CHAPTER V

RESULTS AND DISSCUSION

Part I. Identification of standard (+)-tetrandrine

This compound was obtained as white crystal (in methanol). It is soluble in methanol, ethanol, chloroform, dichloromethane, and water.

$[\alpha]_D^{24}$: + 272° (c, 1.00 in $CHCl_3$)

^{1}H-NMR : δ_H ppm, 400 MHz, in $CDCl_3$, Table 6 and Figure 10.

^{13}C-NMR : δ_C ppm, 100 MHz, in $CDCl_3$, Table 6 and Figure 11.

Proton and Carbon-13 nuclear magnetic resonance (^{1}H and ^{13}C-NMR) chemical shifts of this compound were similar to ^{1}H and ^{13}C-NMR of (+)-tetrandrine (Table 2).Tetrandrine shown positive $[\alpha]_D^{24}$ of 274 (c, 2.16 in $CHCl_3$) (Southon *et al.*, 1989) similarly to positive $[\alpha]_D^{24}$ of 272 (c, 1.00 in $CHCl_3$) showed by this compound. These data confirmed this compound as (+)-tetrandrine.

Table 6. ^{1}H-NMR spectral and ^{13}C-NMR spectral of standard (+)-tetrandrine

Proton	Chemical shift (ppm)	Carbon	Chemical shift (ppm)
H-1	3.733(d, overlapped signal)	C-1	61.335
NMe	2.328(s)	NMe	42.214
H-3α	2.896(m)	C-3	43.996
H-3ß	3.512(m)	C-4	21.904
H-4α	2.401(m)	C-4a	127.924
H-4ß	2.924(m)	C-5	105.626
H-5	6.296 (s broad)	C-6	151.305
6-OMe	3.721(s)	C-7	137.770
7-OMe	3.183(s)	C-8	148.418
H-8	-	C-8a	122.900
H-α(b)	2.513(d, 12.4)	C-α	41.897
H-α(a)	2.694(t, partly overlapped signal)	C-9	134.933
H-10	6.547(d, 1.68)	C-10	116.125
H-11	-	C-11	149.294
12-OMe	3.926(s)	C-12	146.927
H-13	6.866(d,8.0)	C-13	111.426
H-14	6.866(dd,8.0,1.16)	C-14	122.629
H-1'	3.866(dd,5.6,11.2)	C-1'	63.851
N'Me	2.613(s)	N'Me	42.593
H-3'ß	2.866(m)	C-3'	45.204
H-3'α	3.429(m)	C-4'	25.192
H-4'ß	2.715(m)	C-4a'	128.039
H-4'α	2.947(m)	C-5'	112.623
H-5'	6.505 (s broad)	C-6'	148.525
6'-OMe	3.369(s)	C-7'	143.677
H-8'	5.993(s)	C-8'	120.105
H-α'	2.238(t,12.0, 11.6)	C-8a'	128.077
H-α'	3.236(dd, 12.4,5.6)	C-α'	38.223
H-10'	6.309(dd,8.0,2.0)	C-9'	135.212
H-11'	6.807 (dd,8.2, 2.4,)	C-10'	132.593
H-13'	7.138(dd, 2.4,8.0)	C-11'	121.922
H-14'	7.341(dd, 2.0,8.0)	C-12'	153.637
		C-13'	121.853
		C-14'	130.077
		6-OMe	55.730
		7-OMe	60.234
		12-OMe	56.070
		6'-OMe	55.776

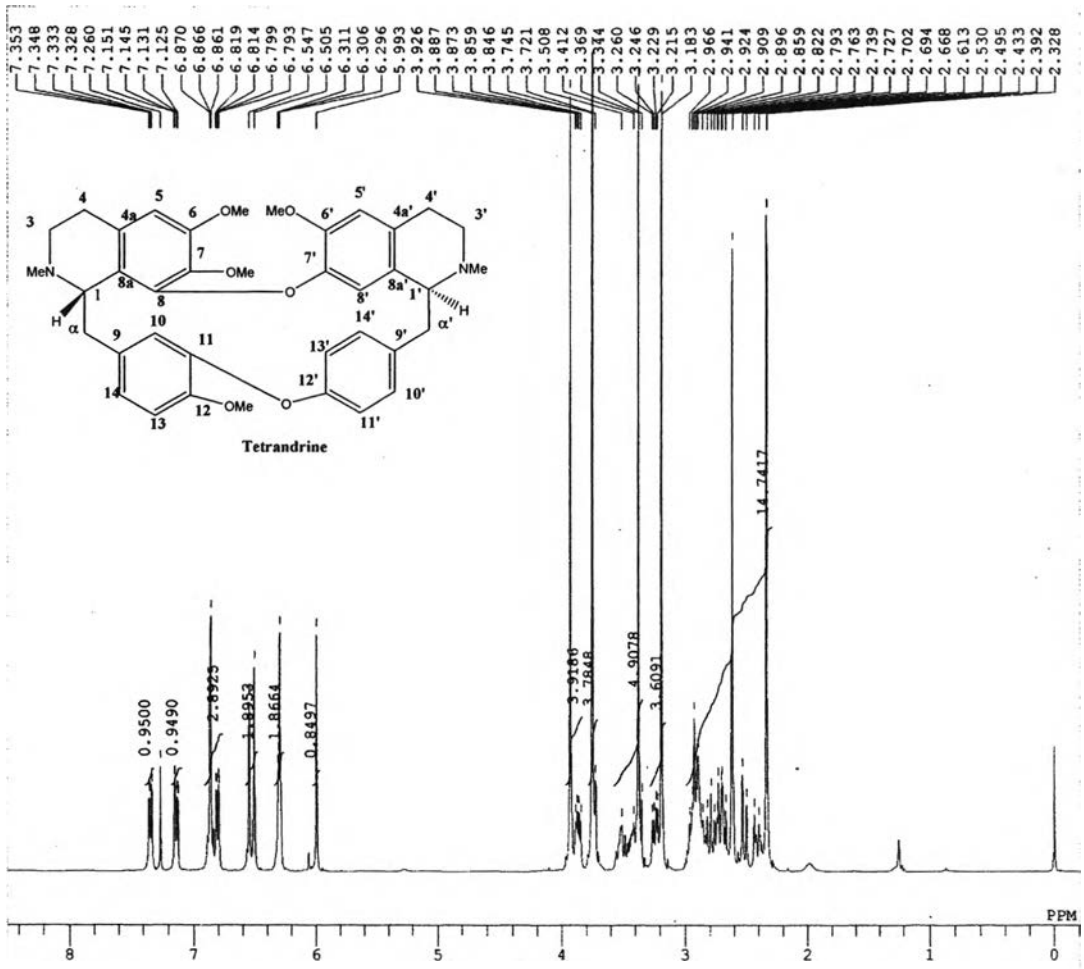


Figure 10. The 400 MHz ^{1}H NMR spectrum of standard (+)-tetrandrine in $CDCl_3$

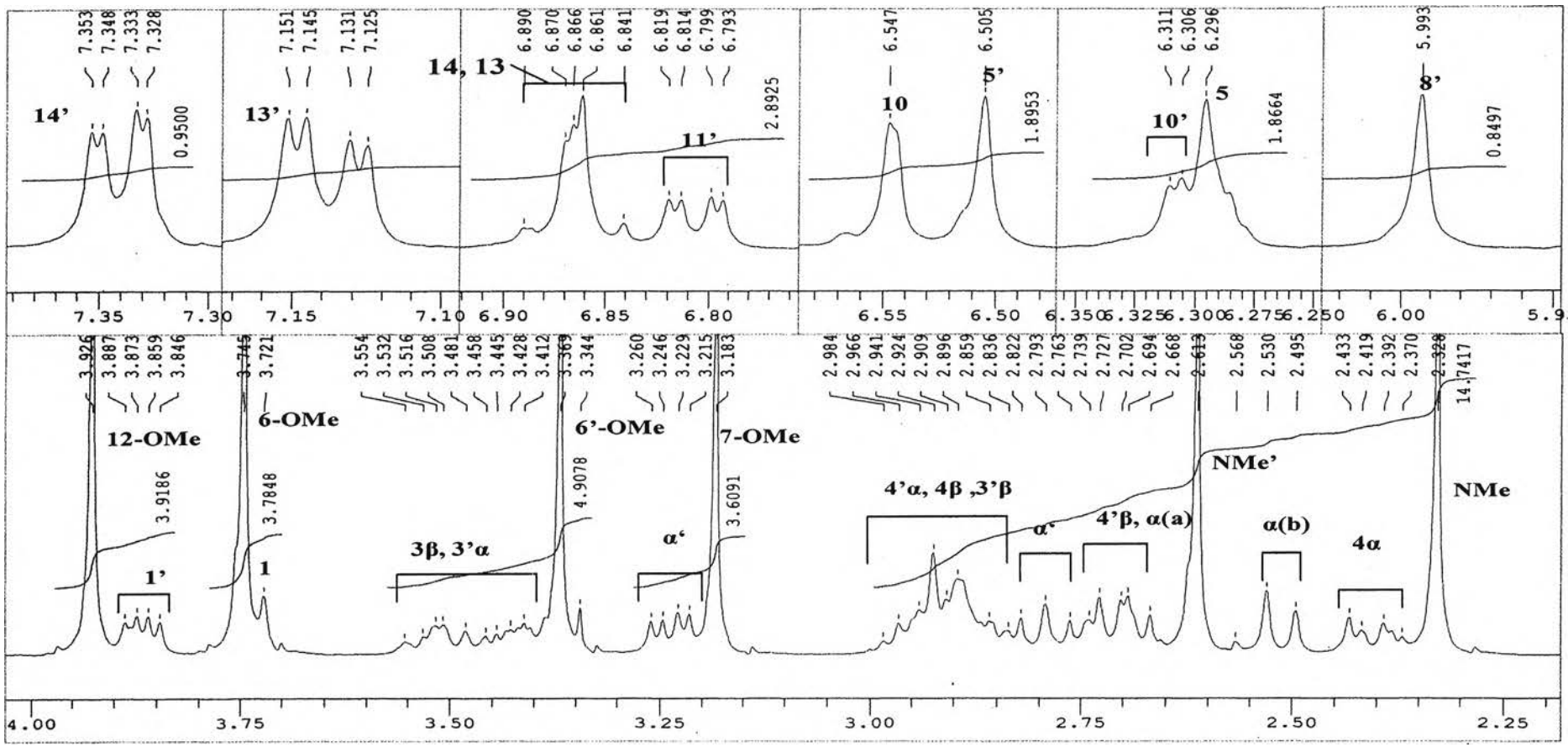


Figure 10. The 400 MHz ^{1}H NMR spectrum of standard (+)-tetrandrine in $CDCl_3$ (Continued).

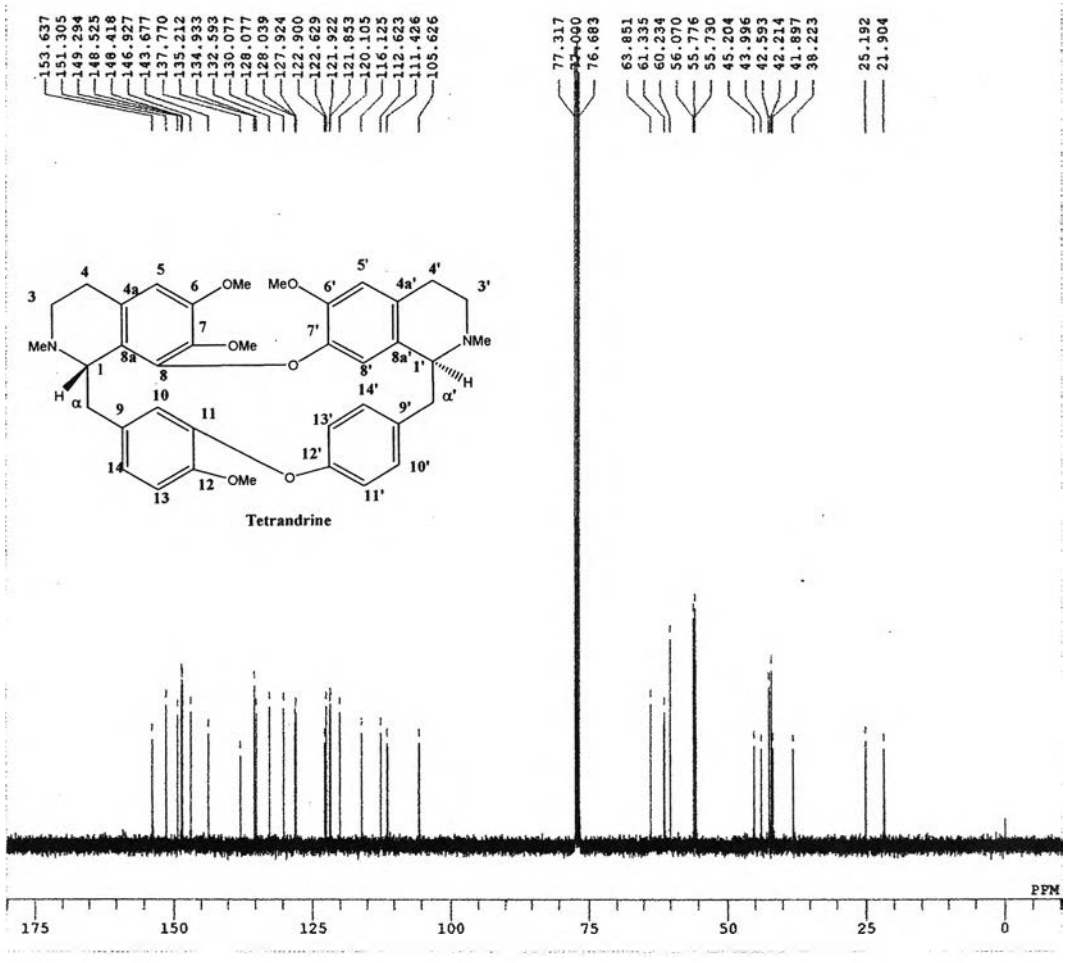


Figure 11. The 100 MHz ^{13}C NMR spectrum of standard (+)-tetrandrine in $CDCl_3$.

