp++		Terpenoid/ Flavonoid
Ae-9	41	-	-	-	-	Pp++		Flavonoid
Ae-10	43	-	R+	-	Pp++	-		Terpenoid
Ae-11	48	-	-	-	-	Pp+++		Flavonoid
Ae-12	51	-	-	-	Pp+++	-		Terpenoid
Ae-13	55	-	-	-	-	-	Br+	Conjugated bond
Ae-14	60	-	*B+	-	-	-		Unknown
Ae-15	67	-	*G+	-	-	-		Unknown

Key:

Colour:

- = no colour
B = Blue
Bb = Blue-black
Br = Brown
*B = Blue fluorescent
*Bg = Blue-green fluorescent
Y = Yellow
*Y = Yellow fluorescent
G = Green
*G = Green fluorescent
Or = Orange
Pk = Pink
Pp = Purple
R = Red
Ro = Red-orange
*Ro=Red-orange fluorescent

Intensity of colour:

\+ = weak
++ = medium
+++ = strong

Table 4.6: TLC Analysis of *Ervatamia coronaria* (roots) chloroform crude extract in 9:1 (chloroform:methanol)

Label Compounds	Rf x100	Colour		Dragendorff	Vanillin-sulphuric acid	Anisaldehyde-sulphuric acid	Iodine Vapour	Comment
		Visible light	UV-light					
Ac-1	3	-	-	-	Bb++	-		Terpenoid
Ac-2	6	-	-	-	Bb+	-		Terpenoid
Ac-3	13	-	G++	-	-	-		Unknown
Ac-4	16	-	Br++	-	-	-		Unknown
Ac-5	19	-	Br++	-	-	-		Unknown
Ac-6	30	Y++	*B++	Or++	Bb++	Pp++	Br++	Alkaloid/ Terpernoid/ Flavonoid/ Conjugated bond
Ac-7	34	Y+++	G++	Or++	Pp++	Bb++	Br++	Alkaloid/ Terpenoid, Saponin/ Conjugated bond
Ac-8	38	-	*G++	-	Pp+	Pp++	Br++	Terpenoid/ Flavonoid/ Conjugated bond
Ac-9	44	-	-	-	-	-	Br+++	Conjugated bond
Ac-10	48	-	Y++	Or++	-	-		Alkaloid
Ac-11	49	-	-	-	Pp++	-		Terpenoid
Ac-12	50	-	-	-	-	-	Br+	Conjugated bond
Ac-13	54	-	-	-	-	Bb+		Saponin
Ac-14	56	-	Y++	Or++	Pp++	-		Alkaloid/ Terpenoid
Ac-15	59	-	-	-	-	-	Br++	Conjugated bond
Ac-16	61	-	*G++	Or++	Bb+	-		Alkaloid/ Terpenoid
Ac-17	65	-	G++	Or++	-	-	Br++	Alkaloid/Conjugated bond
Ac-18	71	-	-	-	Pp++	Pp+		Terpenoid/ Flavonoid
Ac-19	73	-	G++	-	-	-		Unknown
Ac-20	74	-	-	-	-	-	Br++	Conjugated bond
Ac-21	76	-	B+	-	Bb+	Bb++		Terpenoid/ Saponin
Ac-21	79	-	-	Or+++	-	-		Alkaloid
Ac-22	84	-	-	-	Pp++	Pp++		Terpenoid/ Flavonoid
Ac-23	86	-	-	-	Pp++	Pp++		Terpenoid/ Flavonoid

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- *Bg = Blue-green fluorescent
- G = Green
- *G = Green fluorescent
- Or = Orange
- Pk = Pink
- Pp = Purple
- R = Red
- Y = Yellow

Intensity of colour:

- \+ = weak
- ++ = medium
- +++ = strong

Table 4.7: TLC Analysis of *Ervatamia coronaria* (roots) methanol crude extract in 9:1 (chloroform : methanol)

Label Compounds	Rf x100	Colour		Dragendorff	Vanillin-sulphuric acid	Anisaldehyde-sulphuric acid	Iodine Vapour	Comment
		Visible light	UV-light					
Am-1	4	Br+	B++	Or++	R+	Bb++	Br++	Alkaloid/ Phenol/ Saponin/ Conjugated bond
Am-2	7	-	G++	-	B+		Br+	Terpenoid/ Conjugated bond
Am-3	9	-	G++	-	B+	Bb++	-	Terpenoid/ Saponin
Am-4	17	-	*B++	-	-	-	-	Unknown
Am-5	21	-	*G++	-	-	-	-	Unknown
Am-6	27	Pk+	Y++	-	Pp++	-	-	Terpenoid
Am-7	31	Y+	Y++	Or+	Pp++	Pp++	-	Alkaloid/ Terpenoid/ Flavonoid
Am-8	35	-	*G++	Or+	Pp++	B+	Br+	Alkaloid/ Terpenoid/ Saponin/ Conjugated bond
Am-9	39	-	G++	Or+	R+	Pp++	Br++	Alkaloid/ Phenol/ Flavonoid/ Conjugated bond
Am-10	45	-	-	Or+	Bb+	B+	Br++	Alkaloid/ Terpenoid/ Saponin/ Conjugated bond
Am-11	49	-	-	-	-	Pp+	-	Flavonoid
Am-12	55	-	*B++	-	-	Pp+	-	Flavonoid
Am-13	69	-	Br++	-	Bb+	Pp+++	-	Terpenoid, Flavonoid
Am-14	77	-	Br++	-	Pp+	Pp+	Br+	Terpenoid/ Flavonoid/ Conjugated bond
Am-15	81	-	G++	Or++	-	Pp++	Br++	Alkaloid/ Flavonoid/ Conjugated bond
Am-16	87	-	*B++	Or++	-	-	Br+++	Alkaloid/ Conjugated bond

Key:

Colour:

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- B = Blue
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- Br = Brown
- *B = Blue fluorescent
- G = Green
- *G = Green fluorescent
- Or = Orange
- Pk = Pink
- *Bg = Blue-green fluorescent
- R = Red
- Y = Yellow
- Pp = Purple

Intensity of colour:

- \+ = weak
- ++ = medium
- +++ = strong

Table 4.8: TLC Analysis of *Ervatamia coronaria* (roots) water crude extract in 7:3 (acetone: chloroform)

Label Compounds	Rf x100	Colour		Dragendorff	Vanillin-sulphuric acid	Anisaldehyde-sulphuric acid	Iodine Vapour	Comment
		Visible light	UV-light					
Aw-1	10	-	G+	Or+	-	-	-	Alkaloid
Aw-2	19	-	G+	-	-	-	-	Unknown
Aw-3	31	-	G+	-	-	-	-	Unknown
Aw-4	59	-	*B+	-	-	-	Br+	Conjugated bond
Aw-5	71	-	G+	-	-	-	-	Unknown

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- Br = Brown
- *B = Blue fluorescent
- *Bg = Blue-green fluorescent
- Y = Yellow
- *Y = Yellow fluorescent
- G = Green
- *G = Green fluorescent
- Or = Orange
- Pk = Pink
- Pp = Purple
- R = Red
- Ro = Red-orange
- *Ro=Red-orange fluorescent

Intensity of colour:

- \+ = weak
- ++ = medium
- +++ = strong

4.2.2 Thin Layer Chromatography (TLC) of *Ervatamia coronaria* (stems)

According to Table 4.9, 12 compounds were detected in *Ervatamia coronaria* (stems) petroleum ether extract using dichloromethane solvent system. Thin Layer Chromatography (TLC) revealed the presence of alkaloid, terpenoid, phenol, flavonoid and conjugated bond due to colour appeared when sprayed with dragendorff reagent, vanillin-sulphuric acid reagent, anisaldehyde-sulphuric acid reagent and iodine vapour. One compounds could not be detected using the reagents.

Besides, 15 compounds were detected in *Ervatamia coronaria* (stems) chloroform extract using 9:1 (chloroform:methanol) solvent system (Table 4.10). Thin Layer Chromatography (TLC) revealed the presence of alkaloid, terpenoid, phenol, flavonoid, saponin and conjugated bond due to colour appeared when sprayed with dragendorff reagent, vanillin-sulphuric acid reagent, anisaldehyde-sulphuric acid reagent and iodine vapour. Four compounds could not be detected using the reagents.

In addition, 19 compound were detected in *Ervatamia coronaria* (stems) methanol extract using 9:1 (chloroform: methanol) solvent system (Table 4.11). Thin Layer Chromatography (TLC) revealed the presence of alkaloid, terpenoid, flavonoid, saponin and conjugated bond due to colour appeared when sprayed with dragendorff reagent, vanillin-sulphuric acid reagent, anisaldehyde-sulphuric acid reagent and iodine vapour. Three compounds could not be detected using the reagent.

Only 5 compounds were detected in *Ervatamia coronaria* (stems) crude water extract respectively using 7:3 (acetone: chloroform) solvent system (Table 4.12). Thin Layer Chromatography (TLC) revealed the presence of alkaloid due to colour appeared when sprayed with dragendorff reagent. Four compounds could not be detected using the reagents.

Table 4.9: TLC Analysis of *Ervatamia coronaria* (stems) petroleum ether crude extract in dichloromethane.

Label Compounds	Rf x100	Colour		Dragendorff	Vanillin-sulphuric acid	Anisaldehyde-sulphuric acid	Iodine Vapour	Comment
		Visible light	UV-light					
Be-1	6	Y+	G+	Or++	-	-	Br++	Alkaloid/ Conjugated bond
Be-2	10	-	Or+	-	B+	-	-	Terpenoid
Be-3	16	-	-	-	B+	Pp+	-	Terpenoid/ Flavonoid
Be-4	23	*Bg++	-	-	-	-	-	Unknown
Be-5	26	-	G+	-	R++	Pp++	Br++	Phenol/ Flavonoid/ Conjugated bond
Be-6	31	-	-	Or+	-	-	-	Alkaloid
Be-7	36	-	*Ro+	-	Pp++	Pp++	-	Terpenoid/ Flavonoid
Be-8	41	-	-	-	Pp+++	Pp+++	Br++	Terpenoid/ Flavonoid/ Conjugated bond
Be-9	51	-	G+	-	Pp+	-	-	Terpenoid
Be-10	73	-	R+	-	B+	-	-	Terpenoid
Be-11	83	-	G+	-	Pp+++	Pk++	Br++	Terpenoid/ Flavonoid/ Conjugated bond
Be-12	91	Y+	*B+	-	Pp++	Pp+++	Br+	Terpenoid/ Flavonoid/ Conjugated bond

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- *B = Blue fluorescent
- *Bg = Blue-green fluorescent
- Y = Yellow
- *Y = Yellow fluorescent
- G = Green
- *G = Green fluorescent
- Or = Orange
- Pk = Pink
- Pp = Purple
- R = Red
- Ro = Red-orange
- *Ro=Red-orange fluorescent

Intensity of colour:

- \+ = weak
- ++ = medium
- +++ = strong

Table 4.10 :TLC analysis of *Ervatamia coronaria* (stems) chloroform crude extract 9:1 (chloroform : methanol)

Label Compounds	Rf x100	Colour		Dragendorff	Vanillin-sulphuric acid	Anisaldehyde -sulphuric acid	Iodine Vapour	Comment
		Visible light	UV-light					
Bc-1	8	-	G+	-	-	-	-	Unknown
Bc-2	12	-	G+	-	-	-	-	Unknown
Bc-3	21	G+	G+	-	-	-	-	Unknown
Bc-4	29	G+	*B++	Or+	Pp++	-	-	Alkaloid/ Terpenoid
Bc-5	31	-	-	-	-	Pp++	-	Flavonoid
Bc-6	35	-	*B+	-	-	-	-	Unknown
Bc-7	45	-	G+	Or+	B+	Bb+	Br+	Alkaloid/ Terpenoid/ Saponin/ Conjugated bond
Bc-8	50	-	G++	Or+	Red++	-	Br+	Alkaloid/ Phenol/ Conjugated bond
Bc-9	59	Y+	G+	-	Pp++	Pp++	-	Terpenoid/ Flavonoid
Bc-10	60	-	-	-	Bb++	-	-	Terpenoid
Bc-11	62	-	B++	-	-	Pp+	-	Flavonoid
Bc-12	76	-	R++	Or+	B+	B+	-	Alkaloid/ Terpenoid/ Saponin
Bc-13	83	-	G++	Or+	Pp++	-	-	Alkaloid/ Terpenoid
Bc-14	90	-	-	-	-	-	Br+	Conjugated Bond
Bc-15	91	-	*B++	Or+++	-	-	Br++	Alkaloid/ Conjugated Bond

Key:

Colour:

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- B = Blue
- Bb = Blue-black
- Br = Brown
- *B = Blue fluorescent
- *Bg = Blue-green fluorescent
- Y = Yellow
- G = Green
- *G = Green fluorescent
- Or = Orange
- Pk = Pink
- Pp = Purple
- R = Red

Intensity of colour:

- \+ = weak
- ++ = medium
- +++ = strong

Table 4.11: TLC analysis of *Ervatamia coronaria* (stems) methanol crude extract 9:1 (chloroform : methanol)

Label Compounds	Rf x100	Colour		Dragendorff	Vanillin-sulphuric acid	Anisaldehyde-sulphuric acid	Iodine Vapour	Comment
		Visible light	UV-light					
Bm-1	4	Y+	*B++	-	-	-	Br+++	Conjugated Bond
Bm-2	7	-	*B+++	Or++	-	-	-	Alkaloid
Bm-3	10	Br++	*G++	Or+	-	-	-	Alkaloid
Bm-4	14	-	*B++	-	-	-	-	Unknown
Bm-5	20	Y++	B+	Or++	Pp++	Pp++	-	Alkaloid/ Terpenoid, Flavonoid
Bm-6	22	-	-	-	-	Pk++	-	Flavonoid
Bm-7	23	-	B+	-	-	-	-	Unknown
Bm-8	29	-	-	Or+	-	-	-	Alkaloid
Bm-9	31	-	-	Or+	-	-	-	Alkaloid
Bm-10	44	-	G+	-	-	Bb+	-	Saponin
Bm-11	51	-	G+	Or+	Bb++	-	-	Alkaloid, Terpenoid
Bm-12	59	-	G+	Or+	-	-	Br+	Alkaloid/ Conjugated Bond
Bm-13	64	-	*B++	Or+++	Pp+	-	Br+	Alkaloid/ Terpenoid/ Conjugated bond
Bm-14	71	-	R++	-	-	-	-	Unknown
Bm-15	76	-	*B++	Or+	Pp+	-	Br+++	Alkaloi/ Terpenoid/ Conjugated bond
Bm-16	77	-	-	-	Pp+	Pp++	-	Terpenoid/ Flavonoid
Bm-17	91	-	B++	Or++	Pp++	Pk++	-	Alkaloid/ Terpenoid/ Flavonoid
Bm-18	96	-	R++	Or+	Bb++	-	Br++	Alkaloid/ Terpenoid/ Conjugated bond
Bm-19	99	-	R++	Or+	Bb++	-	Br++	Alkaloid/ Terpenoid/ Conjugated bond

Key:

Colour:

- = no colour
B = Blue
Bb = Blue-black
Br = Brown
*B = Blue fluorescent
*Bg = Blue-green fluorescent
G = Green
*G = Green fluorescent
Or = Orange
Pk = Pink
Pp = Purple
R = Red
Y = Yellow

Intensity of colour:

+ = weak
++ = medium
+++ = strong

Table 4.12: TLC Analysis of *Ervatamia coronaria* (stems) water crude extract in 7:3 (acetone: chloroform)

Label Compounds	Rf x100	Colour		Dragendorff	Vanillin-sulphuric acid	Anisaldehyde-sulphuric acid	Iodine Vapour	Comment
		Visible light	UV-light					
Bw-1	6	Y+	G+	Or+	-	-	-	Alkaloid
Bw-2	14	-	G+	-	-	-	-	Unknown
Bw-3	29	-	G+	-	-	-	-	Unknown
Bw-4	56	-	G+	-	-	-	-	Unknown
Bw-5	60	-	*G++	-	-	-	-	Unknown

Key:

Colour:

- = no colour
- B = Blue
- Bb = Blue-black
- Br = Brown
- *B = Blue fluorescent
- *Bg = Blue-green fluorescent
- Y = Yellow
- *Y = Yellow fluorescent
- G = Green
- *G = Green fluorescent
- Or = Orange
- Pk = Pink
- Pp = Purple
- R = Red
- Ro = Red-orange
- *Ro=Red-orange fluorescent

Intensity of colour:

- \+ = weak
- ++ = medium
- +++ = strong

4.2.3 Thin Layer Chromatography (TLC) of *Ervatamia coronaria* (leaves)

According to Table 4.13, 7 compounds were detected in *Ervatamia coronaria* (leaves) petroleum ether crude extract using dichloromethane solvent system. Thin Layer Chromatography (TLC) revealed the presence of terpenoid and flavonoid due to colour appeared when sprayed with vanillin-sulphuric acid reagent and anisaldehyde-sulphuric acid reagent. Three compounds could not be detected using the reagents.

Besides, 13 compounds were detected in *Ervatamia coronaria* (leaves) chloroform extract using 9:1 (chloroform:methanol) solvent system (Table 4.14). Thin Layer Chromatography (TLC) revealed the presence of alkaloid, terpenoid, phenol, flavonoid and conjugated bond due to colour appeared when sprayed with dragendorff reagent, vanillin-sulphuric acid reagent, anisaldehyde-sulphuric acid reagent and iodine vapour. Five compounds could not be detected using the reagents.

In addition, 12 compounds were detected in *Ervatamia coronaria* (leaves) methanol extract using 9:1 (chloroform:methanol) solvent system (Table 4.15). Thin Layer Chromatography (TLC) revealed the presence of alkaloid, terpenoid, phenol, flavonoid and conjugated bond due to colour appeared when sprayed with dragendorff reagent, vanillin-sulphuric acid reagent, anisaldehyde-sulphuric acid reagent and iodine vapour. One compound could not be detected using the reagents.

Only 4 compounds were detected in *Ervatamia coronaria* (leaves) crude water extract using 7:3 (acetone: chloroform) solvent system (Table 4.16). Thin Layer

Chromatography (TLC) revealed the presence of alkaloid, flavonoid and saponin due to colour appeared when sprayed with dragendorff reagent and anysaldehyde-sulphuric acid reagent.

Table 4.13: TLC Analysis of *Ervatamia coronaria* (leaves) petroleum ether crude extract in dichloromethane

Label Compounds	Rf x100	Colour		Dragendorff	Vanillin-sulphuric acid	Anisaldehyde-sulphuric acid	Iodine Vapour	Comment
		Visible light	UV-light					
De-1	6	-	-	-	Bb+	-	-	Terpenoid
De-2	12	Y++	Y++	-	-	-	-	Unknown
De-3	21	G++	Ro++	-	B++	-	-	Terpenoid
De-4	26	G++	Ro+	-	B++	-	-	Terpenoid
De-5	42	-	B+	-	Pp++	Pp++	-	Terpenoid/ Flavonoid
De-6	48	Y+	-	-	-	-	-	Unknown
De-7	52	-	Or+	-	-	-	-	Unknown

Key:

Colour:

- = no colour
- B = Blue
- Bb = Blue-black
- Br = Brown
- *B = Blue fluorescent
- *Bg = Blue-green fluorescent
- Y = Yellow
- *Y = Yellow fluorescent
- G = Green
- *G = Green fluorescent
- Or = Orange
- Pk = Pink
- Pp = Purple
- R = Red
- Ro = Red-orange
- *Ro=Red-orange fluorescent

Intensity of colour:

- \+ = weak
- ++ = medium
- +++ = strong

Table 4.14: TLC Analysis of *Ervatamia coronaria* (leaves) chloroform crude extract in 9:1 (chloroform:methanol)

Label Compounds	Rf x100	Colour		Dragendorff	Vanillin-sulphuric acid	Anisaldehyde-sulphuric acid	Iodine Vapour	Comment
		Visible light	UV-light					
Dc-1	11	-	Ro+++	-	-	-	Br+	Conjugated bond
Dc-2	14	-	G+	-	-	-	-	Unknown
Dc-3	20	-	Ro+	-	-	-	-	Unknown
Dc-4	26	Y+	G+	-	Pp++	Pp++	Br+	Terpenoid/ Flavonoid/ Conjugated bond
Dc-5	39	-	G+	-	-	-	-	Unknown
Dc-6	43	-	G+	-	-	-	-	Unknown
Dc-7	48	-	*G+	-	-	-	-	Unknown
Dc-8	61	Y++	*G++	Or+	-	Pp+++	-	Alkaloid/ Flavonoid
Dc-9	65	-	-	-	R+	-	-	Phenol
Dc-10	70	-	-	-	-	Pp++	-	Flavonoid
Dc-11	78	G++	*B++	-	Pp+	-	-	Terpenoid
Dc-12	88	-	-	-	Bb++	-	-	Terpenoid
Dc-13	90	-	-	-	-	Pp+++	-	Flavonoid

Key:

Colour:

- = no colour
- B = Blue
- Bb = Blue-black
- Br = Brown
- *B = Blue fluorescent
- *Bg = Blue-green fluorescent
- Y = Yellow
- G = Green
- *G = Green fluorescent
- Or = Orange
- Pk = Pink
- Pp = Purple
- R = Red
- Ro = Red-orange

Intensity of colour:

- \+ = weak
- ++ = medium
- +++ = strong

Table 4.15: TLC Analysis of *Ervatamia coronaria* (leaves) methanol crude extract in 9:1 (chloroform:methanol)

Label Compounds	Rf x100	Colour		Dragendorff	Vanillin-sulphuric acid	Anisaldehyde-sulphuric acid	Iodine Vapour	Comment
		Visible light	UV-light					
Dm-1	5		*B++	Or++	-	-	Br++	Alkaloid/ Conjugated bond
Dm-2	7		*G++	Or+	-	-	-	Alkaloid
Dm-3	12		*G++	Or+	Pp+++	-	-	Alkaloid/ Terpenoid
Dm-4	14		-	-	R++	-	-	Phenol
Dm-5	20		Ro+	-	B+	Pp++	-	Terpenoid/ Flavonoid
Dm-6	31		B+	-	-	Pp++	-	Flavonoid
Dm-7	35		Ro++	-	B++	-	-	Terpenoid
Dm-8	48		G+	-	B++	Pp++	-	Terpenoid/ Flavonoid
Dm-9	55	-	-	-	Pp+	-	-	Terpenoid
Dm-10	63	Y++	Ro+++	-	-	-	-	Unknown
Dm-11	84	-	-	-	-	Pp++	-	Flavonoid
Dm-12	86	G++	Ro+	-	B++	-	-	Terpenoid

Key:

Colour:

- = no colour
- B = Blue
- Bb = Blue-black
- Br = Brown
- *B = Blue fluorescent
- *Bg = Blue-green fluorescent
- Y = Yellow
- G = Green
- *G = Green fluorescent
- Or = Orange
- Pk = Pink
- Pp = Purple
- R = Red
- Ro = Red-orange

Intensity of colour:

- + = weak
- ++ = medium
- +++ = strong

Table 4.16: TLC Analysis of *Ervatamia coronaria* (leaves) water crude extract in 7:3 (acetone: chloroform)

Label Compounds	Rf x100	Colour		Dragendorff	Vanillin-sulphuric acid	Anisaldehyde-sulphuric acid	Iodine Vapour	Comment
		Visible light	UV-light					
Dw-1	5	-	-	Or+	-	-	-	Alkaloid
Dw-2	65	-	-	Or+	-	-	-	Alkaloid
Dw-3	83	-	-	-	-	Pp++	-	Flavonoid
Dw-4	94	-	-	-	-	Bb++	-	Saponin

Key:

Colour:

- = no colour
- B = Blue
- Bb = Blue-black
- Br = Brown
- *B = Blue fluorescent
- *Bg = Blue-green fluorescent
- Y = Yellow
- *Y = Yellow fluorescent
- G = Green
- *G = Green fluorescent
- Or = Orange
- Pk = Pink
- Pp = Purple
- R = Red
- Ro = Red-orange
- *Ro=Red-orange fluorescent

Intensity of colour:

- \+ = weak
- ++ = medium
- +++ = strong

4.2.4 Thin Layer Chromatography (TLC) of *Tinospora crispa* (stems)

According to Table 4.17, 13 compounds were detected in *Tinospora crispa* (stems) petroleum ether crude extract using dichloromethane solvent system. Thin Layer Chromatography (TLC) revealed the presence of terpenoid, flavonoid, saponin and conjugated bond due to colour appeared when sprayed with vanillin-sulphuric acid reagent, anisaldehyde-sulphuric acid reagent and iodine vapour. One compound could not be detected using the reagents.

Based on Table 4.18 and Table 4.19, approximately 14 and 11 compounds were detected in *Tinospora crispa* (stems) chloroform and methanol extract respectively using 9:1 (chloroform:methanol) solvent system. Thin Layer Chromatography (TLC) revealed the presence of alkaloid, terpenoid, phenol, flavonoid and conjugated bond due to colour appeared when sprayed with dragendorff reagent, vanillin-sulphuric acid reagent, anisaldehyde-sulphuric acid reagent and iodine vapour. Two compounds cannot be detected in chloroform extract while one compound could not be detected in methanol extract using the reagents.

In addition, only 3 compounds were detected in *Tinospora crispa* (stems) crude water extract using 7:3 (acetone: chloroform) solvent system (Table 4.20). Thin Layer Chromatography (TLC) revealed the presence of alkaloid, terpenoid and flavonoid due to colour appeared when sprayed with dragendorff reagent, vanillin-sulphuric acid reagent and anysaldehyde-sulphuric acid reagent.

Table 4.17: TLC Analysis of *Tinospora crispa* (stems) petroleum ether crude extract in dichloromethane

Label Compounds	Rf x100	Colour		Dragendorff	Vanillin-sulphuric acid	Anisaldehyde-sulphuric acid	Iodine Vapour	Comment
		Visible light	UV-light					
Te-1	5	Y++	-	-	Bb+	-	Br+	Terpenoid/ Conjugated bond
Te-2	13	Y++	*Ro++	-	-	Bb++	-	Saponin
Te-3	16	Y+	*Ro++	-	Bb++	-	Br++	Terpenoid/ Conjugated bond
Te-4	27	-	*B++	-	Pp+++	Pp+++	Br++	Terpenoid/ Flavonoid, Conjugated bond
Te-5	32	Y++	*Ro++	-	-	-	-	Unknown
Te-6	39	Y++	-	-	Pp++	Pp++	Br++	Terpenoid/ Flavonoid/ Conjugated bond
Te-7	43	-	*G+	-	Pp++	Pp+++	Br++	Terpenoid/ Flavonoid/ Conjugated bond
Te-8	57	-	Pp++	-	Pp+	Pp+	Br+	Terpenoid/Flavonoid/ Conjugated bond
Te-9	60	-	*B++	-	Pp+	Pp+	-	Terpenoid/ Flavonoid
Te-10	71	-	-	-	Pp++	-	-	Terpenoid
Te-11	74	-	G+	-	-	Pp+	Br++	Flavonoid/ Conjugated bond
Te-12	87	-	B+	-	-	-	Br+	Conjugated bond
Te-13	91	-	G+	-	Pp++	Pp+++	-	Terpenoid/ Flavonoid

Key:

Colour:

- = no colour
B = Blue
Bb = Blue-black
Br = Brown
*B = Blue fluorescent
*Bg = Blue-green fluorescent
Y = Yellow
*Y = Yellow fluorescent
G = Green
*G = Green fluorescent
Or = Orange
Pk = Pink
Pp = Purple
R = Red
Ro = Red-orange
*Ro=Red-orange fluorescent

Intensity of colour:

+ = weak
++ = medium
+++ = strong

Table 4.18: TLC Analysis of *Tinospora crispa* (stems) chloroform crude extract in 9:1 (chloroform:methanol)

Label Compounds	Rf x100	Colour		Dragendorff	Vanillin-suplhuric acid	Anisaldehyde-sulphuric acid	Iodine Vapour	Comment
		Visible light	UV-light					
Tc-1	8	-	Y++	Or+	Pp++	-	-	Alkaloid/Terpenoid
Tc-2	15	-	-	-	-	Pp+	Br+	Flavonoid/ Conjugated bond
Tc-3	13	-	-	-	Pk+	-	-	Phenol
Tc-4	17	-	Ro+	-	Pp+++	Pp+	-	Terpenoid/ Flavonoid
Tc-5	21	B+	*B++	-	-	-	Br++	Conjugated bond
Tc-6	26	-	-	Or+	-	-	-	Alkaloid
Tc-7	39	G++	R+	-	-	-	-	Unknown
Tc-8	42	G+	Ro+	-	-	-	-	Unknown
Tc-9	47	-	-	-	-	Pp+++	-	Flavonoid
Tc-10	49	-	-	-	Pp++	-	-	Terpenoid
Tc-11	58	-	*Y++	-	-	Pp+++	-	Flavonoid
Tc-12	68	Y++	*Y++	-	-	Pp+++	-	Flavonoid
Tc-13	78	-	*B++	Or++	Bb+	Pp+++	-	Alkaloid/ Terpenoid/ Flavonoid
Tc-14	84	G++	R+++	Or+	-	-	-	Alkaloid

Key:

Colour:

- = no colour
- B = Blue
- Bb = Blue-black
- Br = Brown
- *B = Blue fluorescent
- *Bg = Blue-green fluorescent
- Y = Yellow
- *Y = Yellow fluorescent
- G = Green
- *G = Green fluorescent
- Or = Orange
- Pk = Pink
- Pp = Purple
- R = Red
- Ro = Red-orange

Intensity of colour:

- \+ = weak
- ++ = medium
- +++ = strong

Table 4.19: TLC Analysis of *Tinospora crispa* (stems) methanol crude extract in 9:1 (chloroform:methanol)

Label Compounds	Rf x100	Colour		Dragendorff	Vanillin-sulphuric acid	Anisaldehyde-sulphuric acid	Iodine Vapour	Comment
		Visible light	UV-light					
Tm-1	3	-	B+	Or+	-	-	-	Alkaloid
Tm-2	6	-	*Bg+	Or+	-	-	-	Alkaloid
Tm-3	9	-	-	-	-	R++	-	Flavonoid
Tm-4	13	-	G+	Or+	-	-	-	Alkaloid
Tm-5	18	-	*G++	-	Pp++	-	-	Terpenoid
Tm-6	31	-	B+	-	Pp++	R++	-	Terpenoid/ Flavonoid
Tm-7	44	-	B+	Or+	-	-	Br++	Alkaloid/Conjugated bond
Tm-8	56	-	-	-	Pk+	R+	-	Phenol/ Flavonoid
Tm-9	70	-	G++	-	Pk+	-	-	Phenol
Tm-10	80	-	B++	-	-	-	-	Unknown
Tm-11	84	-	Ro++	Or+	-	Pp+++	Br++	Alkaloid/Flavonoid/ Conjugated bond

Key:

Colour:

- = no colour
B = Blue
Bb = Blue-black
Br = Brown
*B = Blue fluorescent
*Bg = Blue-green fluorescent
Y = Yellow
*Y = Yellow fluorescent
G = Green
*G = Green fluorescent
Or = Orange
Pk = Pink
Pp = Purple
R = Red
Ro = Red-orange

Intensity of colour:

\+ = weak
++ = medium
+++ = strong

Table 4.20: TLC Analysis of *Tinospora crispa* (stems) water crude extract in 7:3 (acetone: chloroform)

Label Compounds	Rf x100	Colour		Dragendorff	Vanillin-sulphuric acid	Anisaldehyde-sulphuric acid	Iodine Vapour	Comment
		Visible light	UV-light					
Tw-1	6	-	-	Or++	-	-	-	Alkaloid
Tw-2	71	-	-	-	-	Pp++	-	Flavonoid
Tw-3	78	-	-	-	Pp+	-	-	Terpenoid

Key:

Colour:

- = no colour
B = Blue
Bb = Blue-black
Br = Brown
*B = Blue fluorescent
*Bg = Blue-green fluorescent
Y = Yellow
*Y = Yellow fluorescent
G = Green
*G = Green fluorescent
Or = Orange
Pk = Pink
Pp = Purple
R = Red
Ro = Red-orange
*Ro=Red-orange fluorescent

Intensity of colour:

+ = weak
++ = medium
+++ = strong

4.3 Total Phenolic Content

The purpose of this assay was to calculate the content of phenolic compound in the sample based on the calibration curve of the standard.

4.3.1 Gallic Acid as Positive Reference Standard

Gallic acid was used as a positive reference standard in total phenolic content assay. The amount of total phenol was determined with the Folin-Ciocalteu reagent. Gallic acid was used as a standard compound and the total phenols were expressed as mg/g gallic acid equivalent (GAE) using the standard curve equation. Table 4.21 shows the absorbance of standard compound of gallic acid as the increased concentration of gallic acid.

Table 4.21: Absorbance of gallic acid in the total phenolic content assay

Concentration (µg/ml)	Absorbance at 725 nm
	Mean± SD
10	0.084 ± 0.004
20	0.148 ± 0.007
30	0.218 ± 0.005
40	0.266 ± 0.002
50	0.342 ± 0.006

Absorbance are expressed as mean ± standard deviation (SD), n=3

The gallic acid concentrations used were 10 µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml and 50 µg/ml. The absorbance of gallic acid ranged between 0.084 A at 10 µg/ml to 0.349 at 50 µg/ml. The absorbance of gallic acid at concentrations 20 µg/ml, 30 µg/ml and 40 µg/ml were 0.148 A, 0.218 A and 0.266 A respectively.

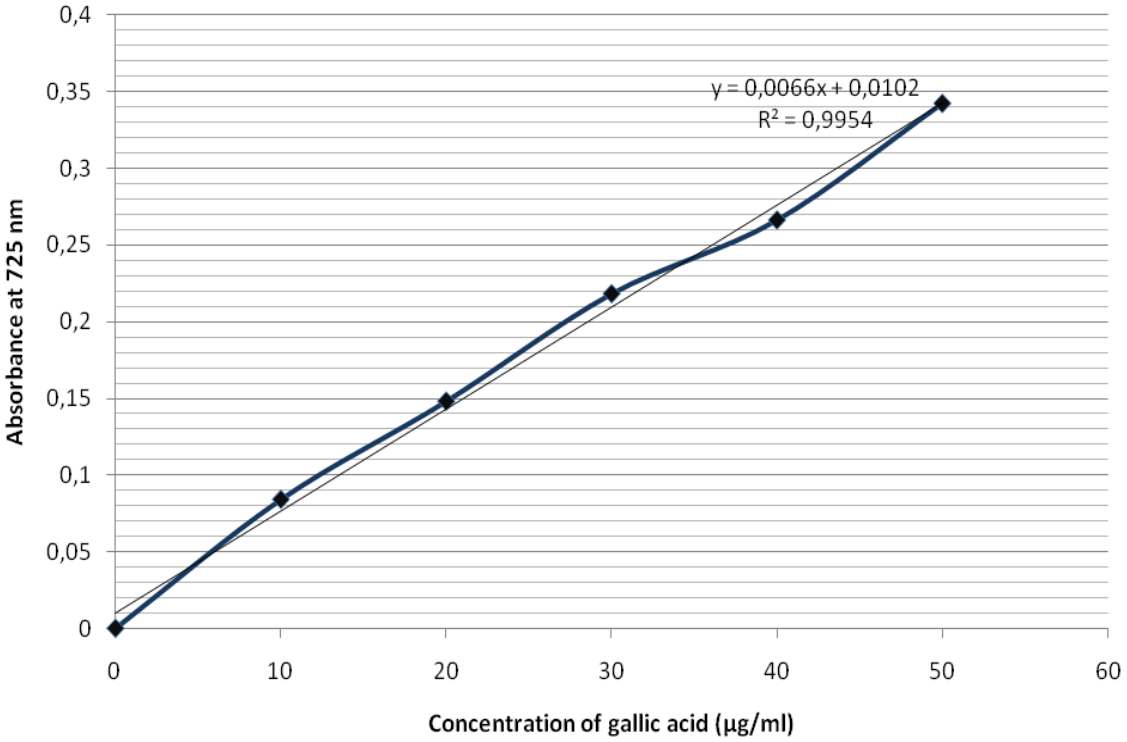

Figure 4.1: A linear calibration curve of gallic acid (standard) in the total phenolic assay

Figure 4.1 above shows a linear calibration curve of gallic acid with r^2 value of 0.9954 and standard curve equation of y = 0.0066x + 0.0102, where y is absorbance at 725 nm and x is concentration of gallic acid in µg/ml.

4.3.2 Total Phenolic Content (TPC) of Crude Extracts

Based on Table 4.22, all the methanol extracts of *Ervatamia coronaria* exhibited higher Total Phenolic Content in comparison to other extracts of this plant particularly methanol extract of leaves which exhibited the highest TPC with 124.61 GAE/mg. While chloroform extract exhibited the highest TPC with 71.53 GAE/mg in *Tinospora crispa* plant.

Table 4.22: Total phenolic content for different type of plants and crude extracts.

Type of crude extracts		Total Phenolic Content (GAE/mg)
		Mean ± Standard Deviation (SD)
Ervatamia coronaria (roots)	Petroleum ether	$22.39^{a} \pm 1.202$
	Chloroform	$57.85^{b} \pm 0.845$
	Methanol	$94.61^{c} \pm 1.409$
	Water	$11.59^{d} \pm 0.964$
Ervatamia coronaria (stems)	Petroleum ether	$11.69^{d} \pm 0.439$
	Chloroform	$75.58^{e} \pm 1.705$
	Methanol	$76.64^{e} \pm 3.108$
	Water	$24.16^{a} \pm 0.229$
Ervatamia coronaria (leaves)	Petroleum ether	$15.22^{d} \pm 0.486$
	Chloroform	$64.01^{b} \pm 0.574$
	Methanol	$124.61^{f} \pm 2.165$
	Water	$60.52^{b} \pm 0.699$
Tinospora crispa (stems)	Petroleum ether	$9.62^{d} \pm 0.463$
	Chloroform	$71.53^{e} \pm 3.725$
	Methanol	$64.67^{b} \pm 1.638$
	Water	$45.12^{g} \pm 5.129$

Different superscript means significantly different ($P < 0.05$)

4.4 Antioxidant Activity Assay

The purpose of this study was to evaluate the potential of *Ervatamia coronaria* (roots, stems and leaves) and *Tinospora crispa* (stems) as antioxidant by using four different assays.

4.4.1 DPPH Radical Scavenging Assay

The potential antioxidant activity of crude petroleum ether, chloroform, methanol and water extracts of *Ervatamia coronaria* and *Tinospora crispa* was determined on the basis of scavenging activity against the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals. The radicals were characterized by a typical deep purple colour and maximum absorbance in the range of 515-520 nm. The DPPH method utilized the UV-Vis spectrophotometer. In the presence of a hydrogen or electron donor (free scavenging antioxidant) the absorption intensity is decreased, and the radical solution is discoloured according to the amount of electron captured (Markowicz Bastos *et al.*, 2007).

