CHAPTER III

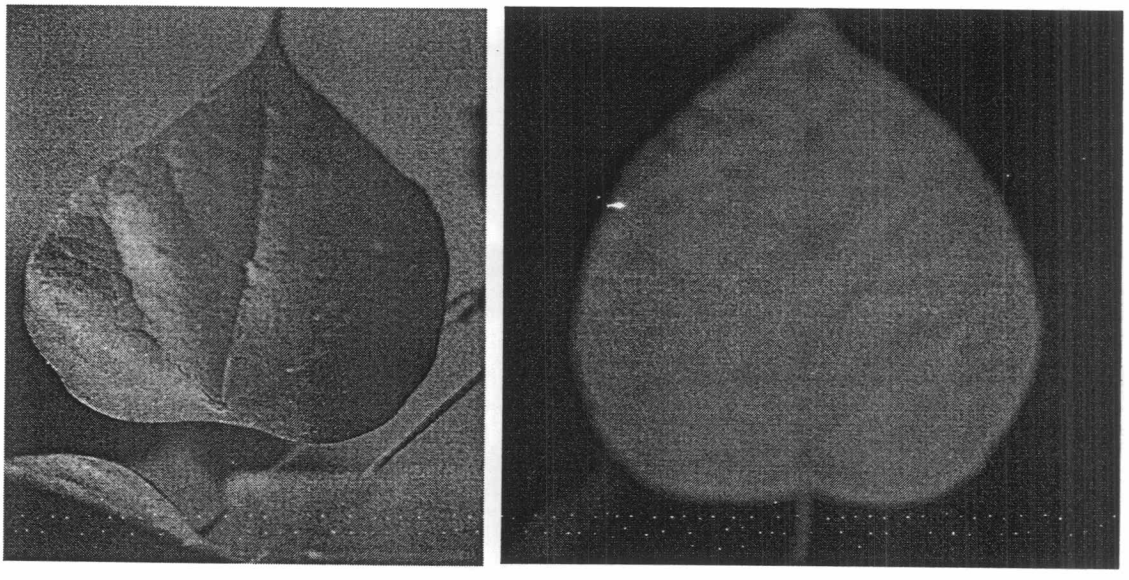
RESULTS AND DISCUSSIONS

1. Macroscopic characterization of HAMM

Leaves are ovate, apex acuminate. Upper surface are glabrescent and smooth. Midrib and other main nerves are sunken. Lower surface are often whitish tomentellous and palmately 5 nerved at base. Petiole (5 cm) are inserted up to 0.8 cm from basal margin of lamina.

Stem is yellow wood. The stem is subcylindrical up to 5 cm in diameter, with occasional enlarged nodes up to 7 cm in diameter. Externally, moderate brown to blackish brown where nearly smooth, irregularly wrinkled and fissures. The transversely cut surface exhibits yellowish brown wood with about 38 radius line structure distinctly between the medullary rays and the lighter xylem. The bark is about 3.5 mm in thickness.

Observation macroscopic and microscopic character of HAMM presently studied is identical to macroscopic and microscopic character of *Coscinium fenestratum*.



Dorsal surface

Ventral surface

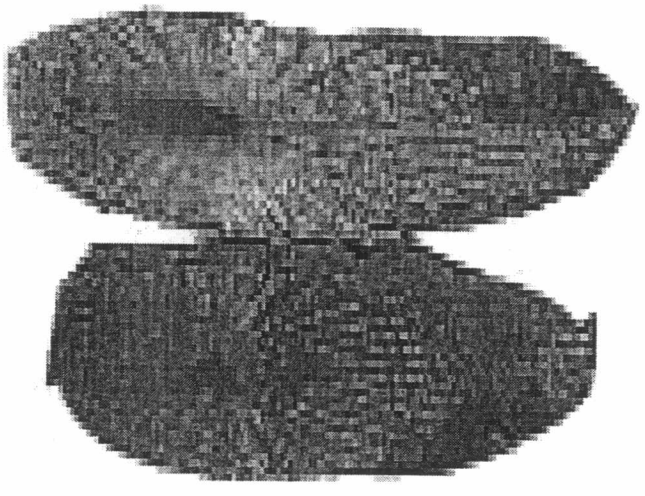


Figure 33. Leaves and stem of HAMM

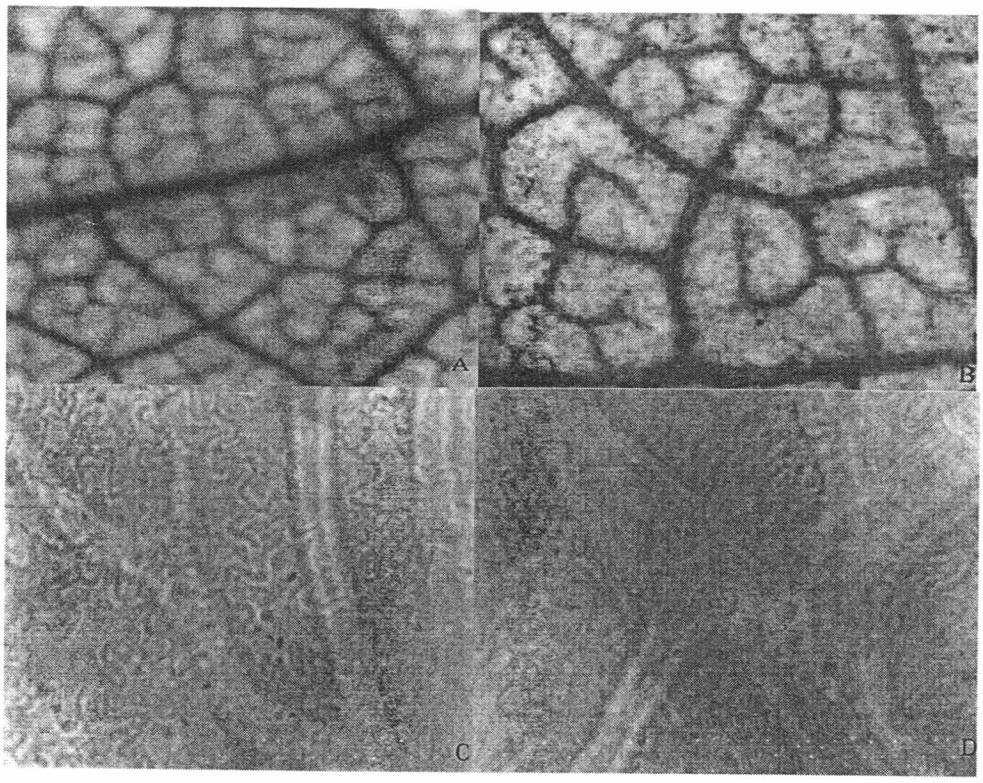


Figure 34. Microscopic character of HAMM leaf.

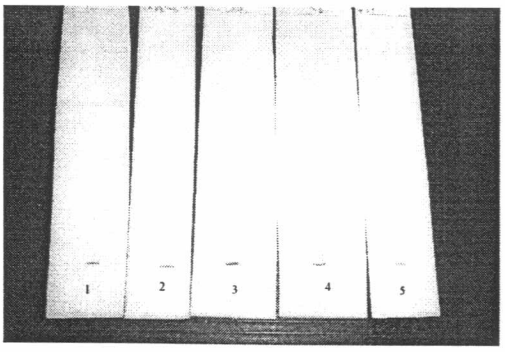
A, B. Vein–islet and veinlet termination

C, D. Upper epidermis with glandular trichome

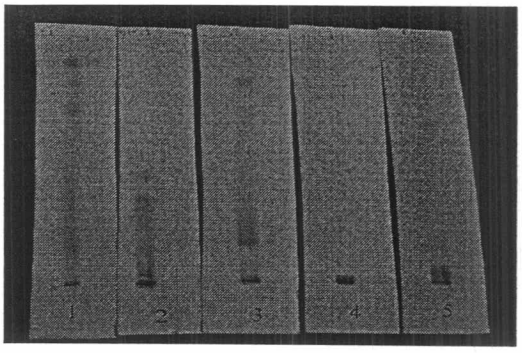
2. Characterization of crude water extract

16 gram of crude water extract was derived from 100 gram of dried HAMM which extracted by distilled water at 65^0C. Character of crude water extract are yellow-brown powder, hygroscopic.

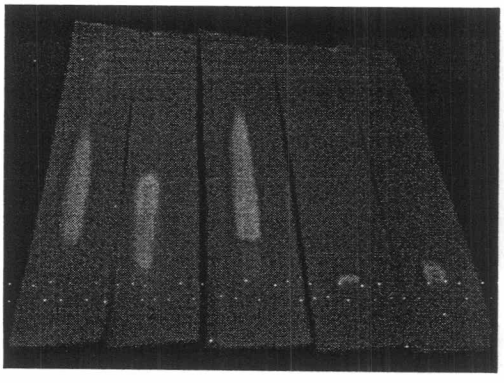
Figure 35. Crude water extract of HAMM.



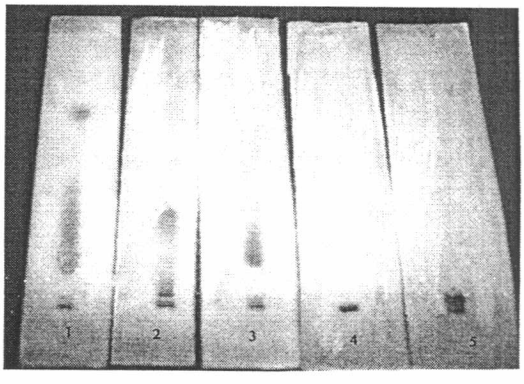
A : Eye observation under visible light



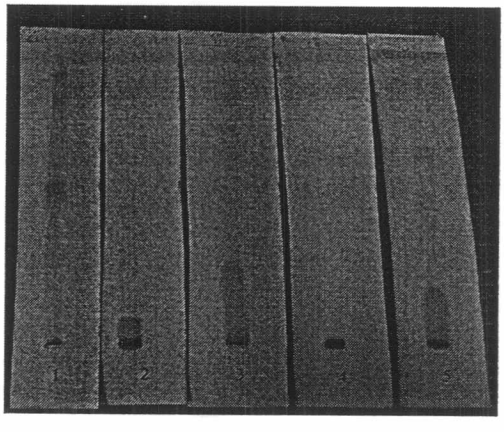
B : Detection with UV 254 nm



C : Detection with UV 350 nm



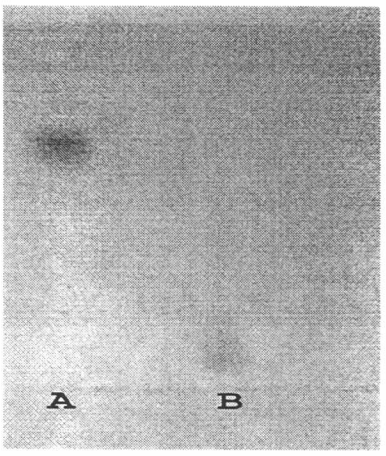
D : Spraying Dragendorff's reagent



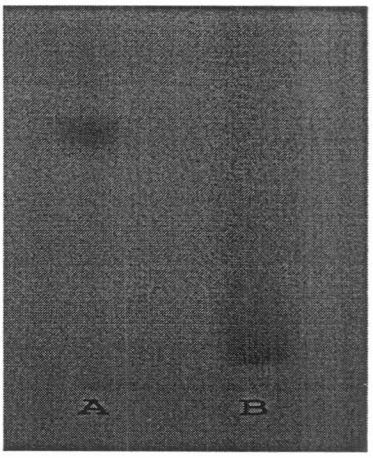
E : Spraying 10% sulfuric acid

Figure 36. TLC System for separation of constituents of CE using different solvent systems 1-5.