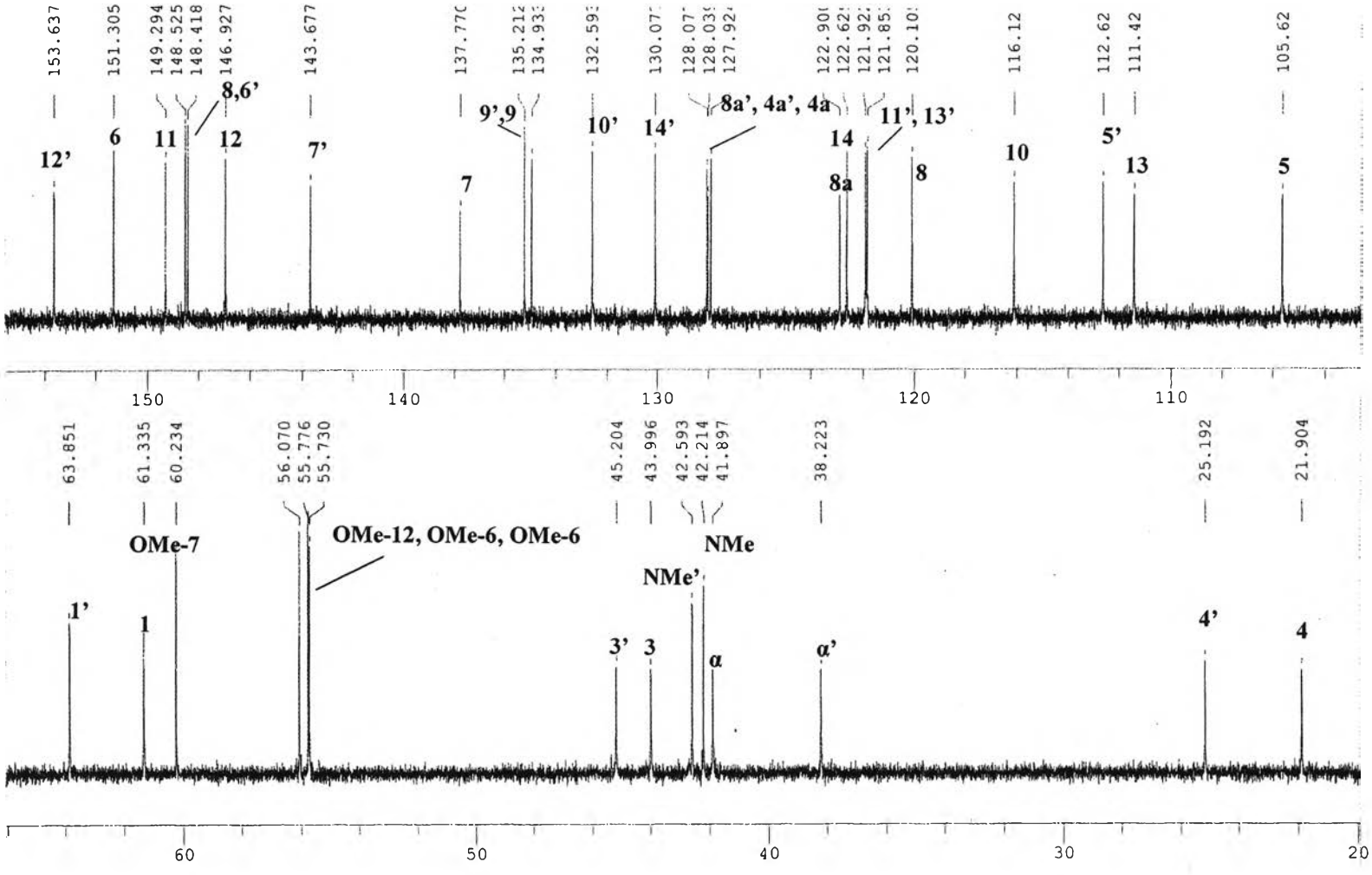


Figure 11. The 100 MHz ^{13} C NMR spectrum of standard (+)-tetrandrine in $CDCl_3$ (Continued).

Part II. Macroscopic and microscopic characterizations of *Cyclea barbata* roots

Macroscopical

The root is longitudinally split into subcylindrical pieces, 2.0 to 7.0 cm. in length and 1.0 to 3.0 cm in diameter. Externally, yellow to brown, where occasionally longitudinal fissures or irrequlary wrinkled. The transversely cut surface show prominent annual rings and distinctly radiate concentric zone of projecting medullar rays. The bark up to 1 mm in thickness (Figure 12).

Microscopical

The powder possessed the diagnostic microscopical characters as follows (Figure13-14).

1. The fragment of brown cork composed of two or three layers of thin-walled cells which are polygonal in surface.

2. The abundant thin-walled parenchyma composed of elongated rectangular or irregularly ovoid containing starch granules.

3. The numerous lignified yellow sclereids, which occur singly or more frequently in small group. Individual cells show considerable variation in size and shape, the cells are rectangular, irregularly ovoid or small polygonal shape. The most cells are moderately thickened walls with numerous pits; some is the thinned wall cell containing starch granules; occasional cells have stratified, pitted, thickened wall with a narrow lumen.

4. The lignified fibers, which occasionally occur singly and mostly found in small group or found associated with vessels; thin-walled and lignified cells with conspicuous simple pits.

5. The lignified vessels, frequently found fragmented of large simple and bordered pitted vessels and also found singly or in small groups vessels with bordered pits. Few vessels also occur with reticulate thickening.

6. The abundant starch granules, which are mostly simple, spherical to ovoid with a circular, slit-shaped or stellate hilum, a number of compound granules also occur with two or three components.

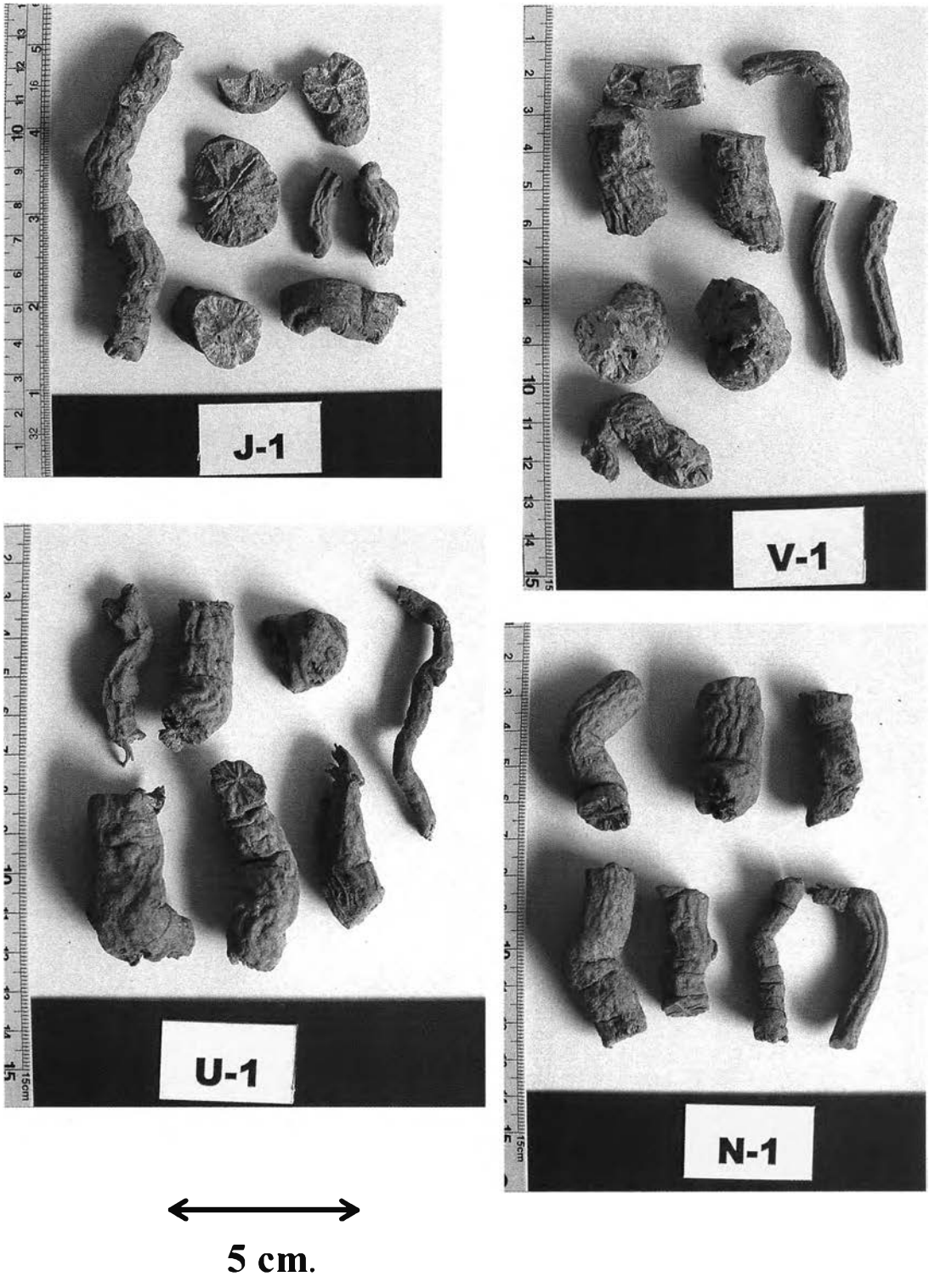


Figure 12. Macroscopic character of *Cyclea barbata* (Wall.)Miers roots.

Samples of "Krung Kha Mao"; Bangkok(Jao-krom-per); J-1, Bangkok(Vechapong); V-1, Ubonrachathanee; U-1, Nakornrachasima; N-1.

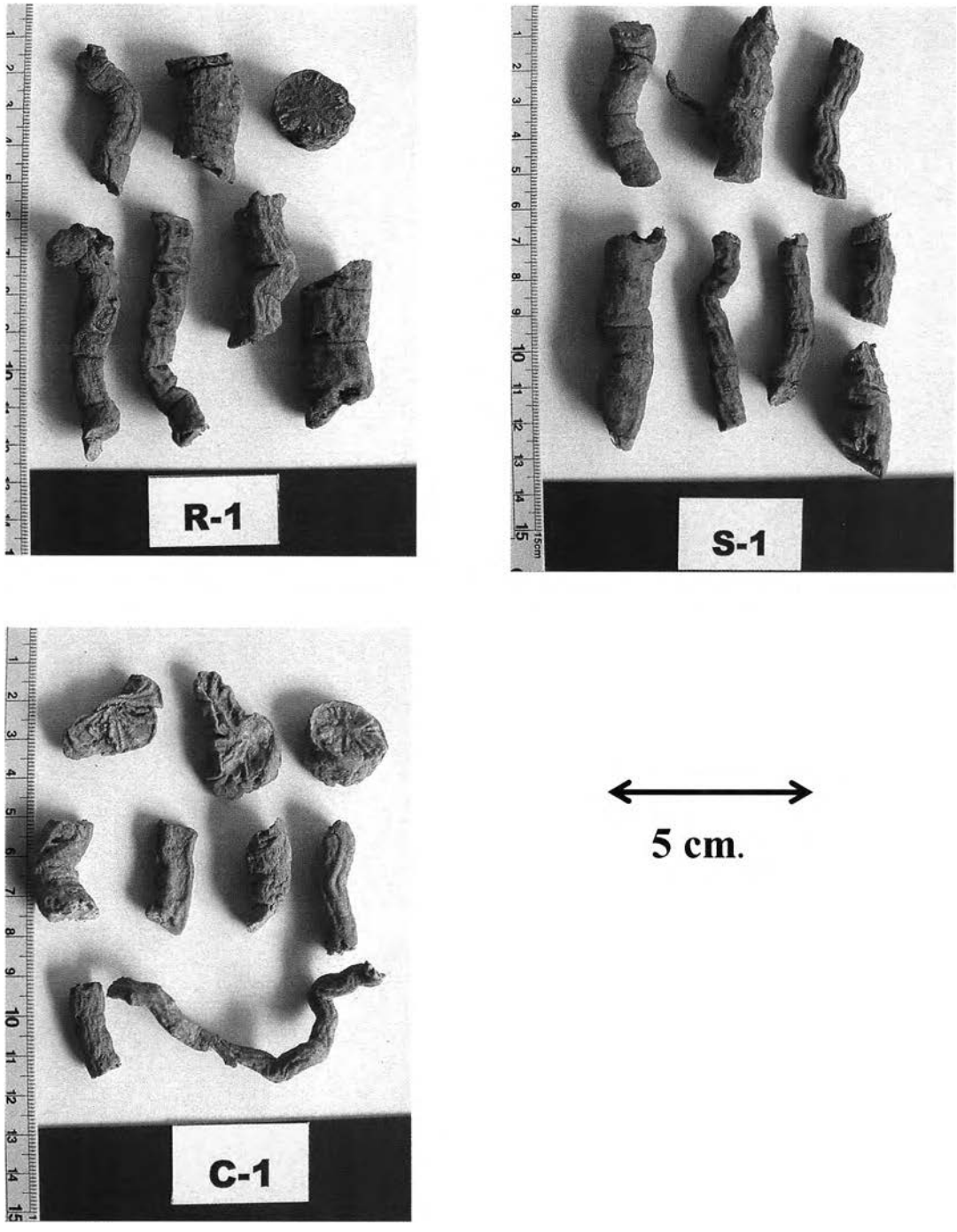


Figure 12. Macroscopic character of *Cyclea barbata* (Wall.)Miers roots (Continued)

Samples of "Krung Kha Mao";Songkla; S-1, Surachthanee; R-1, and Chiang-Mai; C-1.