4.4.1(i) Ascorbic Acid as a Positive Reference Standard

Ascorbic acid or vitamin C is a powerful antioxidant with the ability to scavenge free radicals. An easier way to present antioxidant activity in plants is by comparing with a common reference standard. Ascorbic acid was used as the positive reference standard for the DPPH radical scavenging assay in this study. The scavenging effects of the ascorbic acid on DPPH radicals were shown in Table 4.23.

Table 4.23: Percentage of inhibition of DPPH radical by ascorbic acid

Concentration of Ascorbic acid (mg/ml)	Percentages of inhibition (%)
	Mean ± SD
0.0025	49.76 ± 2.527
0.0125	81.43 ± 0.125
0.0375	87.11 ± 0.451
0.125	96.33 ± 0.069
0.25	96.84 ± 0.412
0.5	96.97 ± 0.193

Percentage of inhibition are expressed as mean ± standard deviation (SD), n=3

The radical scavenging activity of ascorbic acid increased with increasing concentrations. The percentage of inhibition of ascorbic acid ranged between 49.76% at a concentration of 0.025 mg/ml to 96.97% at 0.5 mg/ml. The percentage of inhibition of ascorbic acid at concentrations 0.0125 mg/ml, 0.0375 mg/ml, 0.125 mg/ml and 0.25 mg/ml were 81.43%, 87.11%, 96.33 and 96.85% respectively.

4.4.1(ii) Radical Scavenging Activity of *Ervatamia coronaria (*roots)

The results of DPPH radical scavenging assay for crude petroleum ether, chloroforms, methanol and water extracts of *Ervatamia coronaria* (roots) at concentrations 0.0025, 0.0125, 0.0375, 0.125, 0.25 and 0.5 mg/ml are shown in Table 4.24.

Table 4.24: Radical scavenging activity of the crude petroleum ether, chloroform, methanol, and water extracts of *Ervatamia coronaria* (roots) in the DPPH radical scavenging assay

Concentration of Crude Extracts (mg/ml)	Percentage of Inhibition (%)			
	Crude Petroleum Ether Extracts	Crude Chloroform Extracts	Crude Methanol Extracts	Crude Water Extracts
0.0025	2.15 ± 0.000	8.96 ± 1.423	9.52 ± 1.213	0.01 ± 0.001
0.0125	2.61 ± 0.398	11.44 ± 1.047	13.21 ± 0.830	2.05 ± 0.887
0.0375	2.72 ± 0.693	15.22 ± 2.382	16.11 ± 0.937	4.40 ± 0.078
0.125	7.16 ± 1.135	40.83 ± 1.033	26.82 ± 1.894	7.05 ± 0.815
0.25	18.99 ± ` 0.701	65.13 ± 0.491	43.60 ± 1.026	14.60 ± 0.984
0.5	33.79 ± 0.657	76.53 ± 0.580	63.25 ± 3.656	21.28 ± 0.248

Percentage of inhibition (%) of crude extracts are expressed as mean ± standard deviation, n=3

The percentage of inhibition of DPPH radical scavenging activity by crude petroleum ether, chloroform, methanol and water extracts of *Ervatamia coronaria* (roots) increased with increasing concentration of the extracts. Among the four extracts, crude chloroform extract exhibited the highest inhibition of DPPH radical scavenging activity with 76.53% inhibition at 0.5 mg/ml followed by crude methanol, petroleum ether and water extracts with 63.25%, 33.79% and 21.28% inhibition of DPPH radical scavenging activity at the same concentration.

Figure 4.2 illustrated the dose-response curves showing the DPPH radical scavenging activity of standard ascorbic acid, crude petroleum ether, chloroform, methanol and water extracts of *Ervatamia coronaria* (roots).

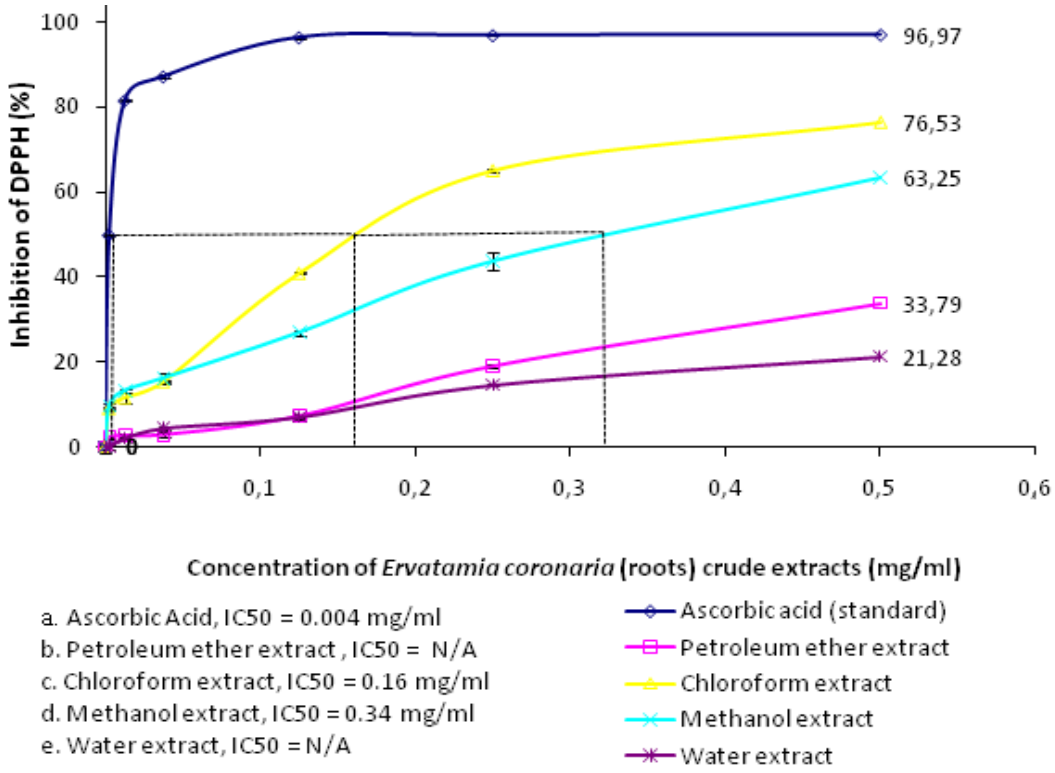

Figure 4.2: Dose-response curves showing radical scavenging activity of *Ervatamia coronaria* (roots) crude extracts and ascorbic acid in the DPPH radical scavenging assay.

In comparison with the positive reference standard ascorbic acid, the radical scavenging activity of the crude chloroform extract was strong and crude methanol extract was moderate strong at highest concentration tested. While the radical scavenging activity of both crude petroleum ether and water extracts were weak at all concentrations tested. IC_{50} is the concentration of an inhibitor where the response (or binding) is reduced by half which can be obtained from graph extrapolation. The IC_{50} of standard ascorbic acid were 0.004 mg/ml followed by crude chloroform and methanol extracts with 0.16 mg/ml and 0.34 mg/ml respectively. The IC_{50} of crude petroleum ether and water extracts could not be determined at the concentrations evaluated due to inhibition percentage of DPPH radical did not reach 50% even at the highest concentration tested.

4.4.1(iii) Radical Scavenging Activity of *Ervatamia coronaria (*stems)

The results of DPPH radical scavenging assay for crude petroleum ether, chloroform, methanol and water extracts of *Ervatamia coronaria* (stems) at concentration 0.0025, 0.0125, 0.0375, 0.125, 0.25 and 0.5 mg/ml are shown in Table 4.25.

Table 4.25: Radical scavenging activity of the crude petroleum ether, chloroform, methanol, and water extracts of *Ervatamia coronaria (*stems) in DPPH radical scavenging assay.

Concentration of Crude Extracts (mg/ml)	Percentage of Inhibition (%)			
	Crude Petroleum Ether Extracts	Crude Chloroform Extracts	Crude Methanol Extracts	Crude Water Extracts
0.0025	0.77 ± 0.127	1.30 ± 0.393	9.03 ± 1.840	1.01 ± 0.237
0.0125	1.25 ± 0.156	5.07 ± 0.676	18.03 ± 0.801	1.32 ± 0.000
0.0375	1.55 ± 0.468	7.63 ± 0.503	21.34 ± 0.465	3.64 ± 0.808
0.125	6.50 ± 1.663	29.56 ± 0.569	41.90 ± 0.620	14.15 ± 0.732
0.25	18.84 ± 0.836	49.74 ± 1.415	58.47 ± 2.824	22.29 ± 0.642
0.5	26.65 ± 2.070	63.97 ± 1.178	82.78 ± 0.674	26.29 ± 1.318

Percentage of inhibition (%) of crude extracts are expressed as mean ± standard deviation, n=3

The percentage of inhibition of DPPH radical scavenging activity by crude petroleum ether, chloroform, methanol and water extracts of *Ervatamia coronaria* (stems) increased with increasing concentration of the extracts. Among the four extracts, crude methanol extract exhibited the highest inhibition of DPPH radical scavenging activity with 82.78% inhibition at 0.5 mg/ml followed by crude chloroform, petroleum ether and water extracts with 63.97%, 26.65% and 26.29% inhibition of DPPH radical scavenging activity respectively at the same concentration.

Figure 4.3 illustrated the dose response curve of DPPH radical scavenging activity of standard ascorbic acid, crude petroleum ether, chloroform, methanol and water extracts of *Ervatamia coronaria* (stems).

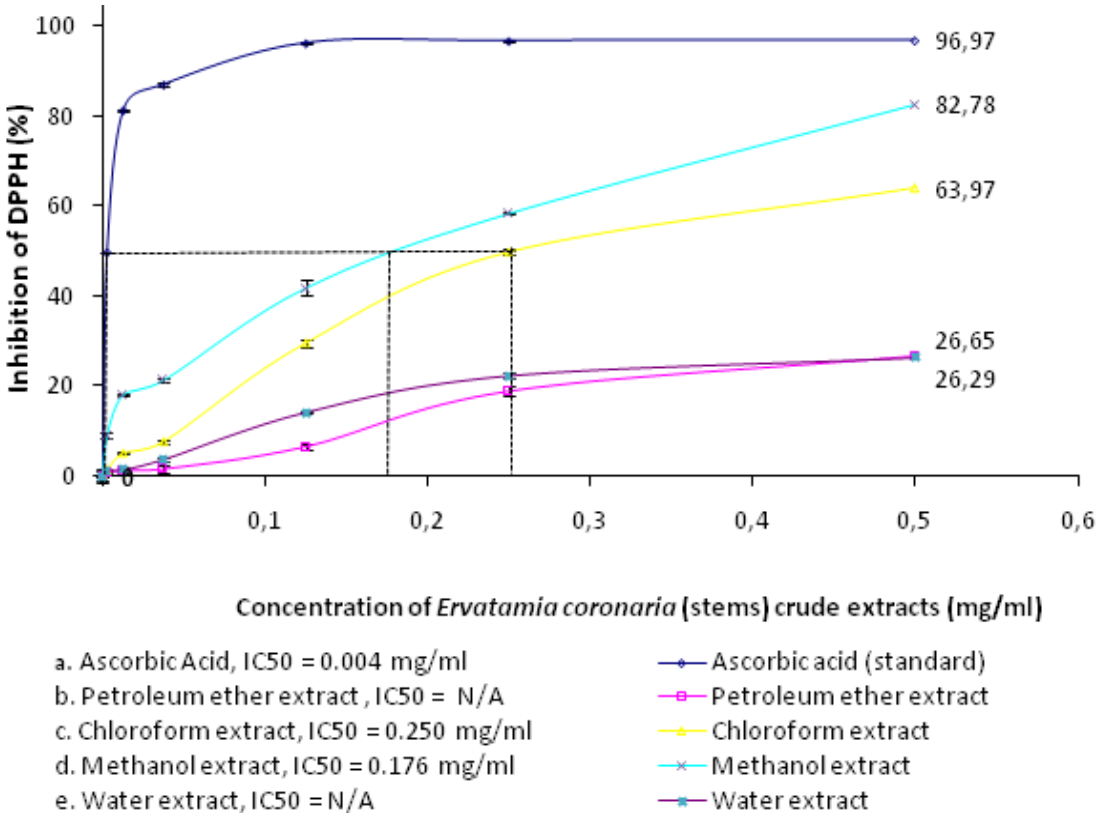

Figure 4.3: Dose-response curves showing radical scavenging activity of *Ervatamia coronaria* (stems) crude extracts and ascorbic acid in the DPPH radical scavenging assay.

In comparison with the positive reference standard ascorbic acid, the radical scavenging activity of the crude methanol extract was strong and crude chloroform extract was moderate strong at highest concentration tested. While the radical scavenging activity of both crude petroleum ether and water extracts were weak at all concentrations tested. The IC_{50} of standard ascorbic acid were 0.004 mg/ml followed by crude methanol and chloroform extracts with 0.176 mg/ml and 0.250 mg/ml respectively. The IC_{50} of crude petroleum ether and water extracts could not be determined at the concentrations evaluated due to inhibition percentage of DPPH radical did not reach 50% even at the highest concentration tested.

4.4.1(iv) Radical Scavenging Activity of *Ervatamia coronaria* (leaves)

The results of DPPH radical scavenging assay for crude petroleum ether, chloroforms, methanol and water extracts of *Ervatamia coronaria* (leaves) at concentration 0.0025, 0.0125, 0.0375, 0.125, 0.25 and 0.5 mg/ml are shown in Table 4.26

Table 4.26: Radical scavenging activity of the crude petroleum ether, chloroform, methanol, and water extracts of *Ervatamia coronaria* (leaves) in the DPPH radical scavenging assay.

Concentration of Crude Extracts (mg/ml)	Percentage of Inhibition (%)			
	Crude Petroleum Ether Extracts	Crude Chloroform Extracts	Crude Methanol Extracts	Crude Water Extracts
0.0025	0.51 ± 0.520	1.90 ± 0.469	3.51 ± 0.331	1.42 ± 0.294
0.0125	1.73 ± 0.395	5.10 ± 0.439	6.25 ± 0.853	4.43 ± 1.223
0.0375	7.29 ± 1.613	9.14 ± 0.705	11.54 ± 0.502	9.11 ± 0.502
0.125	16.27 ± 0.163	34.64 ± 0.730	36.37 ± 0.847	20.95 ± 0.322
0.25	22.35 ± 0.000	70.09 ± 2.523	73.32 ± 1.245	23.07 ± 0.412
0.5	24.64 ± 1.433	72.22 ± 1.111	90.04 ± 0.639	27.44 ± 0.747

Percentage of inhibition (%) of crude extracts are expressed as mean ± standard deviation, n=3

The percentage of inhibition of DPPH radical scavenging activity by crude petroleum ether, chloroform, methanol and water extracts of *Ervatamia coronaria* (leaves) increased with increasing concentration of the extracts. Among the four extracts, crude methanol extract exhibited the highest inhibition of DPPH radical scavenging activity with 90.04% inhibition at 0.5 mg/ml concentration followed by crude chloroform, water and petroleum ether extracts with 72.22%, 27.44% and 24.64% inhibition of DPPH radical scavenging activity respectively.

Figure 4.4 illustrated the dose response curve of DPPH radical scavenging activity of standard ascorbic acid, crude petroleum ether, chloroform, methanol and water extracts of *Ervatamia coronaria* (leaves).

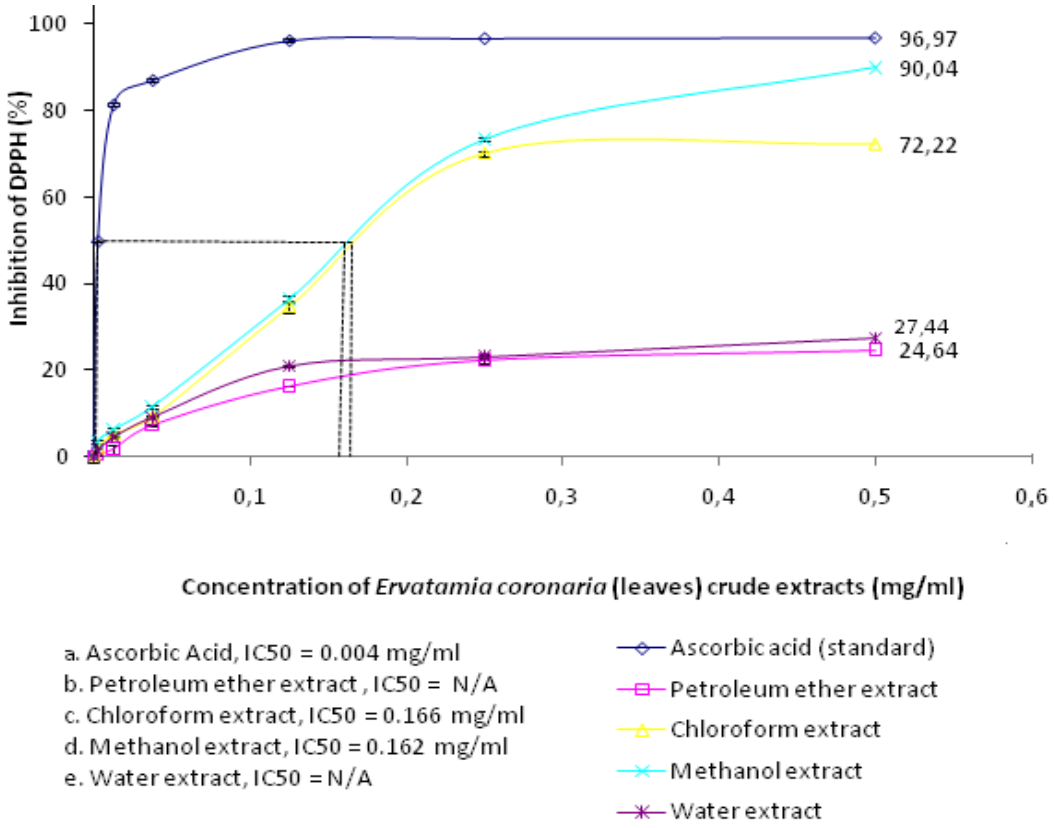

Figure 4.4: Dose-response curves showing radical scavenging activity of *Ervatamia coronaria* (leaves) crude extracts and ascorbic acid in the DPPH radical scavenging assay.

In comparison with the positive reference standard ascorbic acid, the radical scavenging activity of the crude methanol extract was very strong and crude chloroform extract was moderate strong at highest concentration tested. While the radical scavenging activity of both crude petroleum ether and water extracts were weak at all concentrations tested. The IC_{50} of standard ascorbic acid were 0.004 mg/ml followed by crude methanol and chloroform extracts with 0.162 mg/ml and 0.166 mg/ml respectively. The IC_{50} of crude petroleum ether and water extracts could not be determined at the concentrations evaluated due to inhibition percentage of DPPH radical did not reach 50% even at the highest concentration tested.

4.4.1(v) Radical Scavenging Activity of *Tinospora crispa* (stems)

The results of DPPH radical scavenging assay for crude petroleum ether, chloroforms, methanol and water extracts of *Tinospora crispa* (stems) at concentration 0.0025, 0.0125, 0.0375, 0.125, 0.25 and 0.5 mg/ml are shown in Table 4.27.

Table 4.27: Radical scavenging activity of the crude petroleum ether, chloroform, methanol, and water extracts of *Tinospora crispa* (stems) in DPPH radical scavenging assay.

Concentration of Crude Extracts (mg/ml)	Percentage of Inhibition (%)			
	Crude Petroleum Ether Extracts	Crude Chloroform Extracts	Crude Methanol Extracts	Crude Water Extracts
0.0025	1.36 ± 0.435	15.00 ± 1.480	14.35 ± 1.420	7.89 ± 1.316
0.0125	4.27 ± 0.351	18.47 ± 0.379	16.89 ± 1.533	15.38 ± 1.628
0.0375	8.10 ± 0.310	22.23 ± 2.940	24.03 ± 1.211	19.79 ± 0.485
0.125	12.86 ± 1.500	46.83 ± 0.153	52.37 ± 0.592	23.59 ± 1.836
0.25	23.01 ± 1.890	55.47 ± 0.681	86.47 ± 0.530	34.53 ± 1.974
0.5	46.19 ± 3.610	59.90 ± 1.400	90.85 ± 0.666	37.72 ± 1.697

Percentage of inhibition (%) of crude extracts are expressed as mean ± standard deviation, n=3

The percentage of inhibition of DPPH radical scavenging activity by crude petroleum ether, chloroform, methanol and water extracts of *Tinospora crispa* (stems) increased with increasing concentration of the extracts. Among the four extracts, crude methanol extract exhibited the highest inhibition of DPPH radical scavenging activity with 90.85% inhibition at 0.5 mg/ml concentration followed by crude chloroform, petroleum ether and water extracts with 59.90%, 46.19% and 37.72% inhibition of DPPH radical scavenging activity respectively.

Figure 4.5 illustrated the dose response curve of DPPH radical scavenging activity of standard ascorbic acid, crude petroleum ether, chloroform, methanol and water extracts of *Tinospora crispa* (stems).

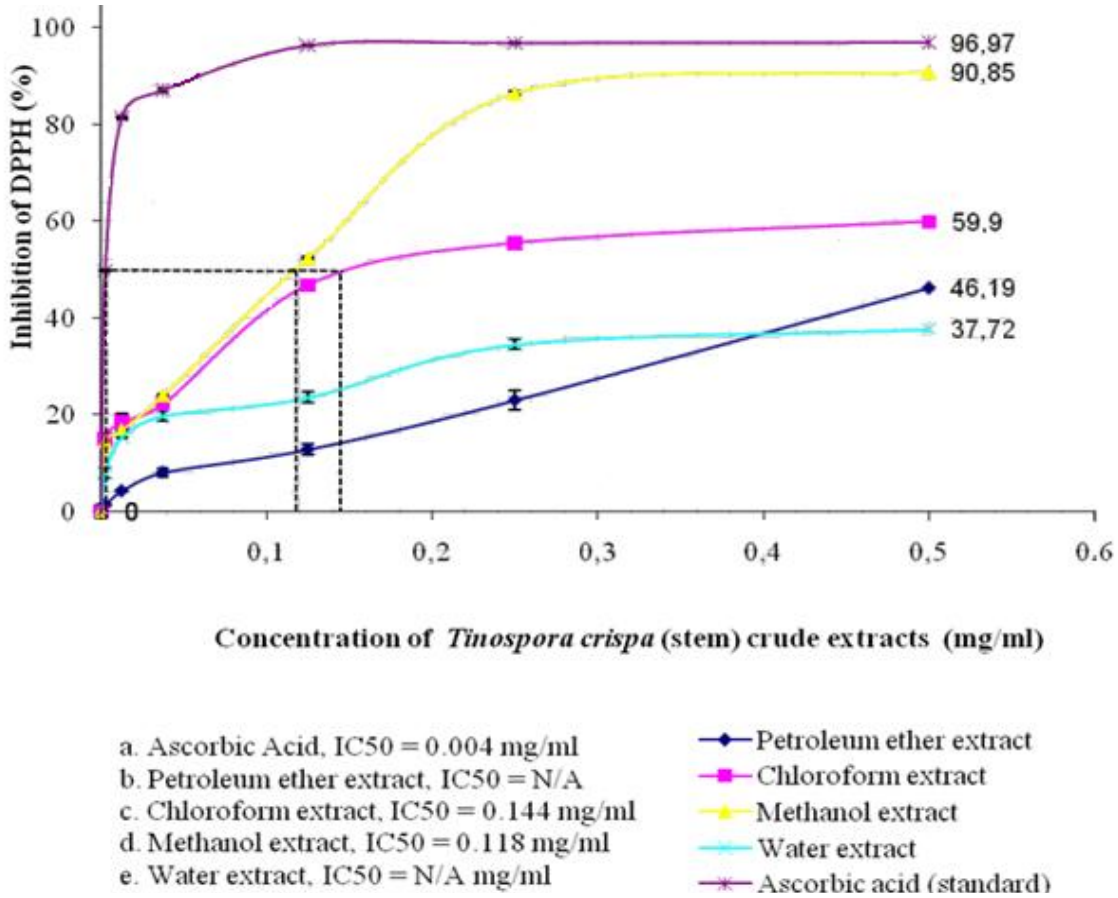

Figure 4.5: Dose-response curves showing radical scavenging activity of *Tinospora crispa* (stems) crude extracts and ascorbic acid in the DPPH radical scavenging assay.

In comparison with the positive reference standard ascorbic acid, the radical scavenging activity of the crude methanol extract was very strong and crude chloroform extract was moderate at highest concentration tested. While the radical scavenging activity of both crude petroleum ether and water extracts were moderate weak at all concentrations tested. The IC_{50} of standard ascorbic acid were 0.004 mg/ml followed by crude methanol and chloroform extracts with 0.118 mg/ml and 0.144 mg/ml respectively. The IC_{50} of crude petroleum ether and water extracts could not be determined at the concentrations evaluated due to inhibition percentage of DPPH radical did not reach 50% even at the highest concentration tested

4.4.2 Metal Chelating Assay

The purpose of metal chelating assay is to evaluate the ability of *Ervatamia coronaria* and *Tinospora crispa* extracts to chelate ferrous ion and preventing the formation of ferrozine-Fe^{2+} complex.

4.4.2(i) Metal Chelating Activity of EDTA as Positive Reference Standard

EDTA (Ethylenediaminetetra acetic acid) was used as the positive reference standard in the metal chelating assay. Table 4.28 shows the percentage of inhibition of ferrozine-Fe^{2+} complex formation by EDTA at concentrations of 0.0625 mg/ml, 0.125 mg/ml, 0.25 mg/ml, 0.5 mg/ml and 1 mg/ml.

Table 4.28: The percentage of inhibition of ferrozine-Fe^{2+} complex formation by EDTA in metal chelating assay.

Concentration of EDTA (mg/ml)	Percentage of inhibition (%)
	Mean ± standard deviation (SD)
0.0625	96.27 ± 0.412
0.125	97.15 ± 0.369
0.25	97.46 ± 0.453
0.5	97.86 ± 1.481
1	98.51 ± 1.561

The percentage of inhibition of ferrozine-Fe^{2+} complex formation by EDTA increased with the increasing concentrations. Very strong chelating activity was observed at all concentrations tested. At concentration of 1 mg/ml, EDTA obtained the highest percentage of chelating activity with 98.51% followed by 97.86%, 97.46%, 97.15% and 96.27% at 0.5 mg/ml, 0.25 mg/ml, 0.125 mg/ml and 0.0625 respectively.

4.4.2(ii) Metal Chelating Activities of *Ervatamia Coronaria* (roots) Extracts

The results of metal chelating assay for crude petroleum ether, chloroform, methanol and water extracts of *Ervatamia coronaria* (roots) at concentrations 0.0625 mg/ml, 0.125 mg/ml, 0.25 mg/ml, 0.5 mg/ml and 1 mg/ml are shown in Table 4.29.

Table 4.29: Metal Chelating activities by crude petroleum ether, chloroform, methanol and water extracts of *Ervatamia coronaria* roots in the metal chelating assay.

Concentration of Crude Extracts (mg/ml)	Percentage of Inhibition (%)			
	Crude Petroleum Ether Extracts	Crude Chloroform Extracts	Crude Methanol Extracts	Crude Water Extracts
0.0625	0.86 ± 0.254	3.40 ± 0.445	3.29 ± 1.154	3.68 ± 0.577
0.125	1.50 ± 0.473	9.06 ± 0.000	8.53 ± 0.906	5.36 ± 1.727
0.25	3.94 ± 0.885	11.41 ± 0.081	10.32 ± 0.000	36.19 ± 2.015
0.5	3.97 ± 1.172	27.97 ± 1.406	30.24 ± 3.498	76.36 ± 3.491
1.0	4.08 ± 0.775	67.25 ± 1.415	51.56 ± 4.029	92.62 ± 2.048

All percentage of inhibition (%) are expressed as mean ± standard deviation, n=3

Crude water extract was the best metal chelator compared to other extracts with percentage of inhibition of 92.62%, 76.36, 36.19%, 5.36% and 3.68% at 1.0 mg/ml, 0.5 mg/ml, 0.25 mg.ml, 0.125 mg/ml and 0.0625 mg/ml respectively. Crude petroleum ether exhibited the lowest metal chelating activity with only 4.08% chelating activity at 1.0 mg/ml. Metal chelating activities of crude chloroform and methanol extracts showed moderate activity with chelating activity inhibition of 67.25% and 51.56% respectively at 1.0 mg/ml.

Figure 4.6 illustrated the dose-response curves showing the metal chelating activities of all four extracts of *Ervatamia coronaria* (roots) and EDTA as positive standard.

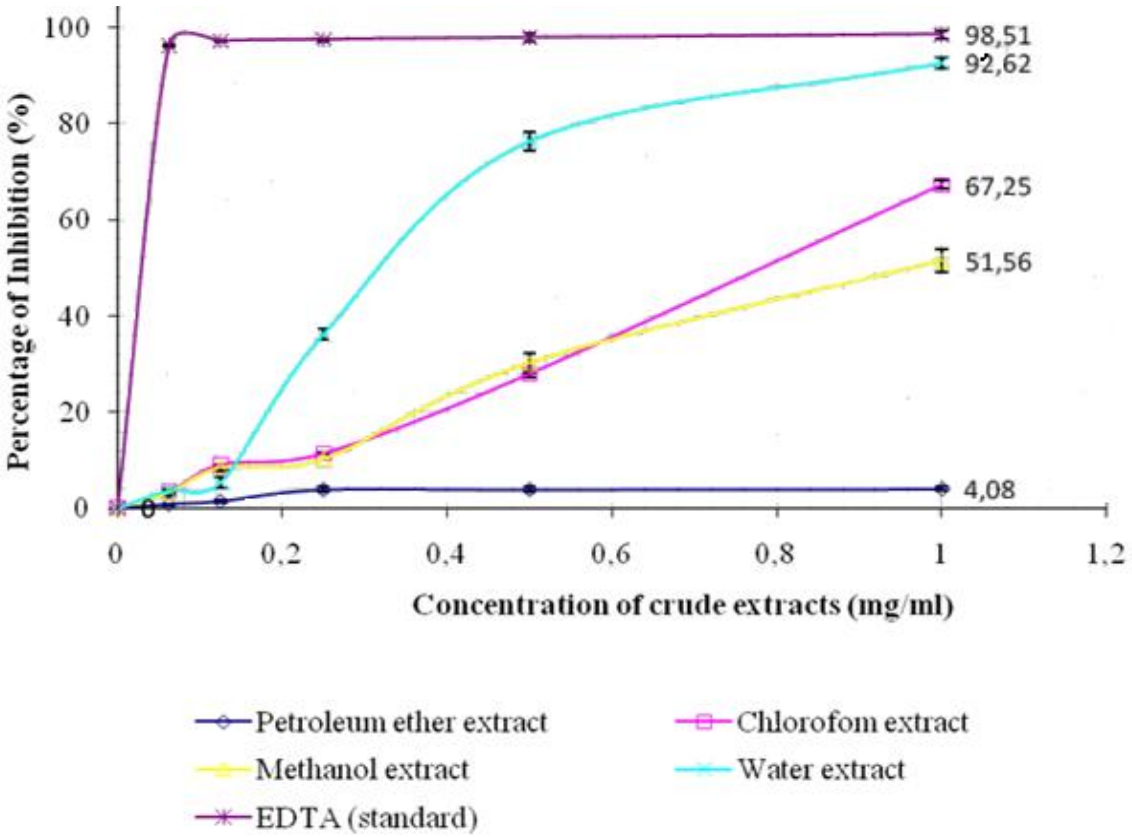

Figure 4.6: Dose-response curve showing the metal chelating activities of *Ervatamia coronaria* (roots) crude extracts and standard EDTA in the metal chelating assay.

The percentage of inhibition of ferrozine-Fe^{2+} by all extracts and positive standard increased with increasing concentrations. In comparison with standard EDTA, the chelating activity of crude water extract was very strong with 92.62% inhibition of ferrozine-Fe^{2+} at the highest concentration. Crude chloroform and methanol extracts exhibited moderate strong and moderate chelating activity respectively at the highest concentrations when compared with standard EDTA. Petroleum ether extract exhibited very weak chelating activity at all concentration tested.

4.4.2(iii) Metal Chelating Activities of *Ervatamia Coronaria* (stems) Extracts.

The results of metal chelating assay for crude petroleum ether, chloroform, methanol and water extracts of *Ervatamia coronaria* (stems) at concentrations 0.0625 mg/ml, 0.125 mg/ml, 0.25 mg/ml, 0.5 mg/ml and 1 mg/ml are shown in Table 4.30.

Table 4.30: Metal chelating activities by crude petroleum ether, chloroform, methanol and water extracts of *Ervatamia coronaria* (stems) in the metal chelating assay.

Concentration of Crude Extracts (mg/ml)	Percentage of Inhibition (%)			
	Crude Petroleum Ether Extracts	Crude Chloroform Extracts	Crude Methanol Extracts	Crude Water Extracts
0.0625	1.02 ± 0.007	1.75 ± 0.006	1.35 ± 0.335	2.73 ± 0.479
0.125	1.65 ± 0.635	2.96 ± 0.508	2.65 ± 0.722	2.81 ± 0.468
0.25	1.68 ± 0.583	11.45 ± 0.606	7.03 ± 2.408	12.82 ± 3.767
0.5	2.46 ± 0.826	37.94 ± 3.361	12.06 ± 2.888	40.59 ± 1.882
1.0	2.94 ± 0.797	85.24 ± 4.752	36.70 ± 2.770	77.65 ± 3.341

All percentage of inhibition (%) are expressed as mean ± standard deviation, n=3

Crude chloroform extract exhibited the best metal chelating activity with percentage of inhibition of ferrozine-Fe^{2+} complex of 85.24% at 1.0 mg/ml followed by crude water extract with 77.65% at the same concentration. Crude methanol extract exhibited lower metal chelating activity compared to crude chloroform extract and crude water extract while crude petroleum ether extract exhibited the lowest metal chelating activity at all concentrations tested compared to the other extracts.

Figure 4.7 illustrated the dose-response curves showing the metal chelating activities of all four extracts of *Ervatamia coronaria* (stems) and EDTA as positive standard.

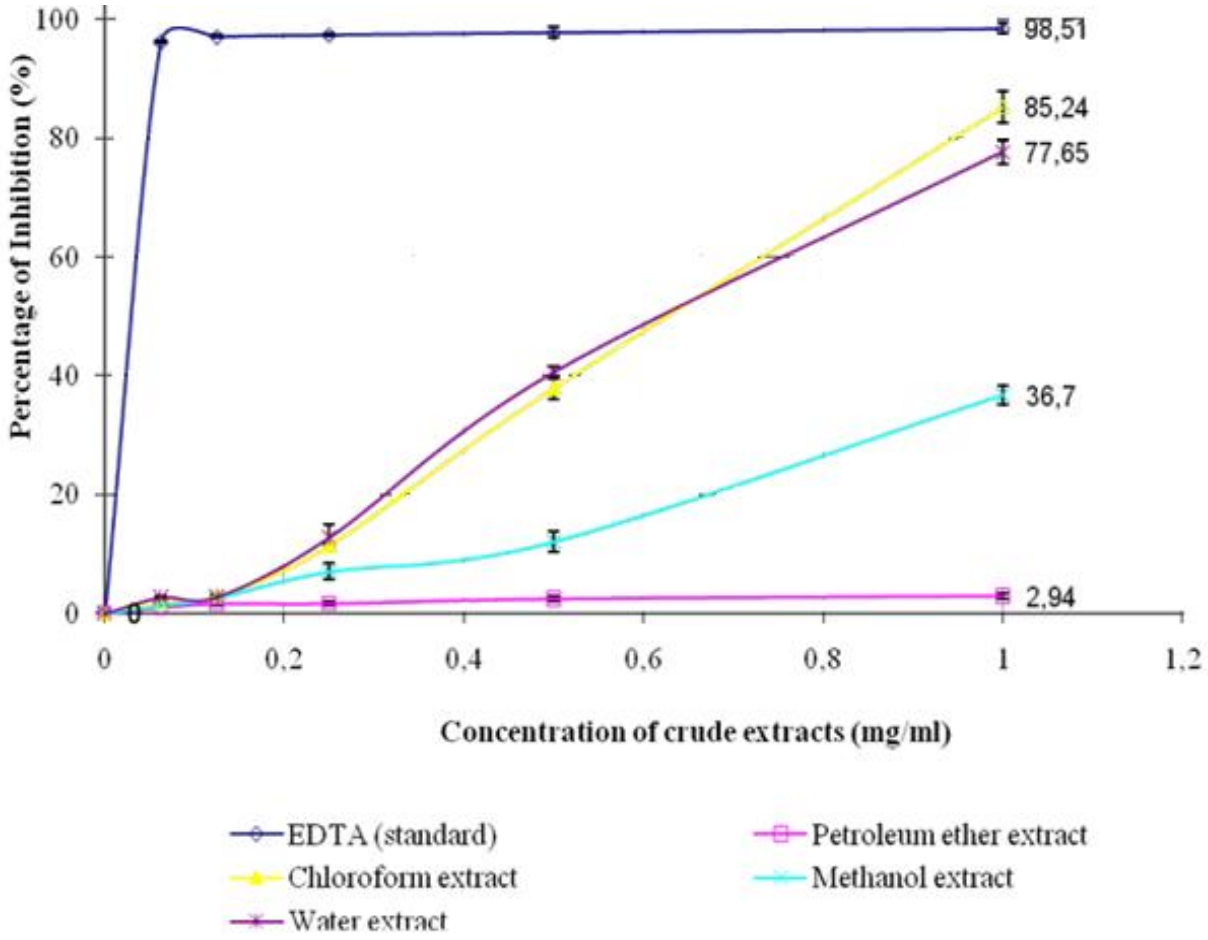

Figure 4.7: Dose-response curves showing the metal chelating activities of *Ervatamia coronaria* (stems) crude extracts and standard EDTA in the metal chelating assay.

The percentage of inhibition of ferrozine-Fe^{2+} of all extracts and positive standard increased with increased of concentrations. In comparison with standard EDTA, the chelating activity of crude chloroform extract and crude water extract were strong with 85.24% and 77.65% inhibition of ferrozine-Fe^{2+} respectively at the highest concentration. Crude methanol extract exhibited moderate weak chelating activity at the highest concentrations while crude petroleum ether extract exhibited very weak chelating activity at all concentration tested when compared with standard EDTA.

4.4.2(iv) Metal Chelating Activities of *Ervatamia Coronaria* (leaves) Extracts

The results of metal chelating assay for crude petroleum ether, chloroform, methanol and water extracts of *Ervatamia coronaria* (leaves) at concentrations 0.0625 mg/ml, 0.125 mg/ml, 0.25 mg/ml, 0.5 mg/ml and 1 mg/ml are shown in Table 4.31.

Table 4.31: Metal chelating activities by crude petroleum ether, chloroform, methanol and water extracts of *Ervatamia coronaria* (leaves) in the metal chelating assay.