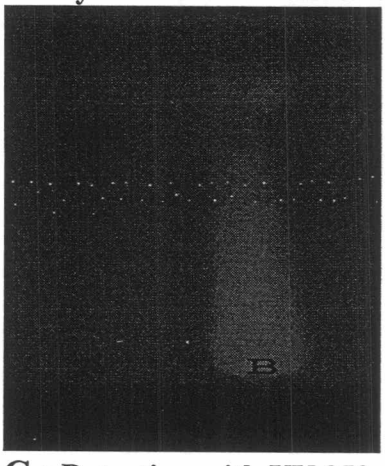
1. MeOH : H_2O: NH_3 (8:1:1)
2. CH Cl_3 : MeOH (1:1)
3. $CHCl_3$: MeOH (8:2)
4. EtOAC : MeOH (7:3)
5. Acetone: MeOH (6 : 4)



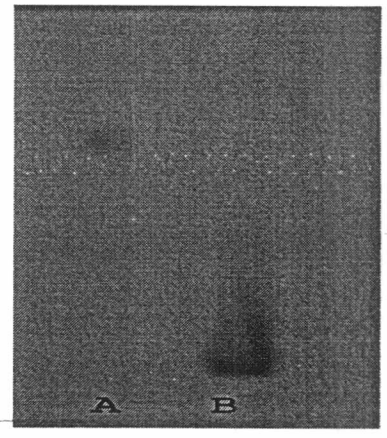
A : Eye observation under visible light



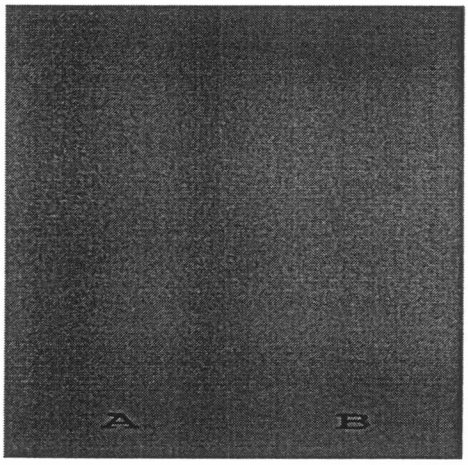
B : Detection with UV 254 nm



C : Detection with UV 350 nm



D : Spraying Dragendorff's reagent



E : Spraying 10% sulfuric acid

Figure 37. TLC pattern of Cf fractions

A : Cf_2-crystal

B : Cf_3 fraction

3. Melting point

Melting point of Cf_2 crystals and Cf_3 were determined using a Melting point Apparatus (Gallenkamb). The compound was packed in a capillary tube and melting temperature of the compound was observed. Melting point of Cf_2 crystals was 208^0C. Melting point of Cf_3 was 206^0C.

4. UV Spectra

In neutral condition, 0.05 mg of Cf_2 crystals / ml of ethanol showed maxima peak at UV λ max/nm (EtOH) (log ε) 227 (1.20), 266 (1.14), 349 (1.15).

0.05 mg of Cf_3 crystals / ml of ethanol showed maxima peak at UV λ max/nm (EtOH) (log ε) 230 (1.12), 266 (1.11), 349 (1.09).

0.00625 mg of standard berberine hemisulfate/ ml of ethanol showed maxima peak at UV λ max/nm (EtOH) (log ε) 230 (1.97), 266 (1.96), 349 (1.94).

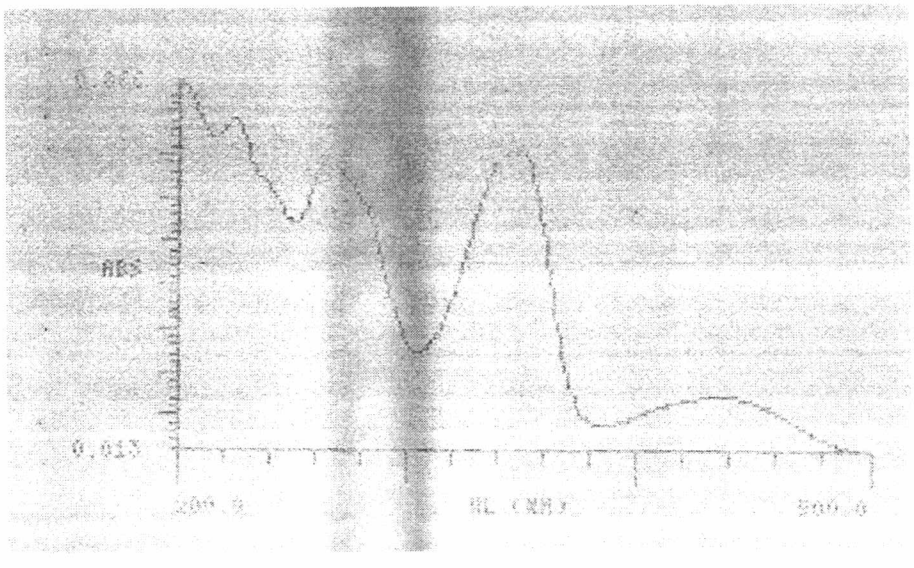


Figure 38. UV spectrum of Cf_2-crystal

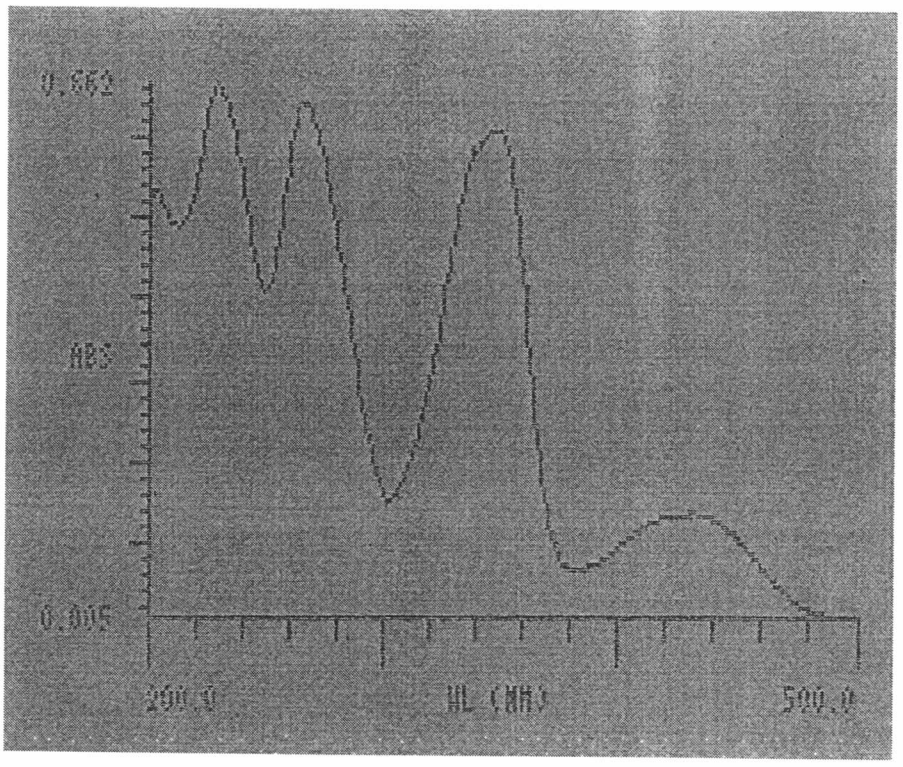


Figure 39. UV spectrum of Cf_3 fraction.

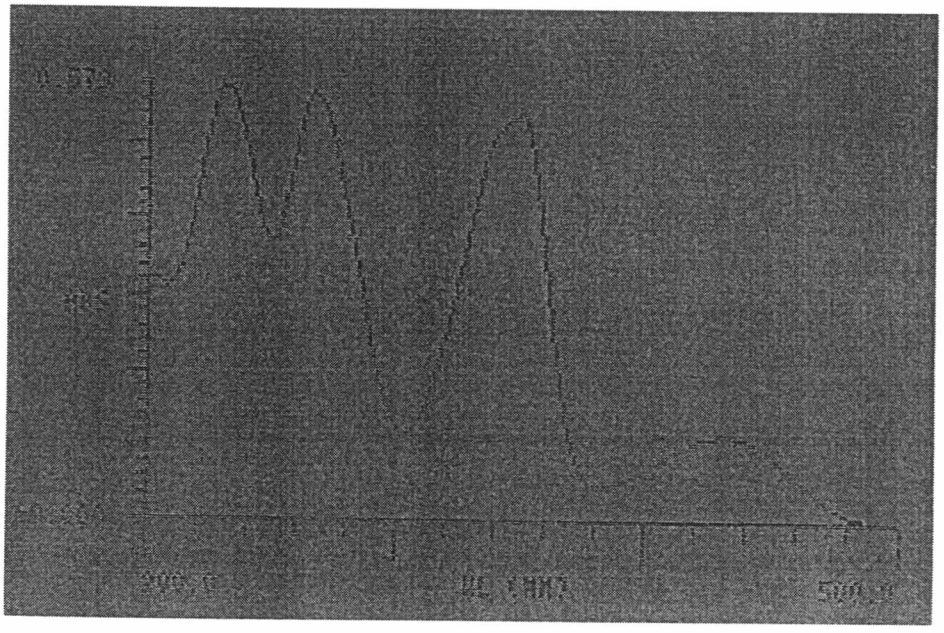


Figure 40. UV spectrum of berberine hemisulfate.

5. Infrared Spectra

Infrared spectra were determined by using a Fourier Transform Infrared Spectrometry (FT-IR). Infrared spectra of Cf_2 crystals are illustrated in Figure 41. IR spectra of Cf_2 crystals showed peaks at (cm^{-1})1110 (-C-N-, -C-O-C-), 1443 (CH_3), 1600 (C=C).

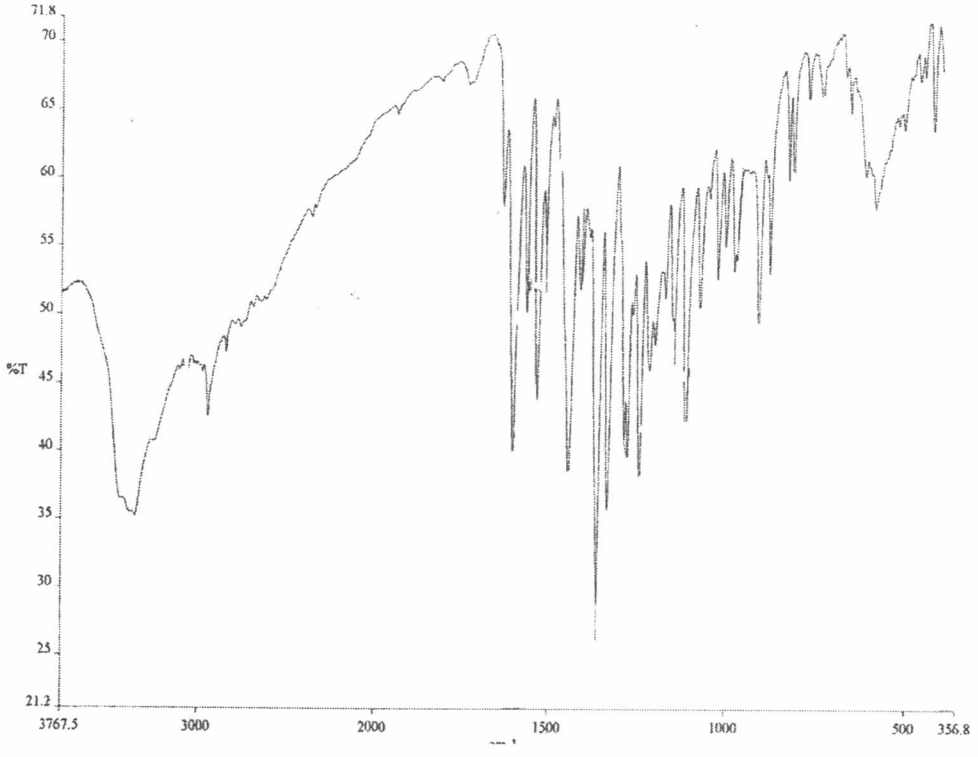


Figure 41. IR spectrum of Cf_2 -crystal in KBr.