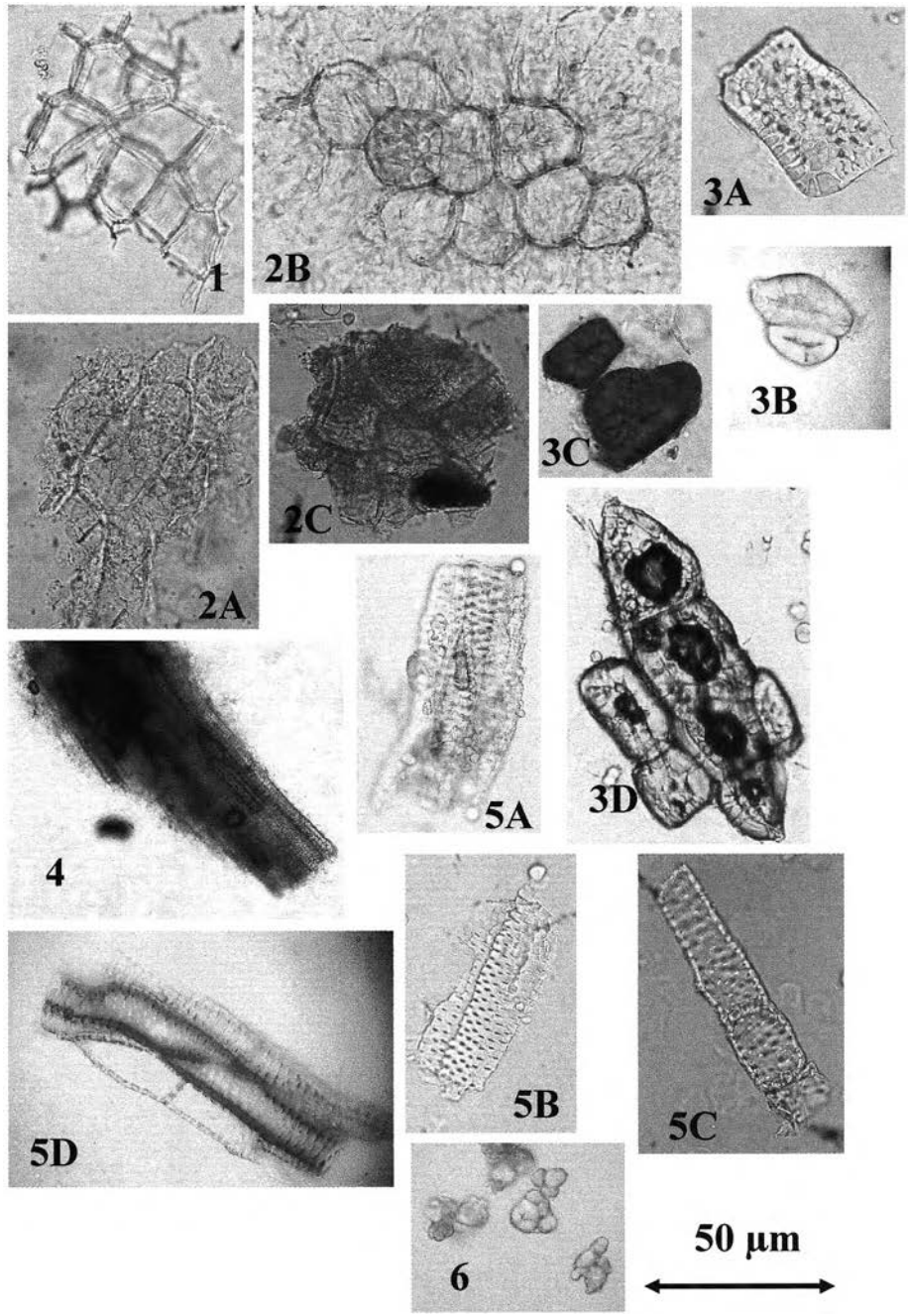


Figure 13. Microscopic character of *Cyclea barbata* (Wall.)Miers roots

Cork cells (1), Parenchyma cells (2A-2B), Parenchyma containimg starch granules (2C), Thinned wall sclereids containing starch granules (3A), Yellow thicken wall sclereids (3B), Lignified sclereids detected with phlroglucinol solution and hydrochloric acid (3C),Group of yellow thicken wall sclereids (3D), Group of lignified fibers (4), Bordered pits vessels (5A-5C), Lignified vessels detected with phlroglucinol solution and hydrochloric acid (5D) and Starch granules (6)

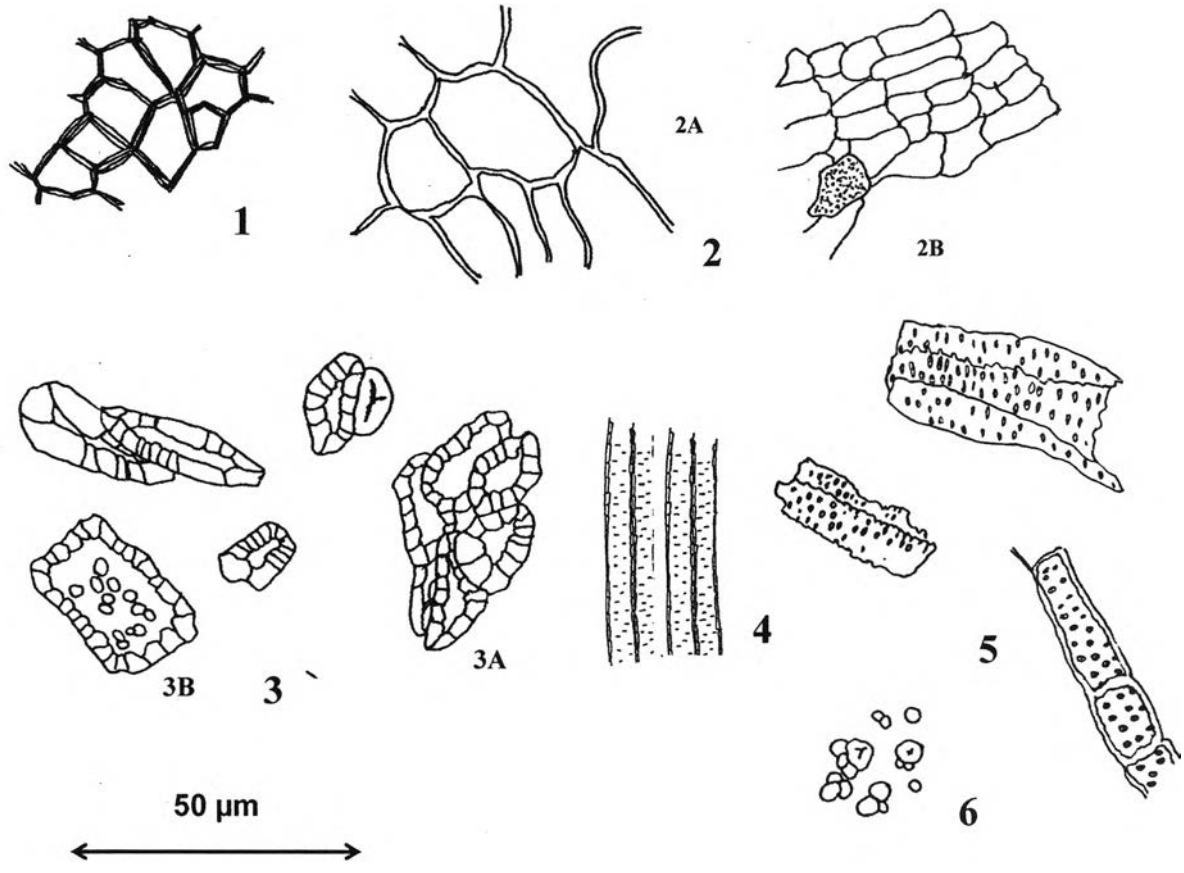


Figure 14. Microscopic character of *Cyclea barbata* (Wall.)Miers roots

Cork cells (1), Parenchyma cells (2A-2B),Lignified Thicken wall sclereids (3A), Thinned wall sclereids containing starch granules (3B), Lignified fibers(4), Lignified vessels and Bordered pits vessels (5), and Starch granules (6)

Part III. Thin-layer chromatographic pattern of roots extract

The results of one-dimensional TLC of crude extract are shown in figure 15-19.

System 1: Dichloromethane: Hexane: Methanol: Ammonia (5:4:1:0.3)

System 2: Ethylacetate: Hexane: Methanol: Ammonia (4:4:1:0.3)

System 3: Toluene: Ethylacetate: Methanol: Ammonia (10:10:5:0.3)

System 4: Chloroform: Methanol: Ammonia (5: 1: 0.3)

Labelled on TLC plate,

T = 0.02% Tetrandrine in methanol

S = Crud drug sample extract

A = Authentic sample (*Cyclea barbata* roots extract)

J = Sample from Bangkok (Jao-krom-per drugstore)

V = Sample from Bangkok (Vechapong drugstroge)

C = Sample from Chiang-Mai

R = Sample from Surachthanee

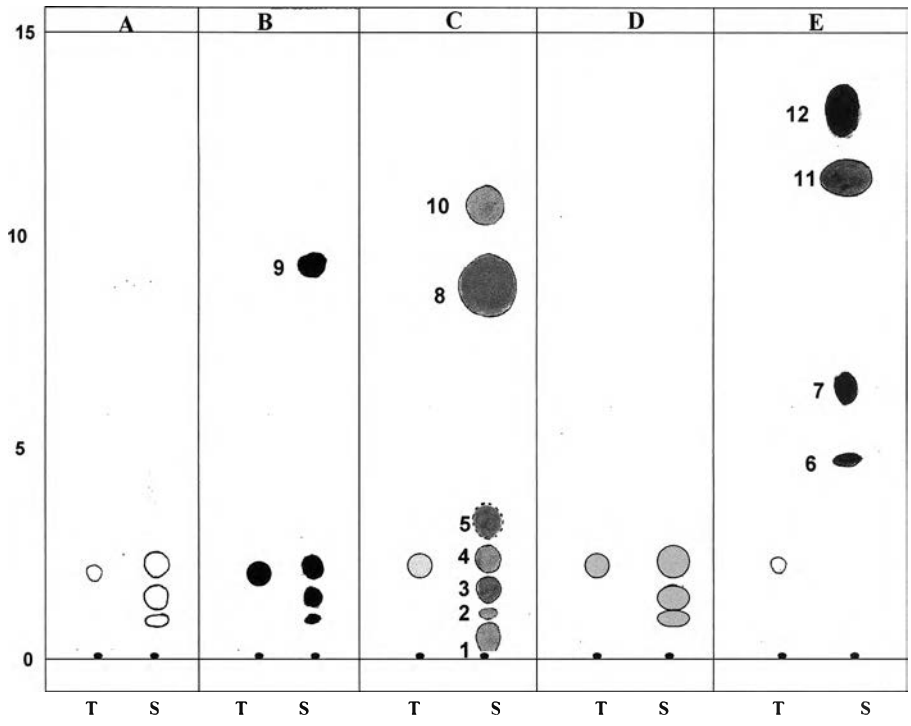
N = Sample from Nakornrachasima

S = Sample from Songkla

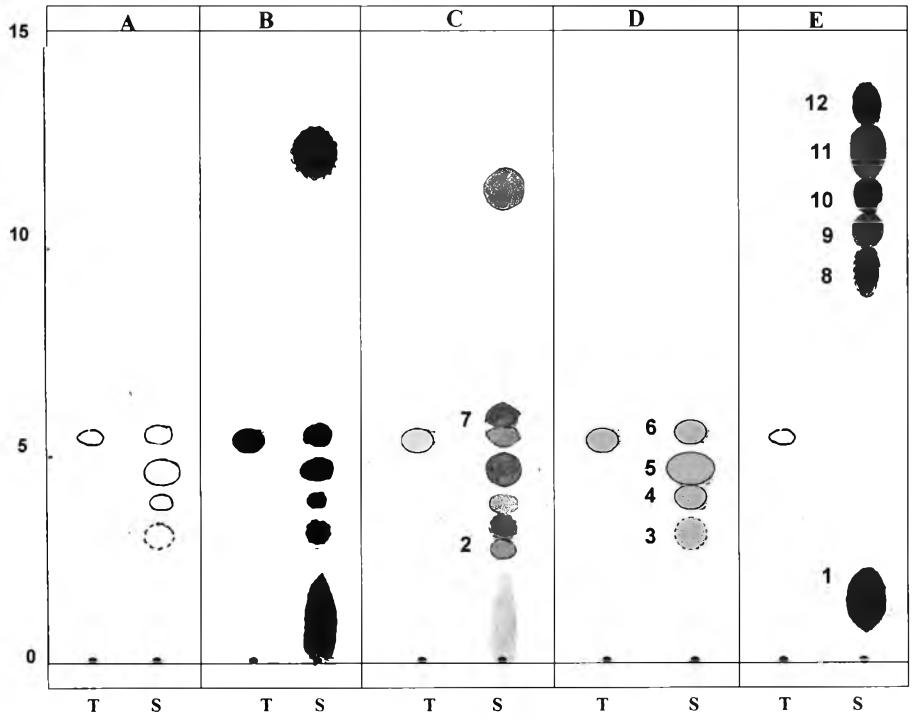
U = Sample from Ubonrachathanee

_____ = spot was shown in every sample.

- - - - - - - = spot was shown in some sample.