Concentration of Crude Extracts (mg/ml)	Percentage of Inhibition (%)			
	Crude Petroleum Ether Extracts	Crude Chloroform Extracts	Crude Methanol Extracts	Crude Water Extracts
0.0625	NV	9.54 ± 0.184	5.53 ± 0.010	0.87 ± 0.551
0.125	NV	10.24 ± 0.756	7.93 ± 1.812	1.70 ± 0.318
0.25	NV	11.22 ± 2.431	14.38 ± 0.006	3.31 ± 0.872
0.5	NV	20.27 ± 0.432	21.61 ± 5.379	19.65 ± 6.949
1.0	NV	37.97 ± 1.405	69.64 ± 4.953	46.11 ± 4.256

All percentage of inhibition (%) are expressed as mean ± standard deviation, n=3;NV= Not valid

Crude methanol extract exhibited the best metal chelating activity compared to other extracts with percentage of inhibition of ferrozine-Fe^{2+} complex of 69.64% at 1.0 mg/ml concentration. Crude water extract and crude chloroform extracts exhibited lower metal chelating activity with 46.11% and 37.97% respectively at 1.0 mg/ml when compared with crude methanol extract. The result for crude petroleum ether extract was not valid because the percentage of inhibition was very low.

Figure 4.8 illustrated the dose-response curves showing the metal chelating activities of three extracts of *Ervatamia coronaria* (leaves) and positive standard of EDTA.

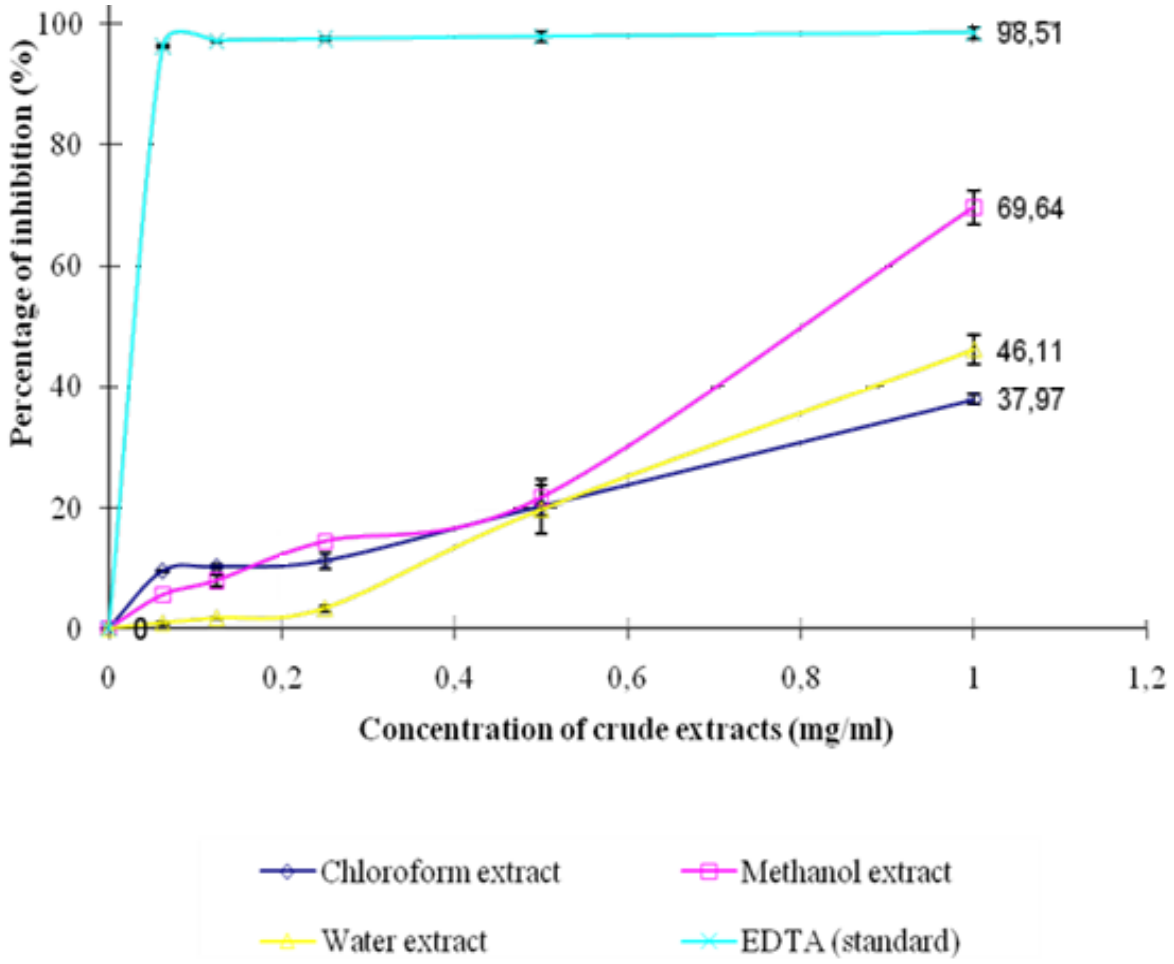

Figure 4.8: Dose-response curves showing the metal chelating activities of *Ervatamia coronaria* (leaves) crude extracts and standard EDTA in the metal chelating assay.

The percentage of inhibition of ferrozine-Fe^{2+} of three extracts and positive standard increased with increasing concentrations. In comparison with standard EDTA, the chelating activity of crude chloroform extract was moderate strong with 69.64% inhibition of ferrozine-Fe^{2+} at the highest concentration. Crude water extract exhibited moderate chelating activity at the highest concentration while crude petroleum ether extract exhibited moderate weak chelating activity at all concentration tested when compared with standard EDTA.

4.4.2(v) Metal Chelating Activities of Crude Extracts of *Tinospora crispa* (stems)

The results of metal chelating assay for crude petroleum ether, chloroform, methanol and water extracts of *Tinospora crispa* (stems) at concentrations 0.0625 mg/ml, 0.125 mg/ml, 0.25 mg/ml, 0.5 mg/ml and 1 mg/ml are shown in Table 4.32.

Table 4.32: Metal chelating activities by crude petroleum ether, chloroform, methanol and water extracts of *Tinospora crispa* (stems) in the metal chelating assay.

Concentration of Crude Extracts (mg/ml)	Percentage of Inhibition (%)			
	Crude Petroleum Ether Extracts	Crude Chloroform Extracts	Crude Methanol Extracts	Crude Water Extracts
0.0625	4.16 ± 0.520	2.36 ± 0.548	5.58 ± 0.000	3.10 ± 0.666
0.125	5.09 ± 0.000	3.46 ± 0.006	5.67 ± 0.000	3.55 ± 1.454
0.25	5.17 ± 0.704	5.44 ± 1.210	13.66 ± 1.800	16.51 ± 1.500
0.5	8.47 ± 0.381	14.35 ± 1.172	35.79 ± 0.578	47.99 ± 1.176
1.0	15.27 ± 2.983	28.36 ± 1.337	81.97 ± 3.282	77.13 ± 5.587

All percentage of inhibition (%) are expressed as mean ± standard deviation, n=3

Crude methanol extract exhibited the best metal chelating activity compared to other extracts with percentage of inhibition of ferrozine-Fe^{2+} complex of 81.97% at 1.0 mg/ml concentration followed by crude water extract with 77.13% at the same concentration. Crude chloroform extract and crude petroleum ether extract showed lower chelating activities with 28.36% and 15.27% respectively at 1.0 mg/ml in comparison with other extracts.

Figure 4.9 illustrated the dose-response curves showing the metal chelating activities of all four extracts of *Tinospora crispa* (stems) and positive standard of EDTA.

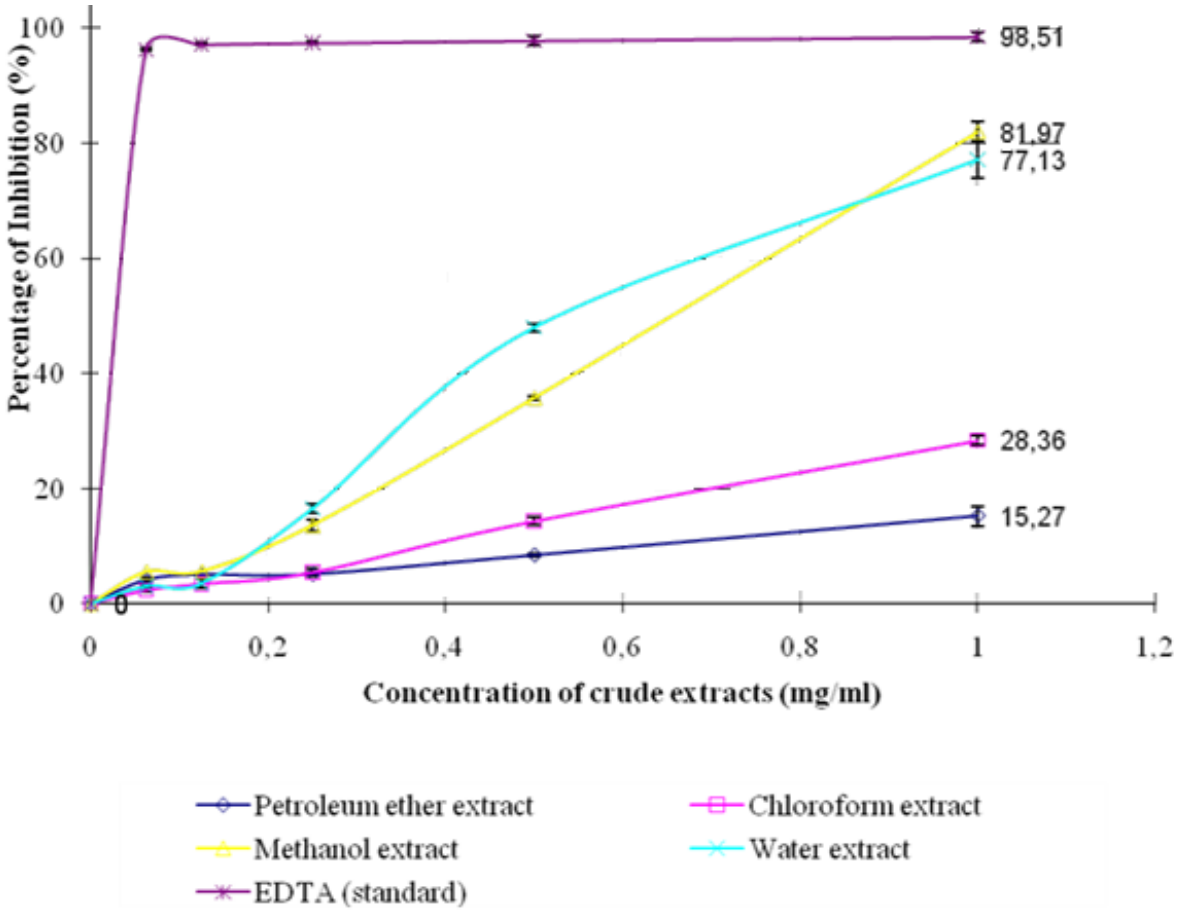

Figure 4.9: Dose-response curves showing the metal chelating assay of *Tinospora crispa* (stems) crude extracts and standard EDTA in the metal chelating assay.

The percentage of inhibition of ferrozine-Fe^{2+} of all extracts and positive standard increased with increasing concentrations. In comparison with standard EDTA, the chelating activities of crude methanol extract and crude water extract were strong with 81.97% and 77.13% inhibition of ferrozine-Fe^{2+} at the highest concentration. Crude chloroform extract and crude petroleum ether extract exhibited weak chelating activity at all concentrations tested when compared with standard EDTA.

4.4.3 Reducing Power Assay

The purpose of reducing power assay is to evaluate the ability of *Ervatamia coronaria* and *Tinospora crispa* extracts to reduce Fe^{3}+ to Fe^{2}+.

4.4.3(i) Reducing Power of BHA as Positive Reference Standard

BHA (Butylated hydroxyanisole) was used as the positive reference standard in the reducing power assay. Table 4.33 shows the absorbance of reducing power of BHA at different concentrations of 0.0625 mg/ml, 0.125 mg/ml, 0.25 mg/ml, 0.5 mg/ml and 1 mg/ml.

Table 4.33 : The absorbance of BHA at 700 nm in reducing power assay

Concentration of BHA (mg/ml)	Absorbance at 700 nm (A)
	Mean ± standard deviation (SD)
0.0625	0.56 ± 0.005
0.125	1.04 ± 0.021
0.25	1.72 ± 0.054
0.5	2.61 ± 0.061
1	2.79± 0.081

The absorbance of BHA at concentration 1 mg/ml, 0.5 mg/ml, 0.25 mg/ml, 0.125 mg/ml and 0.0625 mg/ml were 2.794A, 2.610A, 1.719A, 1.044A and 0.557A respectively. Higher absorbance indicated greater reducing power. Thus, the reducing power of BHA increased with increasing concentration.

Figure 4.10 illustrated the dose-response curve showing the reducing power activity of standard BHA at concentrations 1 mg/ml, 0.5 mg/ml, 0.25 mg/ml, 0.125 mg/ml and 0.0625 mg/ml.

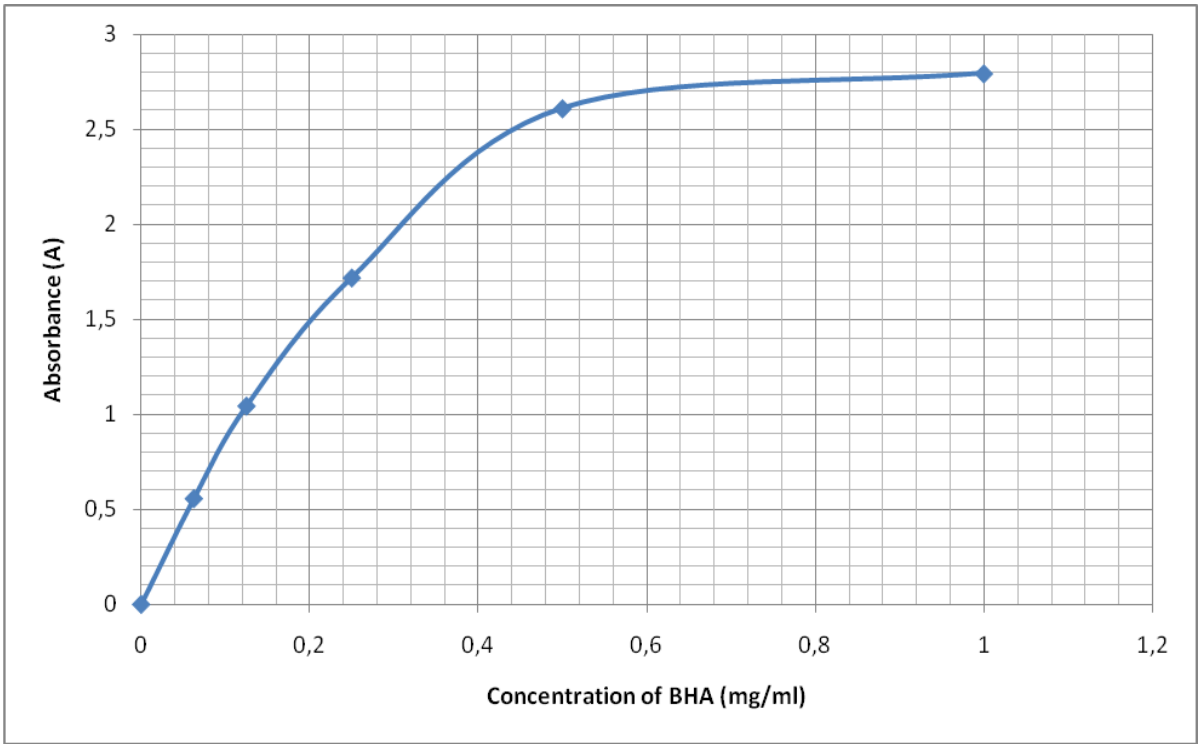

Figure 4.10 : Reducing power of BHA in the reducing power assay.

As shown in Figure 4.10, the reducing power of BHA tremendously increased at concentration 0.0625 mg/ml, 0.125 mg/ml and 0.25 mg/ml; slightly increased at concentrations 0.5 mg/ml and 1.0 mg/ml. The higher the absorbance the greater reducing power.

4.4.3(ii) Reducing Power of *Ervatamia Coronaria* (roots) Extracts

The results of the reducing power assay for crude petroleum ether, chloroform, methanol and water extracts of *Ervatamia coronaria* (roots) at concentrations 0.0625 mg/ml, 0.125 mg/ml, 0.25 mg/ml, 0.5 mg/ml and 1 mg/ml are shown in Table 4.34.

Table 4.34: Reducing power of crude petroleum ether, chloroform, methanol and water extracts of *Ervatamia coronaria* (roots) in the reducing power assay.

Concentration of Crude Extracts (mg/ml)	Absorbance at 700 nm (A)			
	Crude Petroleum Ether Extracts	Crude Chloroform Extracts	Crude Methanol Extracts	Crude Water Extracts
0.0625	0.08 ± 0.011	0.22 ± 0.020	0.15 ± 0.007	0.09 ± 0.003
0.125	0.11 ± 0.009	0.28 ± 0.003	0.16 ± 0.014	0.20 ± 0.008
0.25	0.16 ± 0.014	0.41 ± 0.008	0.21 ± 0.018	0.22 ± 0.003
0.5	0.22 ± 0.018	0.63 ± 0.028	0.30 ± 0.007	0.27 ± 0.017
1.0	0.23 ± 0.006	0.89 ± 0.047	0.47 ± 0.035	0.35 ± 0.006

The absorbance at 700 nm of crude extracts are expressed as mean ± standard deviation, n=3

The reducing power of all four extracts increased with increasing concentrations. Among all the extracts, crude chloroform extract exhibited the highest reducing power with 0.89 A followed by crude methanol extract and crude water extract with 0.47 A and 0.35 A respectively at 1.0 mg/ml. The lowest reducing power was exhibited by crude petroleum ether extract with 0.230 A at 1.0 mg/ml.

Figure 4.11 illustrated the dose-response curves showing the reducing power activities of all four extracts of *Ervatamia coronaria* (roots) and positive standard of BHA.

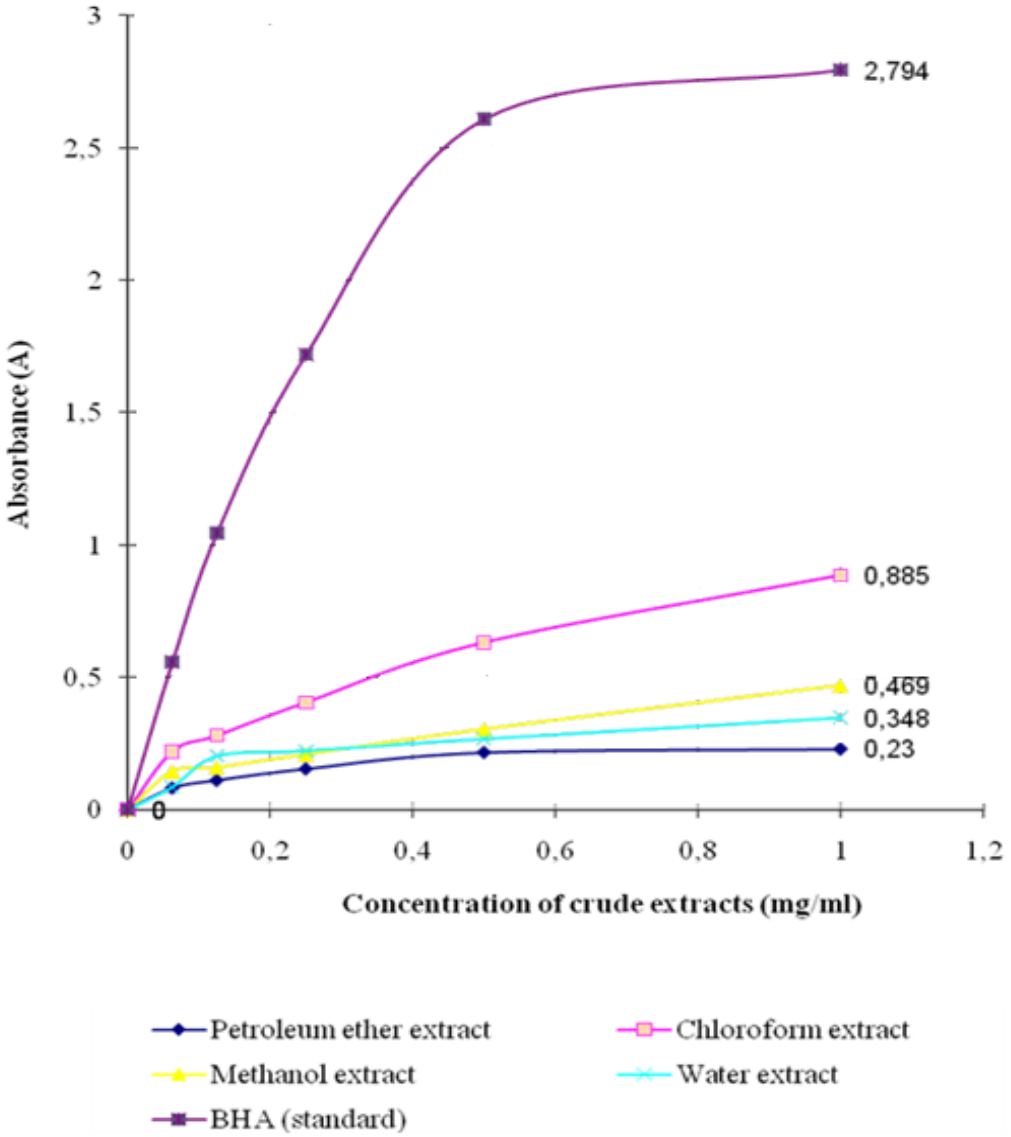

Figure 4.11: Dose-response curves showing the reducing power activities of *Ervatamia coronaria* (roots) crude extracts and BHA in the reducing power assay.

The reducing power (absorbance) of all extracts and positive standard of BHA increased with increasing concentrations. In comparison with positive standard of BHA, the reducing activity of crude chloroform extract was moderate weak with 0.885 A at 1 mg/ml while crude methanol extract, crude water extract and crude petroleum ether extract exhibited weak reducing power activities at all concentrations tested.

4.4.3(iii) Reducing Power of *Ervatamia Coronaria* (stems) Extracts

The results of the reducing power assay for crude petroleum ether, chloroform, methanol and water extracts of *Ervatamia coronaria* (stems) at concentrations 0.0625 mg/ml, 0.125 mg/ml, 0.25 mg/ml, 0.5 mg/ml and 1 mg/ml are shown in Table 4.35.

Table 4.35: Reducing power of crude petroleum ether, chloroform, methanol and water extracts of *Ervatamia coronaria* (stems) in the reducing power assay.

Concentration of Crude Extracts (mg/ml)	Absorbance at 700 nm (A)			
	Crude Petroleum Ether Extracts	Crude Chloroform Extracts	Crude Methanol Extracts	Crude Water Extracts
0.0625	0.12 ± 0.006	0.16 ± 0.000	0.16 ± 0.015	0.22 ± 0.001
0.125	0.14 ± 0.006	0.24 ± 0.006	0.23 ± 0.014	0.23 ± 0.004
0.25	0.22 ± 0.006	0.36 ± 0.127	0.32 ± 0.013	0.26 ± 0.007
0.5	0.35 ± 0.018	0.53 ± 0.042	0.46 ± 0.027	0.34 ± 0.003
1.0	0.62 ± 0.053	0.85 ± 0.061	0.82 ±0.024	0.46 ± 0.006

The absorbance at 700 nm of crude extracts are expressed as mean ± standard deviation, n=3

The reducing power of all four extracts increased with the increased of concentrations. Among all the extracts, crude chloroform extract exhibited the highest reducing power with 0.85 A followed by crude methanol extract, crude petroleum ether extract and crude water extracts with 0.82 A, 0.62 A and 0.46 A respectively at 1.0 mg/ml.

Figure 4.12 illustrated the dose-response curves showing the reducing power activities of all four extracts of *Ervatamia coronaria* (stems) and positive standard of BHA.

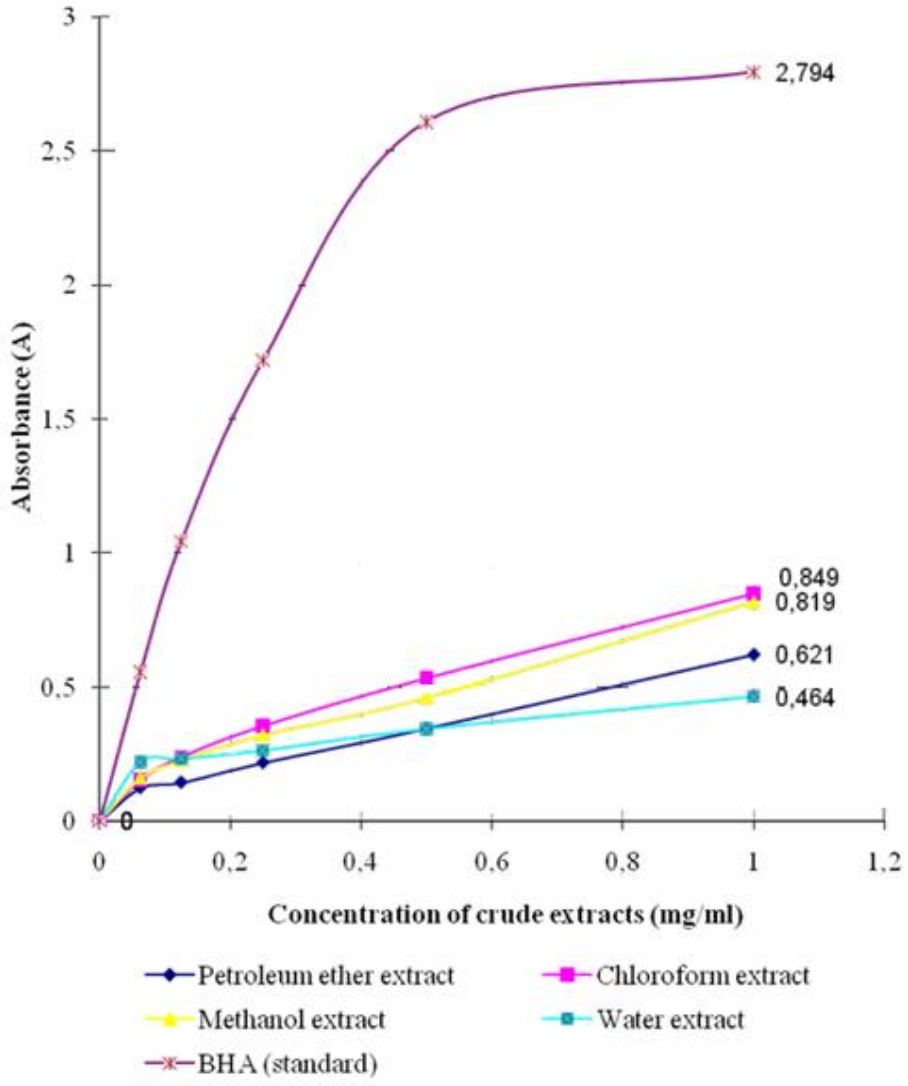

Figure 4.12: Dose-response curves showing the reducing power activities of *Ervatamia coronaria* (stems) crude extracts and BHA in the reducing power assay.

The reducing power (absorbance) of all extracts and positive standard of BHA increased with increased concentrations. In comparison with positive standard of BHA, the reducing activities of crude chloroform extract and crude methanol extract was moderate weak with 0.849 A and 0.819 A respectively at 1 mg/ml while crude petroleum ether extract and crude water extract exhibited weak reducing power activities at all concentrations tested.

4.4.3(iv) Reducing Power of *Ervatamia Coronaria* (leaves) Extracts

The results of the reducing power assay for crude petroleum ether, chloroform, methanol and water extracts of *Ervatamia coronaria* (leaves) at concentrations 0.0625 mg/ml, 0.125 mg/ml, 0.25 mg/ml, 0.5 mg/ml and 1 mg/ml are shown in Table 4.36.

Table 4.36: Reducing power of crude petroleum ether, chloroform, methanol and water extracts of *Ervatamia coronaria* (leaves) in the reducing power assay.

Concentration of Crude Extracts (mg/ml)	Absorbance at 700 nm (A)			
	Crude Petroleum Ether Extracts	Crude Chloroform Extracts	Crude Methanol Extracts	Crude Water Extracts
0.0625	0.10 ± 0.005	0.21 ±0.012	0.15 ± 0.018	0.14 ± 0.004
0.125	0.13 ± 0.019	0.25 ± 0.010	0.16 ± 0.012	0.17 ± 0.006
0.25	0.15 ± 0.004	0.35 ± 0.006	0.25 ± 0.015	0.28 ± 0.013
0.5	0.22 ±0.009	0.56 ± 0.017	0.41 ± 0.041	0.38 ± 0.006
1.0	0.39 ± 0.009	0.87 ± 0.052	0.65 ± 0.030	0.62 ± 0.013

The absorbance at 700 nm of crude extracts are expressed as mean ± standard deviation, n=3

The reducing power of all four extracts increased with the increasing concentrations. Among all the extracts, crude chloroform extract exhibited the highest reducing power with 0.87 A followed by crude methanol extract, crude water extract and crude petroleum ether extracts with 0.65 A, 0.62 A and 0.39 A respectively at 1.0 mg/ml.

Figure 4.13 illustrated the dose-response curves showing the reducing power activities of all four extracts of *Ervatamia coronaria* (leaves) and positive standard of BHA.

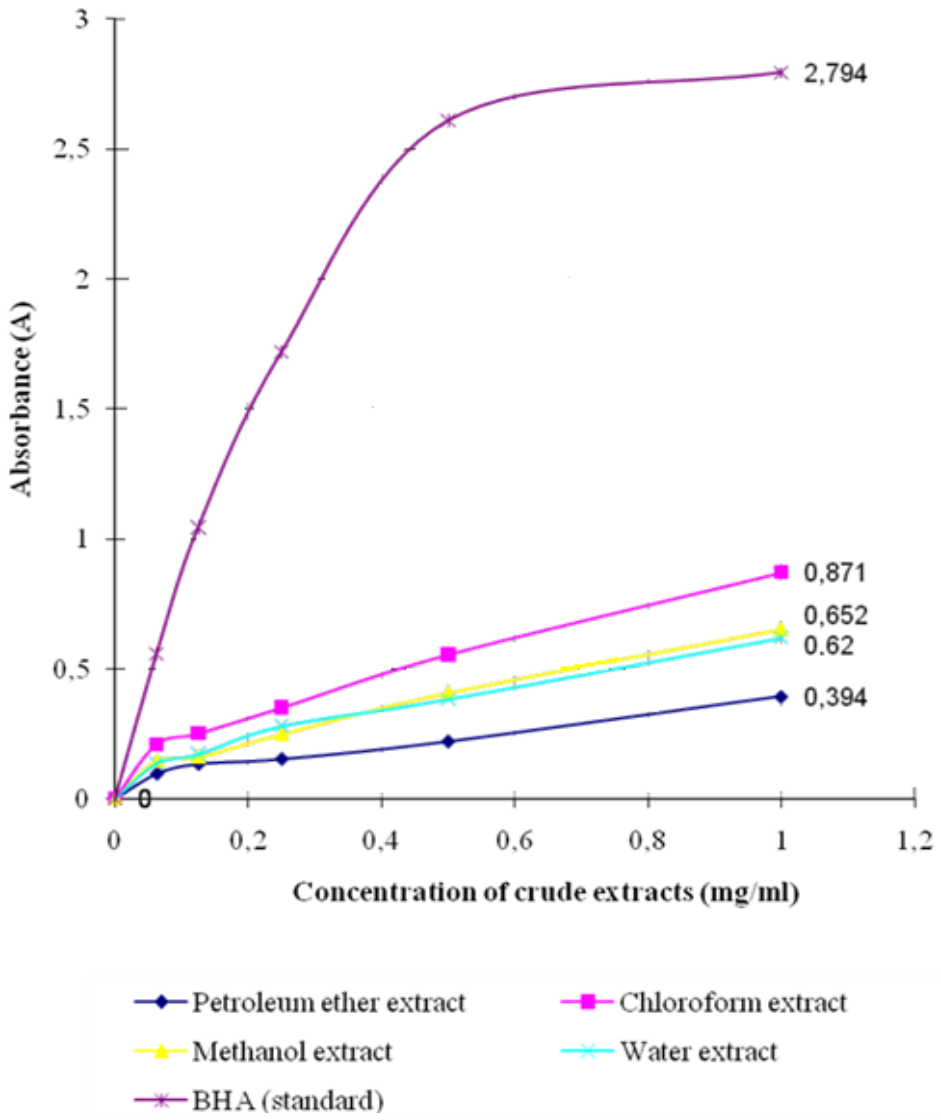

Figure 4.13: Dose-response curves showing the reducing power activities of *Ervatamia coronaria* (leaves) crude extracts and BHA in the reducing power assay.

The reducing power (absorbance) of all extracts and positive standard of BHA increased with increased concentrations. In comparison with positive standard of BHA, the reducing activity of crude chloroform extract was moderate weak with 0.871 A at 1 mg/ml while crude methanol extract, crude water extract and crude petroleum ether extract exhibited weak reducing power activities at all concentrations tested.

4.4.3(v) Reducing Power of *Tinospora crispa* (stems) Extracts

The results of the reducing power assay for crude petroleum ether, chloroform, methanol and water extracts of *Tinospora crispa* (stems) at concentrations 0.0625 mg/ml, 0.125 mg/ml, 0.25 mg/ml, 0.5 mg/ml and 1 mg/ml are shown in Table 4.37.

Table 4.37: Reducing power of crude petroleum ether, chloroform, methanol and water extracts of *Tinospora crispa* (stems) in the reducing power assay.

Concentration of Crude Extracts (mg/ml)	Absorbance at 700 nm (A)			
	Crude Petroleum Ether Extracts	Crude Chloroform Extracts	Crude Methanol Extracts	Crude Water Extracts
0.0625	0.05 ± 0.017	0.27 ± 0.031	0.29 ± 0.006	0.21 ± 0.004
0.125	0.08 ± 0.019	0.30 ± 0.016	0.33 ± 0.010	0.23 ± 0.004
0.25	0.11 ± 0.003	0.40 ± 0.015	0.49 ± 0.060	0.26 ± 0.007
0.5	0.22 ± 0.016	0.51 ± 0.050	0.60 ± 0.023	0.31 ± 0.005
1.0	0.39 ± 0.033	0.77 ± 0.125	0.96 ± 0.040	0.41 ± 0.012

The absorbance at 700 nm of crude extracts are expressed as mean ± standard deviation, n=3

The reducing power of all four extracts increased with the increasing concentrations. Among all the extracts, crude methanol extract exhibited the highest reducing power with 0.96 A followed by crude chloroform extract, crude water extract and crude petroleum ether extract with 0.77 A, 0.41 A and 0.39 A respectively at 1.0 mg/ml.

Figure 4.14 illustrated the dose-response curves showing the reducing power activities of all four extracts of *Tinospora crispa* (stems) and positive standard of BHA.

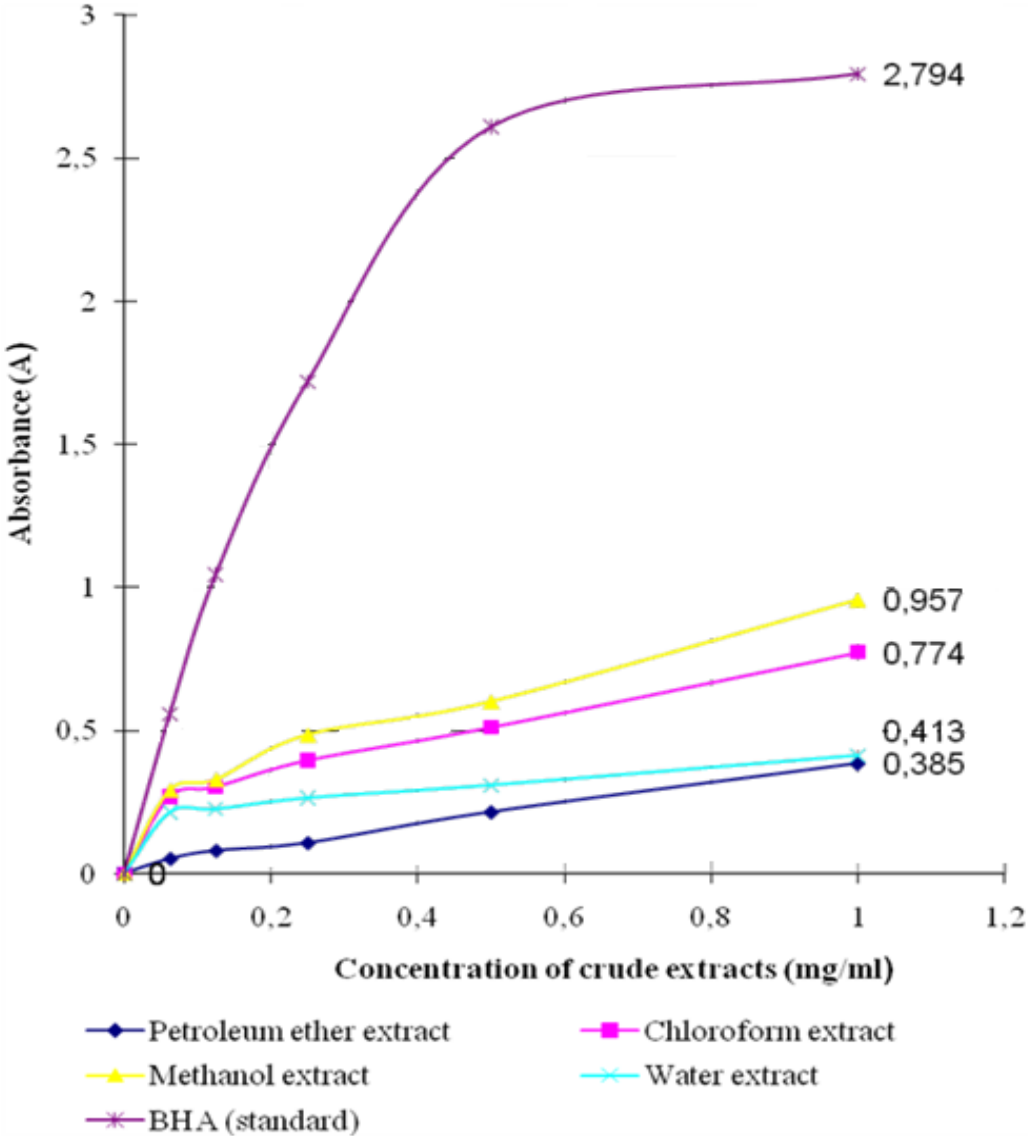

Figure 4.14: Dose-response curves showing the reducing power activities of *Tinospora crispa* (stems) crude extracts and BHA in the reducing power assay.

The reducing power (absorbance) of all extracts and positive standard of BHA increased with increased concentrations. In comparison with positive standard of BHA, the reducing activities of crude methanol extract and crude chloroform extract were moderate weak with 0.957 A and 0.774 A respectively at 1 mg/ml while crude water extract and crude petroleum ether extract exhibited weak reducing power activities at all concentrations tested.