Infrared spectra of Cf_3 fraction are illustrated in Figure 42. IR spectra of Cf_3 crystals showed peaks at (cm^{-1}) 1105 (-C-N-, -C-O-C-), 1506 (phenyl), 1600 (C=C).

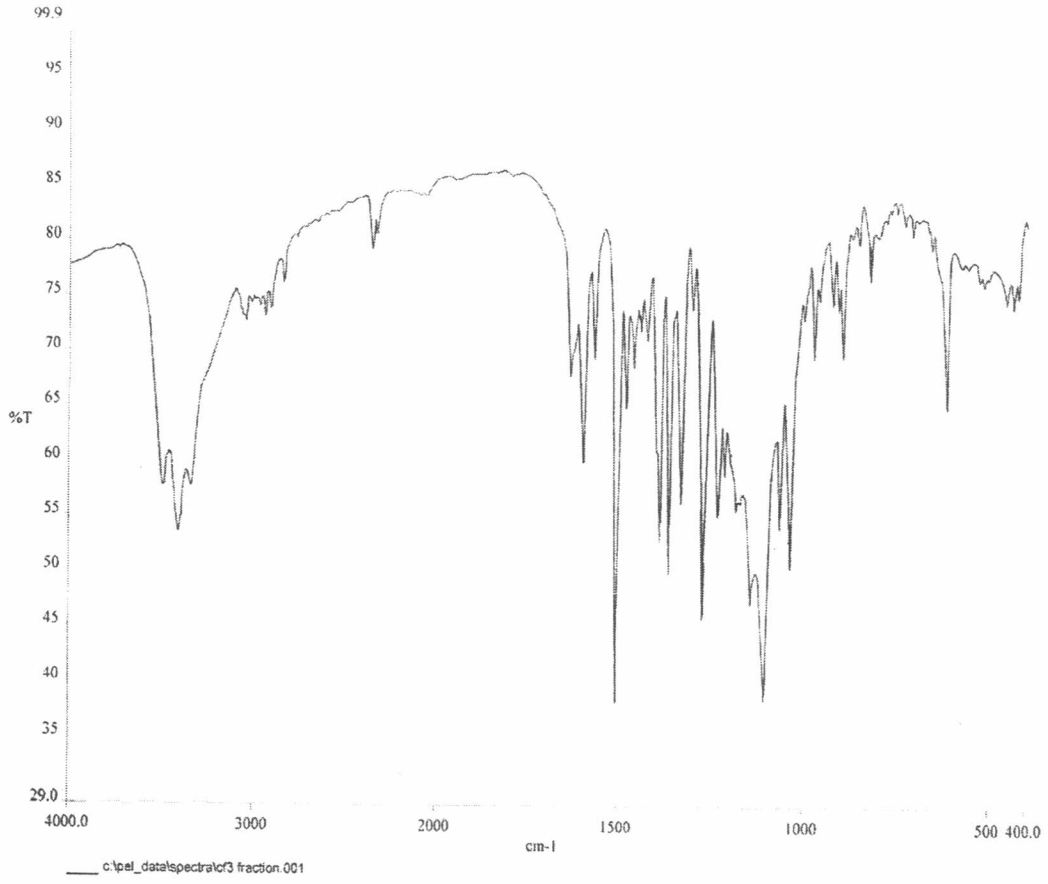


Figure 42. IR spectrum of Cf_3 fraction in KBr.

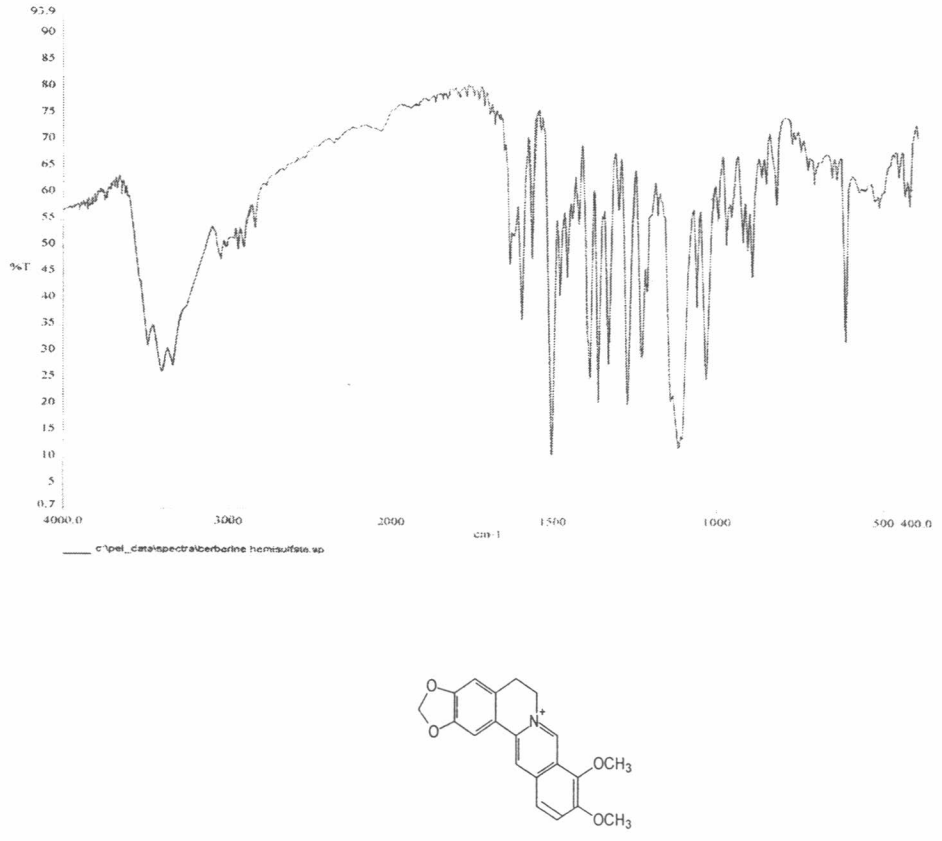


Figure 43. IR spectrum of Berberine hemisulfate in KBr.

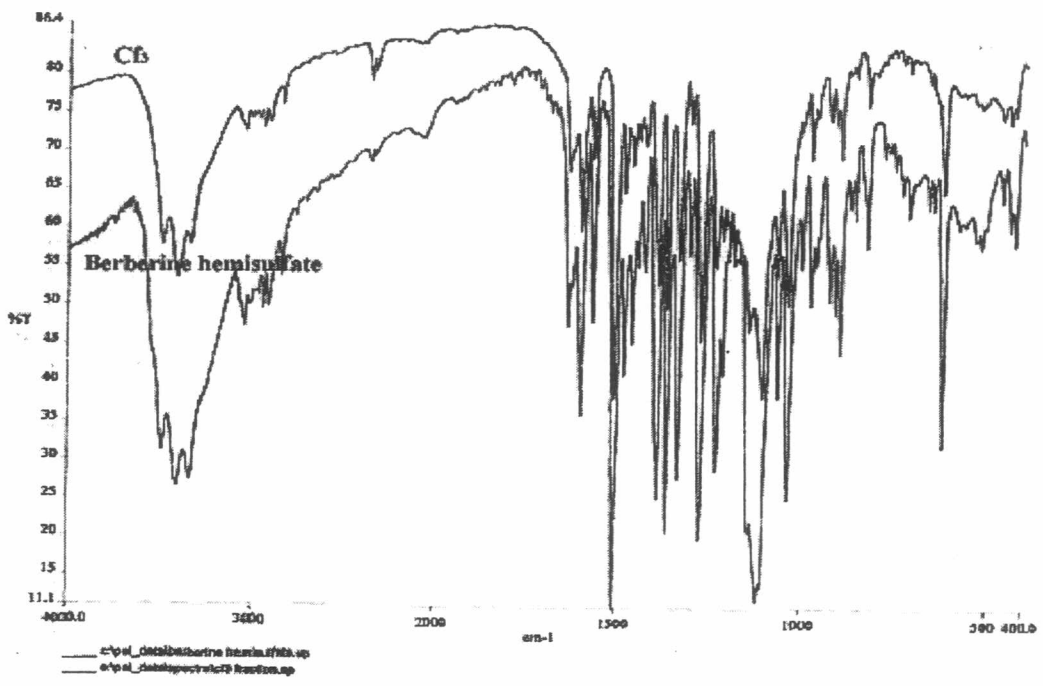


Figure 44. IR spectrum of Cf_3 fraction and Berberine hemisulfate in KBr.

6. NMR Spectra

Chemical structure of Cf_2 fraction was identified by NMR analysis. The compound was redissolved in tetradeuteromethanol (CD_3OD) and performed NMR technique for ^1H and ^{13}C. The result of NMR scanned, ^1H NMR δ 9.72 (s,H_8), δ 8.75 (s, H_{13}), δ 8.08 (d, H_{12}, $J_{11\text{-}12}$ = 9.15 Hz), δ 7.95 (d, H_{11}, $J_{11\text{-}12}$ =9.15 Hz), δ 7.645 (s, H_1), δ 6.865 (s, H_4), δ 4.91 (t, C_6-2H, J=6 Hz), δ 4.205 (s, C_9)-OCH_3, δ 4.11 (s, C_{10})-OCH_3, δ 4.025 (s,C_2)- OCH_3, δ 3.21 (t, C_5-2H, J=6 Hz), showed in Table 1 and Figure 45. ^{13}C- NMR data to showed in the Table 2 and Figure 46. Two doublets of Cf_2-crystal are at δ 7.95 ($J_{11\text{-}12}$ = 9.15 Hz) H-11 and δ 8.08 ($J_{11\text{-}12}$ = 9.15 Hz) H-12 which are identical to the two doublets of jatrorrhizine are at δ 7.77 ($J_{11\text{-}12}$ = 9.2 Hz) H-11 and δ 8.01 ($J_{11\text{-}12}$ = 9.2 Hz) H-12, whereas the two doublets of columbamine appeared at δ 7.58 ($J_{11\text{-}12}$ = 8.8 Hz) H-11 and δ 8.11 ($J_{11\text{-}12}$ = 8.8 Hz) H-12. The two triplets of Cf_2-crystal are at δ 4.91 (J = 6 Hz) H-6 and δ 3.21 (J = 6 Hz) H-5 which are identical to the two triplets of jatrorrhizine are at δ 4.90 (J = 6 Hz) H-6 and δ 3.24 (J = 6 Hz) H-5, whereas the two triplets of columbamine appeared at δ 4.97 (J = 6.4 Hz) H-6 and δ 3.30 (J = 6.4 Hz) H-5 (Hsieh et al., 2004; Siwon et al., 1980).