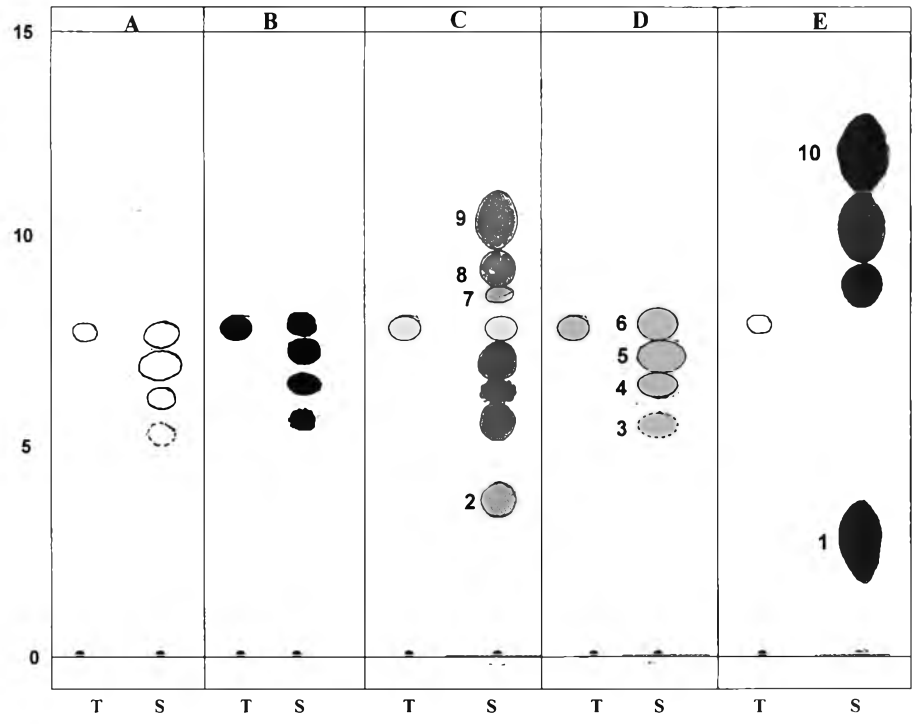


System 1: Dichloromethane: Hexane: Methanol: Ammonia (5:4:1:0.3)

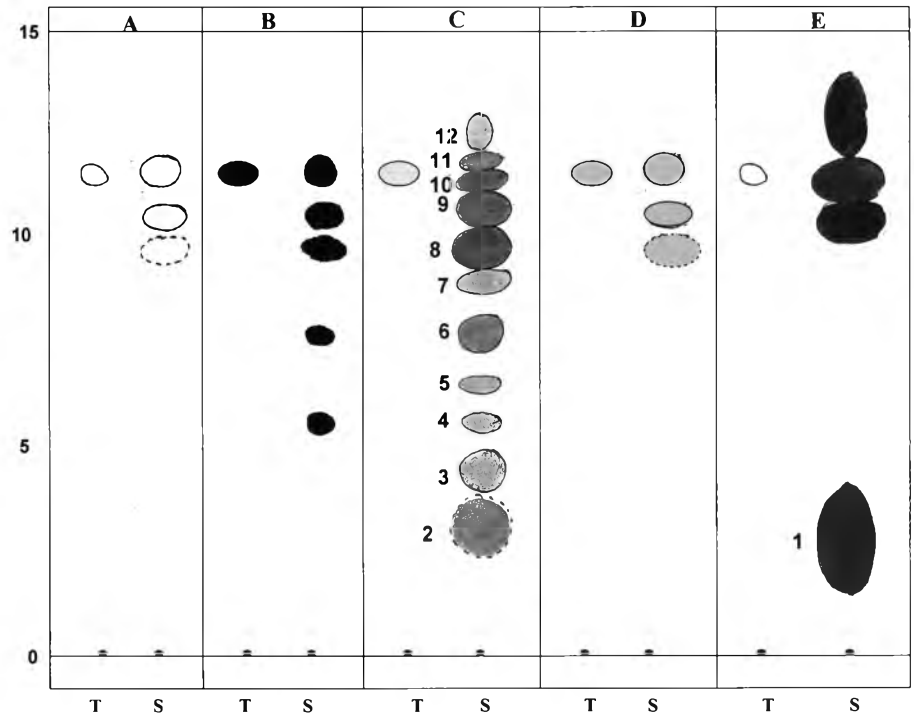


System 2: Ethylacetate: Hexane: Methanol: Ammonia (4:4:1:0.3)

Figure 15. One-dimensional thin-layer chromatogram of *Cyclea barbata* roots extract. Visible in daylight (A), quenching under UV 254 nm (B), fluorescence under UV 365 nm (C), detection with Dragendroff's reagent (D), Detection with Anisaldehyde-sulphuric acid reagent (E).

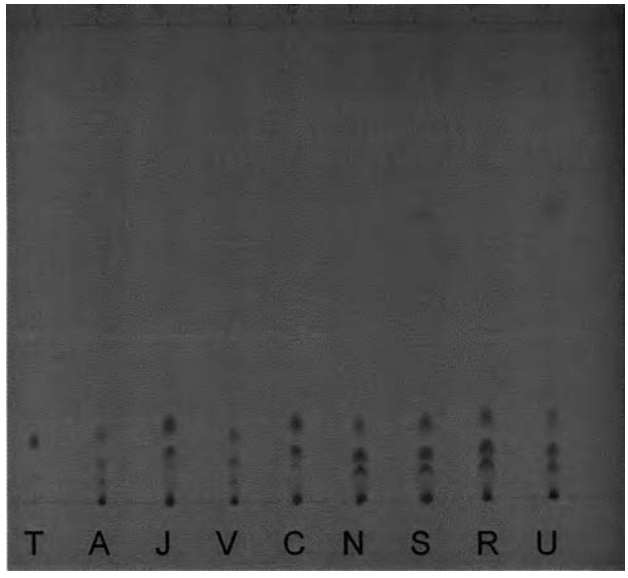


System 3: Toluene: Ethylacetate: Methanol: Ammonia (10:10:5:0.3)



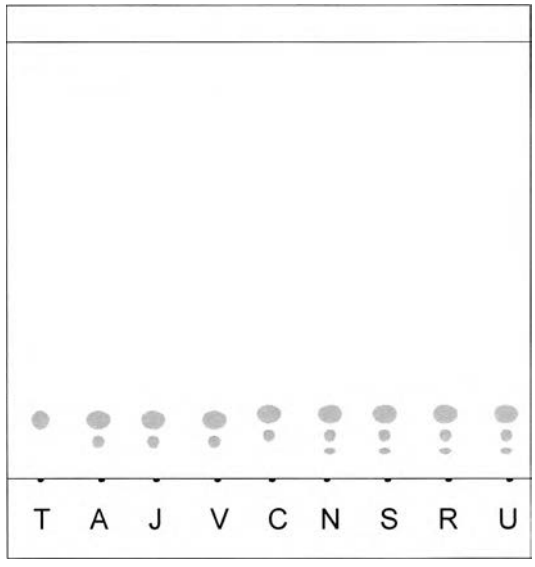
System 4: Chlorofrom: Methanol: Ammonia (5: 1: 0.3)

Figure 15. One-dimensional thin-layer chromatogram of *Cyclea barbata* roots extracts (Continued)

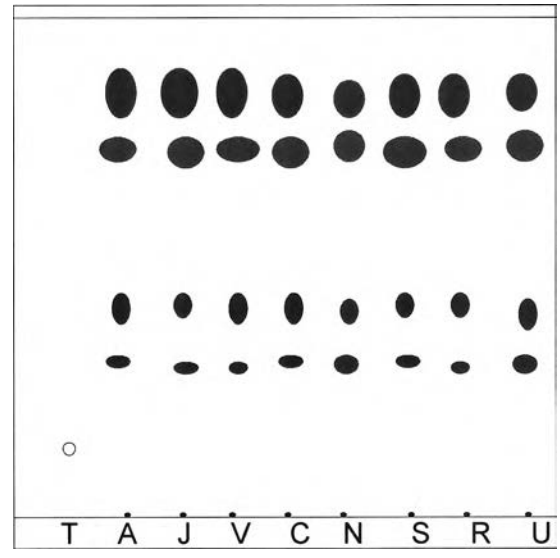


Fluorescence under UV 254 nm

Fluorescence under UV 365 nm



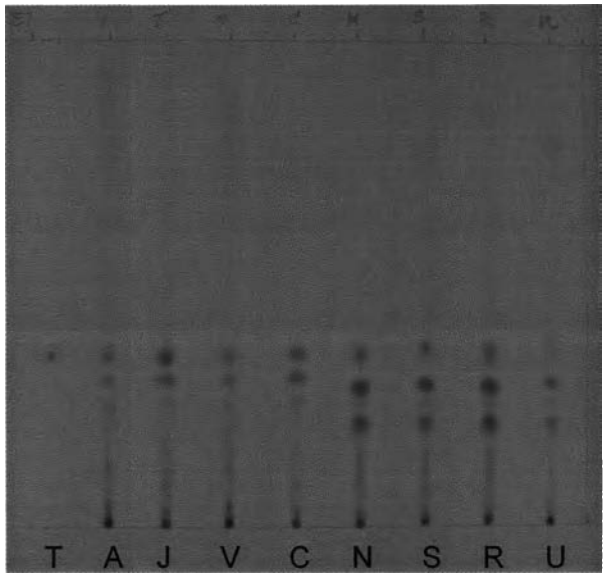
Detection with Dragendorff's reagent



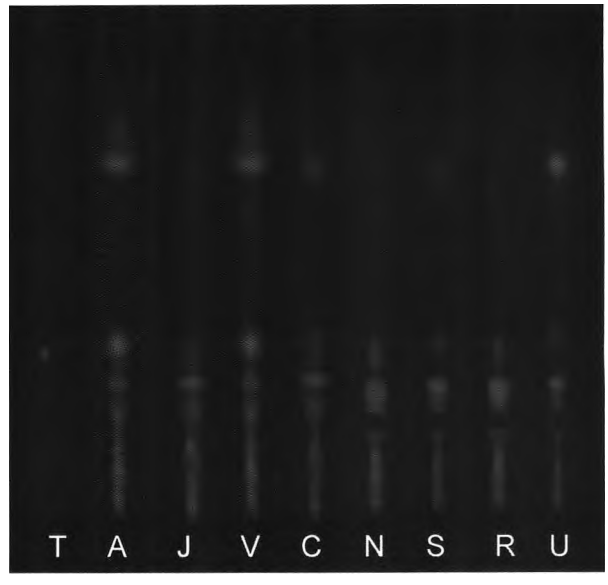
Detection with Anisaldehyde acid reagent

Figure 16. One-dimensional thin-layer chromatogram of *Cyclea barbata* roots extract

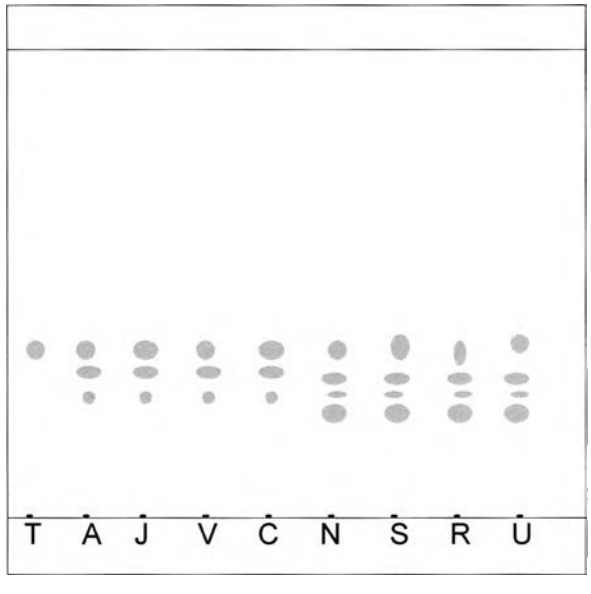
[System 1: Dichloromethane: Hexane: Methanol: Ammonia (5:4:1: 0.3)]



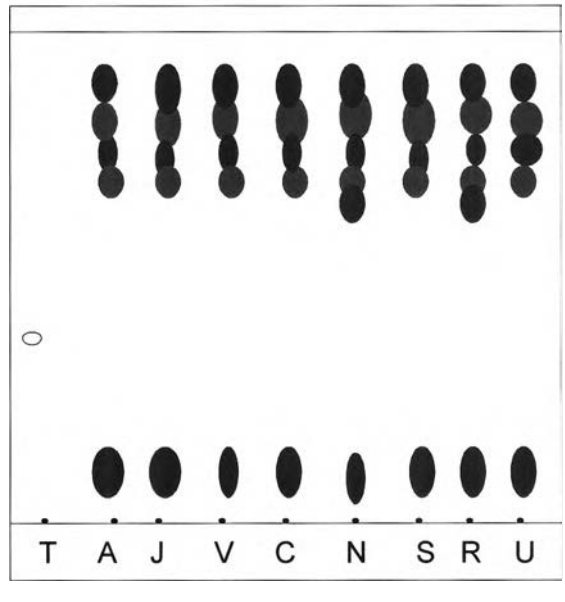
Fluorescence under UV 254 nm



Fluorescence under UV 365 nm

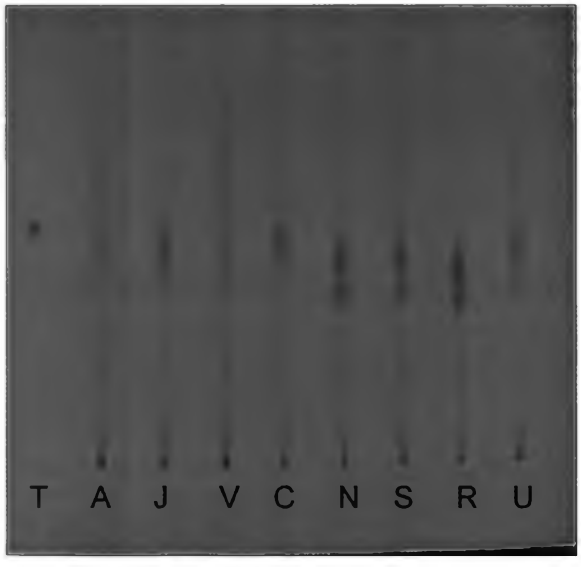


Detection with Dragendorff's reagent



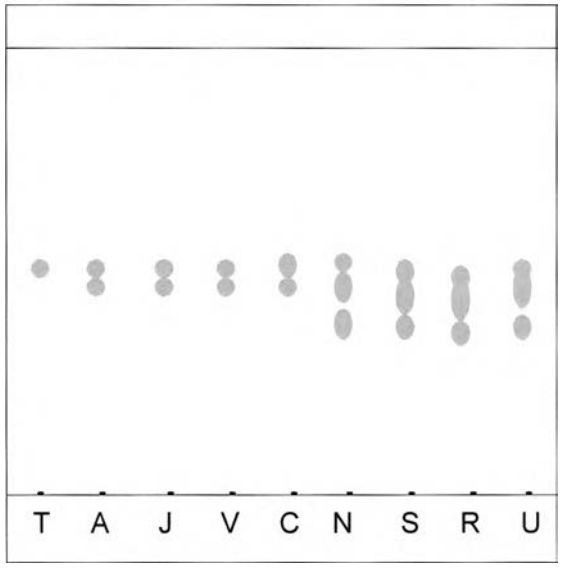
Detection with Anisaldehyde acid reagent

Figure 17. One-dimensional thin-layer chromatogram of *Cyclea barbata* roots extract [System 2: Ethylacetate: Hexane: Methanol: Ammonia (4:4:1:0.3)]