4.4.4 Haemolysate Catalytic Assay

The ability of the *Ervatamia coronaria* and *Tinospora crispa* to reduce hydrogen peroxide (H_2O_2) in synergism with haemolysate catalase were assessed and evaluated in this hemolysate catalytic assay. The hydrogen peroxide (H_2O_2) was reduced into oxygen and water molecule in the presence of H_2O_2 reductant by the catalase and plant extracts.

$$2H_2O_2 \text{ ----> } 2H_2O + O_2$$

The ability of plant crude extracts to reduce the activities of H_2O_2 in synergism with hemolysate catalase was determined by measuring the decrease of H_2O_2 absorbance values. The amount of H_2O_2 reduction was measured using UV-spectrophotometer at 240 nm. The reducing activities of crude petroleum ether, methanol, chloroform and water extracts of the *Ervatamia coronaria* (roots, stems and leaves) and *Tinospora crispa* (stems) were evaluated at varying concentration ranging from 50 µg/ml, 100 µg/ml and 200 µg/ml.

4.4.4(i) Determination of the Concentration of Haemoglobin and Total Weight of Haemoglobin in the Haemolysate.

The concentration and total weight of haemoglobin used was calculated using the principle of Beer-Lambert's law. The extinction coefficient for haemoglobin is 14.6 M^{-1} cm^{-1} at 577 nm was used to calculate the concentration of haemoglobin which can be applied to calculate total weight of haemoglobin in the solution. According to Table 4.38, the absorbance of haem at 577 nm was 0.0518 A and thus giving the concentration of haemoglobin used was 3.55 mM. The total weight of haem in the haemolysate was calculated based on the concentration of the haemolgobin obtained. The molecular weight of haemoglobin is 616.49 amu. Thus the total weight of haemoglobin used in haemolysate catalytic assay was 6.55 mg.

Table 4.38: The concentration of haemoglobin and total weight of haem used in hemolysate catalytic assay.

Haemolysate	Absorbance at 577 nm (A)	Concentration of haemoglobin (mM)	Total weight of haem in 3 ml cuvette (mg)
Blood stock	0.0518	3.55	6.55

4.4.4(ii) Blocked and Unblocked Catalase Activity by Sodium Azide as the Reference Standard

Catalase (CAT) is present in red blood cell haemolysate. The activity of CAT alone from the red blood cell was used as the standard for this hemolysate catalytic assay. Activity of CAT alone was measured using sodium azide-blocked and unblocked CAT. Table 4.39 and Figure 4.15 showed the enzyme kinetics of H_2O_2 reduction by blocked and unblocked CAT activity. The haemolysate with its catalase (CAT) blocked with sodium azide exhibited very poor or slightly no catalytic activity in reducing hydrogen peroxide in the system while obvious decomposition of hydrogen peroxide from time 0-1.5 was seen minutes when CAT was not subjected to any blocking chemical in the system. Therefore, haemolysate with CAT has catalytic activity.

Table 4.39: Enzymatic kinetics of hydrogen peroxide reduction by unblocked and sodium azide blocked catalase activity in the haemolysate catalytic assay.

Time (minutes)	Absorbance of hydrogen peroxide at 240 nm (A)	
	Sodium azide blocked Catalase	Unblocked Catalase
0	1.052 ± 0.0591	1.085 ± 0.1042
0.5	1.051 ± 0.0596	0.380 ± 0.0333
1.0	1.047 ± 0.0575	0.245 ± 0.0399
1.5	1.031 ± 0.0369	0.119 ± 0.0165

The absorbance was measured from time 0 minute, 0.5 minutes, 1.0 minutes and 1.5 minutes. Based on Table 4.39, unblocked CAT showed a decrease in H_2O_2 level as the absorbance values decreased with time. The absorbance values are as follows; 1.085 A at 0

minute, 0.380 A at 0.5 minutes, 0.245 A at 1.0 minutes and 0.119 A at 1.5 minutes. Meanwhile, the mixture containing sodium azide which functions to block CAT activity exhibited almost constant absorbance trend (1.052 A, 1.051 A, 1.047 A and 1.031 A). Hence, the reduction of hydrogen peroxide was indeed due to CAT activity.

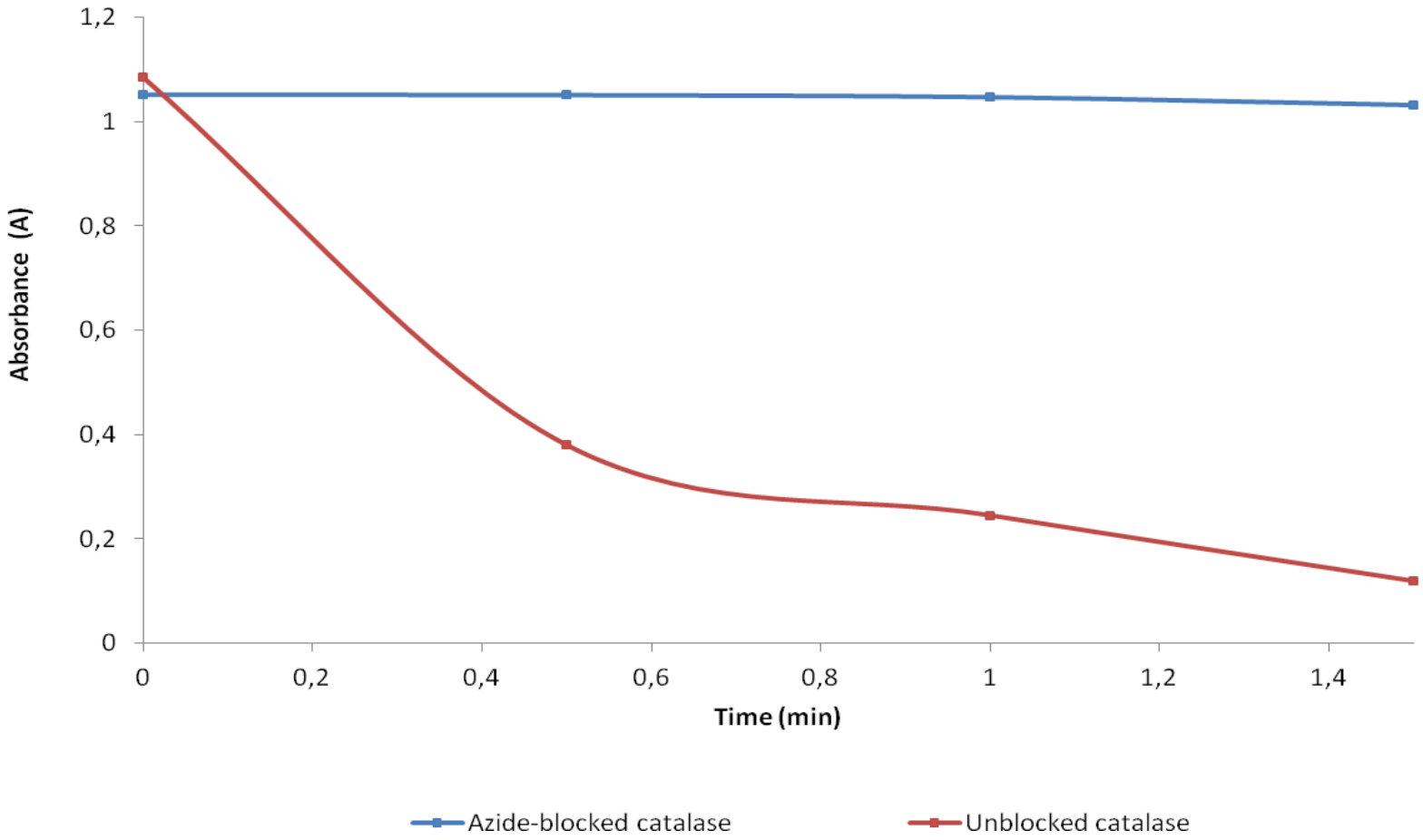

Figure 4.15: Curve showing enzymatic kinetics of hydrogen peroxide reduction by sodium azide blocked and unblocked catalse activity in haemolysate stock in the haemolysate catalytic assay.

4.4.4(iii) Reduction of Hydrogen Peroxide (H_2O_2) by *Ervatamia coronaria* (roots) in Synergism with Haemolysate Catalase (CAT)

The percentage of reduction of H_2O_2 by crude petroleum ether, chloroform, methanol and water extracts of *Ervatamia coronaria* (roots) in synergism with hemolysate catalase (CAT) at three different concentrations of 50 µg/ml, 100 µg/ml and 200 µg/ml are shown in Table 4.40. The dose-response curves showing enhancement of H_2O_2 reduction by the extracts were plotted in Figure 4.16.

Table 4.40: Enhancement of H_2O_2 reducing activities of *Ervatamia coronaria* (roots) crude extracts in haemolysate catalytic assay.

Concentration of Crude Extracts (µg/ml)	Percentage of reduction of H_2O_2 (%)			
	Crude Petroleum Ether Extract	Crude Chloroform Extract	Crude Methanol Extract	Crude Water Extract
0	0.00	0.00	0.00	0.00
50	16.29 ± 0.591	24.36 ± 1.899	16.04 ± 2.042	8.20 ± 0.208
100	17.98 ± 1.828	28.22 ± 8.015	22.57 ± 2.080	15.61 ± 2.408
200	21.38 ± 4.595	32.29 ± 4.457	25.83 ± 2.818	18.13 ± 3.472

Percentage of reduction of H_2O_2 (%) are expressed as mean ± standard deviation; n=3

The *Ervatamia coronaria* (roots) crude extracts enhanced the reduction of H_2O_2 in synergism with haemolysate catalase. The percentage of reduction of H_2O_2 in synergism with haemolysate catalase in each extract increased with increasing concentrations. In comparison with other extracts, crude chloroform extract exhibited the best synergistic effect with haemolysate catalase in reducing H_2O_2 activities at all concentrations with enhancement reduction of H_2O_2 by 24.36% at 50 µg/ml, 28.22 % at 100 µg/ml and 32.29 % at 200 µg/ml for 6.55 mg of haem. Meanwhile, crude water extract exhibited the lowest reduction of H_2O_2 at all concentrations.

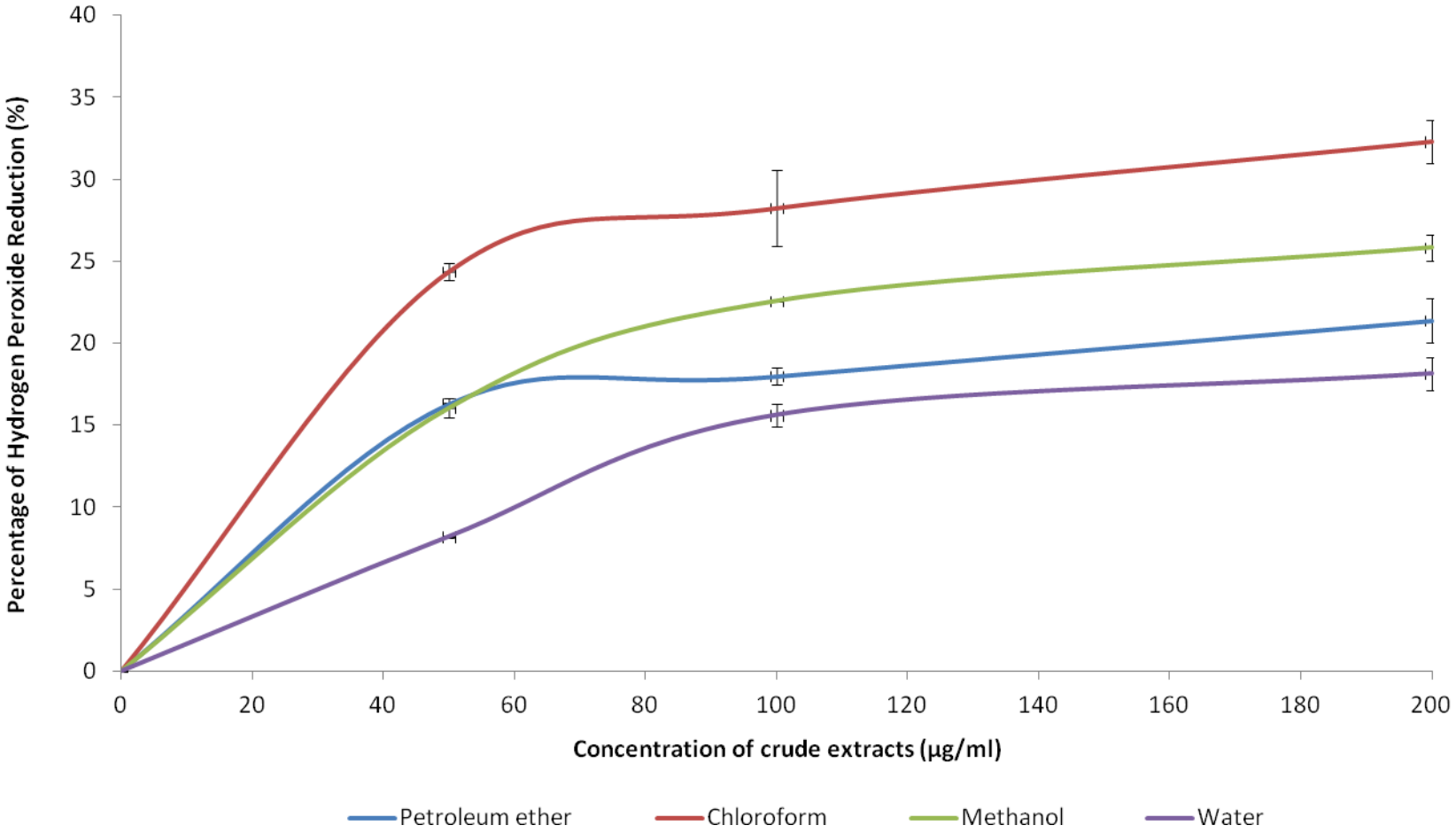

Figure 4.16 : Dose-response curve showing enhancement of H_2O_2 reduction by crude extracts of *Ervatamia coronaria* (roots) in synergism with haemolysate catalase in the hemolysate catalytic assay.

4.4.4(iv) Reduction of Hydrogen Peroxide (H_2O_2) of *Ervatamia coronaria* (stems) in Synergism with Haemolysate Catalase (CAT)

The percentage of reduction of H_2O_2 by crude petroleum ether, chloroform, methanol and water extracts of *Ervatamia coronaria* (stems) in synergism with hemolysate catalase (CAT) at three different concentrations of 50 µg/ml, 100 µg/ml and 200 µg/ml are shown in Table 4.41. The dose-response curves showing enhancement of H_2O_2 reduction was plotted in Figure 4.17.

Table 4.41: Enhancement of H_2O_2 reducing activities of *Ervatamia coronaria* (stems) crude extracts in haemolysate catalytic assay.

Concentration of Crude Extracts (µg/ml)	Percentage of reduction of H_2O_2 (%)			
	Crude Petroleum Ether Extract	Crude Chloroform Extract	Crude Methanol Extract	Crude Water Extract
0	0.00	0.00	0.00	0.00
50	11.67 ± 2.656	13.86 ± 1.403	19.48 ± 0.825	9.62 ± 1.906
100	11.75 ± 0.932	19.77 ± 1.123	23.51 ± 2.875	12.57 ± 1.871
200	17.51 ± 3.741	28.48 ± 2.705	25.35 ± 2.396	18.19 ± 1.530

Percentage of reduction of H_2O_2 (%) are expressed as mean ± standard deviation; n=3

The percentage of reduction of H_2O_2 in synergism with haemolysate catalase in each extract increased with increasing concentrations. Among all the extracts, crude chloroform extract exhibited the highest enhancement of H_2O_2 reduction at concentration 200 µg/ml (28.48%) while crude methanol extract exhibited the highest enhancement of H_2O_2 reduction at concentration 100 µg/ml (23.51%) and 50 µg/ml (19.48%) for 6.55 mg of haem. Meanwhile, both crude water and crude petroleum ether extracts were seen to be almost similar in enhancement of H_2O_2 reducing activities at concentrations 100 µg/ml and 200 µg/ml concentrations. The results revealed that all the extracts were able to enhance the reduction of H_2O_2 in synergism with haemolysate catalase.

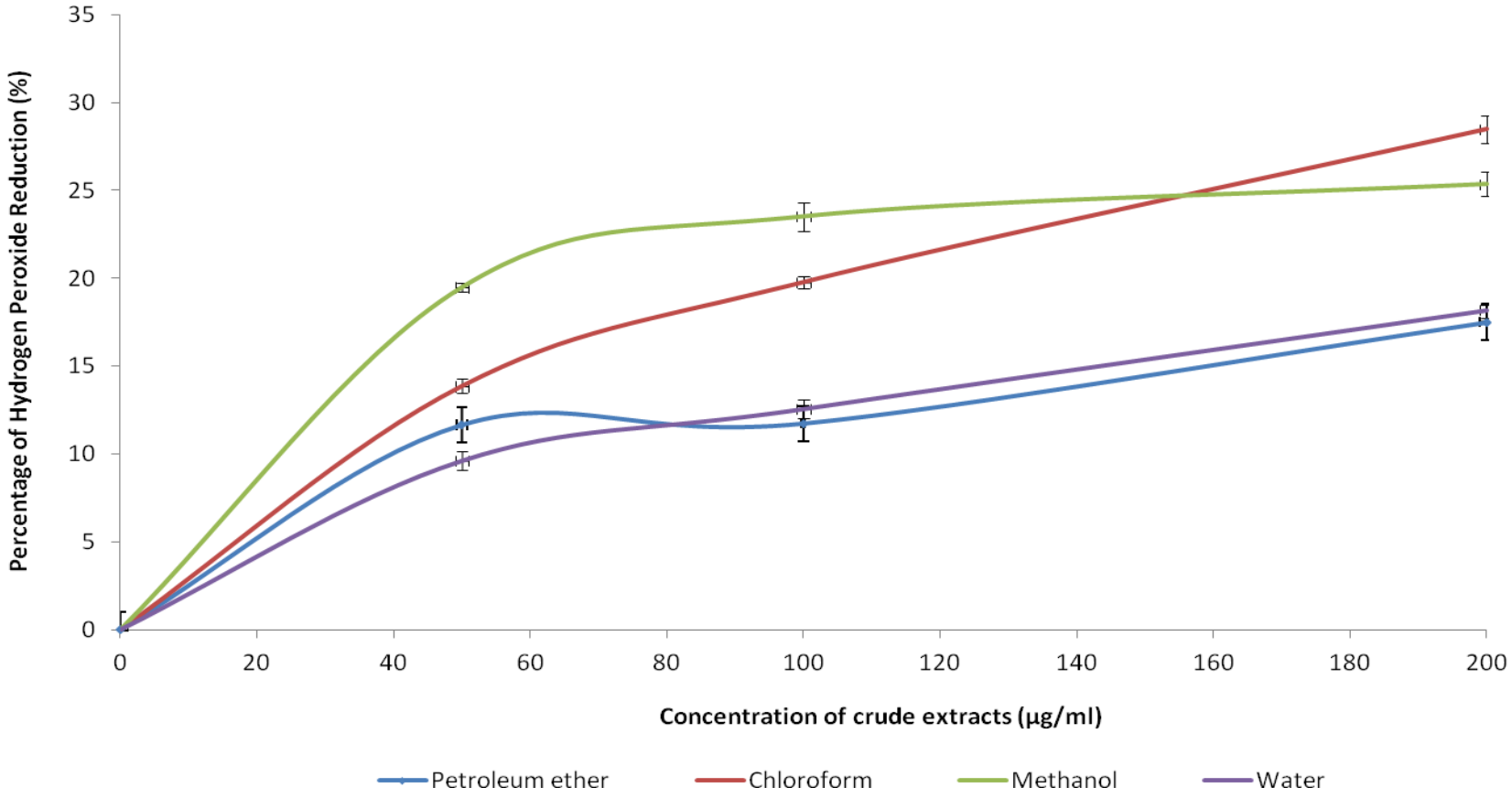

Figure 4.17 : Dose-response curve showing enhancement of H_2O_2 reduction by crude extracts of *Ervatamia coronaria* (stems) in synergism with haemolysate catalse in the hemolysate catalytic assay

4.4.4(v) Reduction of Hydrogen Peroxide (H_2O_2) by *Ervatamia coronaria* (leaves) in Synergism with Haemolysate Catalase (CAT)

The percentage of reduction of H_2O_2 by crude petroleum ether, chloroform, methanol and water extracts of *Ervatamia coronaria* (leaves) in synergism with hemolysate catalase (CAT) at three different concentrations of 50 µg/ml, 100 µg/ml and 200 µg/ml are shown in Table 4.42. The dose-response curves showing enhancement of H_2O_2 reduction was plotted in Figure 4.18.

Table 4.42: Enhancement of H_2O_2 reducing activities of *Ervatamia coronaria* (leaves) crude extracts in haemolysate catalytic assay.

Concentration of Crude Extracts (µg/ml)	Percentage of reduction of H_2O_2 (%)			
	Crude Petroleum Ether Extract	Crude Chloroform Extract	Crude Methanol Extract	Crude Water Extract
0	0.00	0.00	0.00	0.00
50	15.08 ± 0.213	16.87 ± 0.772	13.10 ± 0.762	9.42 ± 2.231
100	16.34 ± 1.236	20.07 ± 0.277	14.91 ± 0.390	16.30 ± 1.043
200	16.97 ± 1.137	20.89 ± 5.190	18.31 ± 1.028	17.09 ± 2.691

Percentage of reduction of H_2O_2 (%) are expressed as mean ± standard deviation; n=3

The *Ervatamia coronaria* (leaves) crude extracts were able to enhance the reduction of H_2O_2 in synergism with haemolysate catalase. The percentage of reduction of H_2O_2 in synergism with haemolysate catalase in each extract increased with increasing concentrations. In comparison with other extracts, crude chloroform extract exhibited the best synergistic effect with haemolysate catalase in reducing H_2O_2 activities by 16.87 % at 50 µg/ml, 20.07 % at 100 µg/ml and 20.89 % at 200 µg/ml for 6.55 mg of haem.

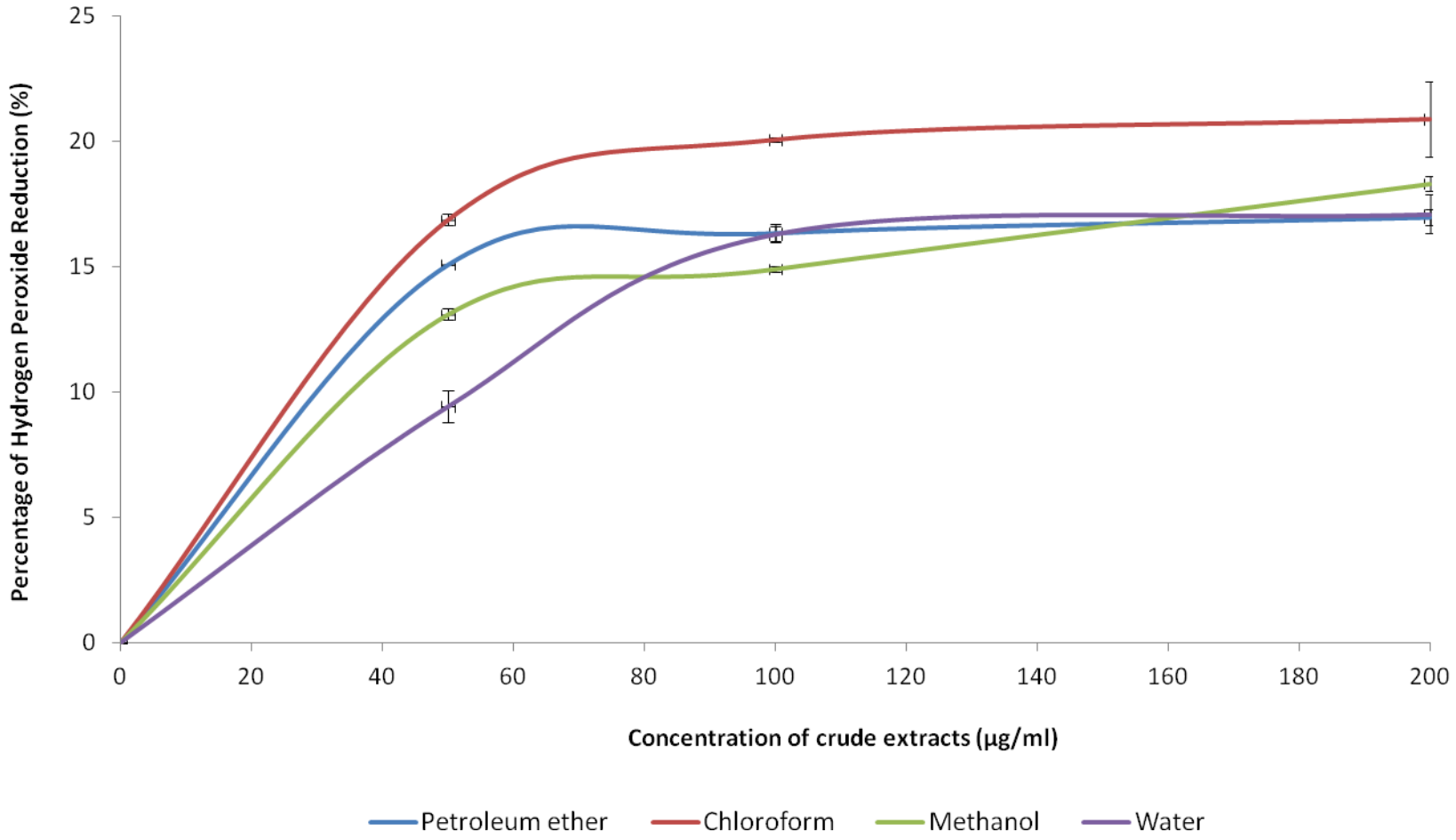

Figure 4.18 : Dose-response curve showing enhancement of H_2O_2 reduction by crude extracts of *Ervatamia coronaria* (leaves) in synergism with haemolysate catalase in the hemolysate catalytic assay

4.4.4(vi) Reduction of Hydrogen Peroxide (H_2O_2) by *Tinospora crispa* (stems) in Synergism with Haemolysate Catalase (CAT)

The percentage of reduction of H_2O_2 by crude petroleum ether, chloroform, methanol and water extracts of *Tinospora crispa* (stems) in synergism with hemolysate catalase (CAT) at three different concentrations of 50 µg/ml, 100 µg/ml and 200 µg/ml are shown in Table 4.43. The dose-response curves showing enhancement of H_2O_2 reduction was plotted in Figure 4.19.

Table 4.43: Enhancement of H_2O_2 reducing activities of *Tinospora crispa* (stems) crude extracts in haemolysate catalytic assay.

Concentration of Crude Extracts (µg/ml)	Percentage of reduction of H_2O_2 (%)			
	Crude Petroleum Ether Extract	Crude Chloroform Extract	Crude Methanol Extract	Crude Water Extract
0	0.00	0.00	0.00	0.00
50	14.93 ± 2.535	21.67 ± 2.933	21.90 ± 0.713	11.31 ± 4.255
100	17.97 ± 1.465	28.48 ± 3.904	26.73 ± 0.939	18.44 ± 1.208
200	20.32 ± 3.354	29.96 ± 2.277	36.71 ± 6.089	20.02 ± 5.791

Percentage of reduction of H_2O_2 (%) are expressed as mean ± standard deviation; n=3

The percentage of reduction of H_2O_2 in synergism with haemolysate catalase in each extract increased with increasing concentrations. Among all the extracts, crude methanol extract exhibited the highest reducing activity of H_2O_2 at concentration 200 µg/ml (36.71%) and 50 µg/ml (21.90%) while crude chloroform extract exhibited the highest reducing activity of H_2O_2 at concentration 100 µg/ml (28.48%) for 6.55 mg of haem. Meanwhile, both crude water and crude petroleum ether extracts were seen to have almost similar H_2O_2 reducing activities at concentrations 100 µg/ml and 200 µg/ml. The results revealed that all the extracts were able to enhance the reduction of H_2O_2 in synergism with haemolysate catalase.

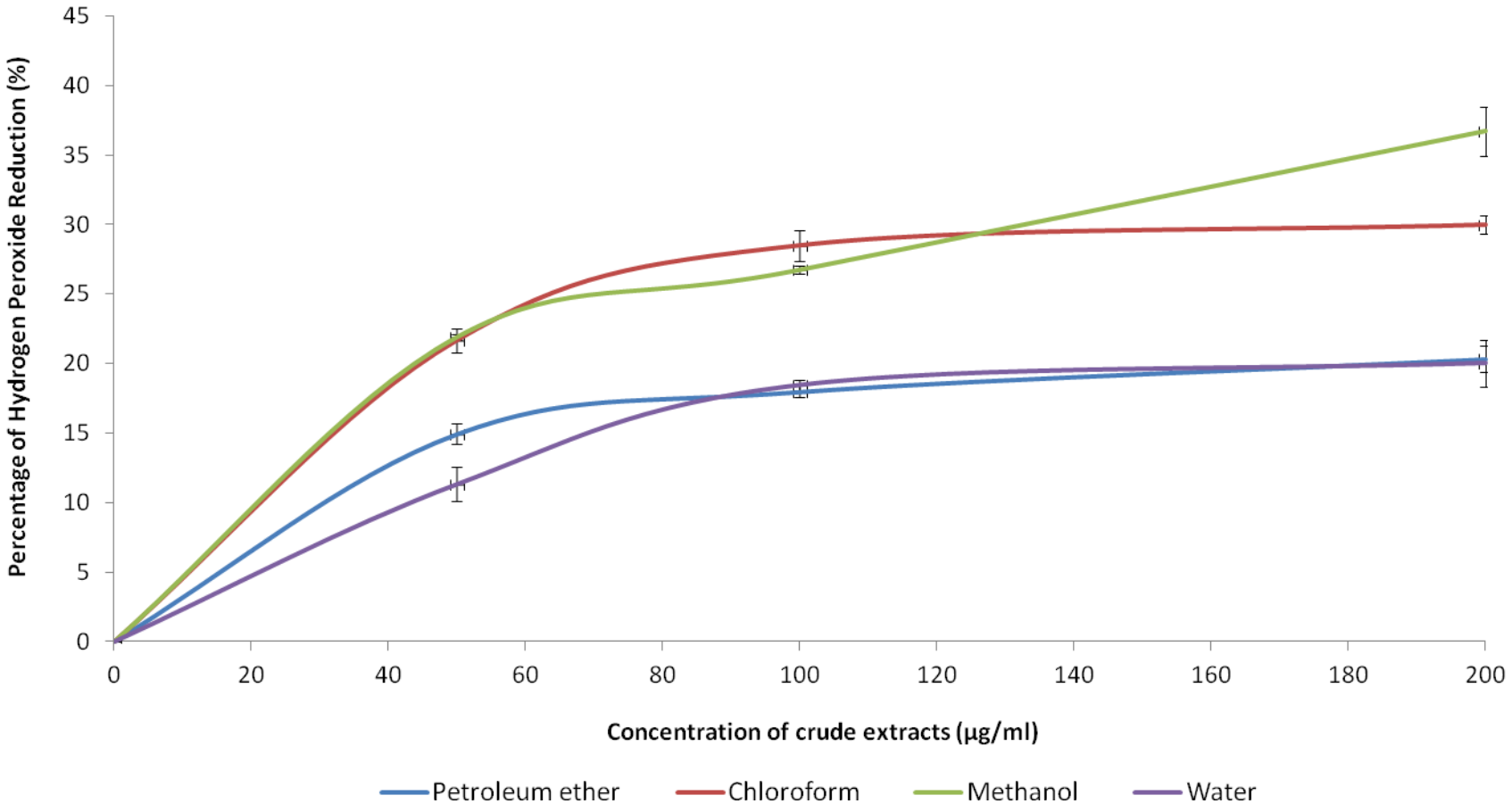

Figure 4.19 : Dose-response curve showing enhancement of H_2O_2 reduction by crude extracts of *Tinospora crispa* (stems) in synergism with haemolysate catalase in the hemolysate catalytic assay

4.5 Correlation among Antioxidant Activities and Total Phenolic Content

Table 4.44 : Correlations among antioxidant activities (DPPH Radical Scavenging, Metal Chelating, Reducing Power, Haemolysate Catalytic Assay) and Total Phenolic Content (TPC), n=16

		Total Phenolic Content (TPC)	DPPH Radical Scavenging Assay	Metal Chelating Assay	Reducing Power Assay	Haemolysate Catalytic Assay
Total Phenolic Content (TPC)	Pearson Correlation	1.000	.788**	.358	.556*	.388
	Sig. (2-tailed)		.000	.174	.025	.137
DPPH Radical Scavenging Assay	Pearson Correlation	.788**	1.000	.282	.751**	.686**
	Sig. (2-tailed)	.000		.289	.001	.003
Metal Chelating Assay	Pearson Correlation	.358	.282	1.000	.281	.302
	Sig. (2-tailed)	.174	.289		.293	.255
Reducing Power Assay	Pearson Correlation	.556*	.751**	.281	1.000	.677**
	Sig. (2-tailed)	.025	.001	.293		.004
Haemolysate Catalytic Assay	Pearson Correlation	.388	.686**	.302	.677**	1.000
	Sig. (2-tailed)	.137	.003	.255	.004	

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Based on the Table 4.44, the significant linear positive correlation was confirmed between the Total Phenolic Content and DPPH Free Radical Scavenging Assay (correlation coefficient, $r = 0.788$, $p < 0.01$, $n=16$). In addition, significant moderate positive correlation was observed between the Total Phenolic Content and Reducing Power Assay (correlation coefficient, $r = 0.556$, $p < 0.05$, $n=16$). Whereas, the correlation coefficient of Total Phenolic Content and Metal Chelating Assay; Haemolysate Catalytic Assay were 0.358 and 0.388 respectively at $p>0.05$ which means no significant difference. Meanwhile, significant linear positive correlation were observed between DPPH Free Radical Scavenging Assay and Reducing Power Assay ($r=0.751$, $p<0.01$) and Haemolysate Catalytic Assay ($r=0.686$, $p<0.01$).

4.6 Antimicrobial Activity Assays

The purpose of this assay is to evaluate antimicrobial activity of *Ervatamia coronaria* and *Tinospora crispa* extracts against sinusitis-causing microorganisms. Microorganism is called susceptible to extract when the infection caused by it is likely to respond to treatment with the extract.

4.6.1 Percentage Susceptibility of Microorganism against Each Extract

Table 4.45: Percentage susceptibility of sinusitis-causing microorganisms against *Ervatamia coronaria* (roots) extract

Solvent	Total no of test organism	No of susceptible strain	Percentage (%) susceptibility
Petroleum Ether	10	1	10
Chloroform	10	5	50
Methanol	10	4	40
Water	10	1	10

Table 4.46: Percentage susceptibility of sinusitis-causing microorganisms against *Ervatamia coronaria* (stems) extract

Solvent	Total no of test organism	No of susceptible strain	Percentage (%) susceptibility
Petroleum Ether	10	2	20
Chloroform	10	4	40
Methanol	10	4	40
Water	10	1	10

Table 4.47: Percentage susceptibility of sinusitis-causing microorganisms against *Ervatamia coronaria* (leaves) extract

Solvent	Total no of test organism	No of susceptible strain	Percentage (%) susceptibility
Petroleum Ether	10	3	30
Chloroform	10	1	10
Methanol	10	2	20
Water	10	1	10

Table 4.48: Percentage susceptibility of sinusitis-causing microorganisms against *Tinospora crispa* (stems) extract

Solvent	Total no of test organism	No of susceptible strain	Percentage (%) susceptibility
Petroleum Ether	10	3	30
Chloroform	10	4	40
Methanol	10	3	30
Water	10	0	0

According to Table 4.45- 4.48, there were 10 microorganisms used in this assay comprising of 9 bacteria and 1 fungus. Number of susceptible strain against each plant extract was different where chloroform extract exhibited the highest number of susceptible strains except for *Ervatamia coronaria* (leaves) in which petroleum ether exhibited the highest number of susceptible strains. Among all the extracts, only the water extract of *Tinospora crispa* (stems) did not have any susceptible microorganisms.

4.6.2 Paper Disc Diffusion Method

Antimicrobial activities in *Ervatamia coronaria* and *Tinospora crispa* were analysed using paper disc diffusion method against 10 microorganisms which included *Klebsiella pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Streptococcus faecalis, Proteus mirabilis, Haemophilus influenza, Streptococcus pneumoniae, Moraxella catarrhalis, and Candida albicans.*

The plants differ significantly in their activity against tested microorganisms strain. The degree of antimicrobial activity was reflected by inhibition zone diameter (IZD) around the disc containing the extracts. The inhibition zone diameter was measured in millimeter.

Blank filter paper-disc and filter paper-disc saturated with solvents were used as negative controls while positive control were filter paper-disc saturated with specific antibiotic. The antibiotics included Chloramphenicol (30 µg), Penicillin (10), Imipinem (10 µg) and Chlorhexidine (0.12% w/v) (Table 4.53). All experiments were done in triplicates and the results were expressed as mean ± standard deviation. The antimicrobial activities shown by the inhibition zone diameter were then classified into the following categories:

1) Weak (7mm-10mm)
2) Medium (11mm-20mm)
3) Strong (21mm-30mm)
4) Very strong (31mm-50mm)

4.6.2(i) Antimicrobial Activity of *Ervatamia coronaria* (roots) Extracts against Sinusitis- Causing Microorganisms

Ervatamia coronaria (roots) crude extracts showed various degree of antimicrobial activity against six test microorganisms namely *Klebsiella penumoniae, Staphylococcus aureus, Psedomonas aeruginosa, Haemophilus influenza, Streptococcus pneumoniae* and *Moraxella catarrhalis*. None of the extracts showed antimicrobial activity against *Escherichia coli, Streptococcus faecalis, Proteus mirabilis* and *Candida albican* (Table 4.49). In comparison to the other extracts, the crude chloroform extract inhibited the highest number of microorganism tested (5 test microorganisms). Crude chloroform extract exhibited medium antimicrobial activity against microorganisms namely *Staphylococcus aureus* (12.7 mm) and *Pseudomonas aeruginosa* (11.0 mm) while weak antimicrobial activity against *Haemophilus influenza* (8.7 mm), *Streptococcus pneumoniae* (9.3 mm), and *Moraxella catarrhalis* (10.0 mm) at the highest concentration tested (50 mg/ml). The crude petroleum ether extract only showed antimicrobial activity against *Moraxella catarrhalis* with inhibition zone diameter of 8 mm (weak activity) at 50 mg/ml while crude water extract only exhibited antimicrobial activity against *Klebsiella penumoniae* with inhibition zone diameter of 23.3 mm (strong activity) at 50 mg/ml. Meanwhile, crude methanol extract inhibited four types of microorganisms namely *Staphylococcus aureus, Haemophilus influenza, Streptococcus pneumoniae,* and *Moraxella catarrhalis*.