Chemical structure of Cf_3 was identified by NMR analysis. The compound was resoluble in tetradeuteromethanol (CD_3OD) and performed NMR technique for ^1H and ^{13}C. The result of NMR scanned, ^1H NMR δ 9.76 (*s*, H-3), δ 8.69 (*s*, H-13), δ 8.11 (*d*, $J_{11\text{-}12}$ = 9.00 Hz) H-11, δ 8.00 (*d*, $J_{11\text{-}12}$ = 9.00 Hz) H-12, δ 7.65 (*s*, H-1), δ 6.96 (*s*, H-4), δ 6.10 (s, -OCH_2O), δ 4.92 (*t*, J = 6 Hz) H_2-6, δ 4.20 (*s*, H-9)-OCH_3, δ 4.15 (*s*, H-10)- OCH_3, δ 3.25 (t, J = 6 Hz) H_2-5, showed in Table 3 and Figure 48. ^{13}C- NMR data to showed in the Table 4 and Figure 51.

Table 1. Summary of ^{1}H- NMR data and assignments for Cf_2-crystal.

Chemical shift δ (ppm)		
^{1}H-NMR of Cf_2	^{1}H-NMR of Jatrorrhizine (Hsieh et al., 2004)	^{1}H-NMR of Columbamine (Hsieh et al., 2004)
9.72 (*s*, H-8)	9.51 (*s*, H-8)	9.45 (*s*, H-8)
8.75 (*s*, H-13)	8.42 (*s*, H-13)	8.45 (*s*, H-13)
8.08 (*d*, $J_{11\text{-}12}$ = 9.15 Hz) H-12	8.01 (*d*, $J_{11\text{-}12}$ = 9.20 Hz) H-12	8.11 (*d*, $J_{11\text{-}12}$= 8.8Hz) H-12
7.95 (*d*, $J_{11\text{-}12}$ = 9.15 Hz) H-11	7.77 (*d*, $J_{11\text{-}12}$ = 9.20 Hz) H-11	7.58 (*d*, $J_{11\text{-}12}$ = 8.8 Hz) H-11
7.66 (*s*, H-1)	7.60 (*s*, H-1)	7.52 (*s*, H-1)
6.87 (s, H_4)	6.83 (*s*, H-4)	6.80 (*s*, H-4)
4.91 (*t*, *J* = 6 Hz)H_2-6	4.90 (*t*, *J* = 6 Hz)H_2-6	4.97 (*t*, *J* = 6.4 Hz) H_2-6
4.21 (*s*, H-9)-OCH_3	4.14 (*s*, H-9)-OCH_3	4.08 (*s*, H-9)-OCH_3
4.11 (*s*, H-10)- OCH_3	4.14 (*s*, H-10)- OCH_3	4.08 (s, H-10)- OCH_3
4.03 (*s*, H-2)- OCH_3	4.02 (*s*, H-2)- OCH_3	3.98 (s, H-2)- OCH_3
3.21 (*t*, *J* = 6 Hz)H_2-5	3.24 (*t*, *J* = 6 Hz)H_2-5	3.30 (*t*, *J* = 6.4 Hz) H_2-5

Table 2. Summary of ^{13}C NMR data and assignments for Cf_2.-crystal.

Carbon	Chemical shift δ (ppm)	
	^{13}C-NMR of Cf_2	^{13}C-NMR of Jatrorrhizine (Keawpradub, 1992)
C (1)	110.03	109.20
C (2)	151.71	149.30
C (3)	145.60	144.50
C (4)	115.89	115.50
C (4a)	135.39	133.90
C (5)	27.63	26.20
C (6)	57.39	56.00
C (8)	146.06	143.40
C (8a)	119.41	118.00
C (9)	151.74	149.40
C (10)	149.63	143.40
C (11)	124.40	123.10
C (12)	128.06	126.80
C (12a)	130.20	129.50
C (13)	120.94	118.00
C (13a)	140.19	139.50
C (13b)	123.15	120.70
2-OCH_3	57.67	57.10
9-OCH_3	62.54	61.80
10-OCH_3	56.98	55.50

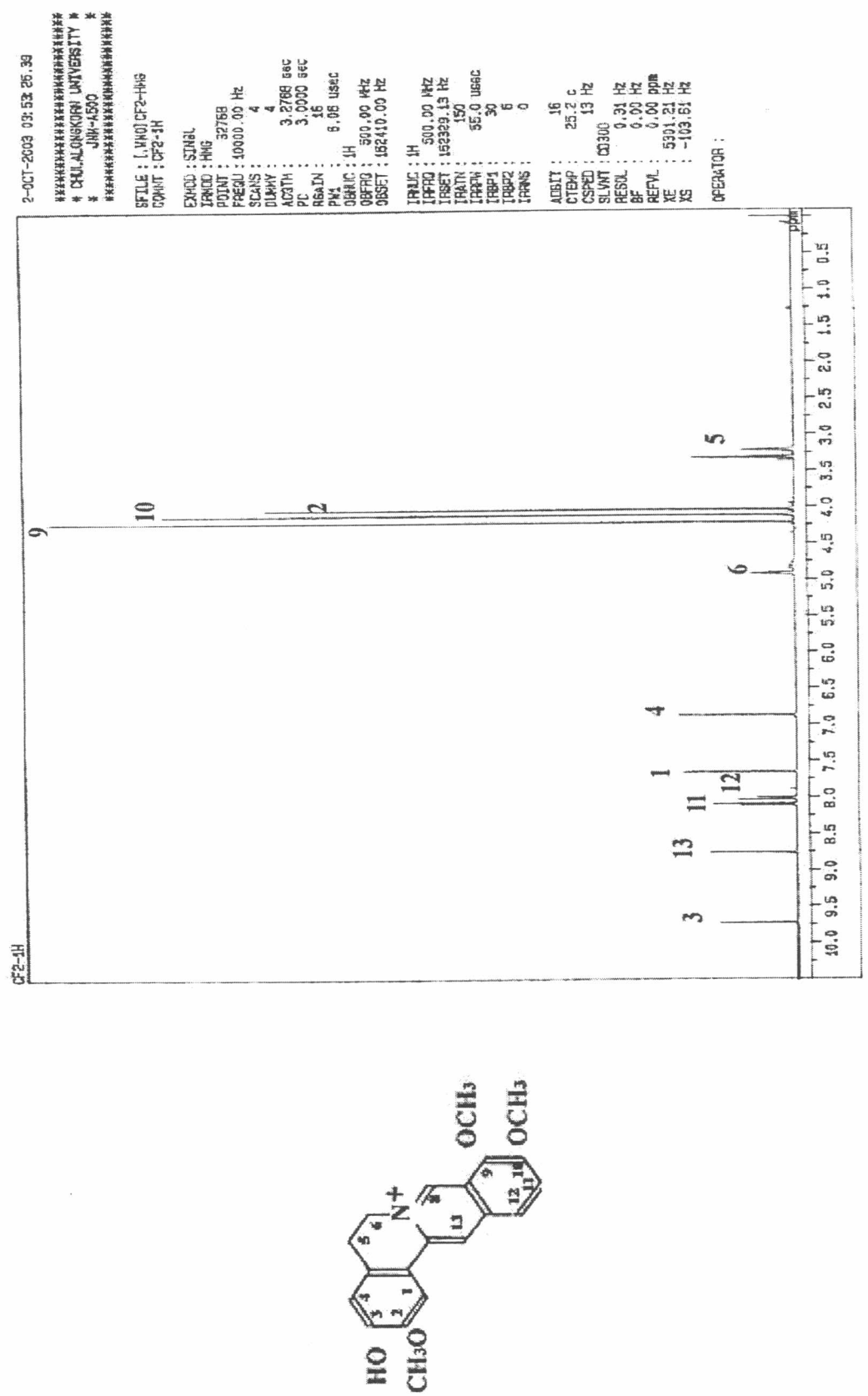


Figure 45. ^{1}H-NMR spectrum of Cf_2 fraction in CD_3OD.

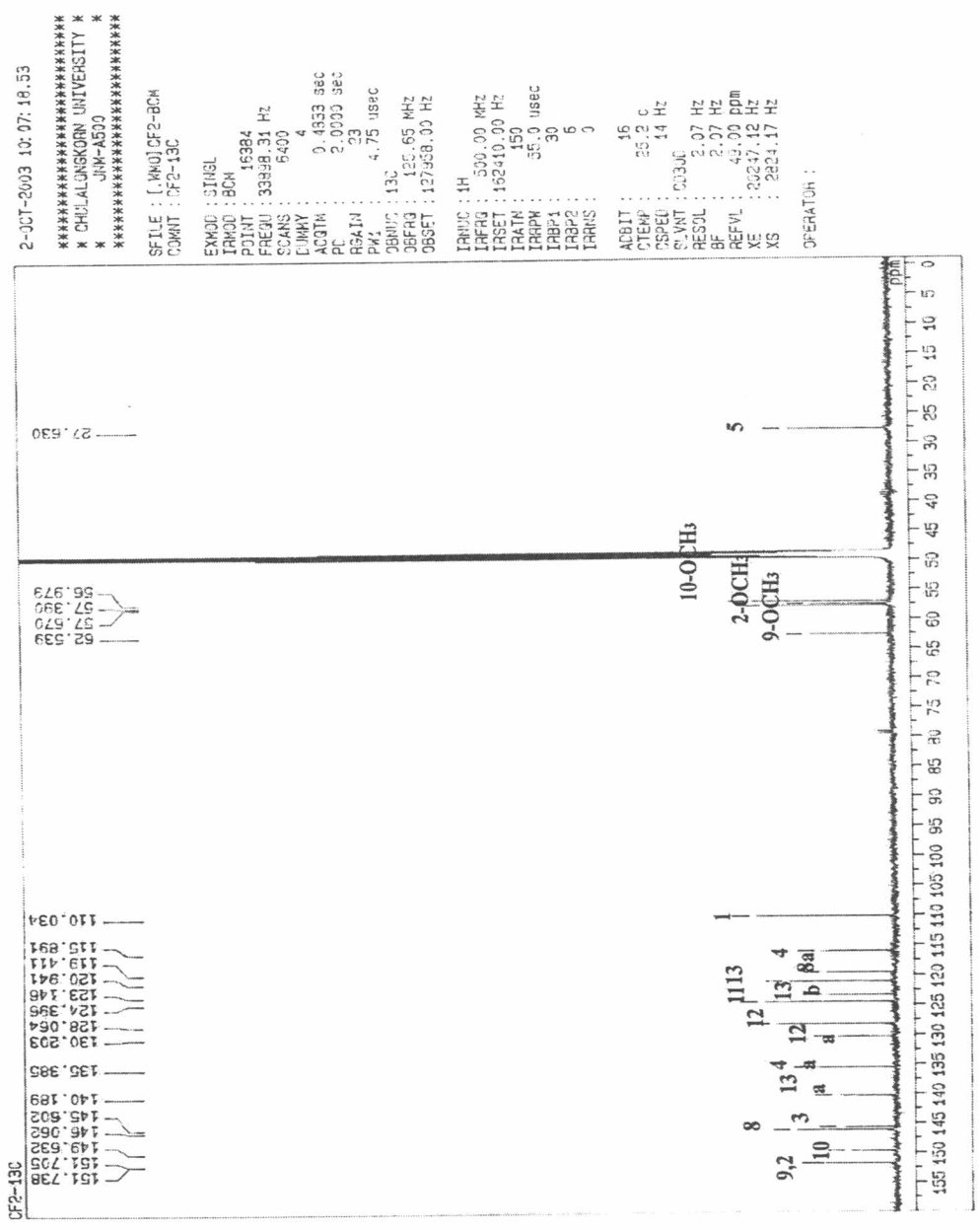


Figure 46. ^{13}**C-NMR Spectrum of Cf$_2$-crystal in CD$_3$OD.**

Figure 46. ^{13}C-NMR Spectrum of Cf_2-crystal in CD_3OD.