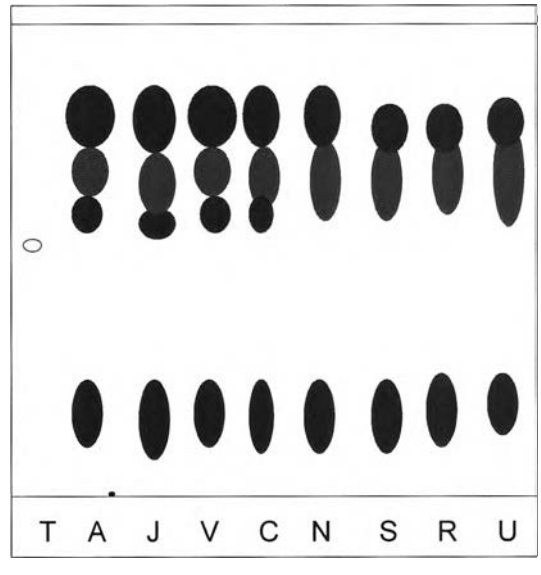


Fluorescence under UV 254 nm

Fluorescence under UV 365 nm



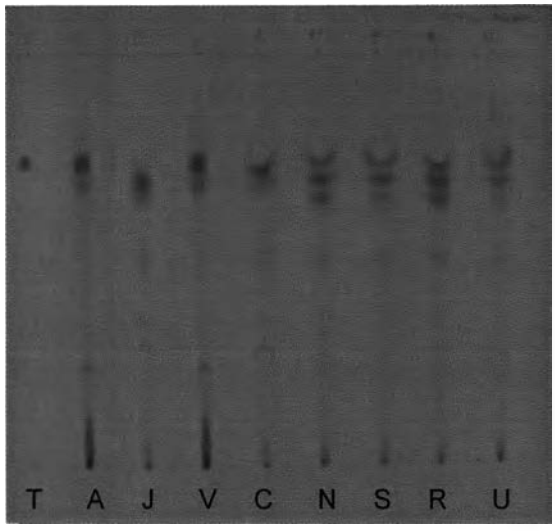
Detection with Dragendorff's reagent



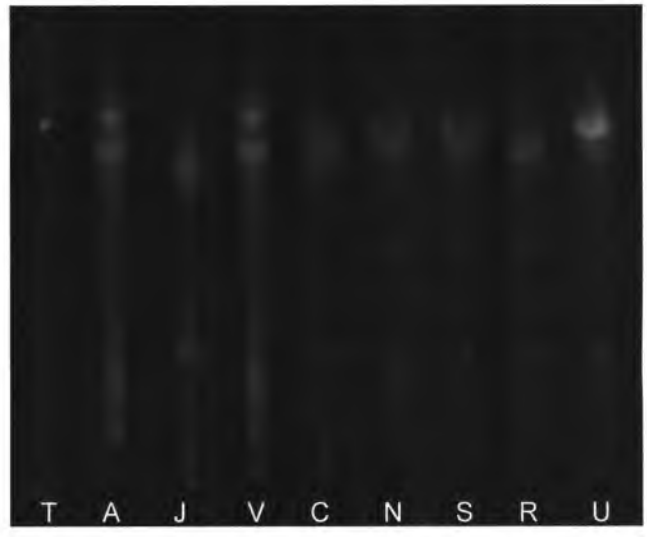
Detection with Anisaldehyde acid reagent

Figure 18.One-dimensional thin-layer chromatogram of *Cyclea barbata* roots extract

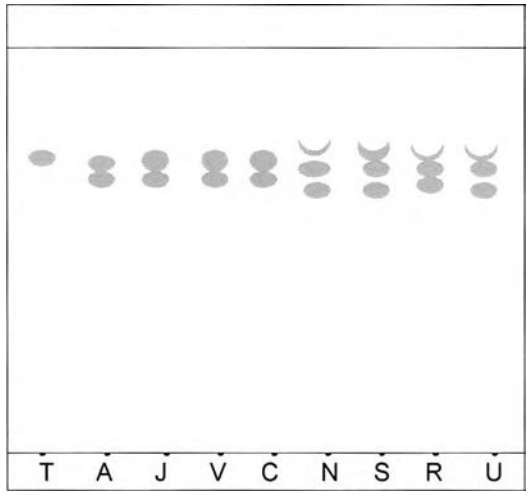
[System 3: Toluene: Ethylacetate: Methanol: Ammonia (10:10:5:0.3)]



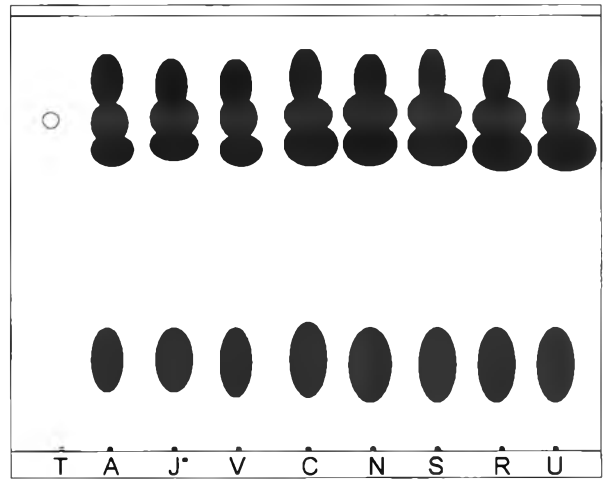
Fluorescence under UV 254 nm



Fluorescence under UV 365 nm



Detection with Dragendorff's reagent



Detection with Anisaldehyde acid reagent

Figure 19. One-dimensional thin-layer chromatogram of *Cyclea barbata* roots extract [System 4: Chloroform: Methanol: Ammonium (10: 2: 0.3)]

Table 7. hR_f value of one-dimensional TLC of *Cyclea barbata* roots extract

[Solvent system- Dichloromethane: Hexane: Methanol: Ammonia (5:4:1:0.3)]

Spot	hR_f	UV 254 nm	UV 365nm	Visible in daylight	Dragendorff 's reagent	Anisladehyde-sulfuric acid reagent
1	2-5	-	Light blue	-	-	-
2	6-7	Quenching	Light blue	Pale yellow	Orange	-
3	8-12	Quenching	Orange	Pale yellow	Orange	-
4*	13-17	Quenching	Blue-green	Pale yellow	Orange	-
5	17-23	-	Orange	-	-	-
6	29-31	-	-	-	-	Purple
7	39-43	-	-	-	-	Purple
8	49-59	-	Blue-green	-	-	-
9	59-62	Quenching	-	-	-	-
10	65-71	-	Light blue	-	-	-
11	70-76	-	-	-	-	Red wine
12	79-87	-	-	-	-	Purple

* (+)-Tetrandrine

Table 8. hR_f value of one-dimensional TLC of *Cyclea barbata* roots extract

[Solvent system- Ethylacetate: Hexane: Methanol: Ammonia (4:4:1:0.3)]

Spot	hR_f	UV 254 nm	UV 365 nm	Visible in daylight	Dragendorff 's reagent	Anisladehyde-sulfuric acid reagent
1	5-15	Quenching	Light blue	-	-	Purple
2	17-18	-	Light blue	-	-	-
3	18-24	Quenching	Orange	Pale yellow	Orange	-
4	24-29	Quenching	Light blue	Pale yellow	Orange	-
5	29-32	Quenching	Orange	Pale yellow	Orange	-
6*	32-37	Quenching	Light blue	Pale yellow	Orange	-
7	37-39	-	Orange	-	-	-
8	58-66	-	-	-	-	Purple
9	66-71	-	-	-	-	Purple
10	71-75	-	-	-	-	Purple
11	75-80	-	Light blue	-	-	Purple
12	80-85	Quenching	-	-	-	Red wine
13	85-92	Quenching	-	-	-	Purple

*(+)-Tetrandrine

Table 9. hR_f value of one-dimensional TLC of *Cyclea barbata* roots extract

[Solvent system- Toluene: Ethylacetate: Methanol: Ammonia (10:10:5:0.3)]

Spot	hR_f	UV 254 nm	UV 365 nm	Visible in daylight	Dragendorff 's reagent	Anisladehyde-sulfuric acid reagent
1	11-24	-	-	-	-	Purple
2	21-27	-	Light blue	-	-	-
3	34-38	Quenching	Orange	Pale yellow	Orange	-
4	40-43	Quenching	Orange	Pale yellow	Orange	-
5	45-49	Quenching	Orange	Pale yellow	Orange	-
6*	50-54	Quenching	Light blue	Pale yellow	Orange	-
7	55-58	-	Light blue	-	-	Purple
8	58-64	-	Light blue	-	-	Purple
9	64-73	-	Light blue	-	-	Purple
10	73-85	-	-	-	-	Red wine

*(+)-Tetrandrine

Table 10. hR_f value of one-dimensional TLC of *Cyclea barbata* roots extract

[Solvent system- Chloroform: Methanol: Ammonia (5: 1: 0.3)]

Spot	hR_f	UV 254 nm	UV 365 nm	Visible in daylight	Dragendorff 's reagent	Anisladehyde-sulfuric acid reagent
1	12-28	-	-	-	-	Purple
2	18-28	-	Blue-green	-	-	-
3	28-34	-	Light blue	-	-	-
4	37-40	Quenching	Light blue	Pale yellow	-	-
5	43-46	-	Light blue	-	-	-
6	50-55	Quenching	Blue-green	Pale yellow	-	-
7	58-61	-	Light blue	-	-	-
8	61-67	-	Red orange	-	-	Purple
9	68-74	Quenching	Orange	Pale yellow	Orange	Purple
10	74-77	-	Orange	-	-	Purple
11*	77-80	Quenching	Light green	-	Orange	Red wine
12	80-86	-	Light blue	-	-	Purple

*(+)-Tetrandrine

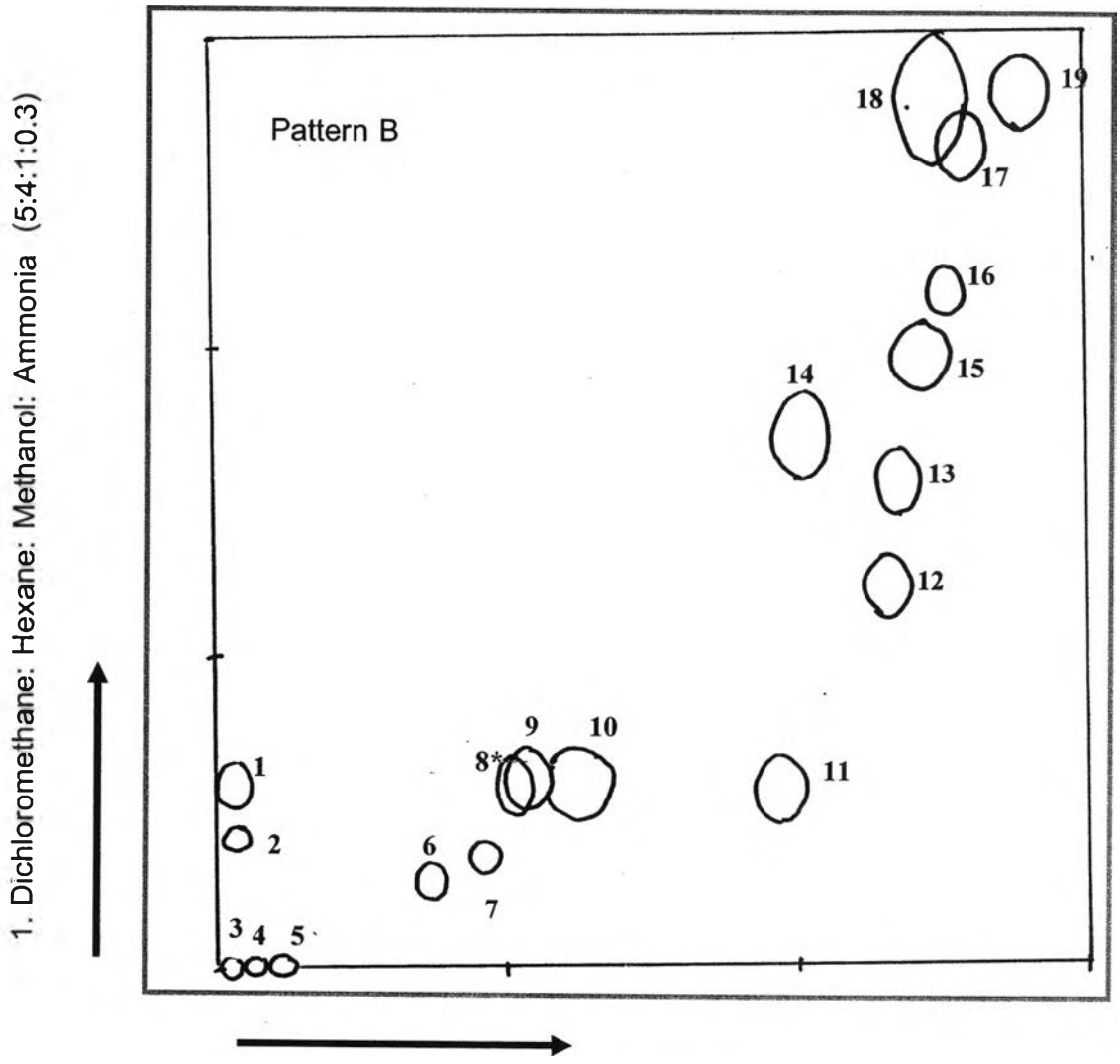


Figure 21. Two-dimensional thin-layer chromatogram of *Cyclea barbata* roots extract

System 1: Dichloromethane: Hexane: Methanol: Ammonia (5: 4: 1: 0.3)

System 2: Ethylacetate: Hexane: Methanol: Ammonia (4: 4: 1: 0.3)

* (+)-Tetrandrine

The results of two-dimensional TLC of crude extract of authentic sample are shown as follows:

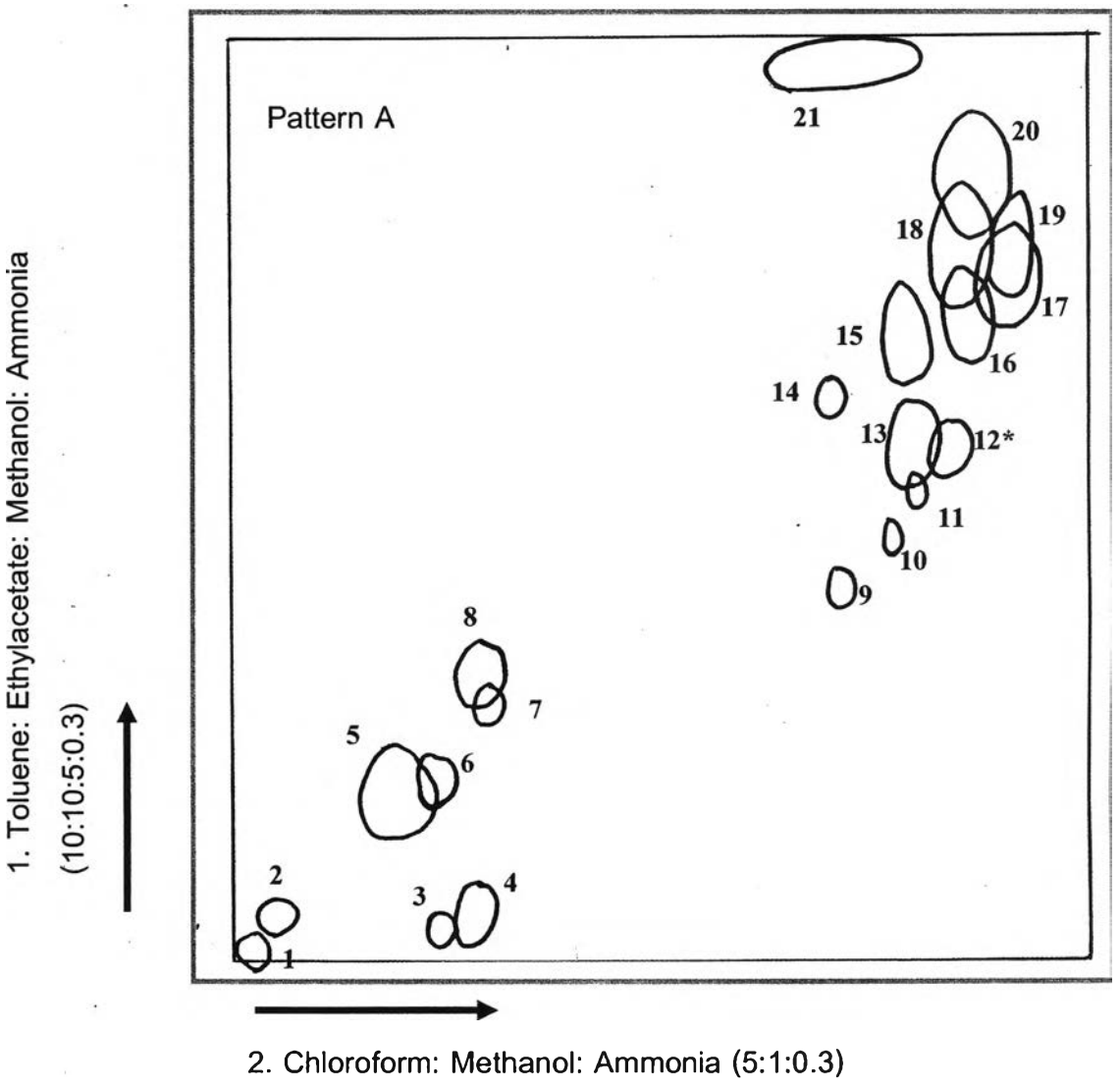


Figure 20. Two-dimensional thin-layer chromatogram C of *Cyclea barbata* roots extract

System 1: Toluene: Ethylacetate: Methanol: Ammonia (10:10:5:0.3)

System 2: Chloroform: Methanol: Ammonia (5: 1: 0.3)

* (+)-Tetrandrine

Table 11. R_f value of two-dimensional TLC of *Cyclea barbata* roots extract

System 1: Toluene: Ethylacetate: Methanol: Ammonia (10:10:5:0.3)

System 2: Chloroform: Methanol: Ammonia (5: 1: 0.3)

Spot	Code	UV 254	UV 365	Dragendorff 's reagent	Anisladehyde-sulfuric acid reagent
1	0000	00	65	00	00
2	0000	00	60	00	00
3	0020	00	20	00	00
4	0028	00	45	00	00
5	1818	00	00	00	75
6	1820	00	20	00	00
7	2828	00	60	00	00
8	2828	00	20	00	00
9	3868	93	00	25	00
10	4070	93	00	25	00
11	4878	95	00	25	00
12*	5080	95	00	25	00
13	5078	00	20	00	00
14	5868	00	20	00	00
15	6878	00	00	00	70
16	6880	00	50	00	00

Spot	Code	UV 254	UV 365	Dragendorff 's reagent	Anisladehyde-sulfuric acid reagent
17	7088	00	20	00	00
18	7880	00	00	00	70
19	7888	00	00	00	70
20	8888	00	00	00	75
21	9078	95	00	00	75

* (+)-Tetrandrine

Table 12. R_f value of two-dimensional TLC of *Cyclea barbata* roots extract

System 1: Dichloromethane: Hexane: Methanol: Ammonia (5: 4: 1: 0.3)

System 2: Ethylacetate: Hexane: Methanol: Ammonia (4: 4: 1: 0.3)

Spot	Code	UV 254	UV 365	Dragendorff 's reagent	Anisladehyde-sulfuric acid reagent
1	1800	00	60	00	00
2	1000	00	20	00	00
3	0000	00	60	00	00
4	0000	00	60	00	00
5	0800	00	60	00	00
6	0820	90	00	25	00
7	0828	95	00	25	00
8*	1830	95	00	25	00
9	1830	00	60	00	00
10	1840	00	45	00	00
11	1860	00	00	00	75
12	3878	00	00	00	75
13	4878	00	45	00	00
14	5068	00	20	00	00
15	6078	00	60	00	00
16	6880	90	00	00	00

Spot	Code	UV 254	UV 365	Dragendorff 's reagent	Anisladehyde-sulfuric acid reagent
17	8080	00	00	00	70
18	9080	90	00	00	00
19	8888	00	00	00	75

* (+)-Tetrandrine

Part IV. General of quality controls

The quality controls of *Cyclea barbata* which were purchased from traditional drugstores are shown as in Table 13.

Table 13. Loss on drying, moisture content, total ash, acid insoluble-ash, extractive values in seven samples of *Cyclea barbata* root.

a) Sample V

Crude drug sample	Loss on drying (%)	Moisture content (%)	Ash content (%)		Extractive value (%)	
			Total ash	Acid insoluble ash	Ethanol	Water
V1	9.87	9.10	5.90	1.97	16.80	16.38
V2	9.62	9.07	5.96	1.99	17.89	15.60
V3	9.74	9.05	5.61	1.98	17.72	15.12
mean	**9.74**	**9.07**	**5.58**	**1.98**	**17.47**	**15.70**
sd	**0.13**	**0.03**	**0.19**	**0.01**	**0.59**	**0.64**

b) Sample J

Crude drug sample	Loss on drying (%)	Moisture content (%)	Ash content (%)		Extractive value (%)	
			Total ash	Acid insoluble ash	Ethanol	Water
J1	9.68	7.13	5.48	1.95	19.53	16.73
J2	9.57	7.83	5.57	1.98	18.96	17.65
J3	9.75	7.28	5.28	1.91	18.87	17.48
mean	**9.67**	**7.41**	**5.44**	**1.94**	**19.12**	**17.29**
sd	**0.09**	**0.37**	**0.15**	**0.04**	**0.36**	**0.49**

c) Sample C

Crude drug sample	Loss on drying (%)	Moisture content (%)	Ash content (%)		Extractive value (%)	
			Total ash	Acid insoluble ash	Ethanol	Water
C1	9.27	8.41	5.45	1.95	18.00	17.58
C2	9.63	8.47	5.66	1.98	18.99	17.96
C3	9.59	8.92	5.28	1.99	18.96	17.99
mean	**9.50**	**8.60**	**5.46**	**1.97**	**18.65**	**17.84**
sd	**0.20**	**0.28**	**0.19**	**0.02**	**0.56**	**0.23**

d) Sample U

Crude drug sample	Loss on drying (%)	Moisture content (%)	Ash content (%)		Extractive value (%)	
			Total ash	Acid insoluble ash	Ethanol	Water
U1	9.77	8.78	5.54	1.28	18.92	18.52
U2	9.74	8.82	5.94	1.26	18.99	18.53
U3	9.94	8.90	5.97	1.24	19.99	19.12
mean	**9.82**	**8.83**	**5.82**	**1.26**	**19.30**	**18.76**
sd	**0.11**	**0.06**	**0.24**	**0.02**	**0.60**	**0.32**

e) Sample N

Crude drug sample	Loss on drying (%)	Moisture content (%)	Ash content (%)		Extractive value (%)	
			Total ash	Acid insoluble ash	Ethanol	Water
N1	9.28	7.10	4.78	0.64	18.87	16.97
N2	9.46	7.27	4.40	0.63	20.87	19.96
N3	9.38	7.27	4.76	0.63	20.80	17.96
mean	**9.37**	**7.21**	**4.64**	**0.63**	**20.18**	**18.30**
sd	**0.09**	**0.10**	**0.21**	**0.01**	**1.13**	**1.52**

f) Sample S

Crude drug sample	Loss on drying (%)	Moisture content (%)	Ash content (%)		Extractive value (%)	
			Total ash	Acid insoluble ash	Ethanol	Water
S1	9.38	8.92	4.47	0.64	20.00	19.96
S2	9.66	8.32	4.44	0.61	20.00	18.92
S3	9.38	8.42	4.43	0.63	19.88	19.34
mean	**9.47**	**8.55**	**4.44**	**0.63**	**19.96**	**19.41**
sd	**0.16**	**0.32**	**0.02**	**0.02**	**0.07**	**0.52**

g) Sample R

Crude drug sample	Loss on drying (%)	Moisture content (%)	Ash content (%)		Extractive value (%)	
			Total ash	Acid insoluble ash	Ethanol	Water
R1	9.62	7.26	5.30	0.99	20.12	19.84
R2	9.87	7.92	5.57	1.31	21.12	19.96
R3	9.87	7.14	5.61	1.32	20.91	19.96
mean	**9.79**	**7.44**	**5.49**	**1.21**	**20.72**	**19.92**
sd	**0.14**	**0.42**	**0.17**	**0.19**	**0.53**	**0.07**

h) Seven samples

Crude drug sample	Loss on drying (%)	Moisture content (%)	Ash content (%)		Extractive value (%)	
			Total ash	Acid insoluble ash	Ethanol	Water
V*	9.74	9.07	5.58	1.98	17.47	15.70
J*	9.67	7.41	5.44	1.94	19.12	17.29
C*	9.50	8.60	5.46	1.97	18.65	17.84
U*	9.82	8.83	5.82	1.26	19.30	18.76
N*	9.37	7.21	4.64	0.63	20.18	18.30
S*	9.47	8.55	4.44	0.63	19.96	19.41
R*	9.79	7.44	5.49	1.21	20.72	19.92
MEAN	**9.63**	**8.16**	**5.30**	**1.38**	**19.34**	**18.17**
SD	**0.19**	**0.78**	**0.54**	**0.61**	**1.00**	**1.31**

V* = mean values of sample V

J* = mean values of sample J

C* = mean values of sample C

U* = mean values of sample U

N* = mean values of sample N

S* = mean values of sample S

R* = mean values of sample R

Part V. Quantitative quality controls

With analyse-spiking and spectrum-matching techniques (Figure 24 and 25), tetrandrine was identified in the samples prepared from the roots of the plant *Cyclea barbata* Miers.

A typical electropherogram is shown in figure 22 and 23. All seven herbal medicine samples gave tetrandrine peak with hR_f values 52.1 in TLC-densitometric method and it gave tetrandrine peak at 6.321 min in CE method.

For quantitation, the external standard method was employed in TLC-densitometric. The calibration curve for tetrandrine was constructed in the concentration range 0.0100-0.0225 mg/ml (50-112.5 ng/5µl) with the linear regression equation and correlation coefficient (Figure 26) being as follows:

$$y = 10.989\,x + 13.625,\ (r^2 = 0.9971)$$

when y is the integrated peak area (integration units) and x is the amount (ng) of tetrandrine in sample.

Similarly, for CE, the calibration curve was constructed in the concentration range of 0.01-0.05 mg/ml (10-50 mg/L) with the linear regression equation and correlation coefficient (Figure 27) being as follows:

$$y = 763.65\,x - 4493.3,\ (r^2 = 0.9986)$$

when y is the integrated peak area (integration units) and x is the concentration (mg/L) of tetrandrine in sample.

Thus, the amounts of tetrandrine in *Cyclea barbata* roots can be quantified according to the above regression lines or equations. Table 14 lists the determined values of tetrandrine in *Cyclea barbata* roots.