Table 4.49: Antimicrobial activity of *Ervatamia coronaria* (roots) extracts against sinusitis- causing microorganisms

Extract and the concentrations tested (mg/ml)	Inhibition Zone Diameter (mm)									
	1	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***	***10***
Petroleum ether extract										
50	-	-	-	-	-	-	-	-	8.0 ± 0.00	-
25	-	-	-	-	-	-	-	-	-	-
12.5	-	-	-	-	-	-	-	-	-	-
6.25	-	-	-	-	-	-	-	-	-	-
3.12	-	-	-	-	-	-	-	-	-	-
Chloroform extract										
50	-	12.7 ± 0.58	11.0 ± 0.00	-	-	-	8.7 ± 0.58	9.3 ± 0.58	10.0 ± 0.00	-
25	-	12.3 ± 0.58	9.3 ± 1.56	-	-	-	7.0 ± 0.00	8.3 ± 0.58	10.0 ± 0.00	-
12.5	-	10.3 ± 0.58	8.3 ± 1.56	-	-	-	-	7.3 ± 0.58	8.0 ± 0.00	-
6.25	-	7.7 ± 0.58	7.7 ± 0.58	-	-	-	-	7.0 ± 1.00	-	-
3.12	-	-	7.0 ± 0.00	-	-	-	-	-	-	-
Methanol extract										
50		10.0 ± 0.00	-	-	-	-	9.0 ± 0.00	8.0 ± 0.00	10.0 ± 0.00	-
25		8.3 ± 0.58	-	-	-	-	7.7 ± 0.58	7.0 ± 0.00	8.0 ± 0.00	-
12.5		-	-	-	-	-	-	-	7.0 ± 0.00	-
6.25		-	-	-	-	-	-	-	-	-
3.12		-	-	-	-	-	-	-	-	-
Water extract										
50	23.3 ± 2.89	-	-	-	-	-	-	-	-	-
25	22.3 ± 4.61	-	-	-	-	-	-	-	-	-
12.5	21.0 ± 0.58	-	-	-	-	-	-	-	-	-
6.25	20.7 ± 0.58	-	-	-	-	-	-	-	-	-
3.12	14.3 ± 2.89	-	-	-	-	-	-	-	-	-

All results are expressed as mean ± standard deviation

Key :
1. *Klebsiella pneumoniae*
2. *Stapylococcus aureus*
3. *Pseudomonas aeruginosa*
4. *Escherichia coli*
5. *Streptococcus faecalis*
6. *Proteus mirabilis*
7. *Haemophilus influenza*
8. *Streptococcus pneumoniae*
9. *Moraxella catarrhalis*
10. *Candida albicans*

Antimicrobial activity (Inhibition Zone Diameter)

Weak (7mm-10mm)
Medium(11mm-20mm)
Strong (21mm-30mm)
Very Strong (31mm-50mm)

4.6.2(ii) Antimicrobial Activity of *Ervatamia coronaria* (stems) Extracts against Sinusitis-Causing Microorganisms

Ervatamia coronaria (stems) crude extracts showed various degree of antimicrobial activity against five test microorganisms namely *Klebsiella penumoniae, Staphylococcus aureus, Haemophilus influenza, Streptococcus pneumoniae* and *Moraxella catarrhalis*. None of the extracts showed antimicrobial activity against *Pseudomonas aeruginosa, Escherichia coli, Streptococcus faecalis, Proteus mirabilis* and *Candida albicans* (Table 4.50). Among all the extracts, chloroform and methanol inhibited the highest number of microorganism tested (4 test microorganisms) namely *Staphylococcus aureus, Haemophilus influenza, Streptococcus pneumoniae*, and *Moraxella catarrhalis.* Crude chloroform extract exhibited medium antimicrobial activity against *Haemophilus influenza* (12.6 mm) and *Moraxella catarrhalis* (15.0 mm) while weak antimicrobial activity against *Staphylococcus aureus* (10.3 mm) and *Streptococcus pneumoniae* (9.7 mm) at 50 mg/ml. Meanwhile, methanol extract exhibited medium antimicrobial activity against *Staphylococcus aureus* (16.7 mm), *Streptococcus pneumoniae* (11.3 mm) and *Moraxella catarrhalis* (13.3 mm) while weak antimicrobial activity against *Haemophilus influenza* (9.0 mm) at the same concentration. The crude petroleum ether extract only showed antimicrobial activity against *Haemophilus influenza* and *Moraxella catarrhalis* with inhibition zone diameter of 8 mm (weak activity) and 9.5 mm (weak activity) respectively at 50 mg/ml .Crude water extract only exhibited antimicrobial activity against *Klebsiella pneumoniae* with inhibition zone diameter of 24.7 mm (strong activity) at 50 mg/ml concentration.

Table 4.50: Antimicrobial activity of *Ervatamia coronaria* (stems) extracts against sinusitis- causing microorganisms

Extract and the concentrations tested (mg/ml)	Inhibition Zone Diameter (mm)									
	1	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***	***10***
Petroleum ether extract										
50	-	-	-	-	-	-	8.0 ± 0.00	-	9.5 ± 0.71	
25	-	-	-	-	-	-	8.0 ± 0.00	-	8.5 ± 0.71	
12.5	-	-	-	-	-	-	-	-	-	
6.25	-	-	-	-	-	-	-	-	-	
3.12	-	-	-	-	-	-	-	-	-	
Chloroform extract										
50	-	10.3 ± 0.58	-	-	-	-	12.6 ± 0.58	9.7 ± 0.58	15.0 ± 0.00	
25	-	9.7 ± 0.58	-	-	-	-	10.0 ± 0.00	8.3 ± 0.58	13.3 ± 0.58	
12.5	-	7.7 ± 0.58	-	-	-	-	9.3 ± 0.58	7.0 ± 0.00	9.7 ± 0.58	
6.25	-	-	-	-	-	-	-	-	7.3 ± 0.58	
3.12	-	-	-	-	-	-	-	-	7.0 ± 0.00	
Methanol extract										
50	-	16.7 ± 1.16	-	-	-	-	9.0 ± 0.00	11.3 ± 0.58	13.3 ± 0.58	
25	-	11.3 ± 0.58	-	-	-	-	7.3 ± 0.58	8.3 ± 0.58	10.7 ± 0.58	
12.5	-	10.3 ± 0.58	-	-	-	-	-	8.0 ± 1.00	9.7 ± 0.58	
6.25	-	9.0 ± 0.00	-	-	-	-	-	7.0 ± 0.00	7.3 ± 0.58	
3.12	-	-	-	-	-	-	-	-	-	
Water extract										
50	24.7 ± 1.16	-	-	-	-	-				
25	19.0 ± 0.00	-	-	-	-	-				
12.5	18.0 ± 0.00	-	-	-	-	-				
6.25	10.7 ± 2.31	-	-	-	-	-				
3.12	10.3 ± 0.58	-	-	-	-	-				

All results are expressed as mean ± standard deviation

Key :
1. *Klebsiella pneumoniae*
2. *Stapylococcus aureus*
3. *Pseudomonas aeruginosa*
4. *Escherichia coli*
5. *Streptococcus faecalis*
6. *Proteus mirabilis*
7. *Haemophilus influenza*
8. *Streptococcus pneumoniae*
9. *Moraxella catarrhalis*
10. *Candida albicans*

Antimicrobial activity (Inhibition Zone Diameter)

Weak (7mm-10mm)
Medium(11mm-20mm)
Strong (21mm-30mm)
Very Strong (31mm-50mm)

4.6.2(iii) Antimicrobial Activity of *Ervatamia coronaria* (leaves) Extracts against Sinusitis-Causing Microorganisms

Ervatamia coronaria (leaves) crude extracts showed various degree of antimicrobial activity against four test microorganisms namely *Klebsiella penumoniae, Staphylococcus aureus, Haemophilus influenza,* and *Moraxella catarrhalis.* On the other hand, none of the extracts showed antimicrobial activity against *Pseudomonas aeruginosa, Escherichia coli, Streptococcus faecalis, Proteus mirabilis, Streptococcus pneumoniae* and *Candida albicans* (Table 4.51). In comparison to the other extracts, crude petroleum ether extract inhibited the highest number of microorganism tested (3 test microorganisms). Crude petroleum ether extract exhibited weak antimicrobial activity against *Staphylococcus aureus* (8.0 mm), *Haemophilus influenza* (9.7 mm) and *Moraxella catarrhalis* (7.7 mm) at the highest concentration tested (50 mg/ml). Meanwhile, crude methanol extract exhibited medium antimicrobial activity against *Klebsiella pneumoniae* (20.0 mm) and *Moraxella catarrhalis*(11.0 mm) at the same concentration. Crude chloroform extract and water extract only inhibited one microorganism namely *Moraxella catarrhalis* and *Klebsiella pneumoniae* respectively.

Table 4.51: Antimicrobial activity of *Ervatamia coronaria* (leaves) extracts against sinusitis- causing microorganisms

Extract and the concentrations tested (mg/ml)	Inhibition Zone Diameter (mm)									
	1	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***	***10***
Petroleum ether extract										
50	-	8.0 ± 0.00	-	-	-	-	9.7 ± 0.58	-	7.7 ± 0.58	-
25	-	7.0 ± 0.00	-	-	-	-	9.3 ± 0.58	-	7.0 ± 0.00	-
12.5	-	-	-	-	-	-	8.0 ± 0.00	-	7.0 ± 0.00	-
6.25	-	-	-	-	-	-	-	-	-	-
3.12	-	-	-	-	-	-	-	-	-	-
Chloroform extract										
50	-	-	-	-	-	-	-	-	7.7 ± 0.58	-
25	-	-	-	-	-	-	-	-	7.0 ± 0.00	-
12.5	-	-	-	-	-	-	-	-	-	-
6.25	-	-	-	-	-	-	-	-	-	-
3.12	-	-	-	-	-	-	-	-	-	-
Methanol extract										
50	20.0 ± 0.00	-	-	-	-	-	-	-	11.0 ± 0.00	-
25	20.0 ± 0.00	-	-	-	-	-	-	-	9.0 ± 0.00	-
12.5	13.0 ± 0.00	-	-	-	-	-	-	-	7.0 ± 0.00	-
6.25	8.7 ± 0.58	-	-	-	-	-	-	-	-	-
3.12	-	-	-	-	-	-	-	-	-	-
Water extract										
50	14.7 ± 1.53	-	-	-	-	-	-	-	-	-
25	13.3 ± 0.58	-	-	-	-	-	-	-	-	-
12.5	10.7 ± 0.58	-	-	-	-	-	-	-	-	-
6.25	-	-	-	-	-	-	-	-	-	-
3.12	-	-	-	-	-	-	-	-	-	-

All results are expressed as mean ± standard deviation

Key :

1. *Klebsiella pneumoniae*
2. *Stapylococcus aureus*
3. *Pseudomonas aeruginosa*
4. *Escherichia coli*
5. *Streptococcus faecalis*
6. *Proteus mirabilis*
7. *Haemophilus influenza*
8. *Streptococcus pneumoniae*
9. *Moraxella catarrhalis*
10. *Candida albicans*

Antimicrobial activity (Inhibition Zone Diameter)

Weak (7mm-10mm)
Medium(11mm-20mm)
Strong (21mm-30mm)
Very Strong (31mm-50mm)

4.6.2(iv) Antimicrobial Activity of *Tinospora crispa* (stems) Extracts against Sinusitis-Causing Microorganisms

Tinospora crispa (stems) crude extracts showed various degree of antimicrobial activity against four test microorganisms namely *Staphylococcus aureus, Haemophilus influenza, Streptococcus pneumoniae* and *Moraxella catarrhalis.* None of the extracts showed activity against *Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli, Streptococcus faecalis, Proteus mirabilis* and *Candida albicans* (Table 4.52). In comparison to the other extracts, the crude chloroform extract inhibited the highest number of microorganism tested (4 test microorganisms). Crude chloroform extract exhibited medium antimicrobial activity against *Staphylococcus aureus* (10.0 mm), *Haemophilus influenza* (10.0 mm), *Streptococcus pneumoniae* (7.7 mm), and *Moraxella catarrhalis* (10.0 mm) at the highest concentration tested (50 mg/ml). Crude petroleum ether extract exhibited medium antimicrobial activity against *Staphylococcus aureus* (16.0 mm) and *Haemophilus influenza* (11.0 mm) while weak antimicrobial activity against *Moraxella catarrhalis* (10.0 mm) at 50 mg/ml. Meanwhile, crude methanol extract exhibited weak antimicrobial activity against three microorganisms namely *Haemophilus influenza* (9.0 mm), *Streptococcus pneumoniae* (8.0 mm), and *Moraxella catarrhalis* (10.0 mm) at 50 mg/ml. Crude water extract did not exhibited antimicrobial activity against any microorganism in this assay.

Table 4.52: Antimicrobial activity of *Tinospora crispa* (stems) extracts against sinusitis- causing microorganisms

Extract and the concentrations tested (mg/ml)	Inhibition Zone Diameter (mm)									
	1	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***	***10***
Petroleum ether extract										
50	-	16.0 ± 0.00	-	-	-	-	11.0 ± 0.00	-	10.0 ± 0.00	-
25	-	16.0 ± 0.00	-	-	-	-	10.0 ± 0.00	-	9.3 ± 0.58	-
12.5	-	14.0 ± 0.00	-	-	-	-	10.0 ± 0.00	-	7.0 ± 0.00	-
6.25	-	13.7 ± 0.00	-	-	-	-	9.0 ± 0.00	-	7.0 ± 0.00	-
3.12	-	8.0 ± 0.00	-	-	-	-	-	-	-	-
Chloroform extract										
50	-	10.0 ± 0.00		-	-	-	10.0 ± 0.00	7.7 ± 0.58	10.0 ± 0.00	-
25	-	10.0 ± 0.00		-	-	-	9.0 ± 0.00	7.0 ± 0.00	9.7 ± 0.58	-
12.5	-	7.0 ± 0.00		-	-	-	8.0 ± 0.00	-	8.0 ± 0.00	-
6.25	-	-		-	-	-	8.0 ± 0.00	-	7.0 ± 0.00	-
3.12	-	-		-	-	-	-	-	-	-
Methanol extract										
50	-	-		-	-	-	9.0 ± 0.00	8.0 ± 0.00	10.0 ± 0.00	-
25	-	-		-	-	-	8.0 ± 0.00	7.0 ± 0.00	7.3 ± 0.58	-
12.5	-	-		-	-	-	7.3 ± 0.58	7.0 ± 0.00	-	-
6.25	-	-		-	-	-	-	-	-	-
3.12	-	-	-	-	-	-	-	-	-	-
Water extract										
50	-	-	-	-	-	-	-	-	-	-
25	-	-	-	-	-	-	-	-	-	-
12.5	-	-	-	-	-	-	-	-	-	-
6.25	-	-	-	-	-	-	-	-	-	-
3.12	-	-	-	-	-	-	-	-	-	-

All results are expressed as mean ± standard deviation

Key :

1. *Klebsiella pneumoniae*
2. *Stapylococcus aureus*
3. *Pseudomonas aeruginosa*
4. *Escherichia coli*
5. *Streptococcus faecalis*
6. *Proteus mirabilis*
7. *Haemophilus influenza*
8. *Streptococcus pneumoniae*
9. *Moraxella catarrhalis*
10. *Candida albicans*

Antimicrobial activity (Inhibition Zone Diameter)

Weak (7mm-10mm)
Medium(11mm-20mm)
Strong (21mm-30mm)
Very Strong (31mm-50mm)

Table 4.53 : Antimicrobial Activity by control

Control and the concentrations tested (mg/ml)	Inhibition Zone Diameter (mm)									
	1	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***	***10***
Blank filter-paper disc	-	-	-	-	-	-	-	-	-	-
Saturated filter paper-disc of solvents	-	-	-	-	-	-	-	-	-	-
Chloramphenicol (30µg)	26	NT	31	20	30	30	34	-	38	-
Penicillin (10)	-	40	24	-	-	30	12	-	41	-
Imipinem (10µg)	NT	NT	NT	NT	NT	39	27	33	45	-
Chlorhexidine (0.12% w/v)	-	-	-	-	-	-	-	-	-	13

Key :
1. *Klebsiella pneumoniae*
2. *Stapylococcus aureus*
3. *Pseudomonas aeruginosa*
4. *Echerichia coli*
5. *Streptococcus faecalis*
6. *Proteus mirabilis*
7. *Haemophilus influenza*
8. *Streptococcus pneumoniae*
9. *Moraxella catarrhalis*
10. *Candida albican*

NT : not tested

Antimicrobial activity (Inhibition Zone Diameter)
Weak (7mm-10mm)
Medium (11mm-20mm)
Strong (21mm-30mm)
Very Strong (31mm-50mm)

4.6.3 Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC)

The MIC for the microorganisms is the lowest concentration that can inhibit the growth of microorganism which was observed in Paper Disk Diffusion Assay. The MBC for the organisms on the other hand, is the lowest concentration of the extracts that can kill the microorganism. MBC was carried out for just microorganisms that showed positive for MIC and at their corresponding concentrations. The concentrations tested in this assay were 3.12 mg/ml, 6.25 mg/ml, 12.5 mg/ml, 25 mg/ml and 50 mg/ml.

Based on Table 4.54, petroleum ether crude extract of *Ervatamia coronaria* (leaves) and chloroform crude extract of *Ervatamia coronaria* (stems) exhibited the lowest minimal inhibitory concentration (MIC) against *Haemophilus influenza* at 12.5 mg/ml while the lowest minimal bactericidal concentration (MBC) was exhibited by petroleum ether crude extract of *Ervatamia coronaria* (leaves) at 12.5 mg/ml compared to other parts and extracts of *Ervatamia coronaria* plant. On the other hand, MIC for *Tinospora crispa* (stems) crude extracts were 6.25 mg/ml (petroleum ether crude extract), 6.25 mg/ml (chloroform crude extract) and 12.5 mg/ml (methanol crude extract) while only petroleum ether crude extract of *Tinospora crispa* (stems) exhibited bactericidal effect against *Haemophilus influenza* at 12.5 mg/ml of MBC.

Based on Table 4.55, chloroform crude extract of *Ervatamia coronaria* (roots) and methanol crude extract of *Ervatamia coronaria* (stems) exhibited the lowest minimal inhibitory concentration (MIC) against *Streptococcus pneumoniae* at 6.25 mg/ml while the lowest minimal bactericidal concentration (MBC) was exhibited by chloroform crude

extract of *Ervatamia coronaria* (roots) at 12.5 mg/ml compared to other parts and extracts of *Ervatamia coronaria* plant. Meanwhile, MIC for *Tinospora crispa* (stems) crude extracts were 25 mg/ml (chloroform crude extract) and 3.12 mg/ml (methanol crude extract) while none of the *Tinospora crispa* extract exhibited bactericidal effect against *Streptococcus peneumoniae.*

Based on Table 4.56, chloroform crude extract of *Ervatamia coronaria* (stems) exhibited the lowest minimal inhibitory concentration (MIC) against *Moraxella catarrhalis* at 3.12 mg/ml compared to other parts and extracts of *Ervatamia coronaria* plant. Meanwhile, at least two extracts of each plant part of *Ervatamia coronaria* exhibited bactericidal effect against *Moraxella catarrhalis*. On the other hand, MIC for *Tinospora crispa*(stems) crude extracts were 6.25 mg/ml (petroleum ether crude extract), 6.25 mg/ml (chloroform crude extract) and 25 mg/ml (methanol crude extract) while only petroleum ether crude extract of *Tinospora crispa* (stems) exhibited bactericidal effect against *Moraxella catarrhalis* at 6.25 mg/ml of MBC.

Based on Table 4.57, chloroform crude extract of *Ervatamia coronaria* (roots) and methanol crude extract of *Ervatamia coronaria* (stems) exhibited the lowest minimal inhibitory concentration (MIC) against *Staphylococcus aureus* at 6.25 mg/ml compared to other parts and extracts of *Ervatamia coronaria* plant. Meanwhile, only chloroform and methanol crude extracts of *Ervatamia coronaria* (roots) exhibited bactericidal effect against *Staphylococcus aureus.*at 25 mg/ml. On the other hand, MIC for *Tinospora crispa* (stems) crude extracts were 3.12 mg/ml (petroleum ether crude extract) and 12.5 mg/ml

(chloroform crude extract) while none of the *Tinospora crispa* (stems) extract exhibited bactericidal effect against *Staphylococcus aureus.*

Based on Table 4.58, water crude extract of *Ervatamia coronaria* (roots) and *Ervatamia coronaria* (stems) exhibited the lowest minimal inhibitory concentration (MIC) at 3.12 mg/ml compared to other parts and extracts of *Ervatamia coronaria* plant while none of *Ervatamia coronaria* crude extracts exhibited bactericidal effect was observed against *Klebsiella pneumoniae.* On the other hand, only methanol crude extract of *Ervatamia coronaria* (roots) exhibited minimal inhibitory concentration against *Pseudomonas aeruginosa* at 3.12 mg/ml (Table 4.59).

Table 4.54: Minimal inhibitory concentration and minimal bactericidal concentration of plant extracts against *Haemophilus Influenzae* ATCC 49247

Type of Extract		**Minimal Inhibitory Concentration (MIC)**	**Minimal Bactericidal Concentration (MBC)**
Ervatamia coronaria (leaves)	Petroleum ether extract	12.5 mg/ml	12.5 mg/ml
	Chloroform extract	-	-
	Methanol extract	-	-
	Water extract	-	-
Ervatamia coronaria (roots)	Petroleum ether extract	-	-
	Chloroform extract	25 mg/ml	-
	Methanol extract	25 mg/ml	-
	Water extract	-	-
Ervatamia coronaria (stems)	Petroleum ether extract	25 mg/ml	25 mg/ml
	Chloroform extract	12.5 mg/ml	25 mg/ml
	Methanol extract	25 mg/ml	25 mg/ml
	Water extract	-	-
Tinospora crispa (stems)	Petroleum ether extract	6.25 mg/ml	12.5 mg/ml
	Chloroform extract	6.25 mg/ml	-
	Methanol extract	12.5 mg/ml	-
	Water extract	-	-

Table 4.55: Minimal inhibitory concentration and minimal bactericidal concentration of plant extracts against *Streptococcus pneumoniae* ATCC 49619

Type of Extract		**Minimal Inhibitory Concentration (MIC)**	**Minimal Bactericidal Concentration (MBC)**
Ervatamia coronaria (leaves)	Petroleum ether extract	–	-
	Chloroform extract	–	-
	Methanol extract	–	-
	Water extract	–	-
Ervatamia coronaria (roots)	Petroleum ether extract	–	-
	Chloroform extract	6.25 mg/ml	12.5 mg/ml
	Methanol extract	25 mg/ml	25 mg/ml
	Water extract	–	-
Ervatamia coronaria (stems)	Petroleum ether extract	–	-
	Chloroform extract	12.5 mg/ml	25 mg/ml
	Methanol extract	6.25 mg/ml	-
	Water extract	–	-
Tinospora crispa (stems)	Petroleum ether extract	–	-
	Chloroform extract	25 mg/ml	-
	Methanol extract	3.12 mg/ml	-
	Water extract	–	-

Table 4.56: Minimal inhibitory concentration and minimal bactericidal concentration of plant extracts against *Moraxella catarrhalis* ATCC 23296

Type of Extract		**Minimal Inhibitory Concentration (MIC)**	**Minimal Bactericidal Concentration (MBC)**
Ervatamia coronaria (leaves)	Petroleum ether extract	12.5 mg/ml	12.5 mg/ml
	Chloroform extract	25 mg/ml	-
	Methanol extract	12.5 mg/ml	12.5 mg/ml
	Water extract	–	-
Ervatamia coronaria (roots)	Petroleum ether extract	50 mg/ml	50 mg/ml
	Chloroform extract	12.5 mg/ml	12.5 mg/ml
	Methanol extract	12.5 mg/ml	-
	Water extract	–	-
Ervatamia coronaria (stems)	Petroleum ether extract	25 mg/ml	25 mg/ml
	Chloroform extract	3.12 mg/ml	-
	Methanol extract	6.25 mg/ml	12.5 mg/ml
	Water extract	–	-
Tinospora crispa (stems)	Petroleum ether extract	6.25 mg/ml	6.25 mg/ml
	Chloroform extract	6.25 mg/ml	-
	Methanol extract	25 mg/ml	-
	Water extract	–	-

Table 4.57: Minimal inhibitory concentration and minimal bactericidal concentration of plant extracts against *Staphylococcus aureus*

Type of Extract		**Minimal Inhibitory Concentration (MIC)**	**Minimal Bactericidal Concentration (MBC)**
Ervatamia coronaria (leaves)	Petroleum ether extract	25 mg/ml	-
	Chloroform extract	–	-
	Methanol extract	–	-
	Water extract	–	-
Ervatamia coronaria (roots)	Petroleum ether extract	–	-
	Chloroform extract	6.25 mg/ml	25 mg/ml
	Methanol extract	25 mg/ml	25 mg/ml
	Water extract	–	-
Ervatamia coronaria (stems)	Petroleum ether extract	–	-
	Chloroform extract	12.5 mg/ml	-
	Methanol extract	6.25 mg/ml	-
	Water extract	–	-
Tinospora crispa (stems)	Petroleum ether extract	3.12 mg/ml	-
	Chloroform extract	12.5 mg/ml	-
	Methanol extract	–	-
	Water extract	–	-

Table 4.58: Minimal inhibitory concentration and minimal bactericidal concentration of plant extracts against *Klebsiella pneumoniae*

Type of Extract		Minimal Inhibitory Concentration (MIC)	Minimal Bactericidal Concentration (MBC)
Ervatamia coronaria (leaves)	Petroleum ether extract	-	-
	Chloroform extract	-	-
	Methanol extract	6.25 mg/ml	-
	Water extract	12.5 mg.ml	-
Ervatamia coronaria (roots)	Petroleum ether extract	-	-
	Chloroform extract	-	-
	Methanol extract	-	-
	Water extract	3.12 mg/ml	-
Ervatamia coronaria (stems)	Petroleum ether extract	-	-
	Chloroform extract	-	-
	Methanol extract	-	-
	Water extract	3.12 mg/ml	-
Tinospora crispa (stems)	Petroleum ether extract	-	-
	Chloroform extract	-	-
	Methanol extract	-	-
	Water extract	-	-

Table 4.59: Minimal inhibitory concentration and minimal bactericidal concentration of plant extracts against *Pseudomonas aeruginosa*

Type of Extract		**Minimal Inhibitory Concentration (MIC)**	**Minimal Bactericidal Concentration (MBC)**
Ervatamia coronaria (leaves)	Petroleum ether extract	-	-
	Chloroform extract	-	-
	Methanol extract	-	-
	Water extract	-	-
Ervatamia coronaria (roots)	Petroleum ether extract	-	-
	Chloroform extract	-	-
	Methanol extract	3.12 mg/ml	-
	Water extract	-	-
Ervatamia coronaria (stems)	Petroleum ether extract	-	-
	Chloroform extract	-	-
	Methanol extract	-	-
	Water extract	-	-
Tinospora crispa (stems)	Petroleum ether extract	-	-
	Chloroform extract	-	-
	Methanol extract	-	-
	Water extract	-	-

4.7 Liquid Chromatography-Mass Spectrometry

Initial investigation using LCMS/MS of methanol extract of *Ervatamia coronaria* (roots) exhibited the presence of 6 alkaloids and 1 terpenoid such as Apparicine, Conodurine, Voacamine, Voacangine, Voacristine, 3-Oxo Coronaridine and Campesterol. Methanol extract of *Ervatamia coronaria* (stems) showed the presence of 5 alkaloids such as Apparicine, Voacamine, Voacristine, 3-Oxo Coronaridine and Conodusarine. Initial investigation on methanol extract of *Ervatamia coronaria* (leaves) showed the presence of 5 alkaloids and 1 phenolic compound such as Apparicine, Lochnericine, Voacristine, Voafinidine, Voafinine and Catechin. Meanwhile, initial investigation of methanol extract of *Tinospora crispa* (stems) revealed the presence of 6 compounds such as 3 alkaloids (Jatrorhizine, Magnoflorine and Norboldine) and 3 phenolic compounds (Apigenin, Brevifolin Carboxylic Acid and Protocatechuic Acid).

4.7.1 Chromatogram (Positive Mode) of *Ervatamia coronaria* (roots)

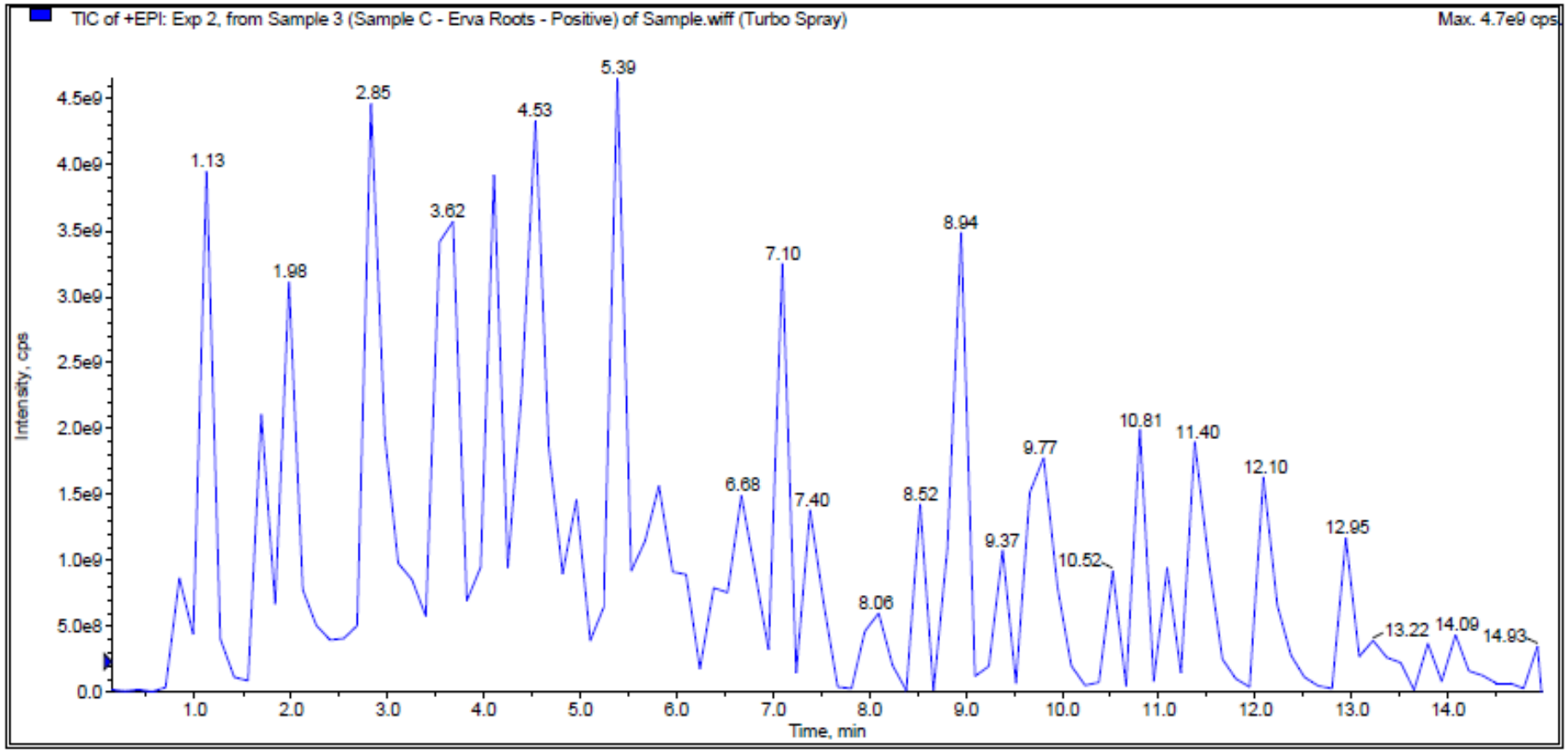

Figure 4.20: Full Positive Chromatogram *Ervatamia coronaria* (roots)

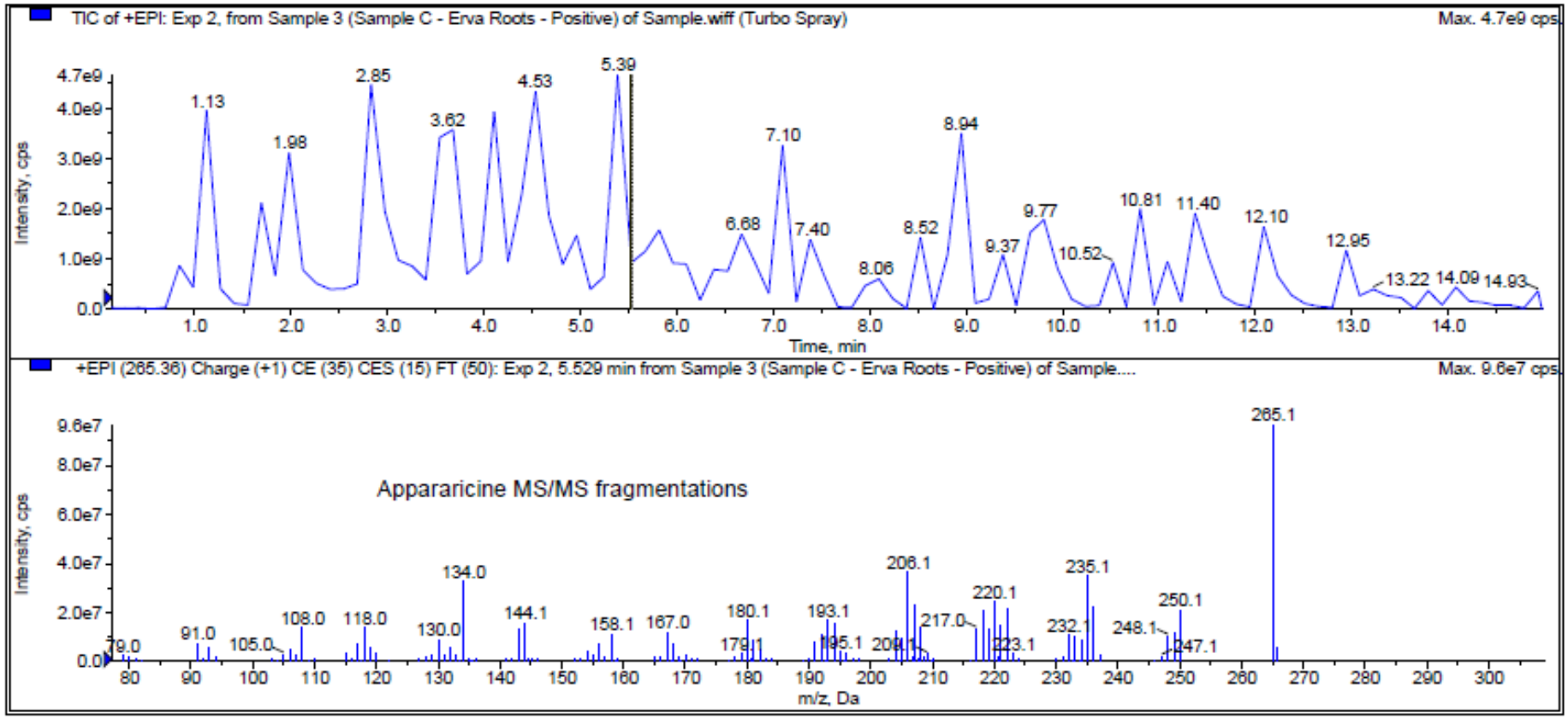

Figure 4.21: Full positive chromatogram of *Ervatamia coronaria* (roots) with Apparicine MS/MS fragmentation (+EPI: 265.36)

Figure above shows full scan (positive mode) of *Ervatamia coronaria* (roots) with MS/MS Apparicine fragmentation. The retention time for Apparicine was 5.529 minute. The mass spectrum fragmentation of Apparicine displayed the following major peaks m/z: 265.1, 250.1, 235.1, 220.1, 206.1 and 134.0. The count per second (cps) of the tallest peak was 9.6 e7 cps.

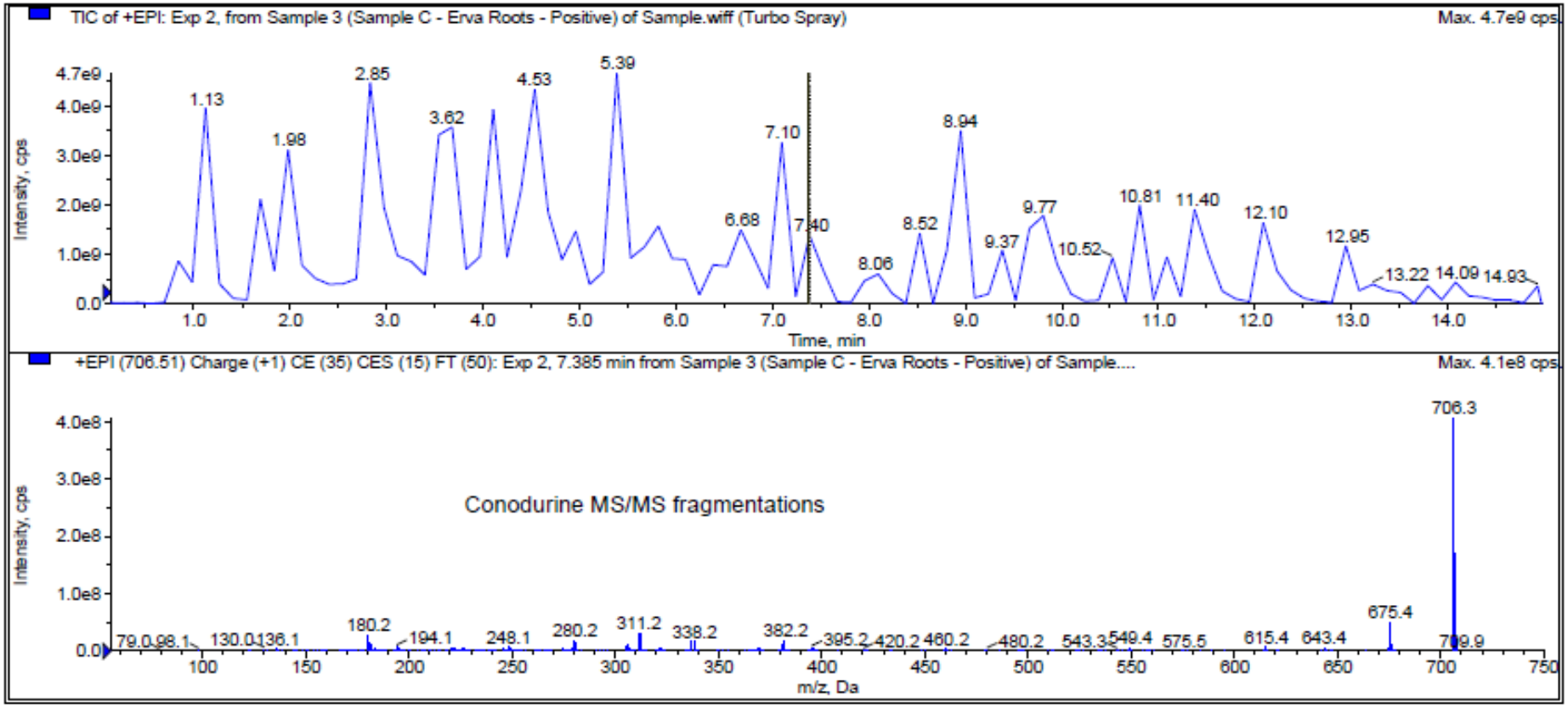

Figure 4.22: Full positive chromatogram of *Ervatamia coronaria* (roots) with Conodurine MS/MS fragmentation (+EPI: 706.51)

Figure above shows full scan (positive mode) of *Ervatamia coronaria* (roots) with Conodurine MS/MS fragmentation. The retention time for Conodurine was 7.385 minute. The mass spectrum fragmentation of Conodurine displayed the following major peaks at m/z: 706.3, 675.4, 311.2, 280.2 and 180.2. The count per second (cps) of the tallest peak was 4.1 e8 cps.