Table 3. Summary of ^{1}H- NMR data and assignments for Cf_3 fraction.

Chemical shift δ (ppm)		
^{1}H-NMR of Cf_3	^{1}H-NMR of Berberine hemisulfate	^{1}H-NMR of Berberine (Siwon, 1980)
9.76 (*s*, H-3)	9.76 (*s*, H-3)	
8.69 (*s*, H-13)	8.61 (*s*, H-13)	8.68(*s*, H-13)
8.11 (*d*, $J_{11\text{-}12}$= 9.00 Hz) H-11	8.07 (*d*, $J_{11\text{-}12}$= 9.20 Hz) H-11	8.11(*d*, $J_{11\text{-}12}$ = 8.90 Hz) H-11
8.00 (*d*, $J_{11\text{-}12}$=9.00 Hz) H-12	7.97(*d*, $J_{11\text{-}12}$ = 9.20 Hz) H-12	7.99 (*d*, $J_{11\text{-}12}$ = 8.90 Hz) H-12
7.65 (*s*, H-1)	7.58 (*s*, H-1)	7.64 (*s*, H-1)
6.96 (*s*, H-4)	6.93 (*s*, H-4)	6.94 (*s*, H-4)
6.10 (*s*, -OCH_2O)	6.08 (*s*, -OCH_2O)	6.12 (*s*, -OCH_2O)
4.92 (*t*, *J* = 6 Hz)H_2-6	4.93 (*t*, *J* = 6 Hz) H_2-6	
4.20 (*s*, H-9)-OCH_3	4.18 (*s*, H-9)-OCH_3	4.23 (*s*, H-9)-OCH_3
4.11 (*s*, H-10)- OCH_3	4.07 (*s*, H-10)- OCH_3	4.12 (*s*, H-10)- OCH_3
3.25 (t, *J* = 6 Hz)H_2-5	3.245 (t, *J* = 6 Hz)H_2-5	

Table 3. Summary of ^{13}C NMR data and assignments for Cf_3 , Berberine hemisulfate.

Carbon	Chemical shift δ (ppm)		
	^{13}C-NMR of Cf_3	^{13}C-NMR of Berberine hemisulfate	^{13}C-NMR of Berberine (Keawpradub, 1992)
C (1)	106.55	106.46	105.50
C (2)	149.95	149.81	147.70
C (3)	152.03	151.97	149.80
C (4)	109.38	109.38	108.50
C (4a)	131.88	131.96	130.70
C (5)	28.19	28.19	26.40
C (6)	57.19	57.21	55.20
C (8)	146.38	146.46	145.50
C (8a)	123.34	123.24	121.40
C (9)	145.78	145.68	143.70
C (10)	152.18	152.08	150.40
C (11)	128.10	127.98	126.70
C (12)	124.50	124.58	123.60
C (12a)	135.19	135.04	132.90
C (13)	121.52	121.39	120.30
C (13a)	139.70	139.55	137.50
C (13b)	121.88	121.81	120.50
2, 3-OCH_2O	103.667	103.634	102.1
9-OCH_3	62.539	62.605	62.0
10-OCH_3	57.653	57.604	57.1

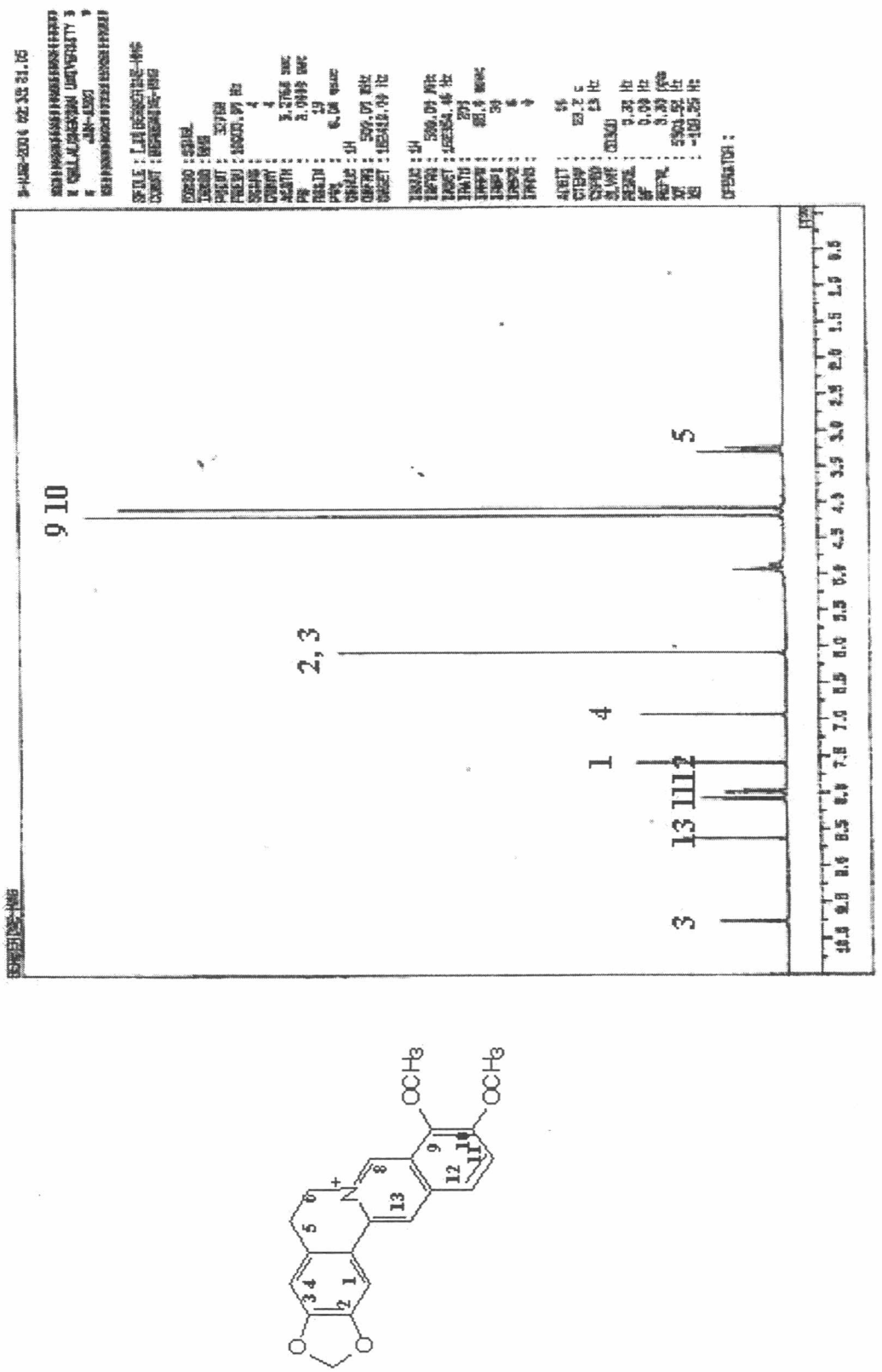


Figure 47. ^{1}H-NMR Spectrum of Berberine hemisulfate in CD_3OD.

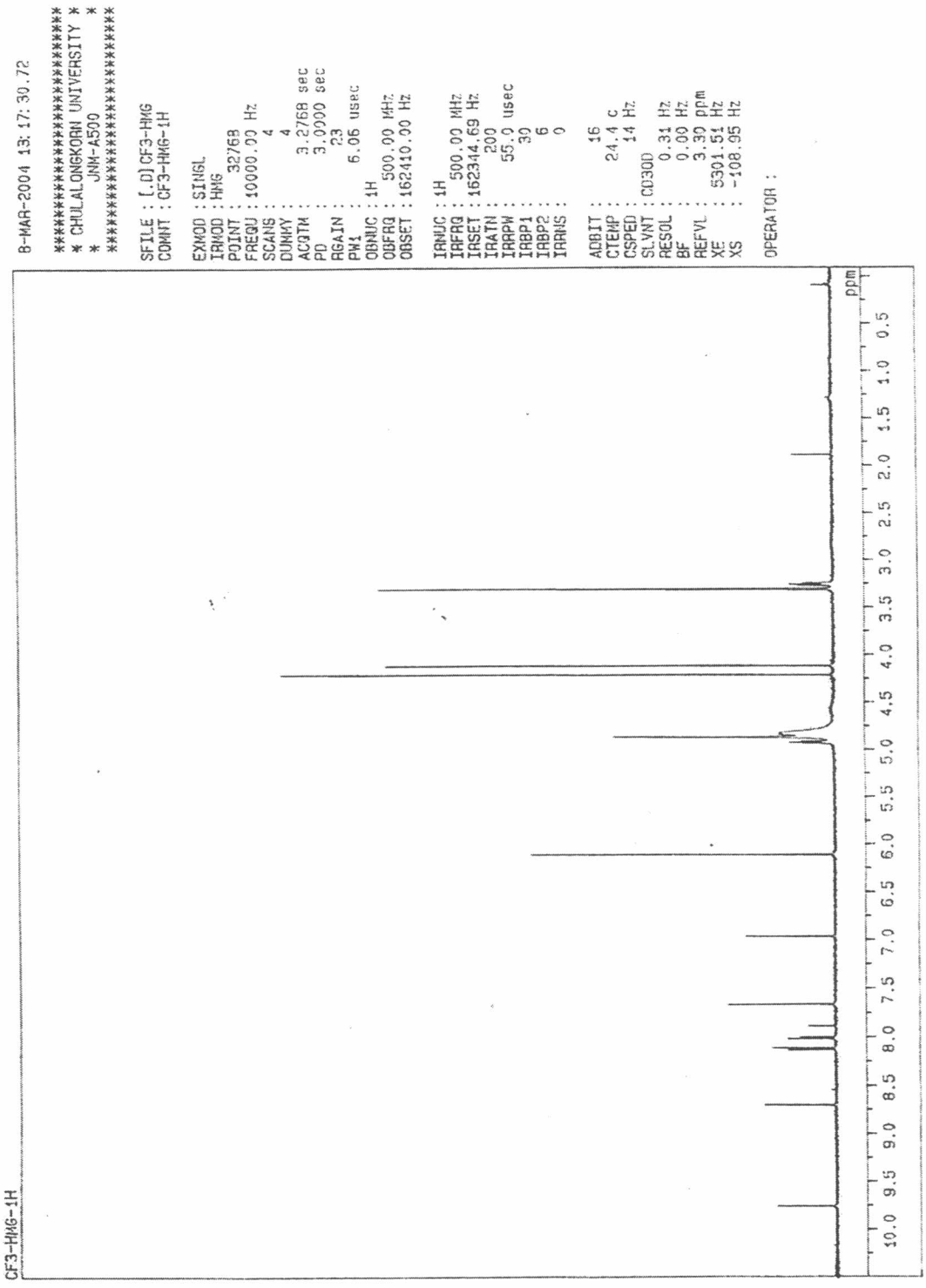


Figure 48. ^{1}H-NMR Spectrum of Cf_3 fraction in CD_3OD.