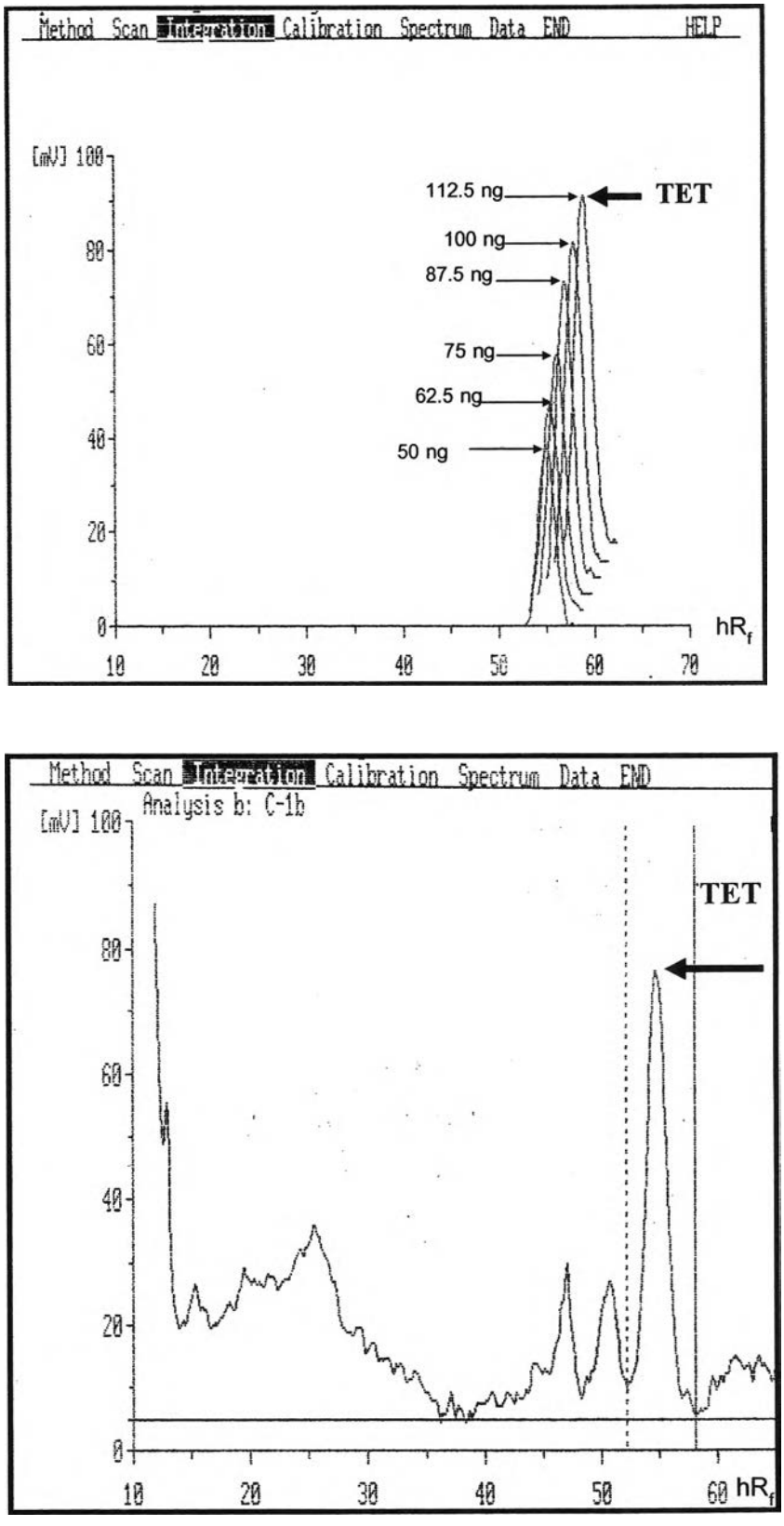


Figure 22. Top: TLC-electropherogram of standard (+)-tetrandrine (hRf value = 52.1) and bottom: (+)-tetrandrine in *Cyclea barbata* roots extract (hR_f value = 52.1).

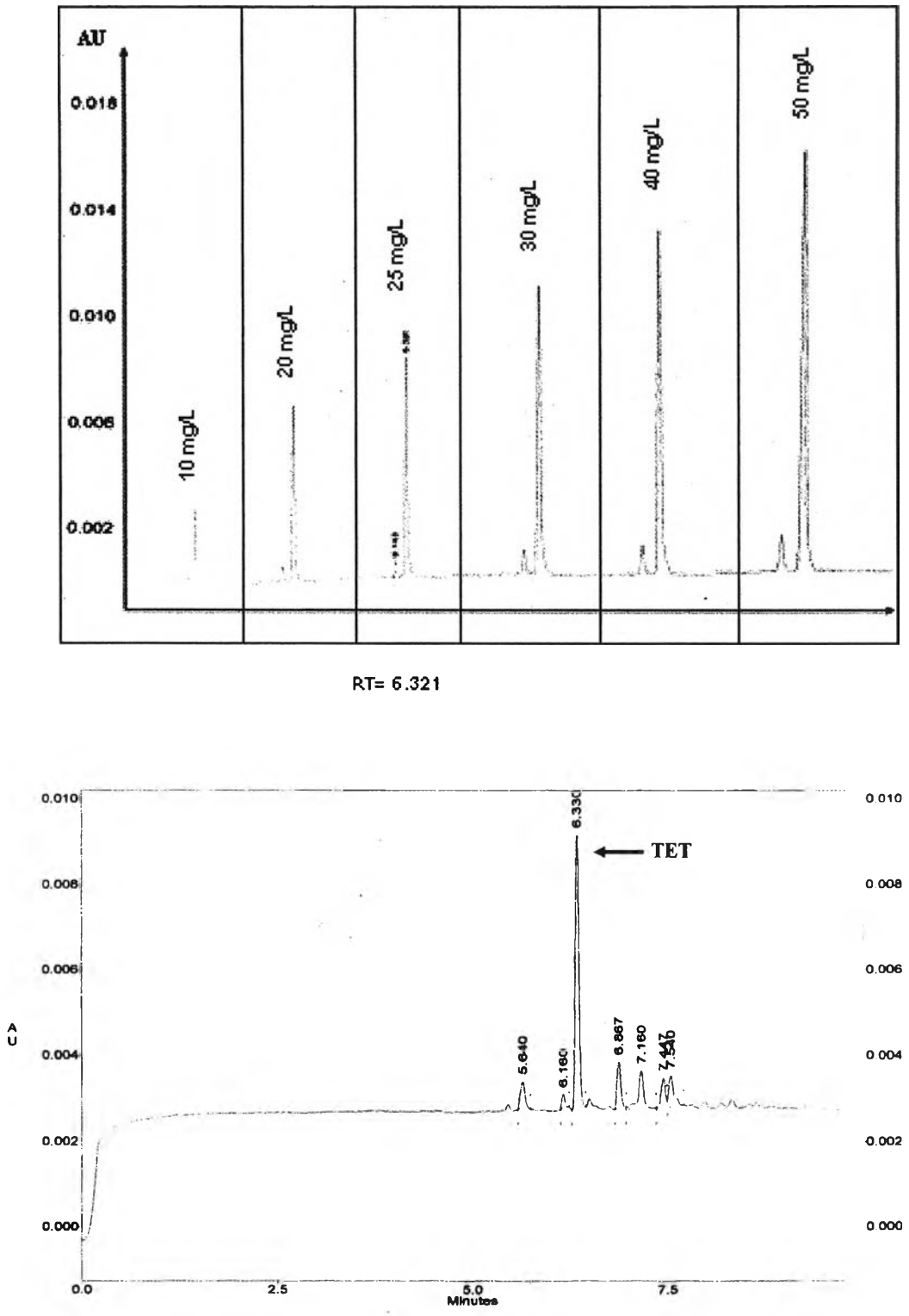


Figure 23. Top: CE-electropherogram of standard (+)-tetrandrine (RT = 6.321 min) and bottom: (+)-tetrandrine in *Cyclea barbata* roots extract (RT = 6.330 min).

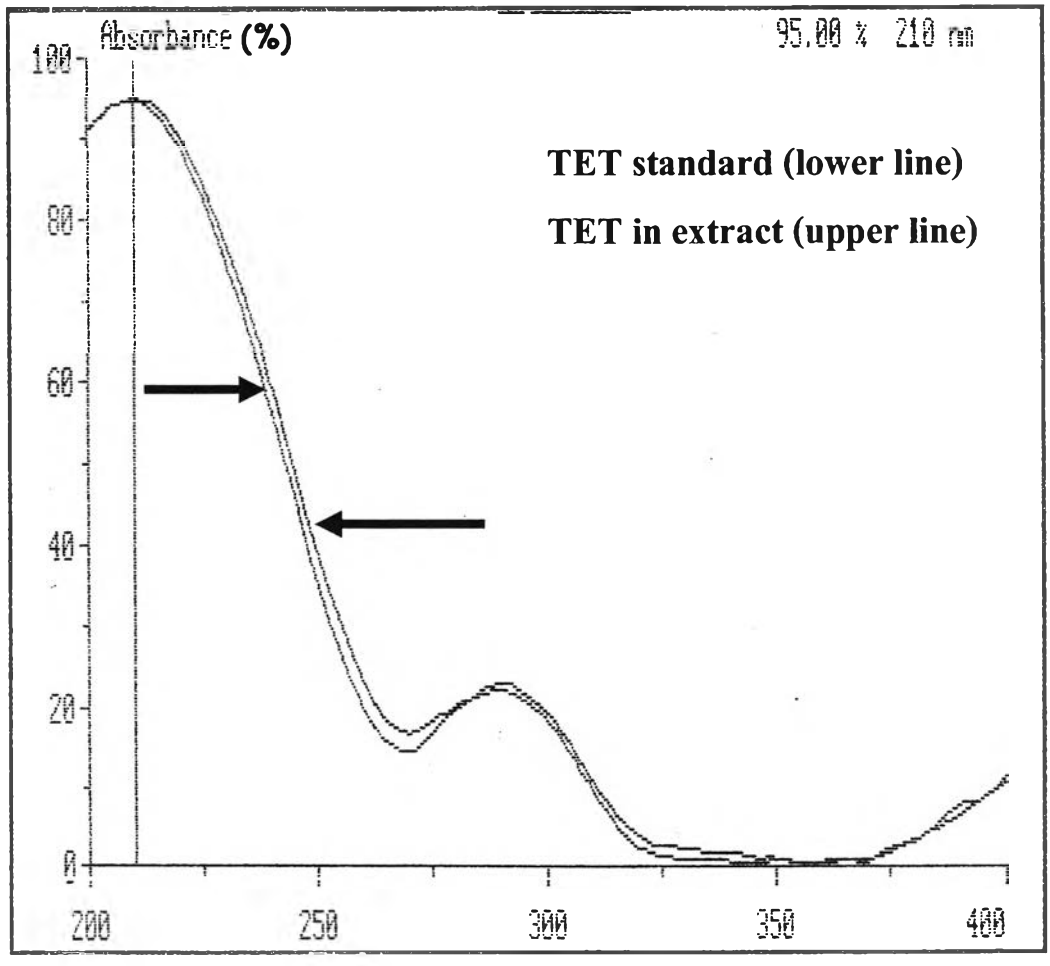
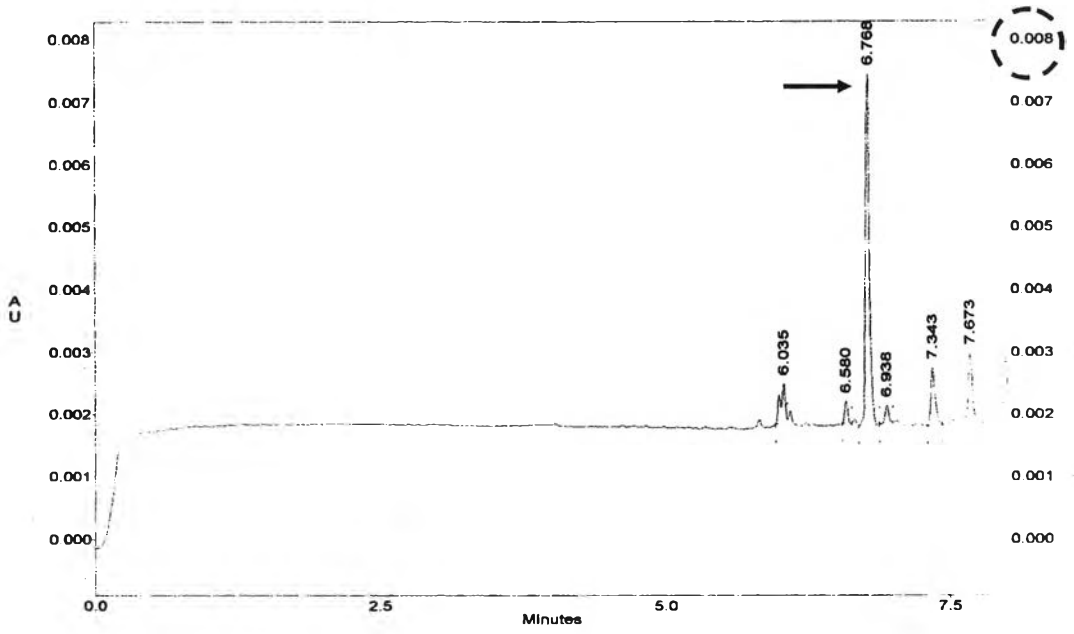
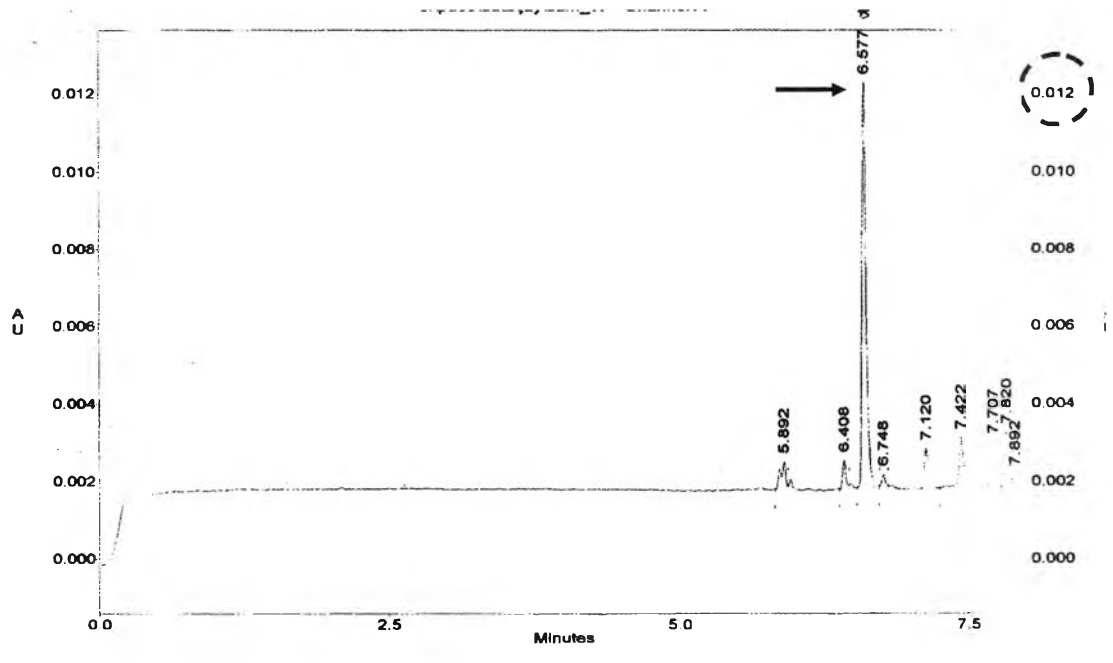


Figure 24. UV-absorption spectra of standard (+)-tetrandrine (lower line) and the compound on TLC plate with similar hR_f value to (+)-tetrandrine in *Cyclea barbata* roots extract (upper line).