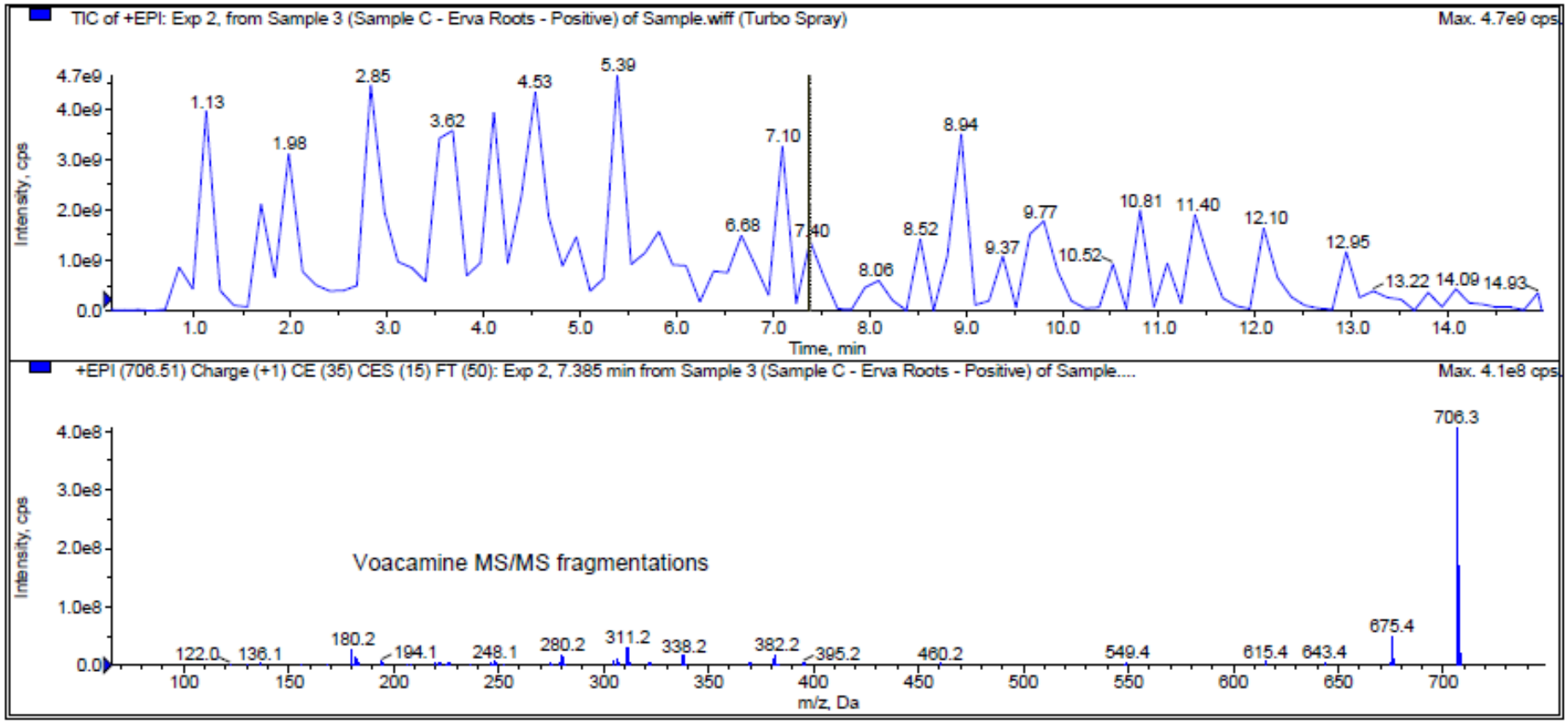

Figure 4.23 : Full positive chromatogram of *Ervatamia coronaria* (roots) with Voacamine MS/MS fragmentation (+EPI: 706.51)

Figure above shows full scan (positive mode) of *Ervatamia coronaria* (roots) with Voacamine MS/MS fragmentation. The retention time for Voacamine was 7.385 minute. The mass spectrum fragmentation of Voacamine displayed the following major peaks at m/z: 706.3, 675.4, 311.2 and 180.2. The count per second (cps) of the tallest peak was 4.1 e8 cps.

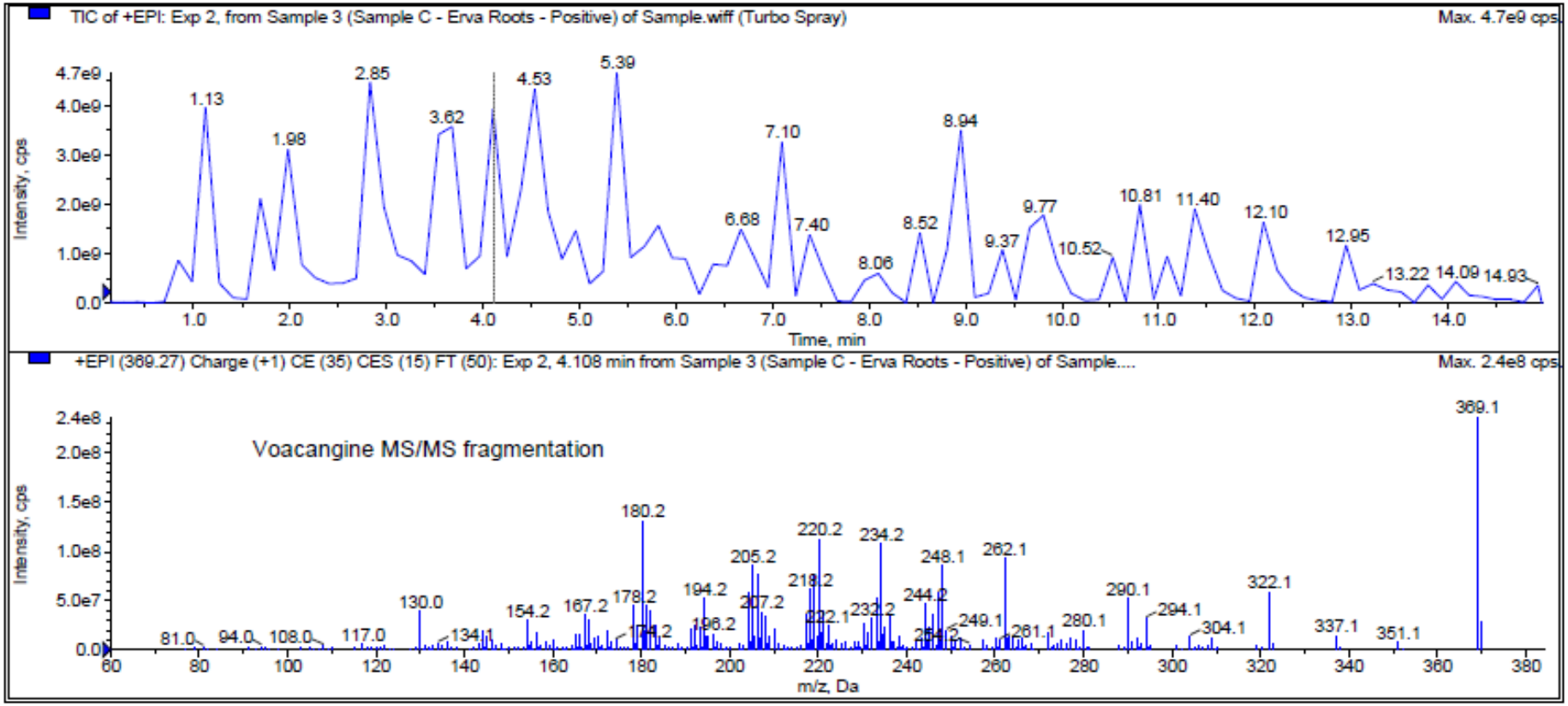

Figure 4.24: Full positive chromatogram of *Ervatamia coronaria* (roots) with Voacangine MS/MS fragmentations (+EPI: 369.27)

Figure above shows full scan (positive mode) of *Ervatamia coronaria* (roots) with Voacangine MS/MS fragmentation. The retention time for Voacangine was 4.108 minute. The mass spectrum fragmentation of Voacangine displayed the following major peaks at m/z: 369.1, 262.1, 234,2, 220.2 and 180.2. The count per second (cps) of the tallest peak was 2.4 e8 cps.

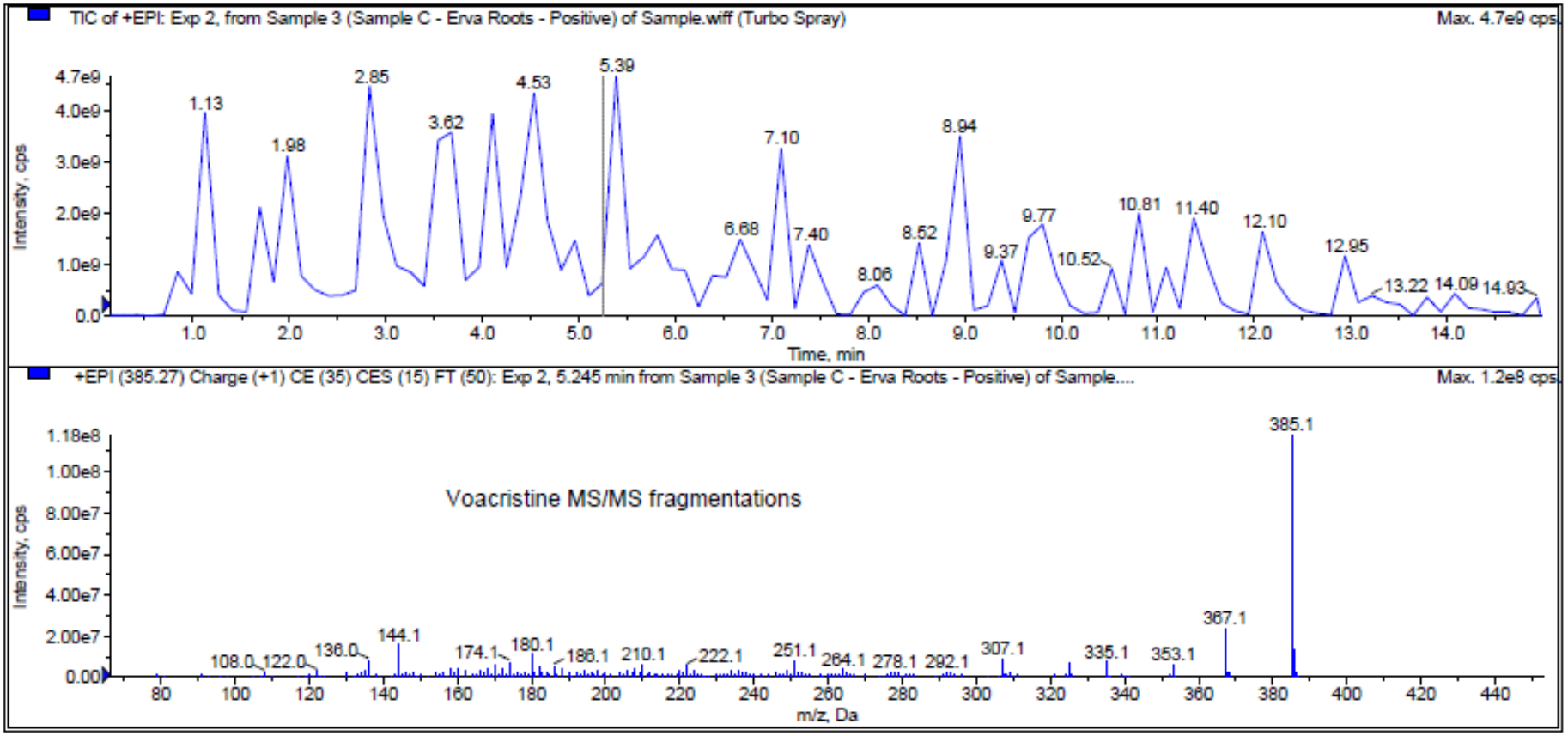

Figure 4.25: Full positive chromatogram of *Ervatamia coronaria* (roots) with Voacristine MS/MS fragmentations (+EPI: 385.27)

Figure above shows full scan (positive mode) of *Ervatamia coronaria* (roots) with Voacristine MS/MS fragmentation. The retention time for Voacristine was 5.245 minute. The mass spectrum fragmentation of Voacristine displayed the following major peaks at m/z: 385.1, 367.1, 180.1 and 144.1. The count per second (cps) of the tallest peak was 1.2 e8 cps.

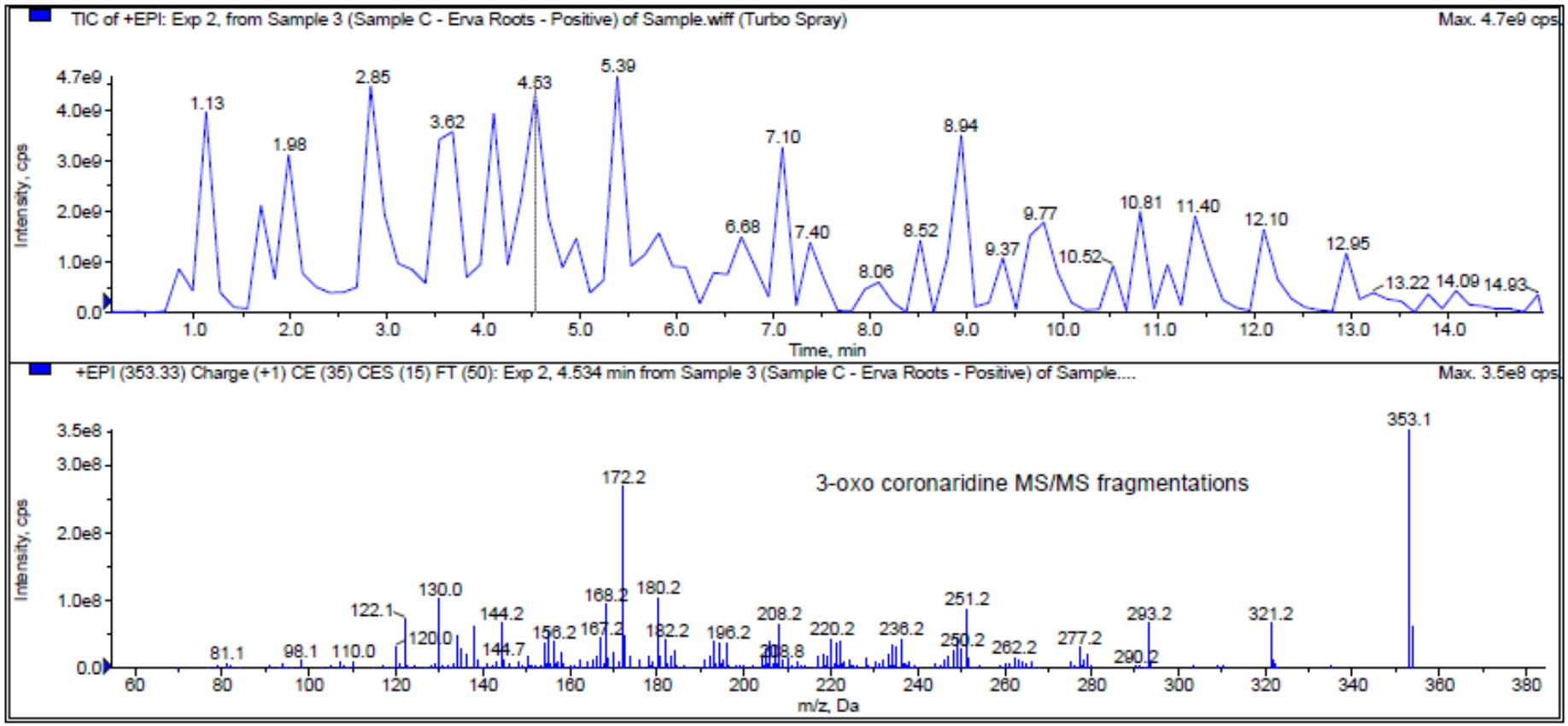

Figure 4.26: Full positive chromatogram of *Ervatamia coronaria* (roots) with 3-oxo coronaridine MS/MS fragmentations (+EPI: 353.33)

Figure above shows full scan (positive mode) of *Ervatamia coronaria* (roots) with 3-oxo coronaridine MS/MS fragmentation. The retention time for 3-oxo coronaridine was 4.534 minute. The mass spectrum fragmentation of 3-oxo coronaridine displayed the following major peaks at m/z: 353.1, 251.2, 180.2, 172.2, 168.2 and 130.0. The count per second (cps) of the tallest peak was 3.5 e8 cps.

4.7.2 Chromatogram (Negative Mode) of *Ervatamia coronaria* (roots)

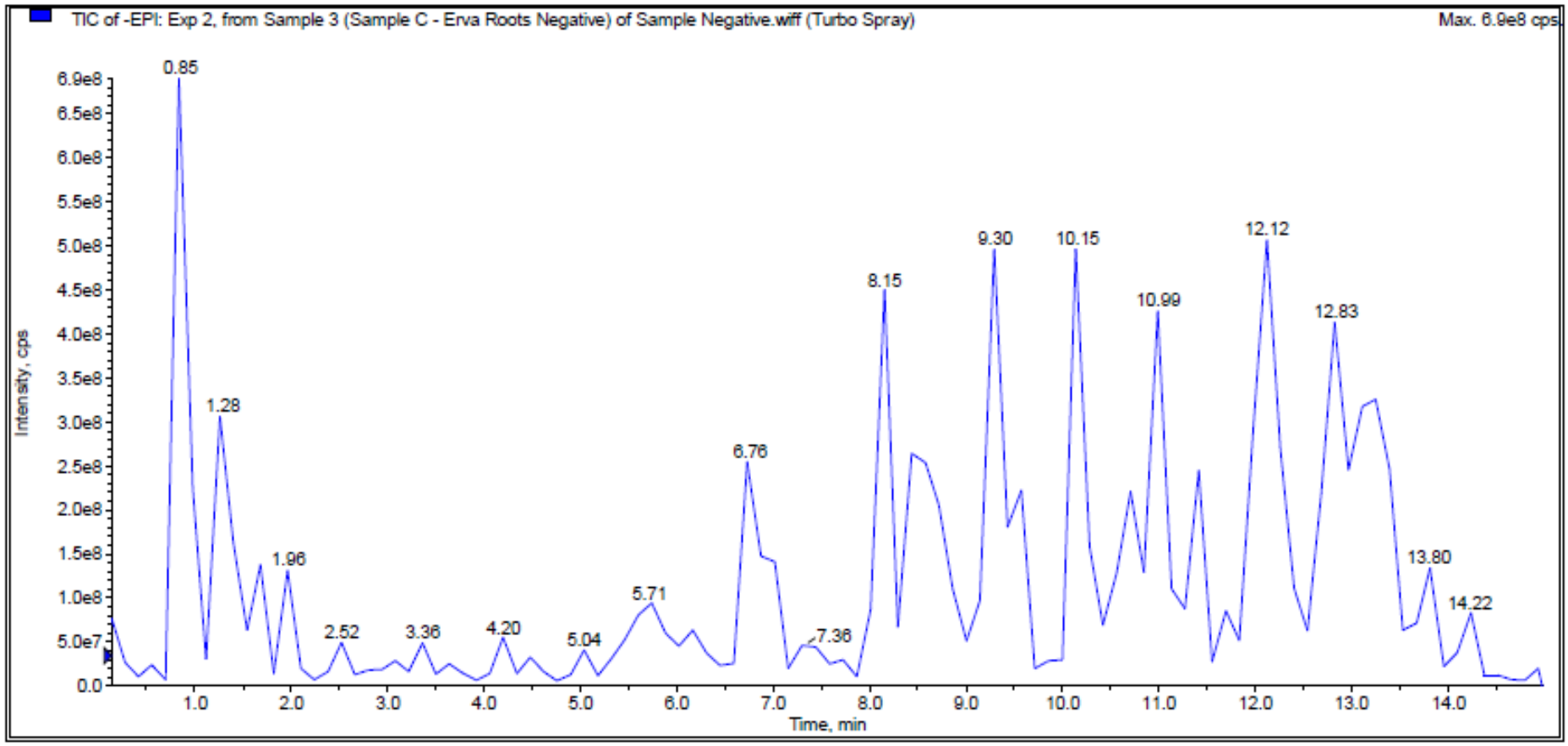

Figure 4.27: Full Negative Chromatogram *Ervatamia coronaria* (roots)

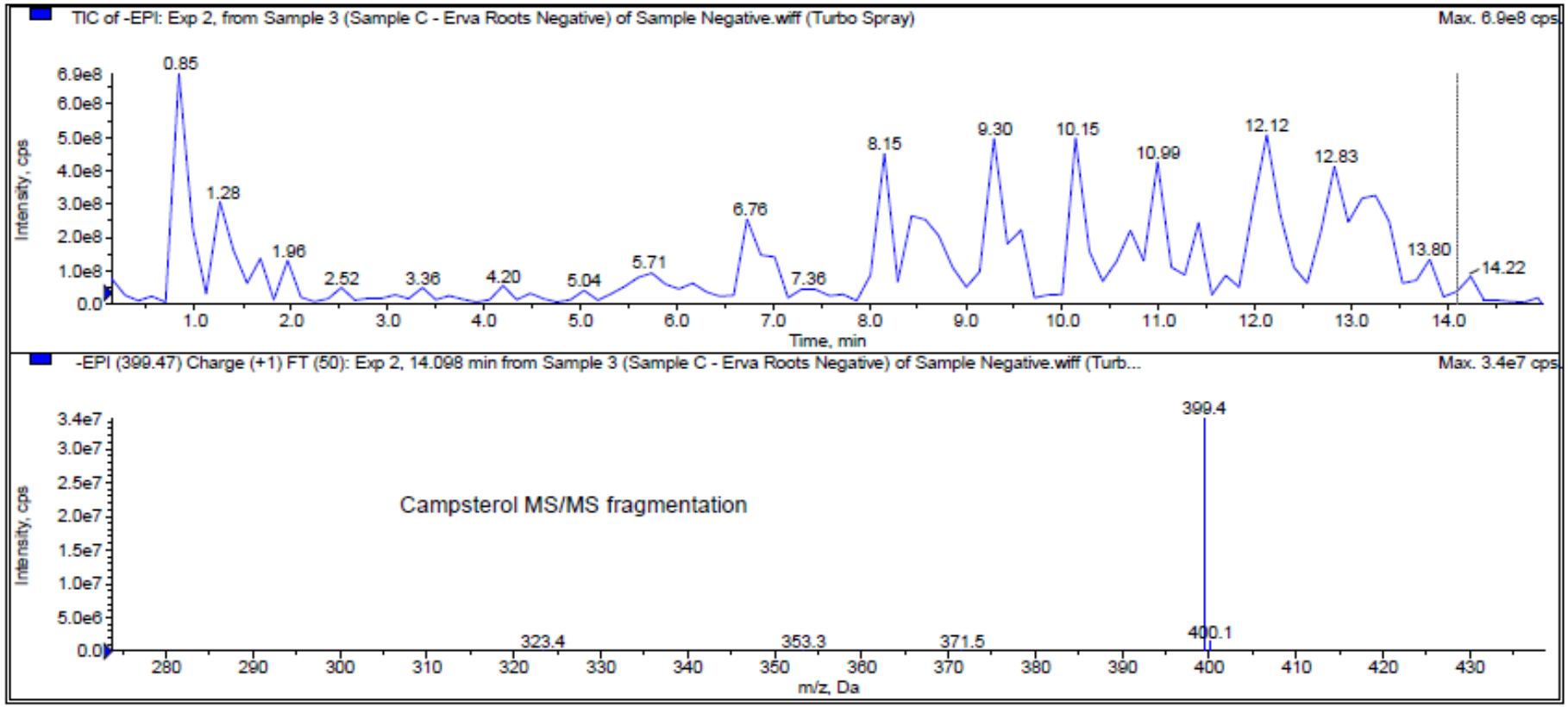

Figure 4.28: Full negative chromatogram of *Ervatamia coronaria* (roots) with Campesterol MS/MS fragmentations (-EPI: 399.47)

Figure above shows full scan (negative mode) of *Ervatamia coronaria* (roots) with Campesterol MS/MS fragmentation. The retention time for Campesterol was 14.098 minute. The mass spectrum fragmentation of Campesterol displayed the following peaks at m/z: 399.4, 371.5, 353.3 and 323.4. The count per second (cps) of the tallest peak was 3.4 e7 cps.

4.7.3 Chromatogram (Positive Mode) of *Ervatamia coronaria* (stems)

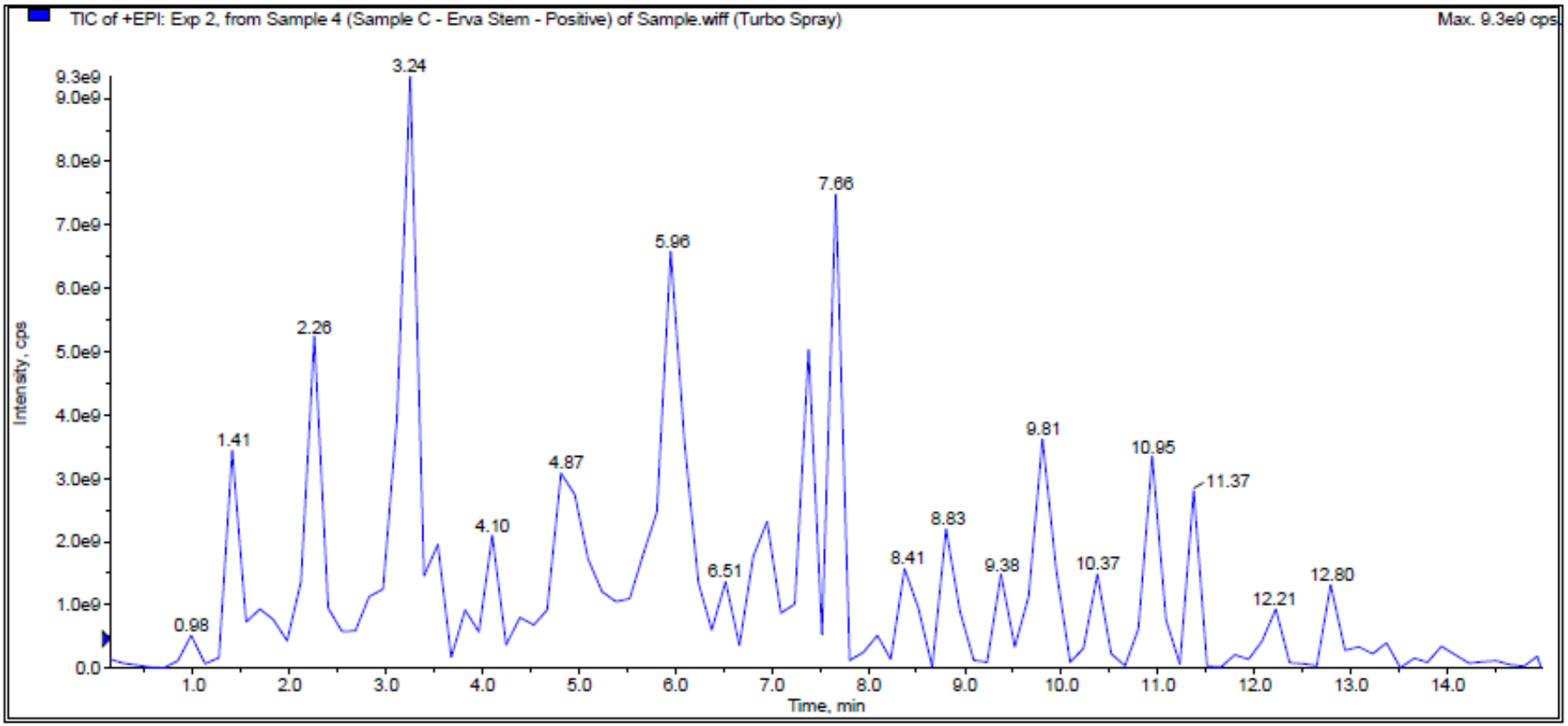

Figure 4.29: Full Positive Chromatogram of *Ervatamia coronaria* (stems)

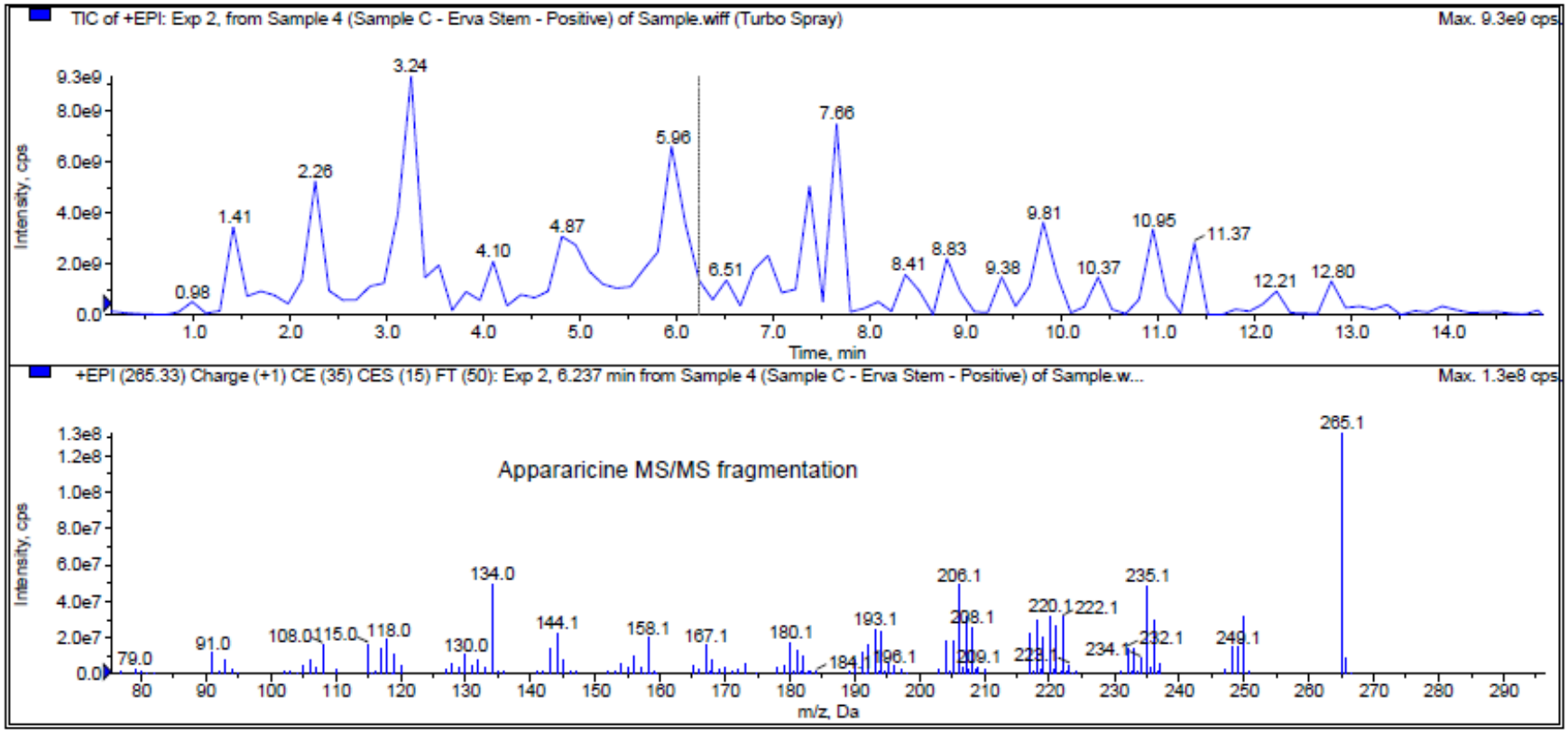

Figure 4.30: Full positive chromatogram of *Ervatamia coronaria* (stems) with Apparicine MS/MS fragmentations (+EPI: 265.33)

Figure above shows full scan (positive mode) of *Ervatamia coronaria* (stems) with Apparicine MS/MS fragmentation. The retention time for Apparicine was 6.237 minute. The mass spectrum fragmentation of Apparicine displayed the following major peaks at m/z: 265.1, 235.1, 208.1, 206.1, and 134.0. The count per second (cps) of the tallest peak was 1.3 e8 cps.

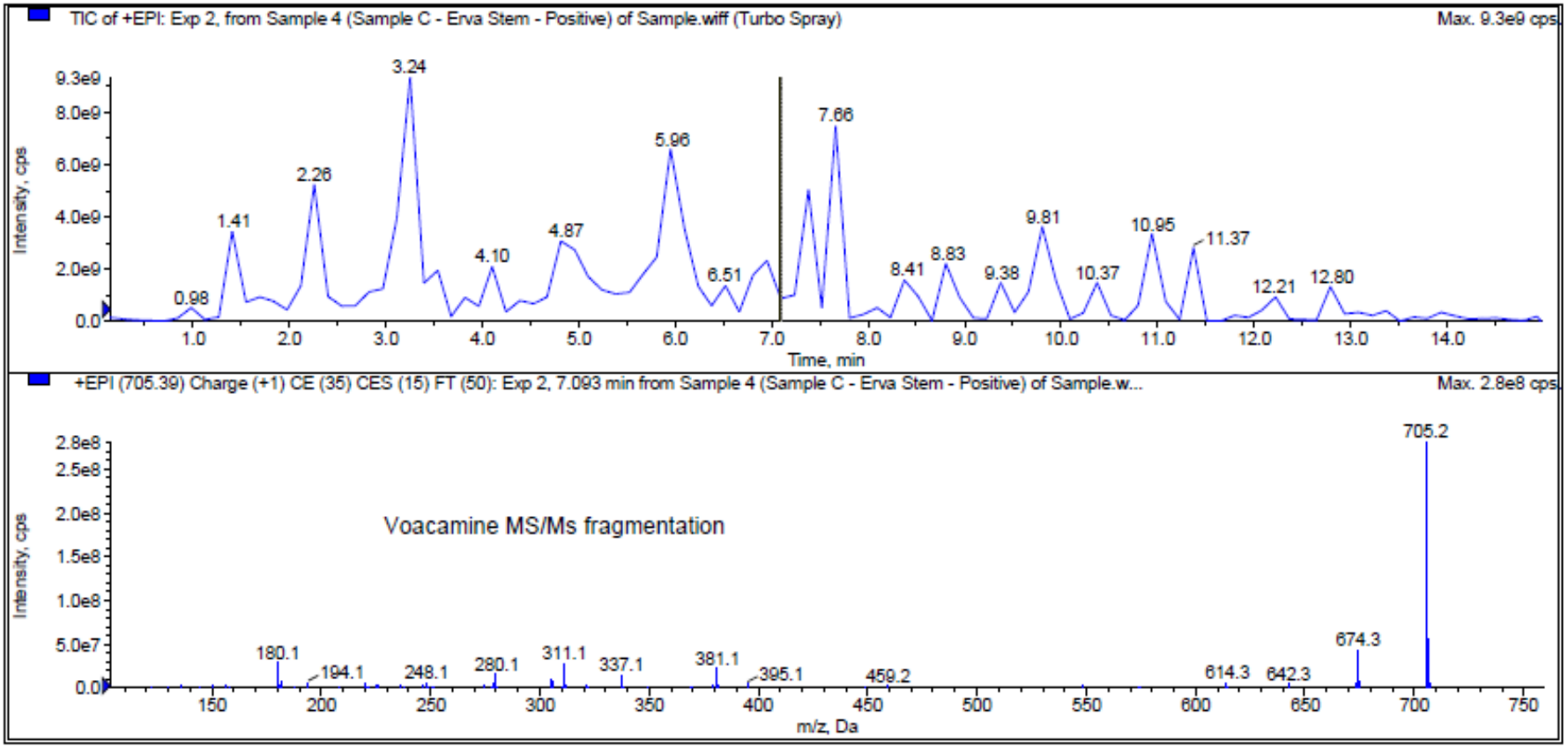

Figure 4.31: Full positive chromatogram of *Ervatamia coronaria* (stems) with Voacamine MS/MS fragmentations (+EPI: 705.39)

Figure above shows full scan (positive mode) of *Ervatamia coronaria* (stems) with Voacamine MS/MS fragmentation. The retention time for Voacamine was 7.093 minute. The mass spectrum fragmentation of Voacamine displayed the following major peaks at m/z: 705.2, 674.3, 381.1, 311.1, 280.1 and 180.1. The count per second (cps) of the tallest peak was 2.8 e8 cps.