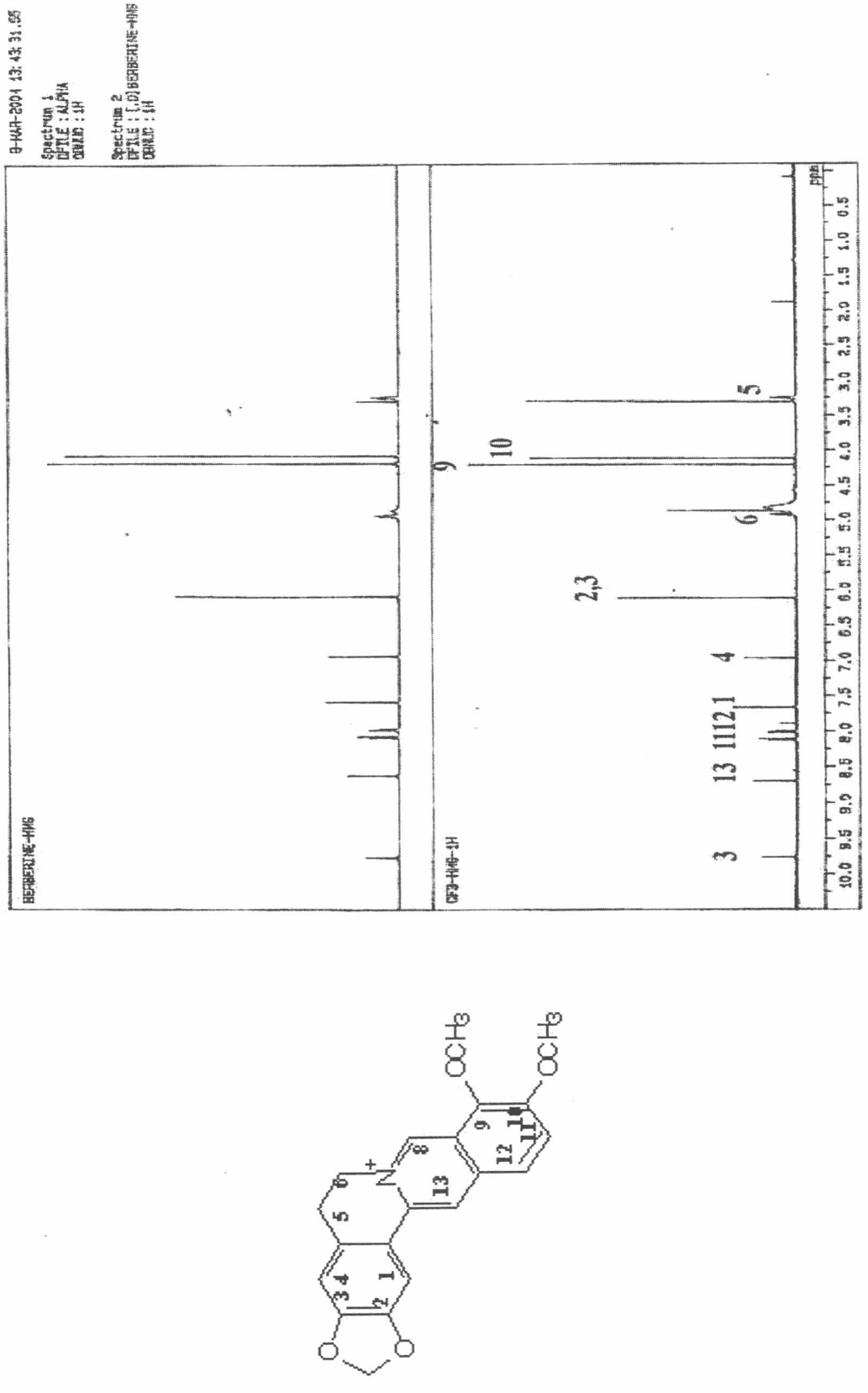


Figure 49. Comparison ^{1}H-NMR Spectrum of Berberine hemisulfate and Cf_3 fraction in CD_3OD.

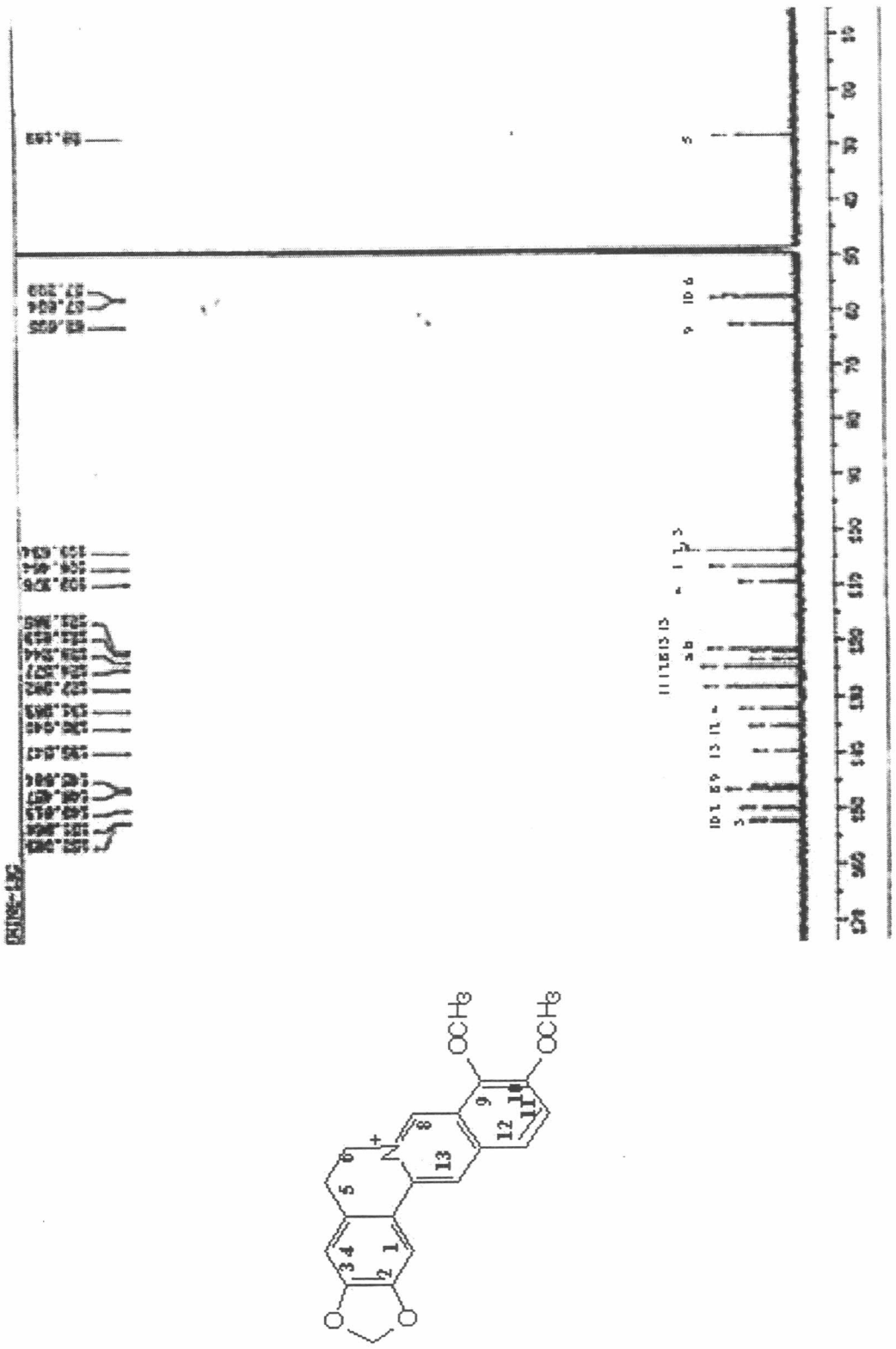


Figure 50. ^{13}C-NMR Spectrum of Berberine hemisulfate in CD_3OD.