Sample V-1



Sample V-1 spiked with TET

Figure 25. Representation of CE-electropherogram illustrating of the extract of *Cyclea barbata* roots to determine the present of tetrandrine in the extract. Top: electropherogram of the extract, bottom: electropherogram of the extract spiked with tetrandrine.

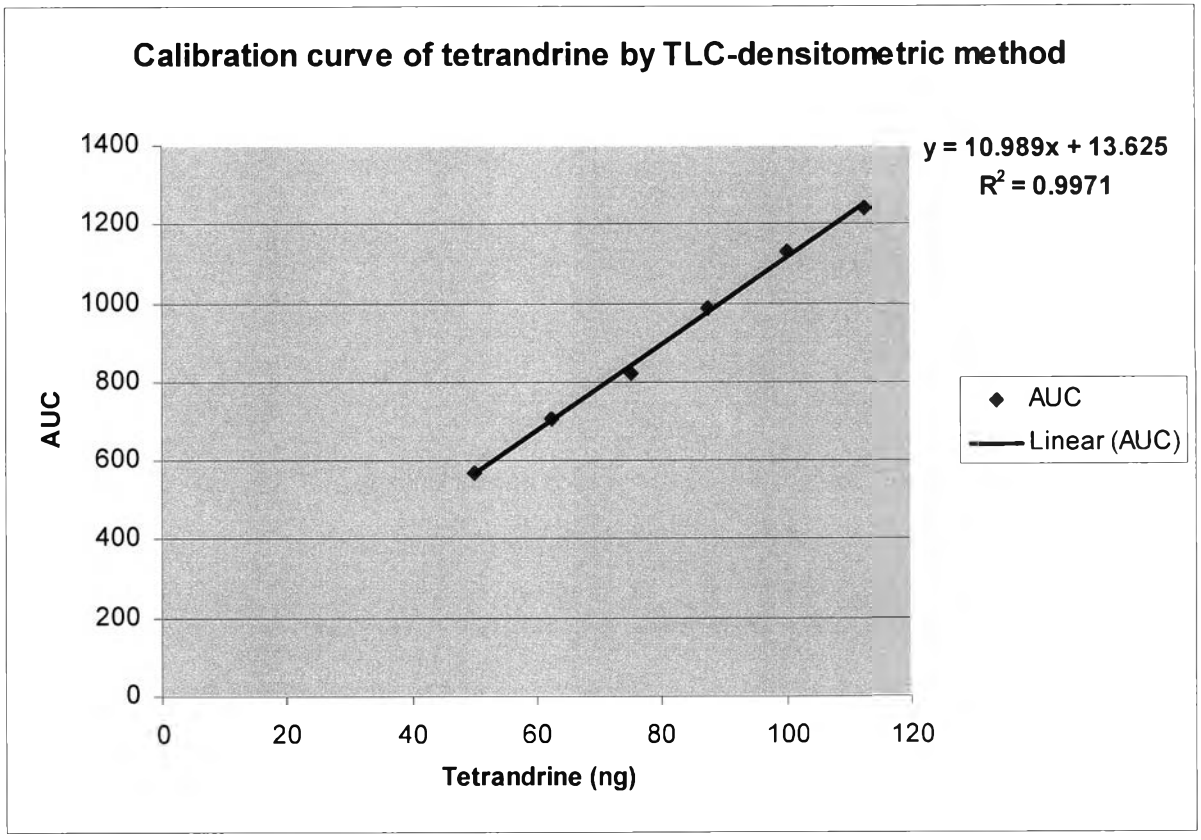


Figure 26. Calibration curve of (+)-tetrandrine by TLC-densitometric method.

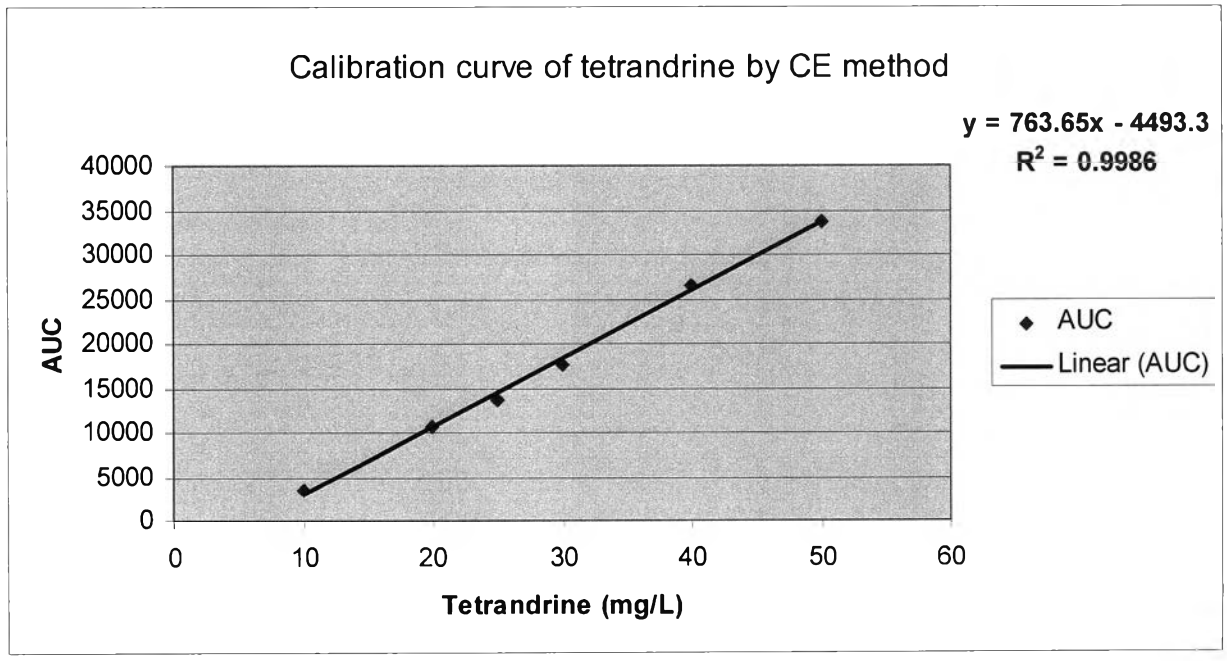


Figure 27. Calibration curve of (+)-tetrandrine by CE method.

Table 14. Tetrandrine content (% w/w) in seven *Cyclea barbata* roots.

Sample	Tetrandrine content (%w/w) TLC-densitometric method*	Tetrandrine content (%w/w) CE method*
J-1	1.15 ± 0.06	1.14 ± 0.04
V-1	1.15 ± 0.05	1.14 ± 0.05
C-1	1.16 ± 0.05	1.06 ± 0.02
R-1	0.39 ± 0.01	0.33 ± 0.01
S-1	0.37 ± 0.01	0.34 ± 0.01
N-1	0.45 ± 0.02	0.45 ± 0.02
U-1	0.35 ± 0.02	0.38 ± 0.02

* Each value represented the mean ± SD of analysis (triplicate in each sample).

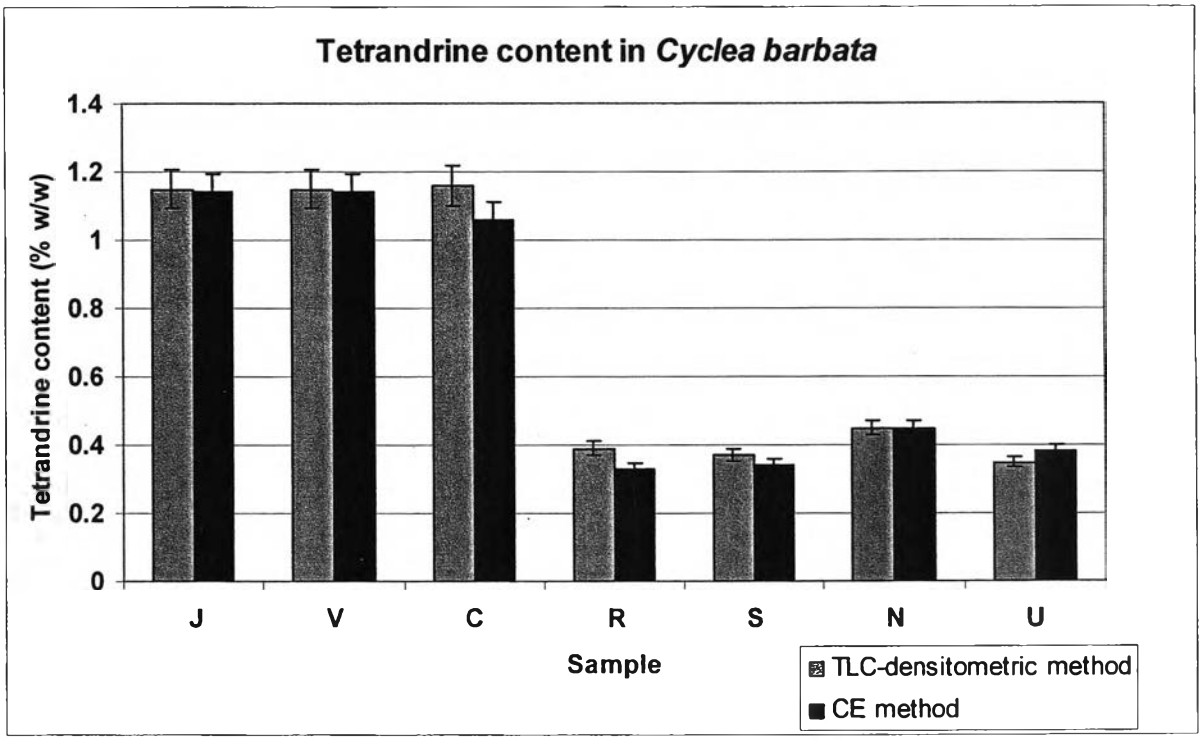


Figure 28. Bar graph of (+)-tetrandrine in *Cyclea barbata* roots determined by TLC-densitometric method and CE method. The values of (+)-tetrandrine content were obtained from Table 14.

Table 15. Paired sample t-test.

a) Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	TLC	0.7234	21	0.36799	0.08030
	CE	0.6931	21	0.37370	0.08155

Paired Samples Statistics shows for each variable, the number of cases, the mean, the standard deviation, and the standard error of the mean

b) Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	TLC & CE	21	0.982	0.000

Paired Samples Correlations shows the correlation between the two variables. The two variables are positively correlated, $r\ (N = 21) = 0.982$, $p = 0.000$.

c) Paired Samples Test

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
Pair 1 TLC - CE	0.0303	0.06984	0.01524	-0.0015	0.0621	1.990	20	0.060

Paired Samples Test shows the t statistics for the paired differences. Compare between content of (+)-tetrandrine determine by TLC-densitometric method and CE method. The mean was less difference, 0.0303, $t\ (20) = 1.990$, $p = 0.060$.

TLC = (+)-tetrandrine content determine by TLC-densitometric method

CE = (+)-tetrandrine content determine by CE method

Discussion

This study dealed with the investigation of pharmacognostic specification of *Cyclea barbata* Miers (Krung Kha Mao) roots. Macroscopic characters of Krung Kha Mao from several sources in this study are slightly differences in length and diameter, due to the preparation of these crude drugs in each source, whilst the microscopic characters are similar including the TLC pattern.

Microscopically, the majority tissues were found as sclereids, corks and starch granules. The TLC patterns showed major constituent of alkaloid especially tetrandrine that were used as the marker for plant identification.

Moisture content is employed to control the water in crude drug. On the other hand, loss on drying controls the loss in weight (due to water and other volatile materials) of crude drug. The excessive content of water in crude drugs and temperature are the promoter factors of fungal and bacterial growth which cause the spoilage. Ash content are accountable for controlling the admixture of foreign inorganic matter due to their storage, container or intentional add to disguise the appearance of crude drug.

The determination of ethanol-soluble extractive value is used to control the constituents of crude drugs which cause inferiority from many factors such as moisture content, temperature, harvesting, drying process, kept duration and storage.

Cyclea barbata roots from several location were determined and concluded the data as an estimated percentage values.The results of quality controls *Cyclea barbata* roots could inform the standardization of this drug as shown in Table 16.

Table 16. General specification of *Cyclea barbata* roots.

	Data interval (%)	Mean ± SD (%)
Loss on drying	9.27-9.94	9.62 ± 0.19
Moisture content	7.10-9.10	8.16 ± 0.78
Total ash	4.40-5.97	5.30 ± 0.54
Acid-insoluble ash	0.61-1.99	1.38 ± 0.61
Ethanol-soluble extractive value	16.38-21.12	19.34 ± 1.00
Water-soluble extractive value	15.12-19.99	18.17 ± 1.31

In order to evaluate its accuracy and precision, TLC-densitometry was compared with the capillary electrophoresis (CE) method. In this study, a number of *Cyclea barbata* root samples from seven sources were analyzed for their (+)-tetrandrine content using the two methods and the results were compared. It can be seen in Table 14-15 and Figure 28 that the values of (+)-tetrandrine content of seven samples determined by TLC-densitometry (M = 0.7234, SD = 0.36799) was closed to values of (+)-tetrandrine content determined by CE method (M = 0.6931, SD = 0.37370, d = 0.982). The two variables are positively correlated, r (N = 21) = 0.982, p = 0.000 and there were not significantly difference (t (20) = 1.990, p >0.060). In terms of precision, the three separate determination of each sample showed narrow range in their standard deviation (SD).

Cyclea barbata roots from several sources contained variable (+)-tetrandrine contents, ranging from 0.33 to 1.21% (w/w) (Table 14, Figure 28). The difference in (+)-tetrandrine contents in the crude drugs might be due to age of plants, geographic and genetic variations. Moreover the season of collection and storage condition might also caused the fluctuation point of view.