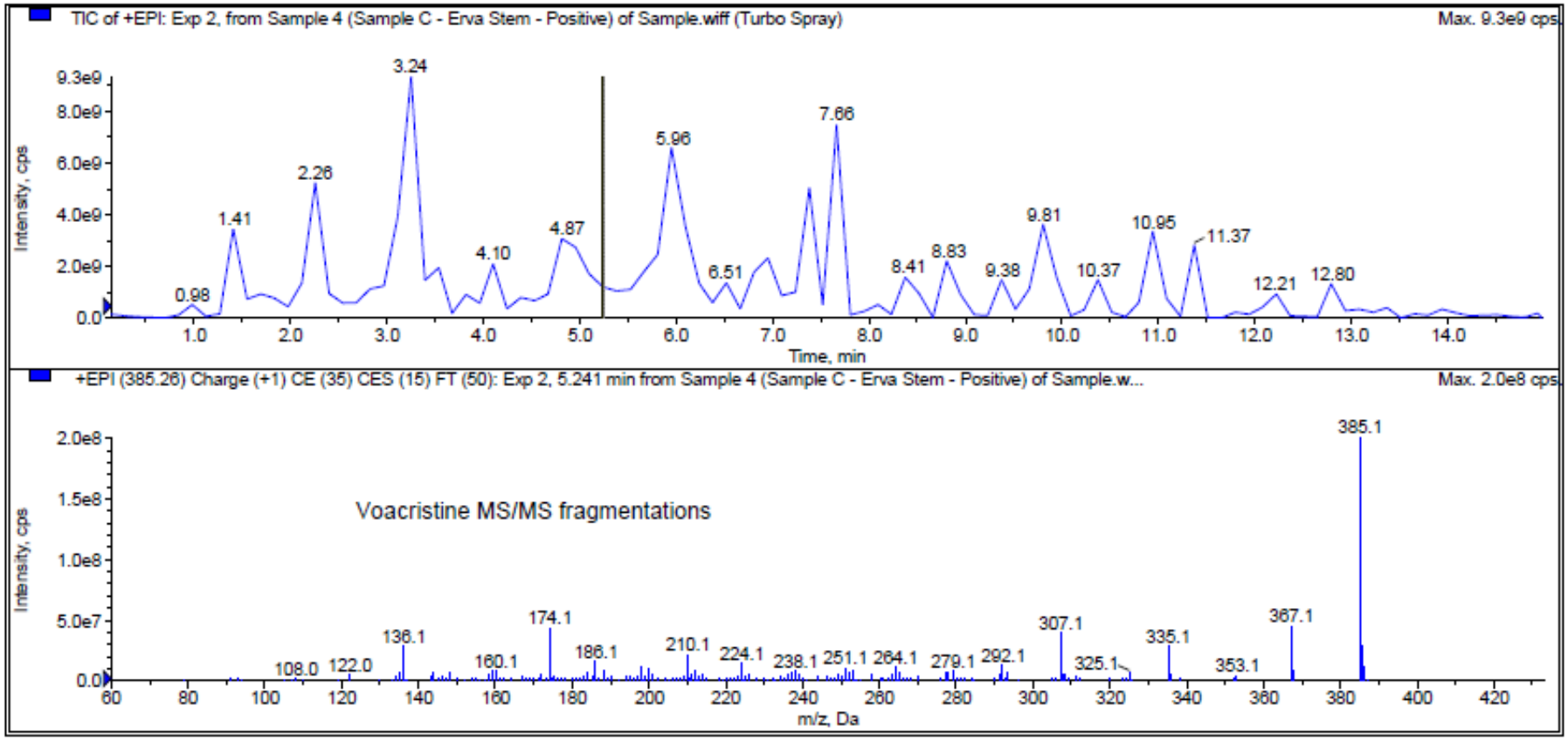

Figure 4.32: Full positive chromatogram of *Ervatamia coronaria* (stems) with Voacristine MS/MS fragmentations (+EPI: 385.26)

Figure above shows full scan (positive mode) of *Ervatamia coronaria* (stems) with Voacristine MS/MS fragmentation. The retention time for Voacristine was 5.241 minute. The mass spectrum fragmentation of voacristine displayed the following major peaks m//z: 385.1, 367.1, 335.1, 307.1, 174.1 and 136.1. The count per second (cps) of the tallest peak was 2.0 e8 cps.

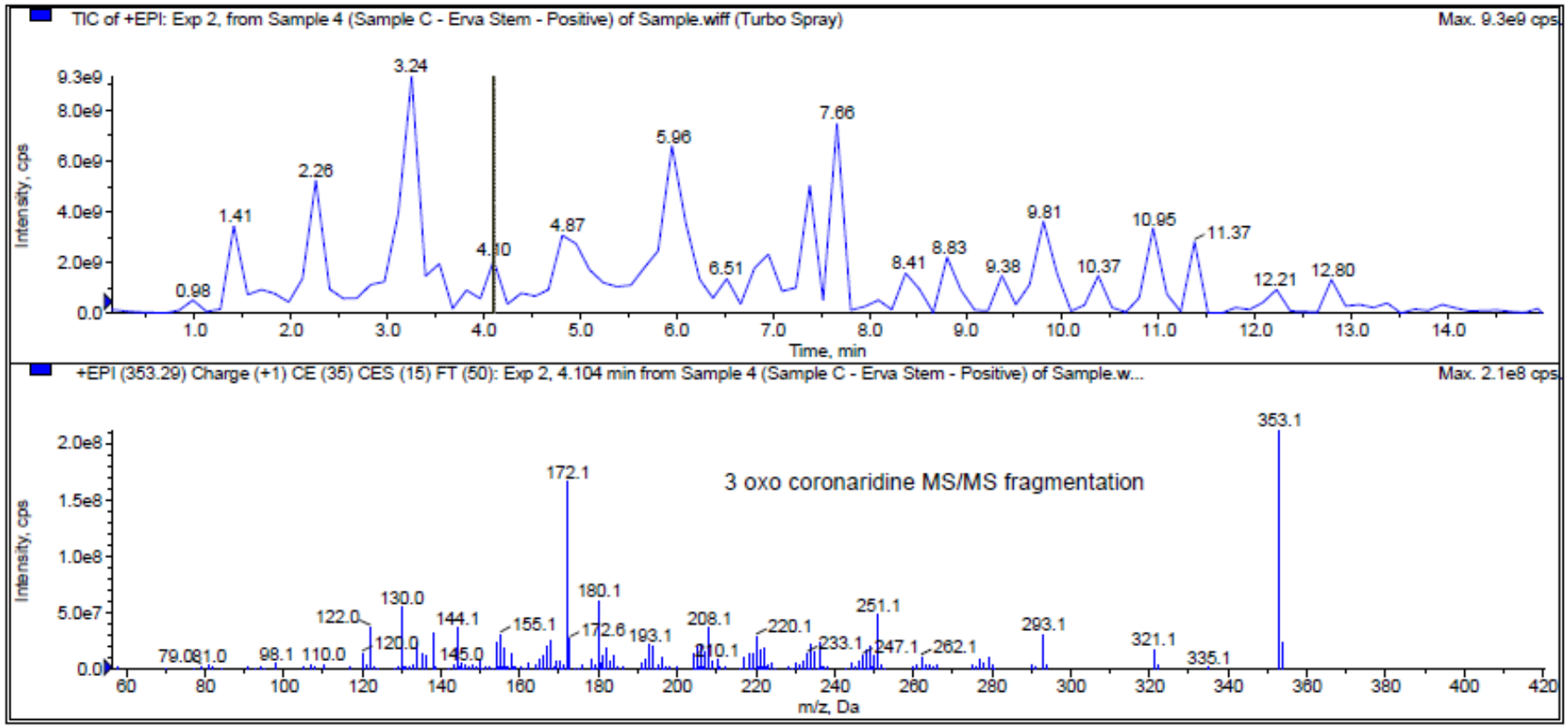

Figure 4.33: Full positive chromatogram of *Ervatamia coronaria* (stems) with 3-Oxo coronaridine MS/MS fragmentations (+EPI: 353.29)

Figure above shows full scan (positive mode) of *Ervatamia coronaria* (stems) with 3-oxo coronaridine MS/MS fragmentation. The retention time for 3-oxo coronaridine was 4.104 minute. The mass spectrum fragmentation of 3-oxo coronaridine displayed the following major peaks at m/z: 353.1, 251.2, 180.2, 172.2 and 130.0. The count per second (cps) of the tallest peak was 2.1 e8 cps.

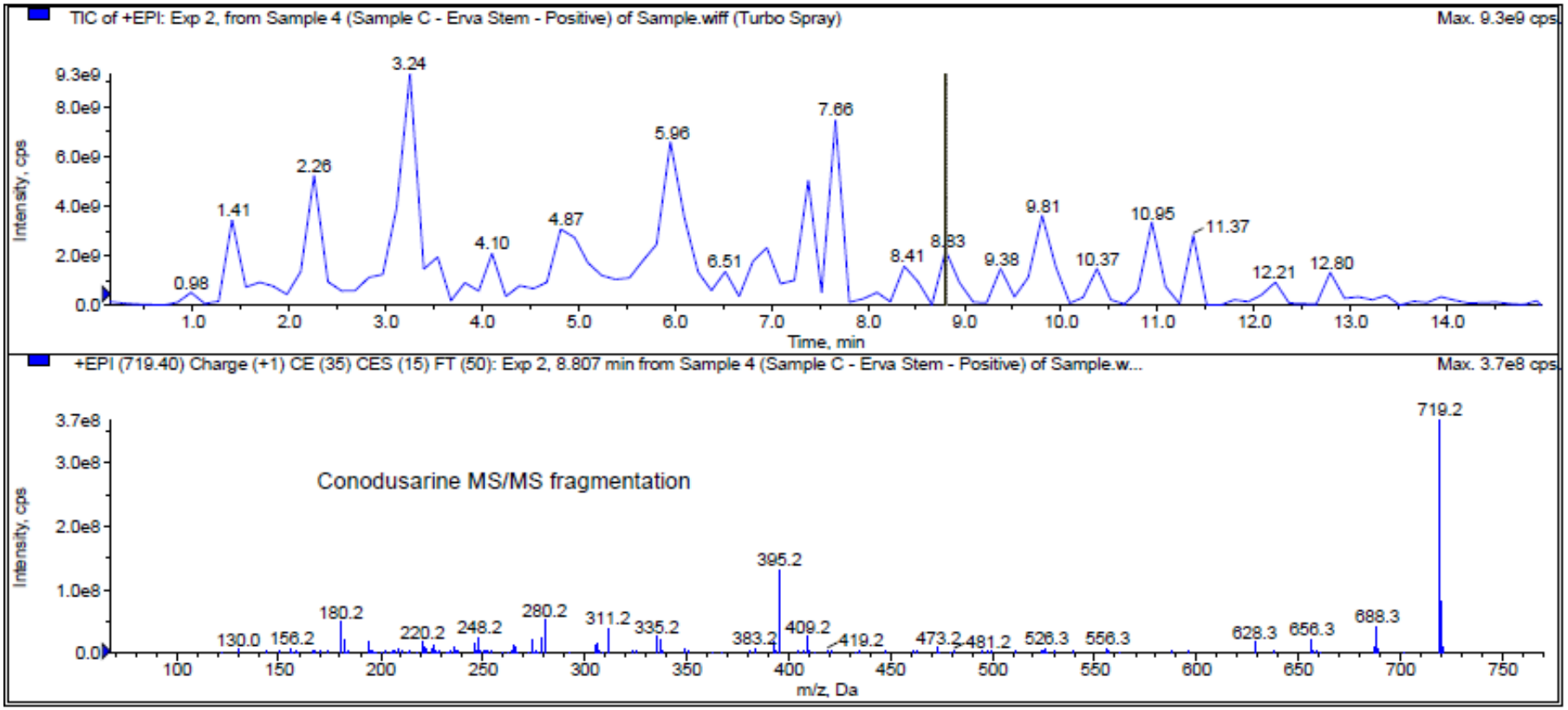

Figure 4.34: Full positive chromatogram of *Ervatamia coronaria* (stems) with Conodusarine MS/MS fragmentations (+EPI: 719.40)

Figure above shows full scan (positive mode) of *Ervatamia coronaria* (stems) with Conodusarine MS/MS fragmentation. The retention time for Conodusarine was 8.807 minute. The mass spectrum fragmentation of Conodusarine displayed the following major peaks at m/z: 719.2, 688.3, 395.2, 311.2, 280.2 and 180.2. The count per second (cps) of the tallest peak was 3.7 e8 cps.

4.7.4 Chromatogram (Negative Mode) of *Ervatamia coronaria* (stems)

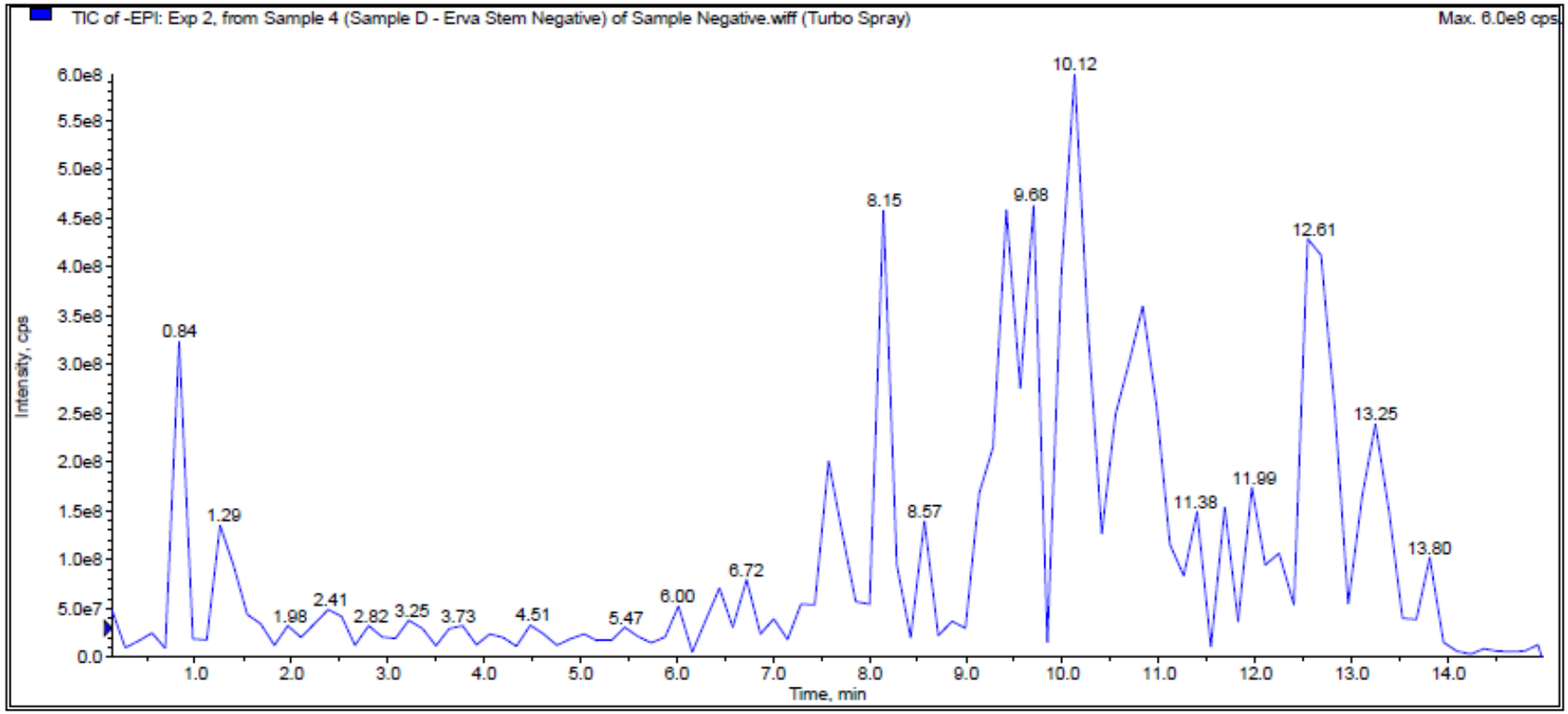

Figure 4.35: Full Negative Chromatogram of *Ervatamia coronaria* (stems)

4.7.5 Chromatogram (Positive Mode) of *Ervatamia coronaria* (leaves)

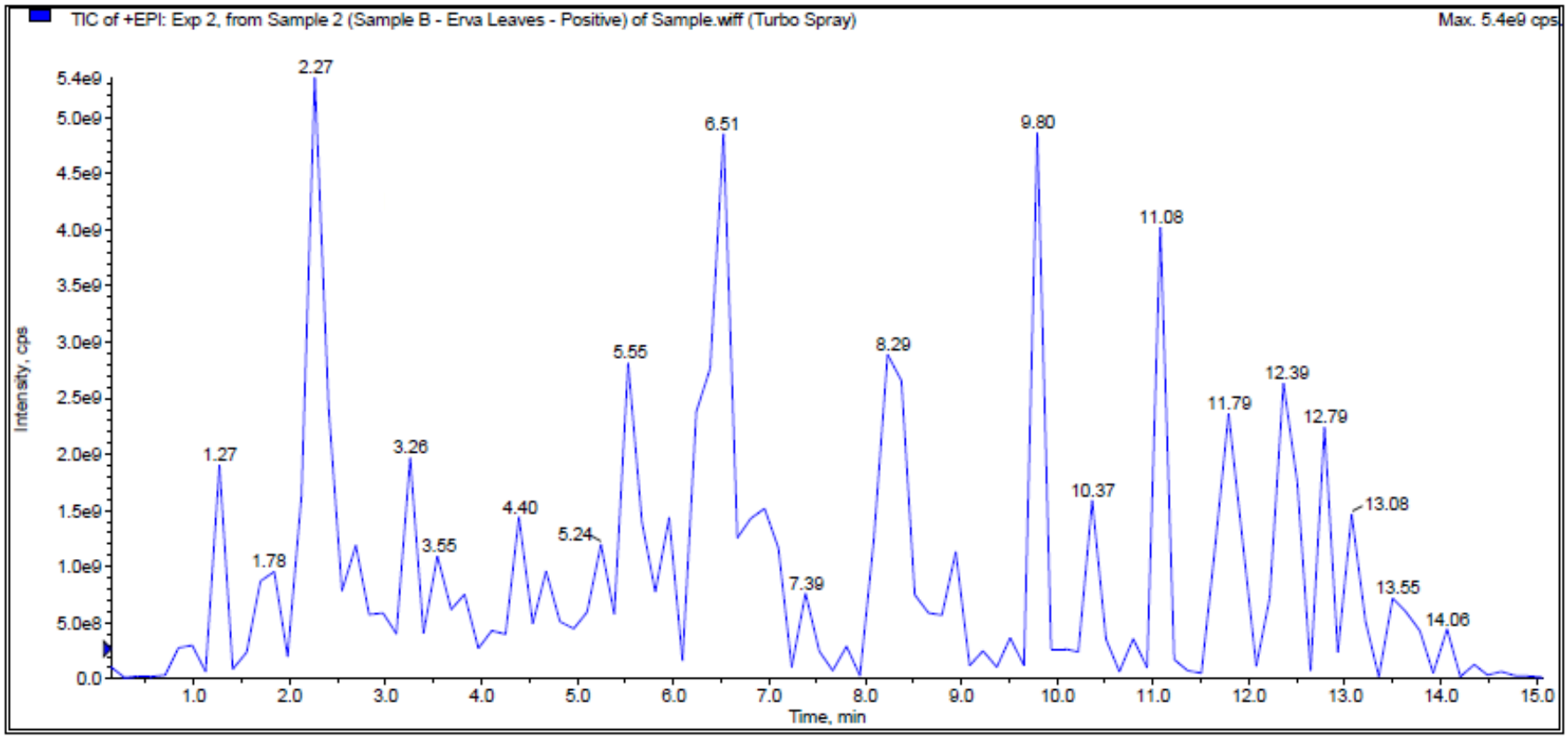

Figure 4.36: Full Positive Chromatogram of *Ervatamia coronaria* (leaves)

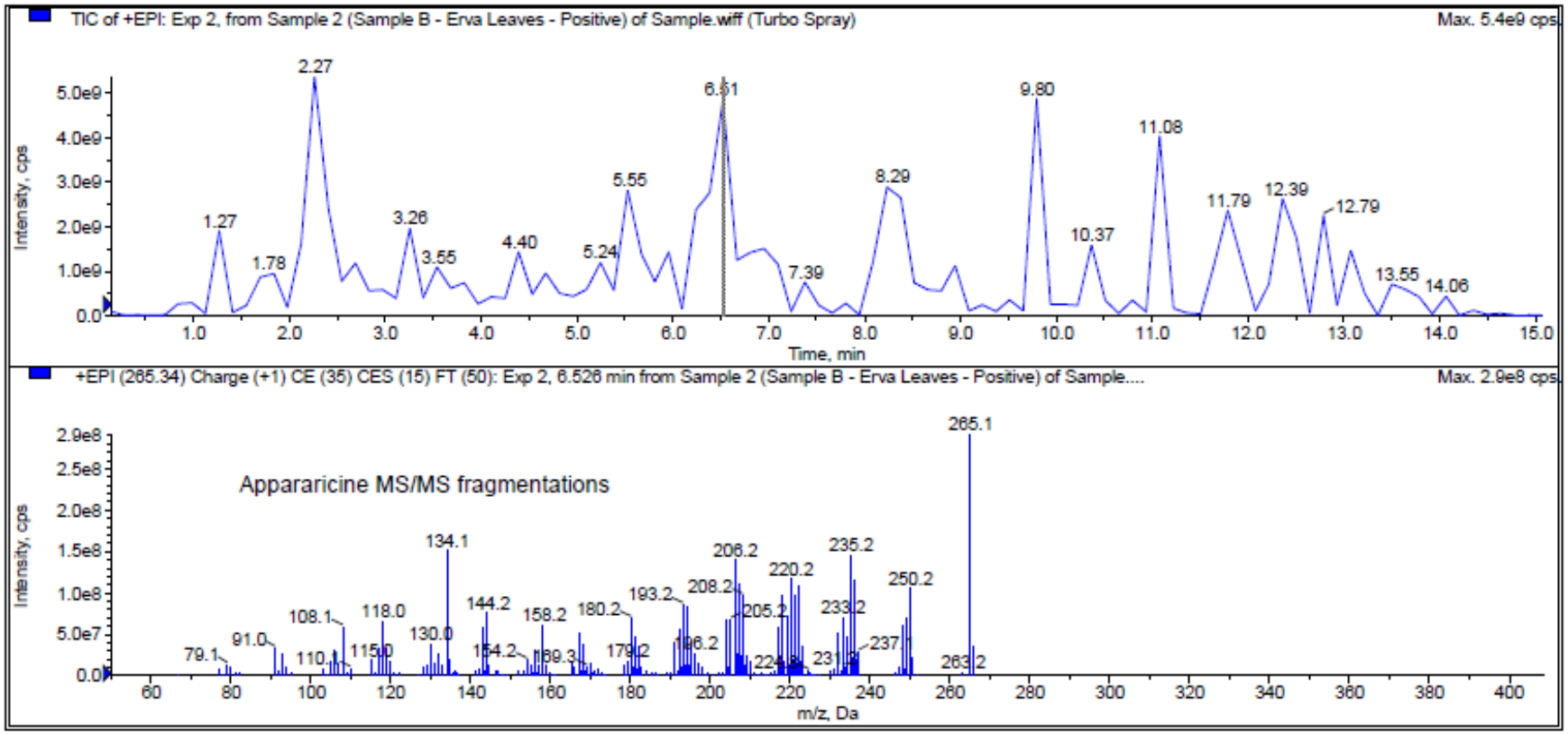

Figure 4.37: Full positive chromatogram of *Ervatamia coronaria* (leaves) with Apparicine MS/MS fragmentations (+EPI: 265.34)

Figure above shows full scan (positive mode) of *Ervatamia coronaria* (leaves) with Apparicine MS/MS fragmentation. The retention time for Apparicine was 6.526 minute. The mass spectrum fragmentation of Apparicine displayed the following major peaks at m/z: 265.1, 250.2, 235.2, 220.2, 206.2 and 134.1. The count per second (cps) of the tallest peak was 2.9 e8 cps.

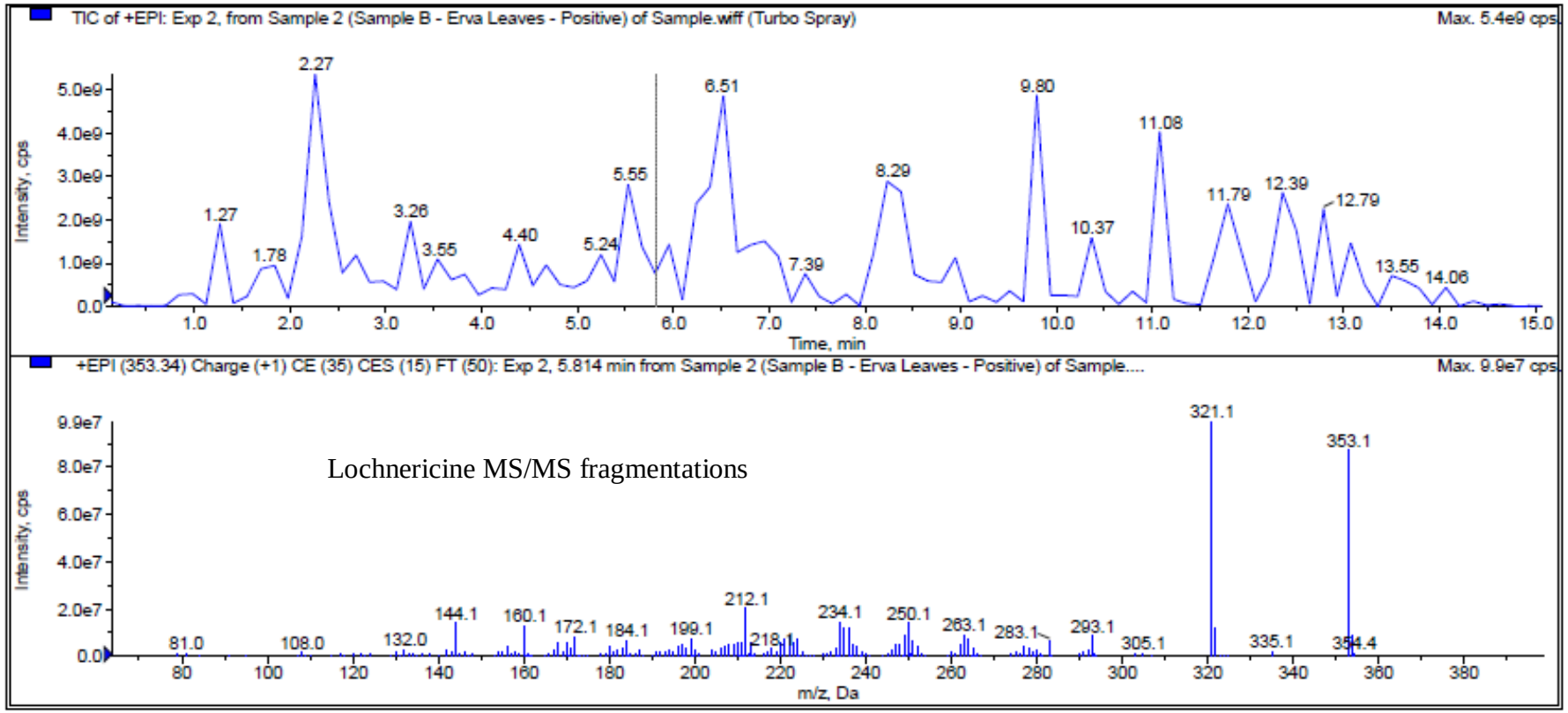

Figure 4.38: Full positive chromatogram of *Ervatamia coronaria* (leaves) with Lochnericine MS/MS fragmentations (+EPI:353.34)

Figure above shows full scan (positive mode) of *Ervatamia coronaria* (leaves) with Lochnericine MS/MS fragmentation. The retention time for Lochnericine was 5.814 minute. The mass spectrum fragmentation of Lochnericine displayed the following major peaks at m/z: 353.1, 321.1, 250.1, 212.1, 160.1 and 144.1. The count per second (cps) of the tallest peak was 9.9 e7 cps.

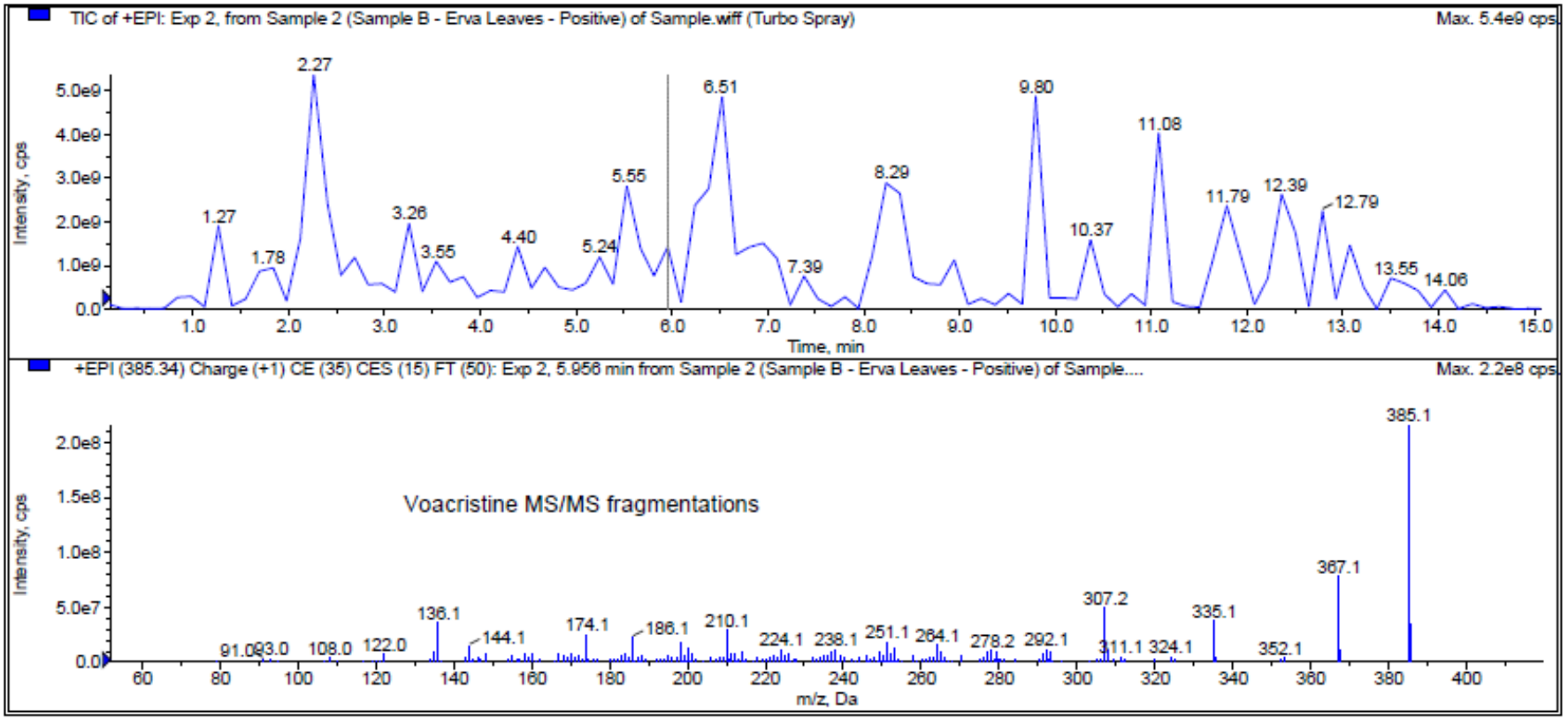

Figure 4.39: Full positive chromatogram of *Ervatamia coronaria* (leaves) with Voacristine MS/MS fragmentations (+EPI: 385.34)

Figure above shows full scan (positive mode) of *Ervatamia coronaria* (leaves) with Voacristine MS/MS fragmentation. The retention time for Voacristine was 5.956 minute. The mass spectrum fragmentation of Voacristine displayed the following major peaks at m/z: 385.1, 367.1, 335.1, 307.2, 210.1 and 136.1. The count per second (cps) of the tallest peak was 2.2 e8 cps.

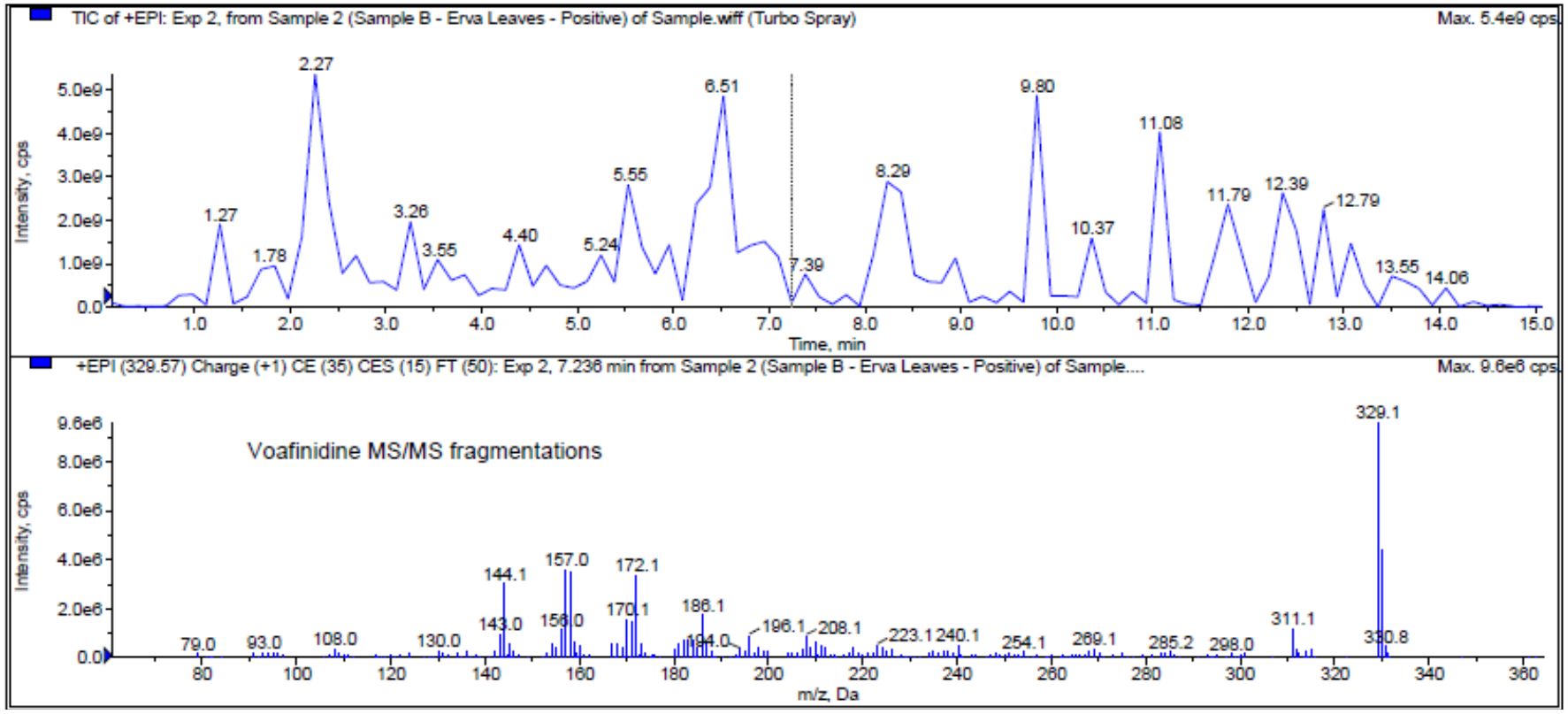

Figure 4.40: Full positive chromatogram of *Ervatamia coronaria* (leaves) with Voafinidine MS/MS fragmentations (+EPI: 329.57)

Figure above shows full scan (positive mode) of *Ervatamia coronaria* (leaves) with Voafinidine MS/MS fragmentation. The retention time for Voafinidine was 7.236 minute. The mass spectrum fragmentation of Voafinidine displayed the following major peaks at m/z: 329.1, 311.1, 186.1, 172.1, 170.1, 157.0 and 144.1. The count per second (cps) of the tallest peak was 9.6 e6 cps.

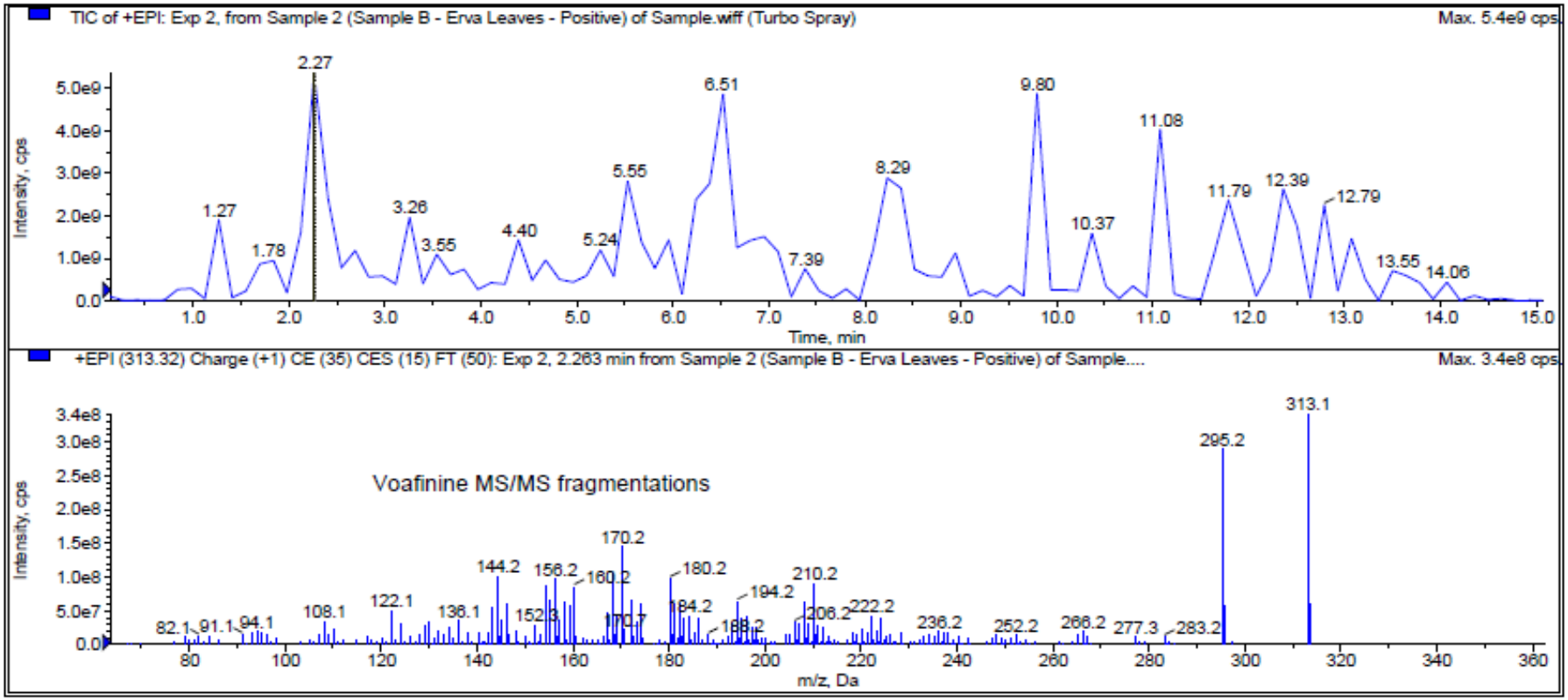

Figure 4.41: Full positive chromatogram of *Ervatamia coronaria* (leaves) with Voafinine MS/MS fragmentations (+EPI: 313.32)

Figure above shows full scan (positive mode) of *Ervatamia coronaria* (leaves) with Voafinine MS/MS fragmentation. The retention time for Voafinine was 2.263 minute. The mass spectrum fragmentation of Voafinine displayed the following major peaks at m/z: 313.1, 295.2, 210.2, 180.2, 170.2, 156.2 and 144.2. The count per second (cps) of the tallest peak was 3.4 e8 cps.