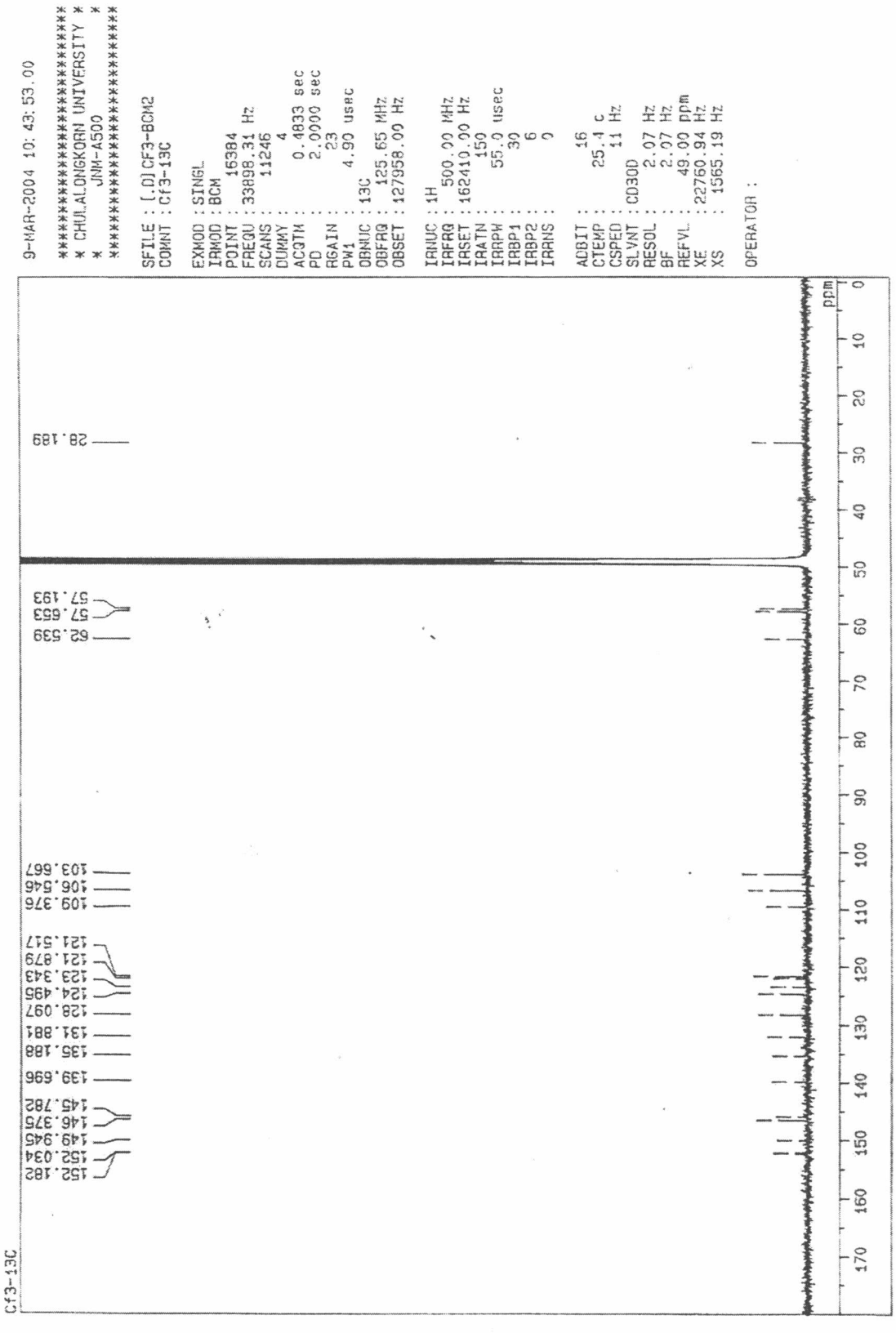


Figure 51. ^{13}**C-NMR Spectrum of** Cf_3 **fraction in** CD_3OD**.**

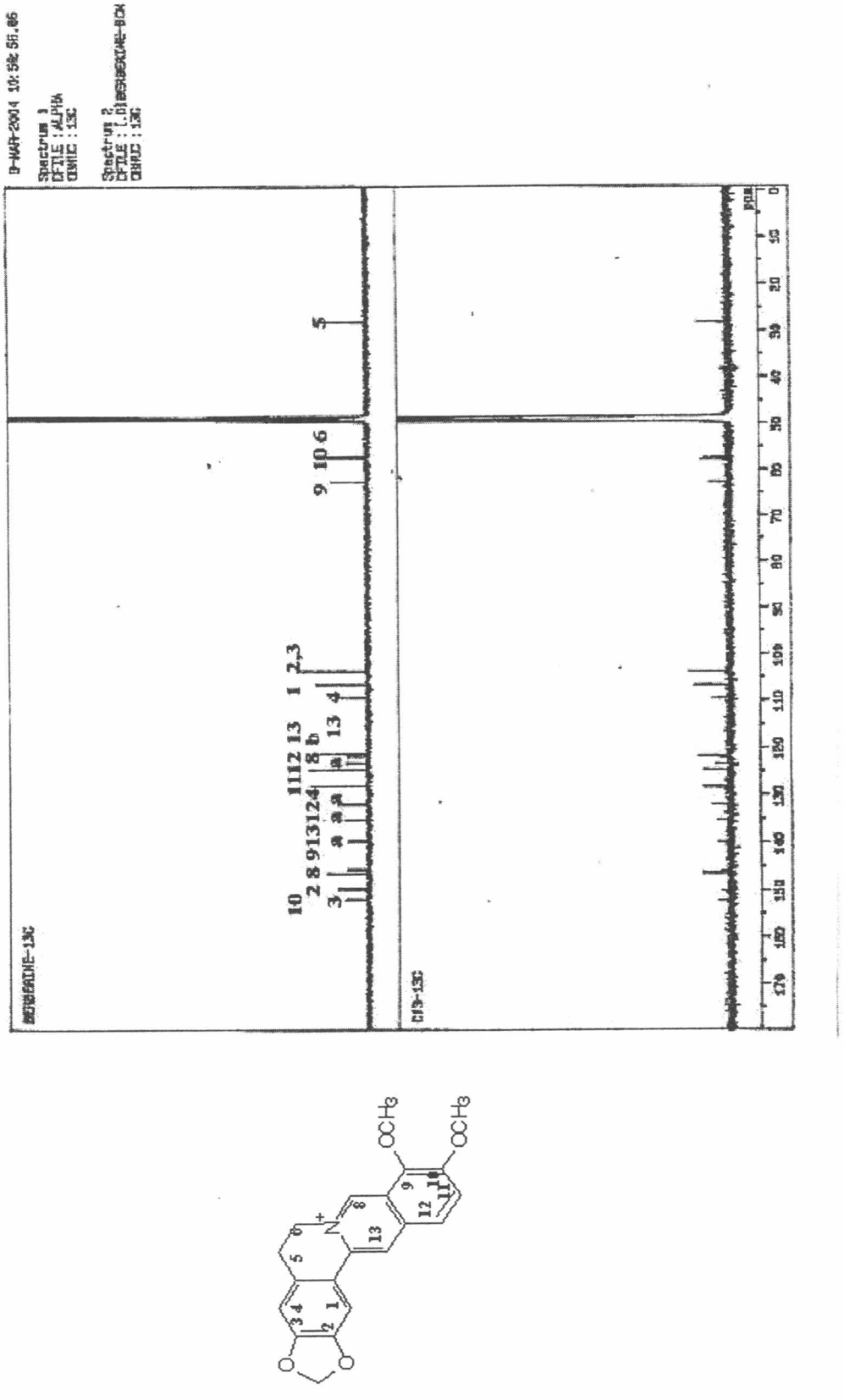


Figure 52. Comparison ^{13}C-NMR Spectrum of Berberine hemisulfate and Cf_3 fraction in CD_3OD.

The 500 MHz ^{1}H-NMR assignments of the isolated Cf fractions based on the nature that they differ from one another in the type, number and placement of various oxygen functions (usually –OH, -OCH_3 and occasionally –OCH_2O) on the two aromatic ring, A and D. The substituents are usually at C (2), C (3) and either at C (9) and C (10). Less frequent is substitution at C (1), C (5), C (8), C (11), C (12) and C (13) .

The substituents carbon of Cf_2-crystal and Cf_3 are different at 2 and 3. The oxygenation of ring D was determined by the multiplicity of the aromatic protons. In the case of 2 and 3-substituents, the H-1 and H-4 signals can readily be recognized as two 1-proton singlets while the *ortho*-coupling signals of H-11 and H-12 appear as doublets.

The ^{1}H-NMR chemical shift assignment of Cf_2-crystal and Cf_3 have 1-proton singlet of H-8 and H13 as shown in spectrum of Cf_2-crystal and Cf_3. The signals of methoxyl substitution can readily be assigned as the 3 protons singlet appeared in the region 4.03 and 4.21 ppm.

The determination for each proton of Cf_2-crystal and Cf_3 are straightforward. The difference of these both fractions are presented by the substitutions at C-2 and C-3. The methylenedioxy group of Cf_3 fraction showed 2 protons singlet at 6.10 ppm., carbon at 103.67 (C-2, 3) ppm. as shown in Figure 48 and 51. The methylenedioxy group of berberine hemisulfate showed a 2 protons singlet at 6.08 ppm., carbon at 103.63 ppm. as shown in Figure 47 and 49. The methoxyl group of Cf_3 fraction showed 3 protons singlet at 4.20 (H-9) and 4.11 (H-10), Carbon (C-9, C-10) at 145.78 and 152.18, and methoxyl group of berberine hemisulfate showed 3 protons singlet at 4.18 (H-9) and 4.07 (H-10), Carbon (C-9, C-10) at 145.68 and 152.08 ppm. The

methylenedioxy and methoxyl group of Cf_3 fraction and berberine hemisulfate showed protons singlet and carbon at the same region.

The methoxyl group of Cf_2-crystal showed 3 protons singlet at 4.21(H-9), 4.11 (H-10) and 4.03 (H-2), Carbon (C-9, C-10 and C-2) at 62.54, 56.98 and 57.67 as shown in Figures 45 and 46.

7. Mass spectrum

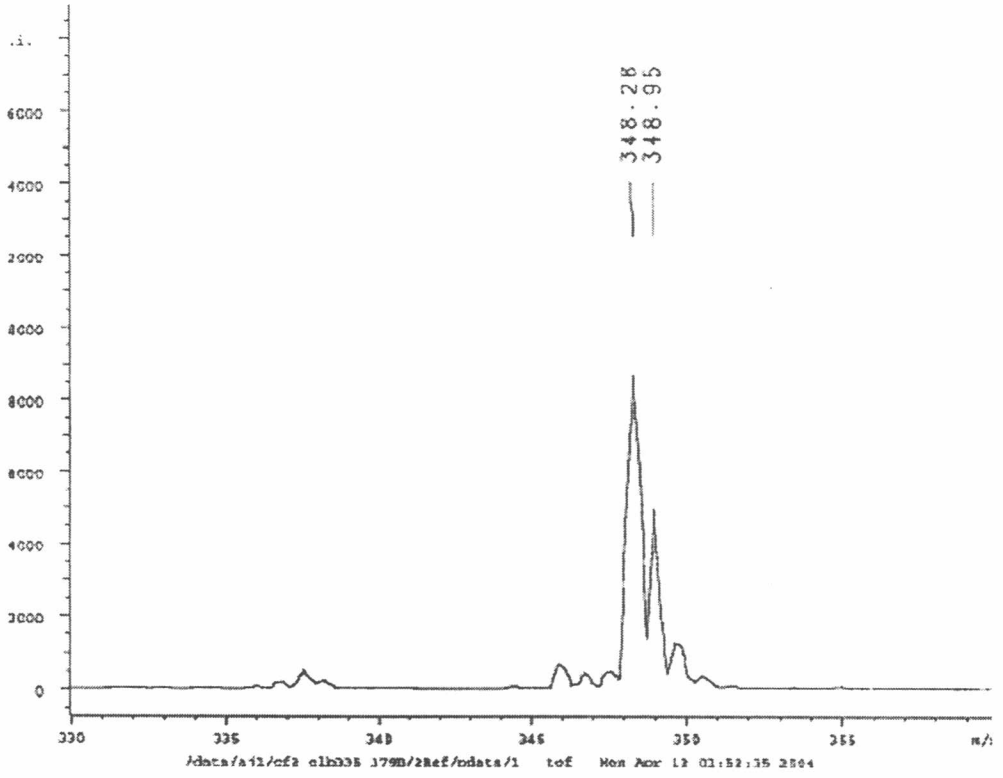


Figure 53. Mass spectrum of Cf_2

Mass spectrum of Cf_2 showed mass at 347 (M-1) as jatrorrhizine ($C_{20}H_{20}O_4N^+$).

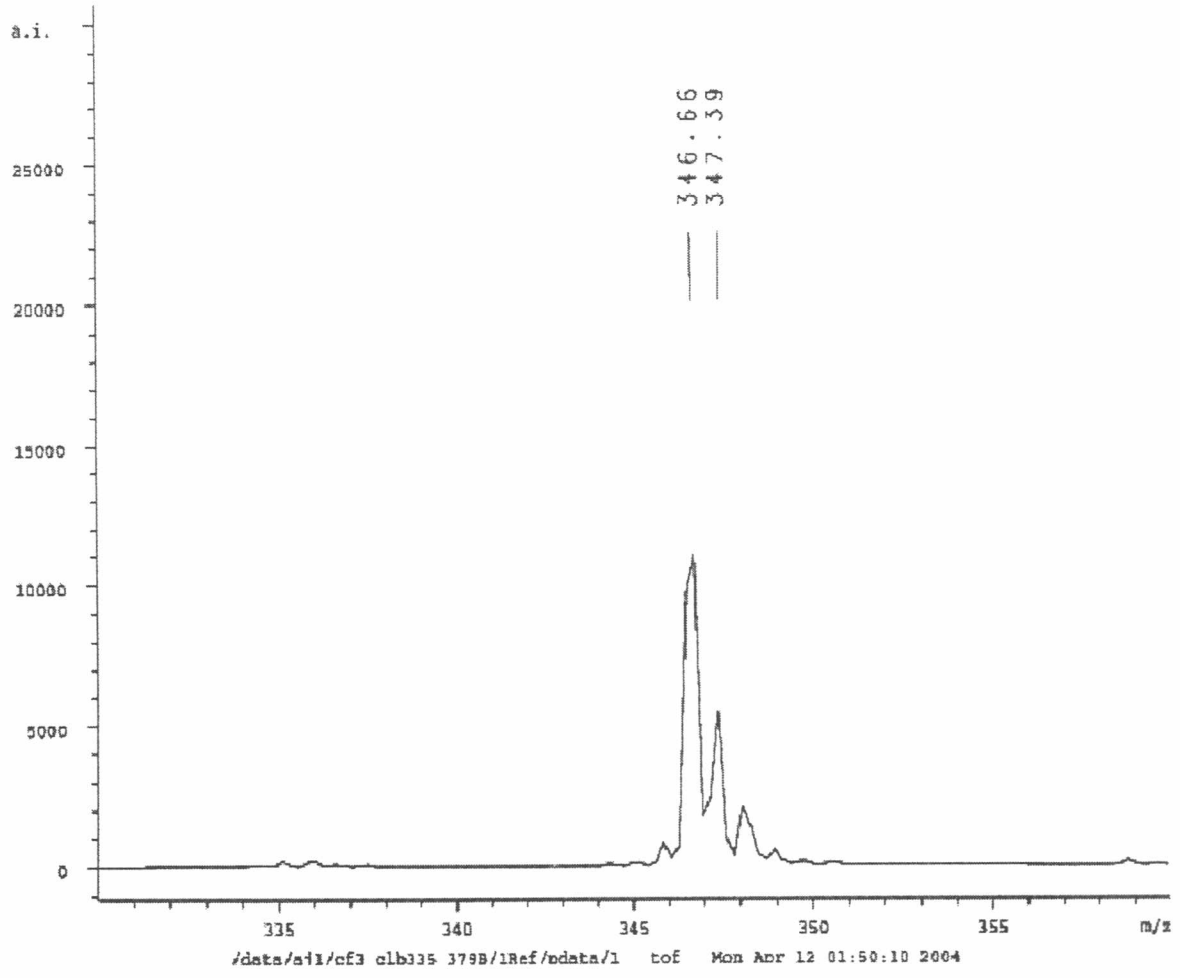


Figure 54. Mass spectrum of Cf_3

Mass spectrum of Cf_3 showed mass at 345 (M-1) as berberine ($C_{20}H_{18}O_4N^+$).