4.7.6 Chromatogram (Negative Mode) of *Ervatamia coronaria* (leaves)

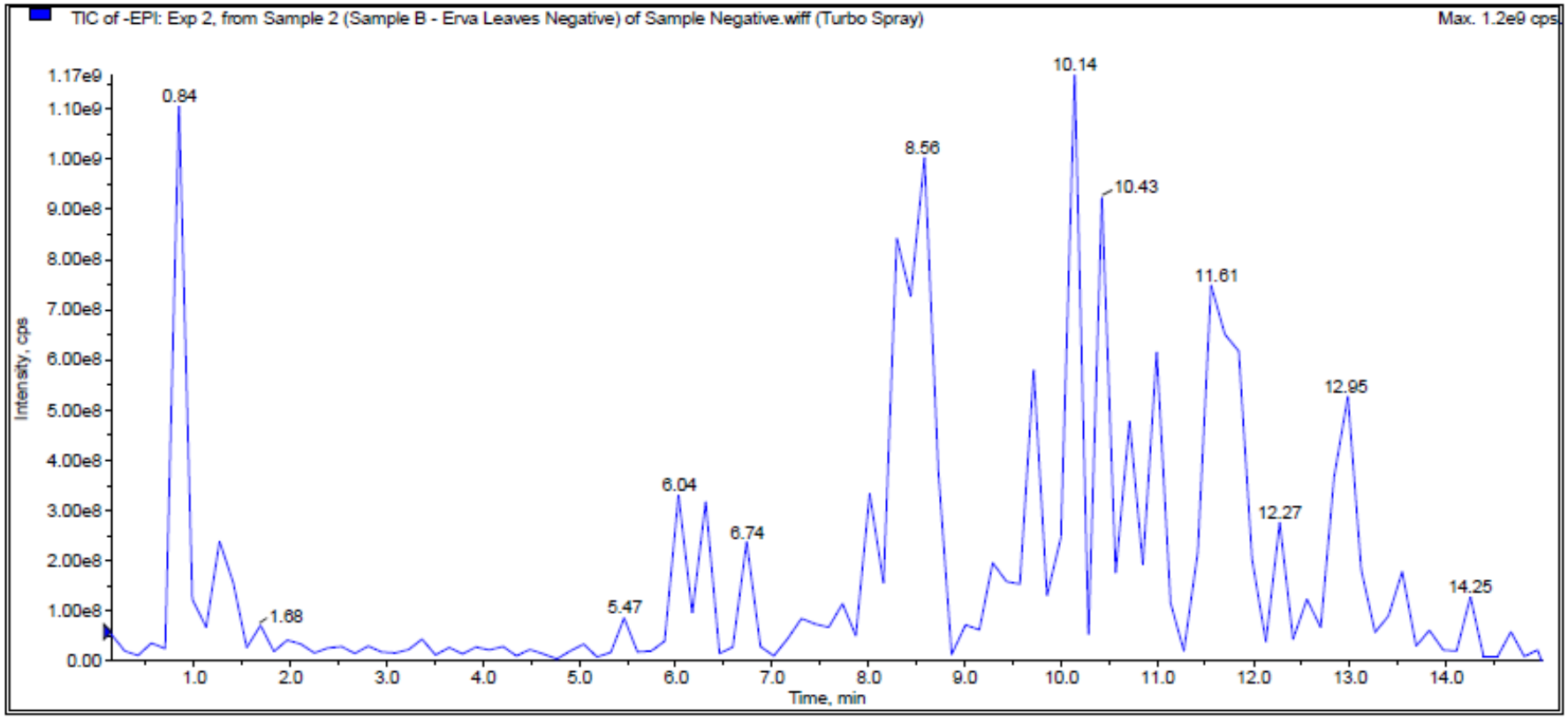

Figure 4.42: Full Negative Chromatogram *Ervatamia coronaria* (leaves)

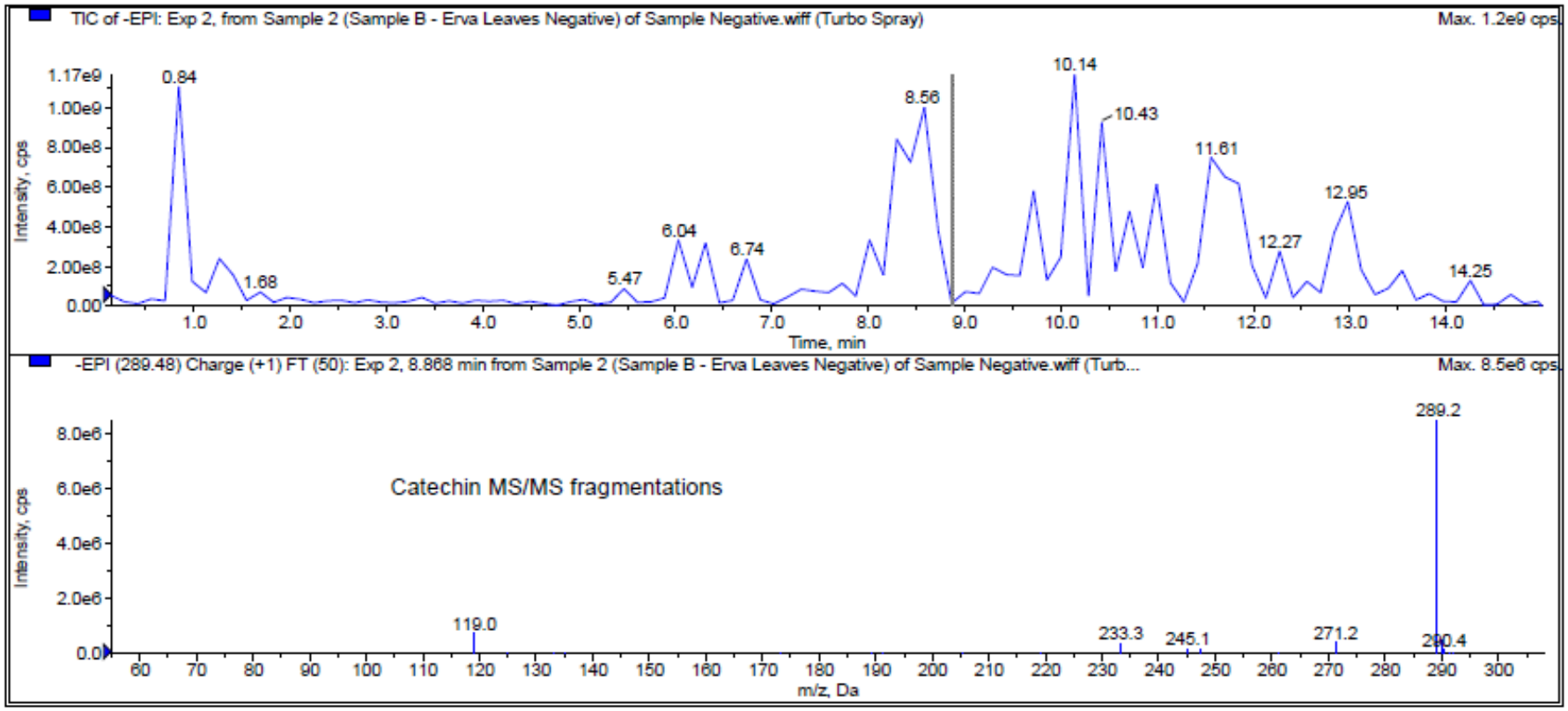

Figure 4.43: Full negative chromatogram of *Ervatamia coronaria* (leaves) with Catechin MS/MS fragmentations (-EPI: 289.48)

Figure above shows full scan (negative mode) of *Ervatamia coronaria* (leaves) with Catechin MS/MS fragmentation. The retention time for Catechin was 8.868 minute. The mass spectrum fragmentation of Catechin displayed the following peaks at m/z: 289.2, 271.2, 245.1, 233.3 and 119.0. The count per second (cps) of the tallest peak was 8.5 e6 cps.

4.7.7 Chromatogram (Positive Mode) of *Tinospora crispa* (stems)

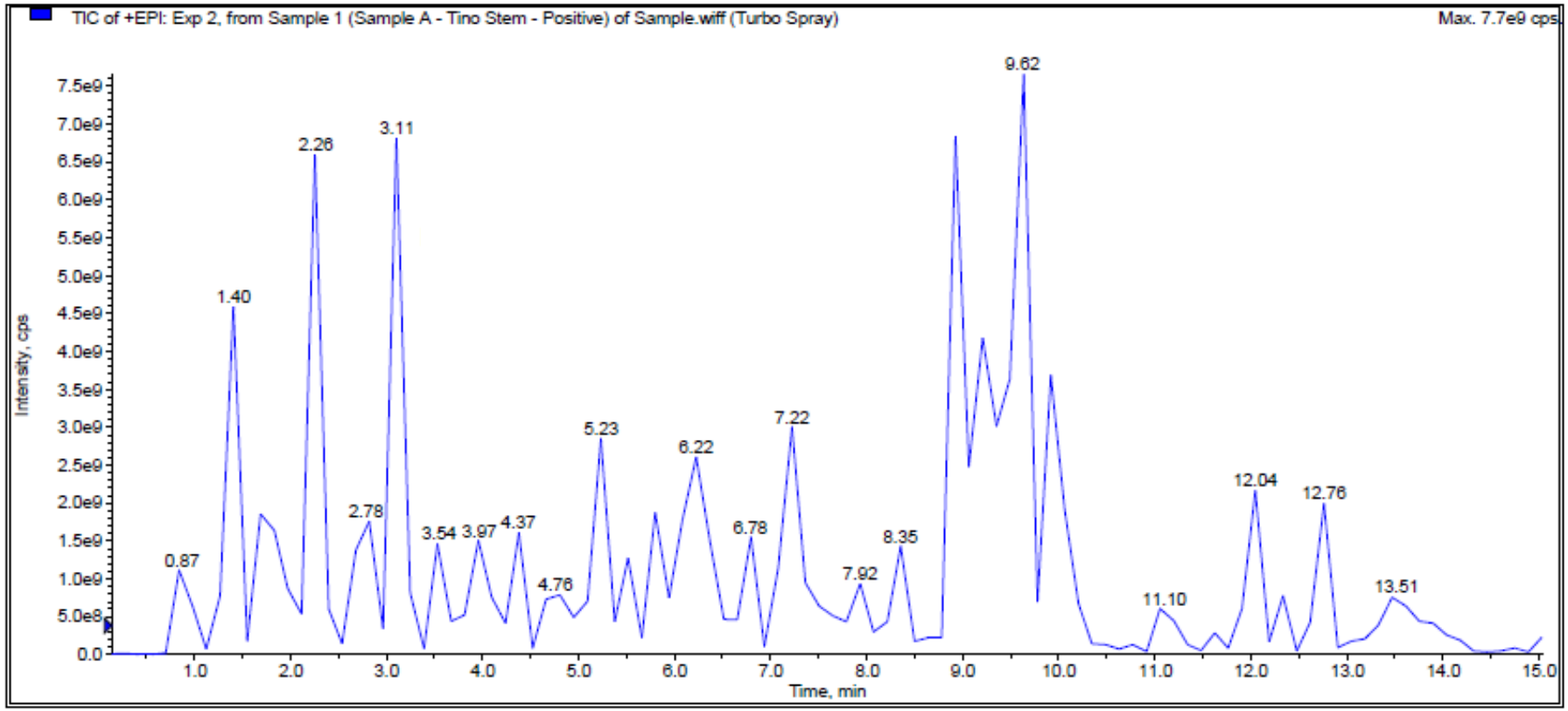

Figure 4.44: Full Positive Chromatogram *Tinospora crispa* (stems)

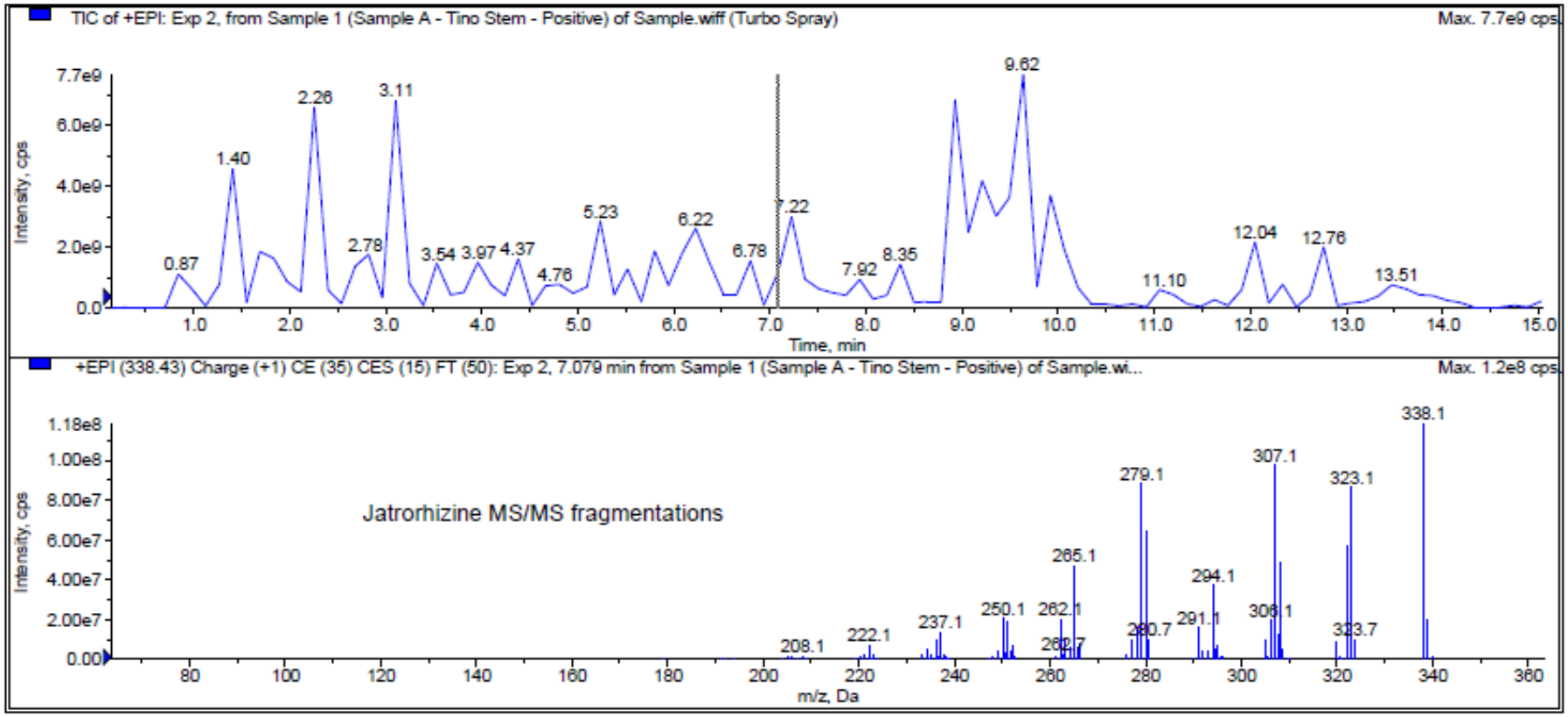

Figure 4.45: Full positive chromatogram of *Tinospora crispa* (stems) with Jatrorhizine MS/MS fragmentations (+EPI: 338.43)

Figure above shows full scan (positive mode) of *Tinospora crispa* (stems) with Jatrorhizine MS/MS fragmentation. The retention time for Jatrorhizine was 7.079 minute. The mass spectrum fragmentation of Jatrorhizine displayed the following major peaks at m/z: 338.1, 323.1, 307.1, 294.1, 279.1 and 265.1. The count per second (cps) of the tallest peak was 1.2 e8 cps.

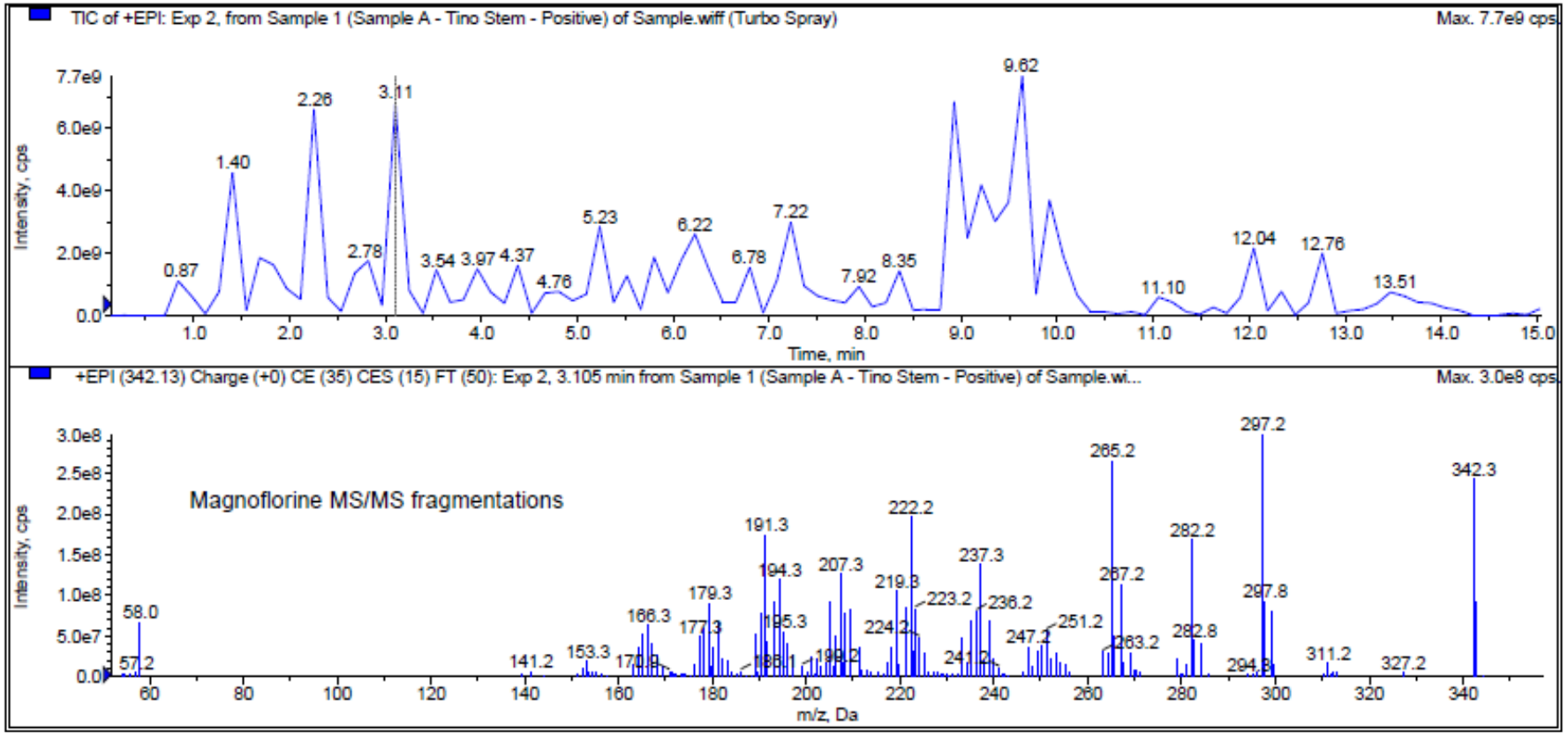

Figure 4.46: Full positive chromatogram of *Tinospora crispa* (stems) with Magnoflorine MS/MS fragmentations (+EPI: 342.13)

Figure above shows full scan (positive mode) of *Tinospora crispa* (stems) with Magnoflorine MS/MS fragmentation. The retention time for Magnoflorine was 3.105 minute. The mass spectrum fragmentation of Magnoflorine displayed the following major peaks at m/z: 342.3, 297.2, 282.2, 265.2, 222.2 and 191.3. The count per second (cps) of the tallest peak was 3.0 e8 cps.

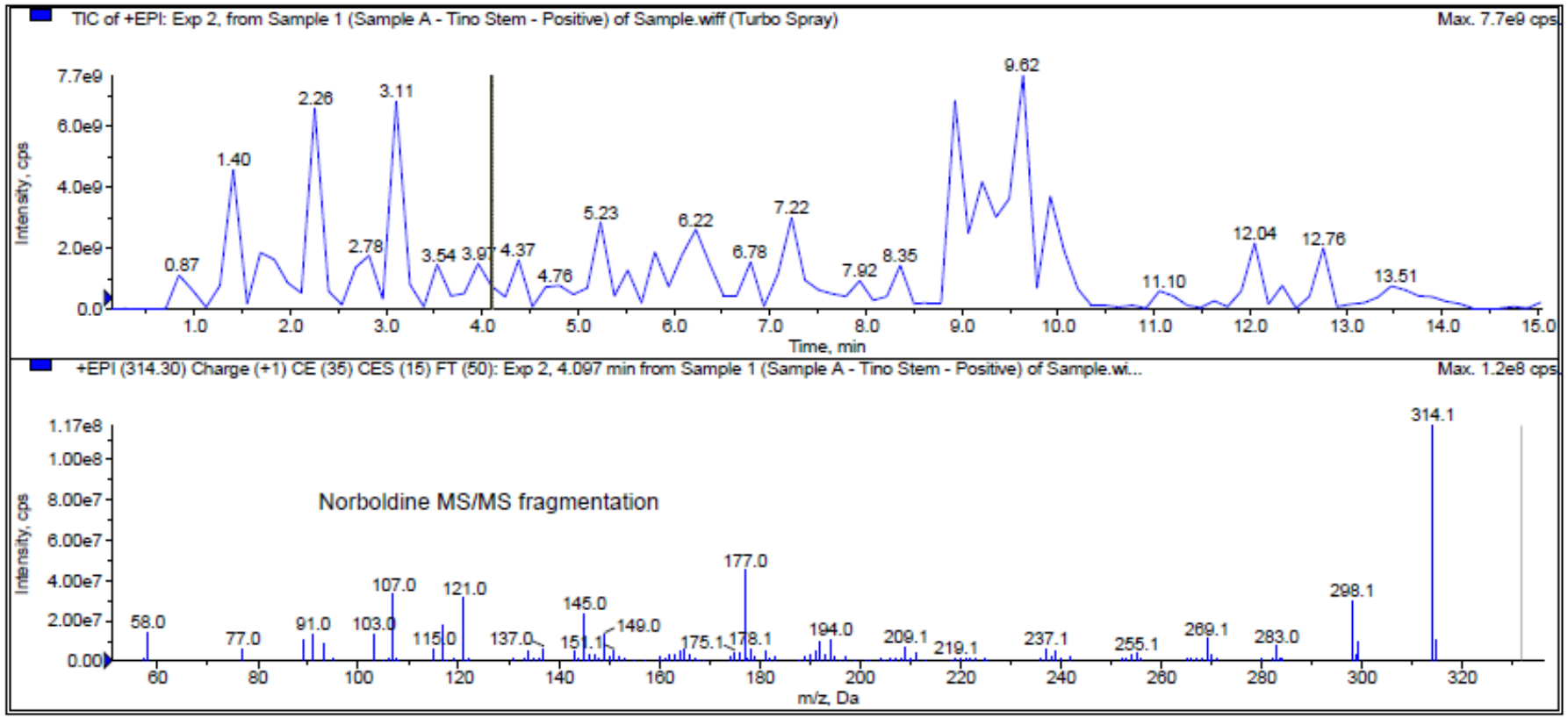

Figure 4.47: Full positive chromatogram of *Tinospora crispa* (stems) with Norboldine MS/MS fragmentations (+EPI: 314.30)

Figure above shows full scan (positive mode) of *Tinospora crispa* (stems) with Norboldine MS/MS fragmentation. The retention time for Norboldine was 4.097 minute. The mass spectrum fragmentation of Norboldine displayed the following major peaks at m/z: 314.1, 298.1, 177.0, 145.0, 121.0 and 107.0. The count per second (cps) of the tallest peak was 1.2 e8 cps.

4.7.8 Chromatogram (Negative Mode) of *Tinospora crispa* (stems)

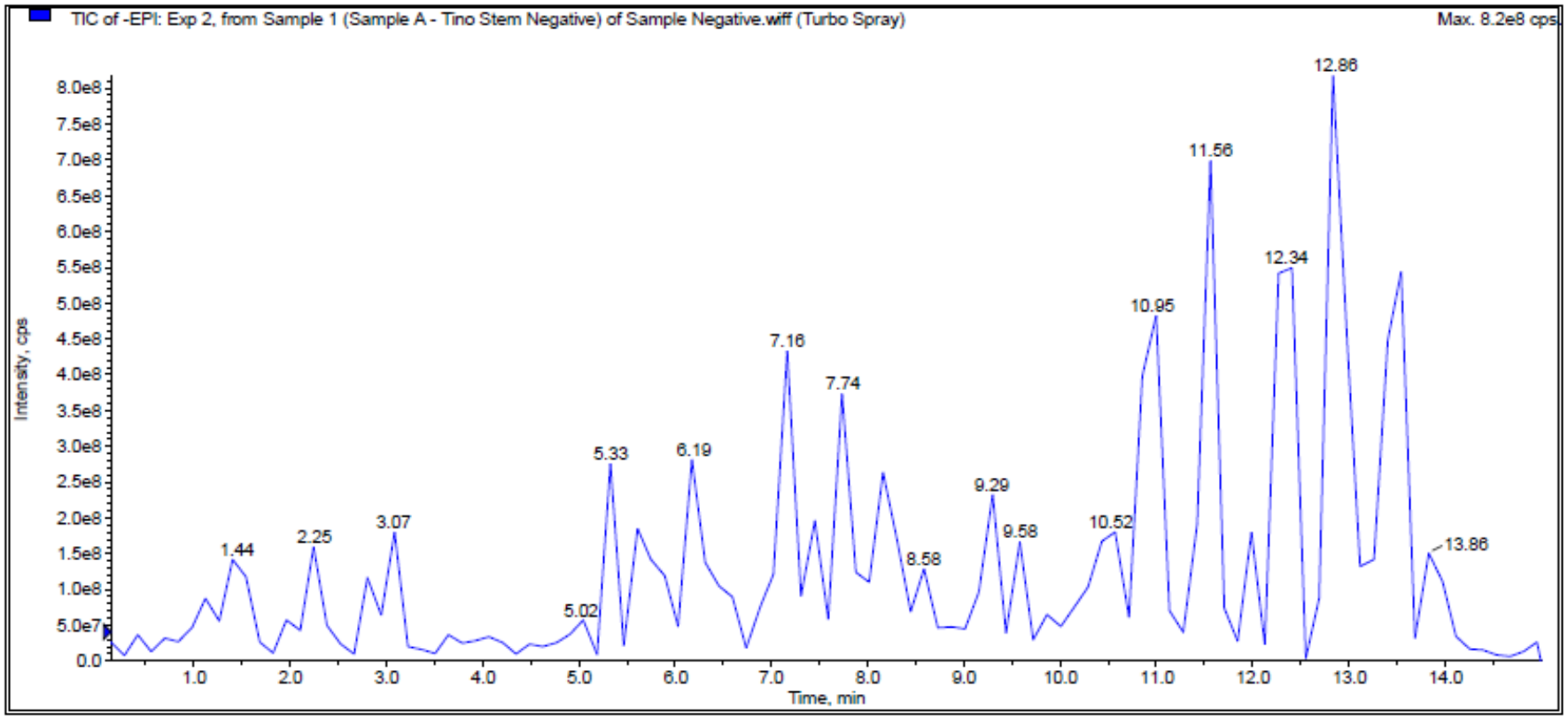

Figure 4.48: Full Negative Chromatogram *Tinospora crispa* (stems)

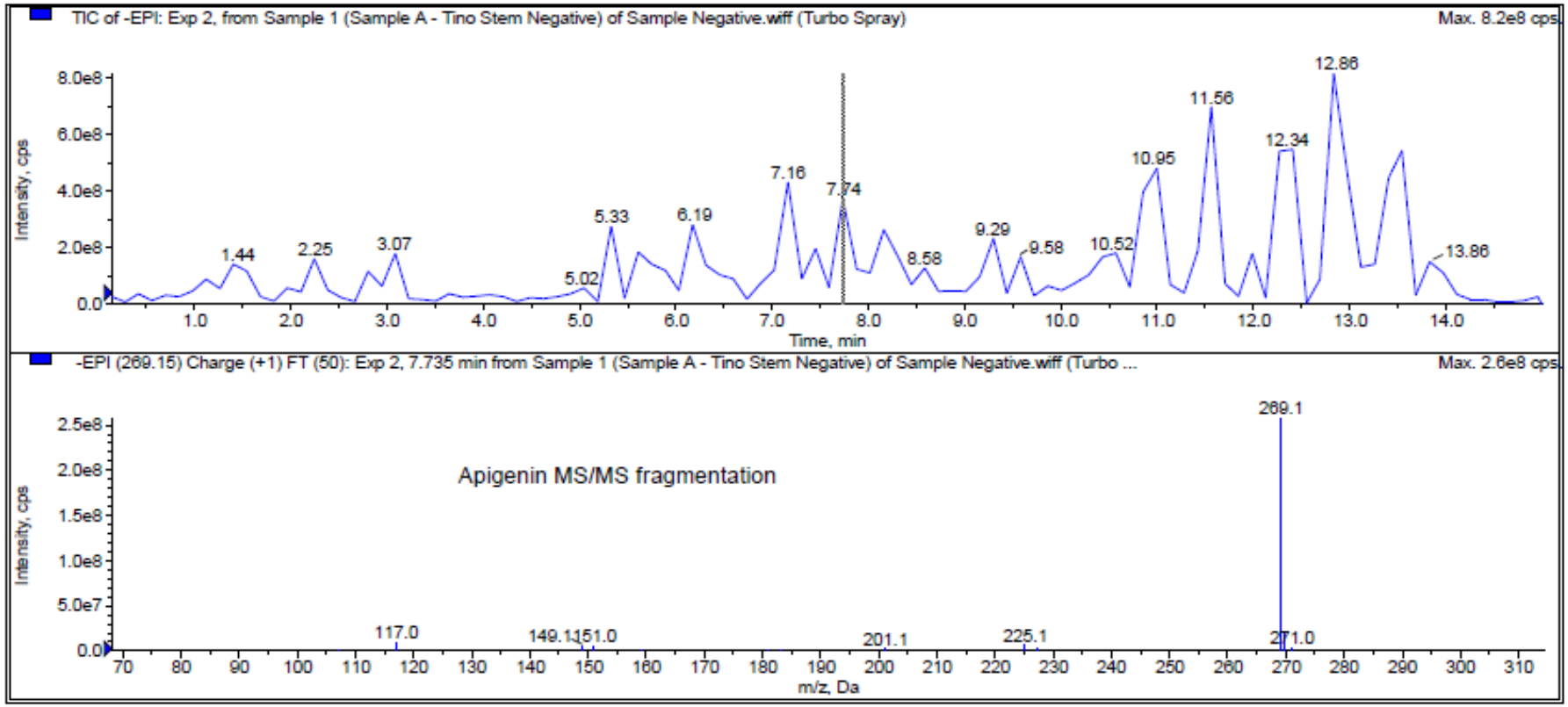

Figure 4.49: Full negative chromatogram of *Tinospora crispa* (stems) with Apigenin MS/MS fragmentations (-EPI: 269.15)

Figure above shows full scan (negative mode) of *Tinospora crispa* (stems) with Apigenin MS/MS fragmentation. The retention time for Apigenin was 7.735 minute. The mass spectrum fragmentation of Apigenin displayed the following peaks at m/z: 269.1, 225.1, 201.1, 151.0, 149.1 and 117.0. The count per second (cps) of the tallest peak was 2.6 e8 cps.

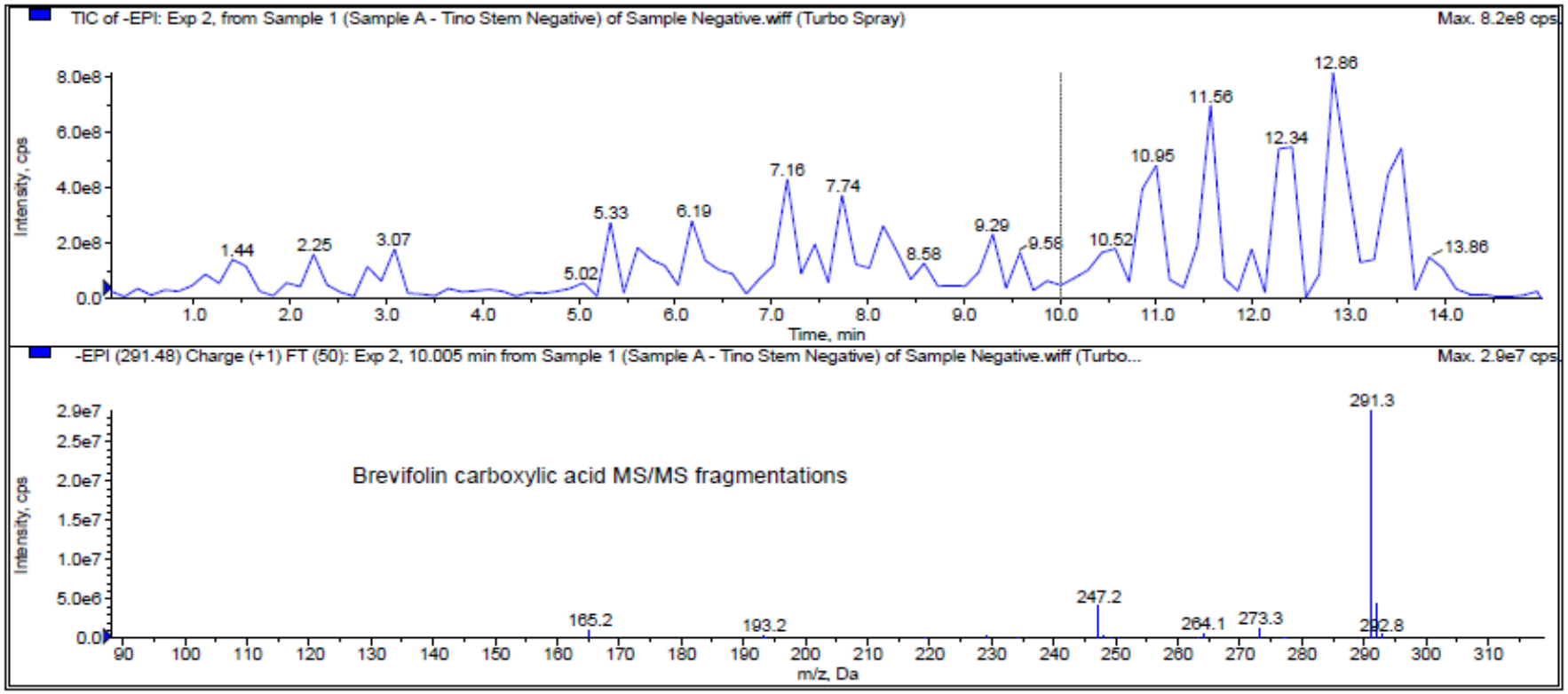

Figure 4.50: Full negative chromatogram of *Tinospora crispa* (stems) with Brevifolin Carboxylic Acid MS/MS fragmentations (-EPI: 291.48)

Figure above shows full scan (negative mode) of *Tinospora crispa* (stems) with Brevifolin Carboxylic Acid MS/MS fragmentation. The retention time for Brevifolin Carboxylic Acid was 10.005 minute. The mass spectrum fragmentation of Brevifolin Carboxylic Acid displayed the following peaks at m/z: 291.3, 273.3, 264.1, 247.2, 193.2 and 165.2. The count per second (cps) of the tallest peak was 2.9 e7 cps.

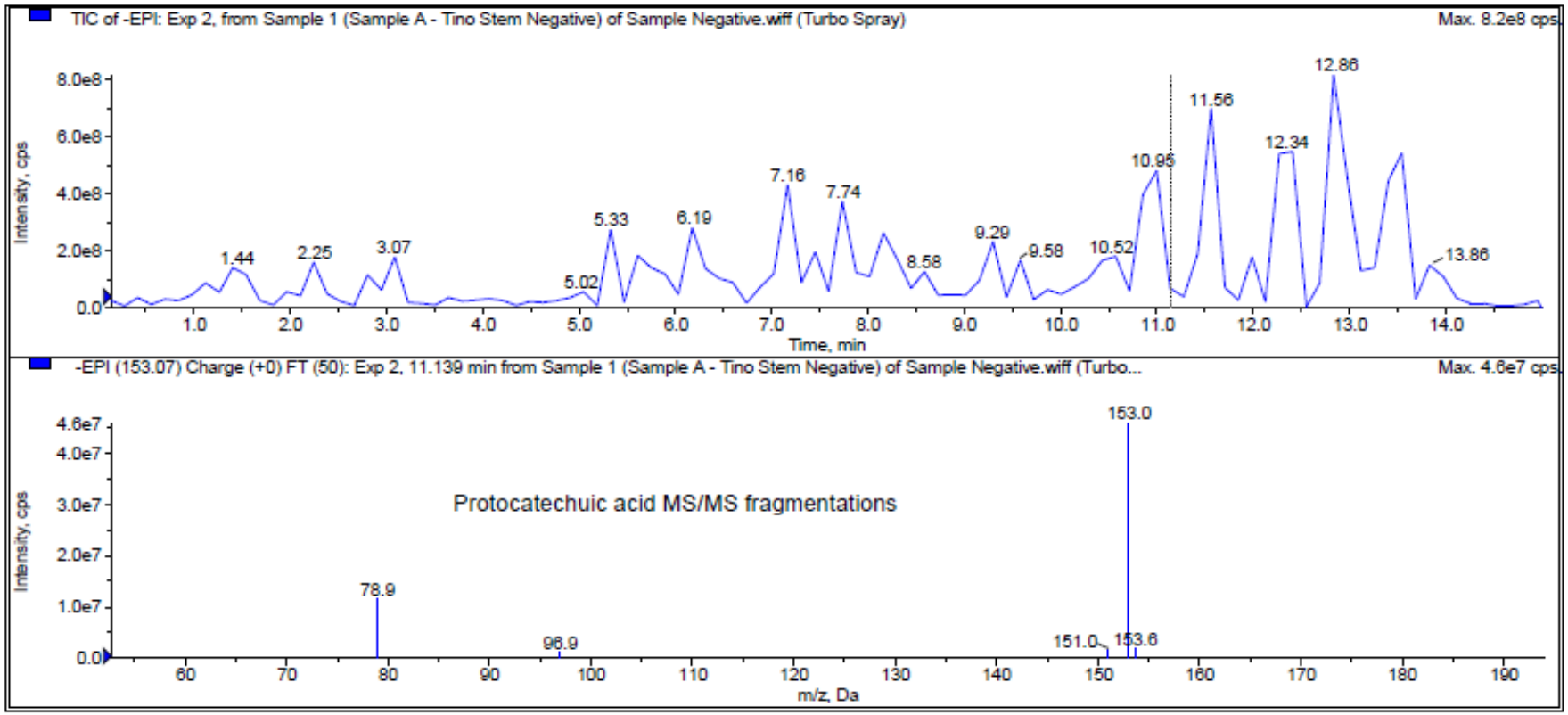

Figure 4.51: Full negative chromatogram of *Tinospora crispa* (stems) with Protocatechuic Acid MS/MS fragmentations (-EPI: 153.07)

Figure above shows full scan (negative mode) of *Tinospora crispa* (stems) with Protocatechuic Acid MS/MS fragmentation. The retention time for Protocatechuic Acid was 11.139 minute. The mass spectrum fragmentation of Protocatechuic Acid displayed the following peaks at m/z: 153.0, 151.0, 96.9 and 78.9. The count per second (cps) of the tallest peak was 4.6 e7 cps.