8. Hypoglycemic effect of *C. fenestratum* fraction in normal male Wistar rats

8.1 Effect of single-oral dose of *C. fenestratum* (Cf_3) fraction, Berberine hemisulfate and crude water extract of *C. fenestratum* (CE) in oral glucose tolerance test (OGTT)

The effect of single oral dose of Cf_3 on blood glucose concentration in normal male Wistar rats had been studied. Treatment group 1, 2, 3 received Cf_3 at dose 20, 60, 180 mg/kg body weight, respectively. Treatment group 4, 5, 6 received berberine hemisulfate at dose 20, 60, 180 mg/kg body weight, respectively. Treatment group 7 received CE at dose 1 g/kg body weight and control group fed distilled water at 30 min before glucose feeding. Cf_3-treated rats showed significantly decrease ($p<0.05$) in blood glucose concentration when compared with control group at 30 min after glucose feeding (Figure 55). Blood glucose concentration of treatment group 1-3 were 141.33 ± 6.257, 110.33 ± 8.14 and 111.5 ± 12.88 mg/dl, respectively and control group was 169 ± 4.54 mg/dl; where percentage decreases were 16.37, 34.71 and 34.02 %, respectively. Blood glucose concentration of treatment groups 4-6 were 138.75 ± 7.45, 130.33 ±5.434 and 125.33 ± 7.309 mg/dl, respectively; where percentage decreases were 17.89, 22.88 and 25.84 %, respectively.

8.2 Effect of single-oral dose of Cf_2 fraction and CE in oral glucose tolerance test (OGTT)

The effect of single oral dose of Cf_2 on blood glucose concentration in normal male Wistar rats had been studied. Treatment group 1 and 2 received Cf_2 at dose 20 and 60 mg/kg body weight, respectively. Treatment group 3 received CE at dose

1g/kg body weight and control group fed distilled water at 30 min before glucose feeding. Blood glucose concentration of treatment groups 1 and 2 (Cf_2) were 141.25 ± 20.76, 142.91 ± 12.28 mg/dl, respectively and control group was 166.5 ± 13.59 mg/dl; where percentage decreases were 15.16 and 14.16%, respectively. Blood glucose concentration of treatment group 3 (CE) was 149.87 ± 9.627 mg/dl.

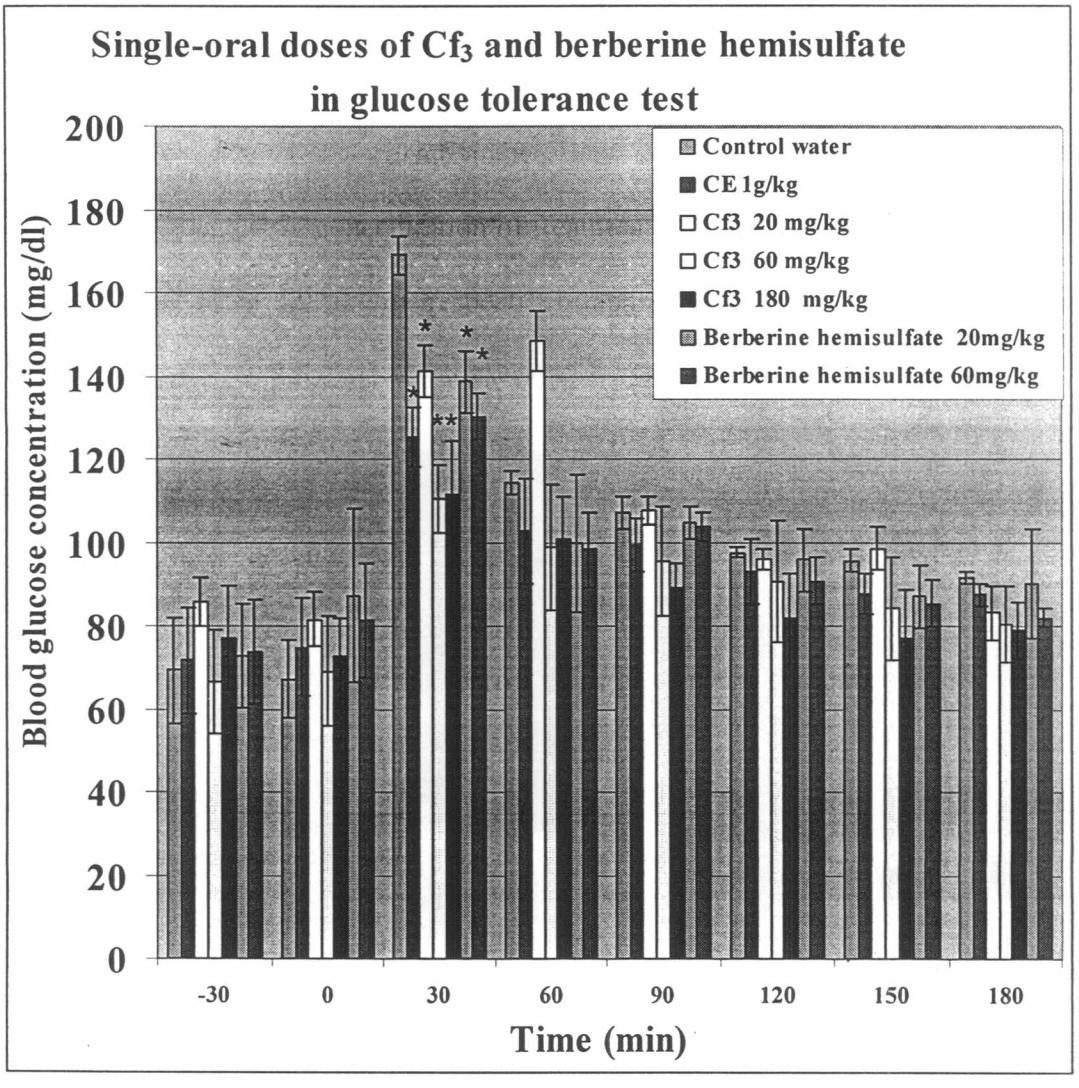


Figure 55. Effect of Cf_3, berberine hemisulfate and CE on blood glucose in normal Wistar rats; 3 hr-duration.

Symbols represents Mean ± S.E.M.

* Significant difference ($p<0.05$) compared to control.

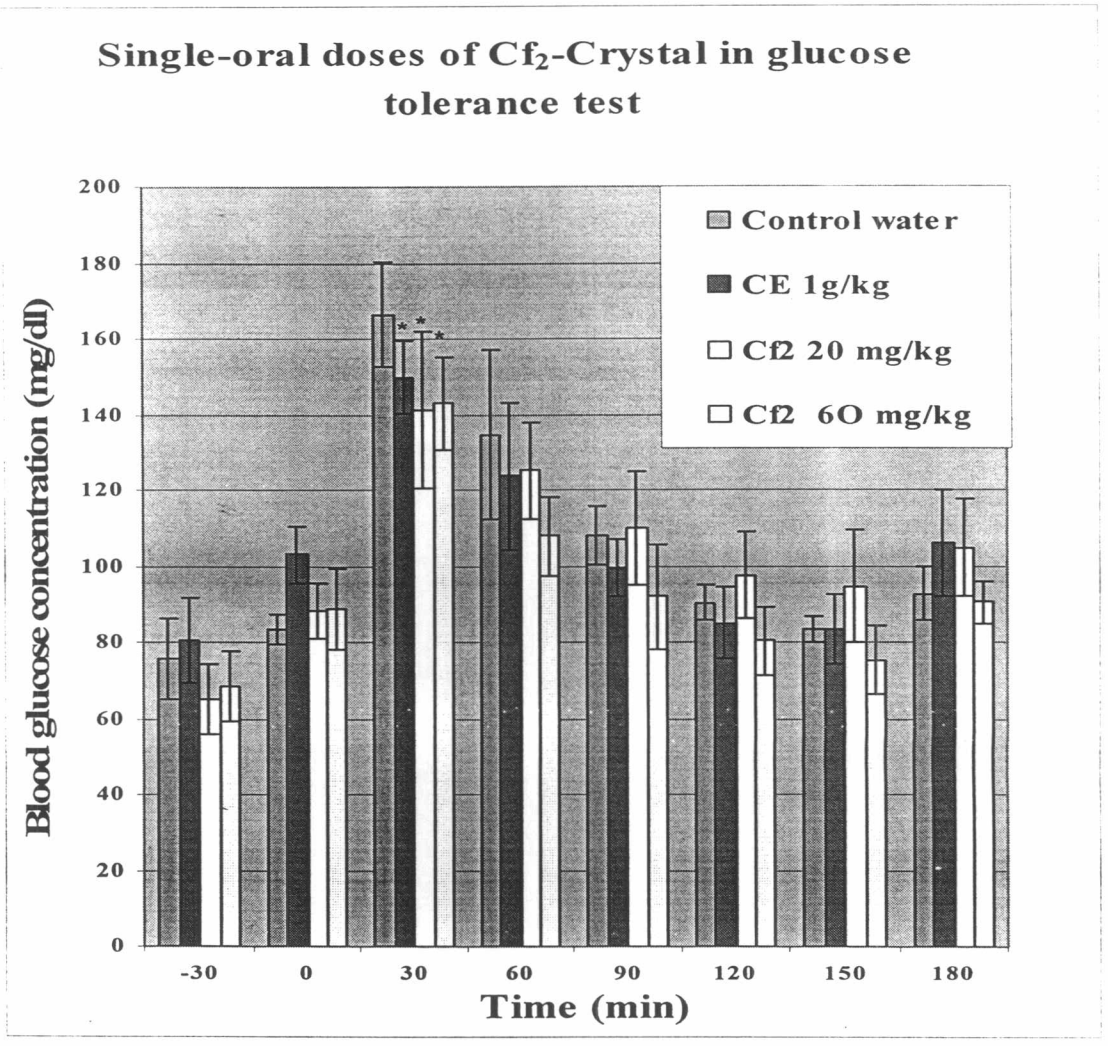


Figure 56. Effect of Cf_2 and CE on blood glucose in normal Wistar rats; 3 hr-duration.

Symbols represents Mean ± S.E.M.

* Significant difference ($p<0.05$) compared to control.