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Identification of Naturally Occurring Carbazole Alkaloids Isolated from *Murraya koenigii* and *Glycosmis pentaphylla* by the Preparation of HPLC Fingerprint

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Abstract

The plants *Murraya koenigii* and *Glycosmis pentaphylla* are rich sources of different carbazole alkaloids. A number of monomeric carbazole alkaloids with C_{13}, $C_{18,}$ and C_{23} carbon frames and a good number of dimeric carbazole alkaloids were isolated from these two plants. Scientists are still working on these two plants in search of more and more novel compounds. Many of these alkaloids have potential biological activities. Scientists have determined the structures of these compounds by detailed analysis of spectral data like UV, IR, Mass,^{1}H NMR, and ^{13}C NMR (1D and 2D). These procedures require expertise in spectral data analysis and huge time, and also these instruments are very costly. In this paper, I report the preparation of the HPLC fingerprint of some known carbazole alkaloids. These HPLC data will be helpful in quick and unambiguous identification of the natural products.

Keywords: Carbazole alkaloids; *Murraya koenigii*; *Glycosmis pentaphylla*; HPLC fingerprint.

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1. Introduction

Desoky [1] has published his work on isolating several phytosterols from *Murraya exotica* using column chromatography accompanied by HPLC. Similarly, several flavonoids from fruits of *Murraya paniculata* were purified by Ferracin *et al*. [2] using reverse-phase - HPLC. The use of HPLC is limited to the separation and purification of organic molecules. However, the HPLC profile can also be used for the identification and authenticity check of the compounds. In 2021, Chang and the group reported screening of anti-lipase components of *Artemisia Argui* leaves based on spectrum-effect relationship and HPLC-MS/MS [3].

Murraya koenigii, commonly known as curry leaf tree, and Glycosmis pentaphylla, commonly known as 'toothbrush plant,' belongs to the family Rutaceae and are rich sources of carbazole alkaloids [4-6]. The monomeric and dimeric carbazole alkaloids isolated from these plants have important biological activities [7-9]. Many scientists working in this field have also prepared several derivatives of these naturally occurring

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carbazole alkaloids [10-13]. In our present work, five carbazole alkaloids viz. murrayanine, girinimbine, koenidine, mahanimbine, and o-methylmurrayamine-A were isolated from the leaves and bark of *Murraya koenigii*. Two more carbazole alkaloids glycozoline and glycozolidine were obtained from the root bark of *Glycosmis pentaphylla*. Structures of all the isolated compounds were determined by a detailed investigation of 1D and 2D NMR spectral data analysis. HPLC fingerprint of each compound was prepared using a Water 515 HPLC pump. In this paper, the details of sample preparation for HPLC, selection of appropriate solvent system, selection of the optimum concentration of the compounds, and their retention time will be discussed. These data will be helpful in the quick and unambiguous identification of natural products.

2. Materials and Methods

2.1. *Drugs and chemicals*

HPLC grade solvents such as methanol, petroleum ether, chloroform, and other necessary chemicals and reagents were collected from Merck, Germany.

2.2. *General experimental procedure*

HPLC fingerprints were prepared using a Waters 515 HPLC pump equipped with model 680 automated gradient controller and Waters 2487 dual λ absorbance detector. Solvents (HPLC grade, Merck) were filtered by using a Millipore system, and analyses were performed on a Waters RP C18 (7 μm) column (300 mm × 7.8 mm, ID) in isocratic mode. For injection into the HPLC system, solutions of the pure compounds were prepared in methanol. Injection volume was 50 μL for all the cases. All the sample solutions were detected at a UV wavelength of 254 nm, using methanol as a mobile phase in each case. ^{1}H NMR spectra were recorded using a BRUKER AVANCE 600 MHz NMR with TLC-cryoprobe using TMS as an internal standard.

2.3. *Plant material*

The leaves and stem bark of *Murraya koenigii* were collected from Shantiniketan, West Bengal, India. The root bark of *Glycosmis pentaphylla* was collected from Jhargram, West Bengal, India.

2.4. *Extraction and separation*

The stem bark of *Murraya koenigii* (1 kg) was dried in air and cut into small pieces. Then, at that point, it was extracted with MeOH at 25 ^{o}C and concentrated utilizing a vacuum evaporator at 40 ^{o}C. Then, at that point, the MeOH extract (8 g) was fractionated into three sections: petroleum ether, ethyl acetate, and water. The ethyl acetate portion was kept undisturbed for 24 h. A solid was precipitated from it, which was insoluble in

chloroform. The solid was filtered, washed with cold chloroform, and dried. The compound was described to be girinimbine **1** by TLC (Benzene) and examination of the spectroscopic information with reference. The EtOAc extract was chromatographed on a column of silica gel (size 60-120). At first, elution was completed with petrol ether (bp 60-80 ºC) trailed by various combinations of petroleum ether and benzene (3:1, 1:1, 1:3 and 100 % benzene) and again various combinations of benzene and chloroform (200 mL each). Parts giving comparable spots were blended. Rehashed chromatography of the parts brought about the isolation of the rest of girinimbine **1** and murrayanine **2** (with Petroleum ether: benzene = 1:1 as eluent).

Likewise, the powdered leaves of *Murraya koenigii* (2 Kg.) were extracted with MeOH at around 25 °C and concentrated utilizing a vacuum evaporator at 40 ºC, and 15 g of concentrate was acquired. The MeOH separate was chromatographed on a column of silica gel (size 60-120). First elution was done with petrol ether (bp 60-80 ºC) trailed by various combinations of petrol ether and benzene (3:1, 1:1, 1:3 and 100 % benzene) and again various combinations of benzene and chloroform (200 mL. each). Divisions giving comparative spots in TLC were blended. Rehashed chromatography of the divisions brought about the separation of koenidine **3**, mahanimbine **4,** and O-methylmurrayamine-A **5** (with benzene).

2 kg of powdered root bark of *Glycosmis pentaphylla* was extracted with pet ether followed by methanol at room temperature. The petrol ether extract (10 g) was chromatographed on a column of neutral alumina. First step elution was done with pet ether followed by various combinations of petrol ether-$CHCl_3$ (3:1, 1:1 and 1:3) and MeOH in $CHCl_3$ (5 %, 10 %, 20 % and 30 %; 200 mL each). Glycozoline **6** and glycozolidine 7 were obtained from this section with a 3:1 combination of pet ether-$CHCl_3$.

3. Results and Discussion

The stem bark of *Murraya koenigi* was extracted with MeOH. The MeOH extract was fractionated into three parts: petroleum ether, ethyl acetate, and aqueous. The EtOAc extract was chromatographed on a silica gel column yielding girinimbine **1** [14] and murrayanine **2** [15]. The air-dried powdered leaves of *Murraya koenigii* were extracted with MeOH. The MeOH extract was chromatographed on a silica gel column, yielding three more known pyrano carbazoles, *viz.* koenidine **3** [16], mahanimbine **4** [17], and O-methylmurrayamine-A **5** [18] (Fig. 1). Structures of these compounds were determined by the detailed NMR spectral data analysis (Fig. 4) and then by comparing these data with that of the reported compounds.

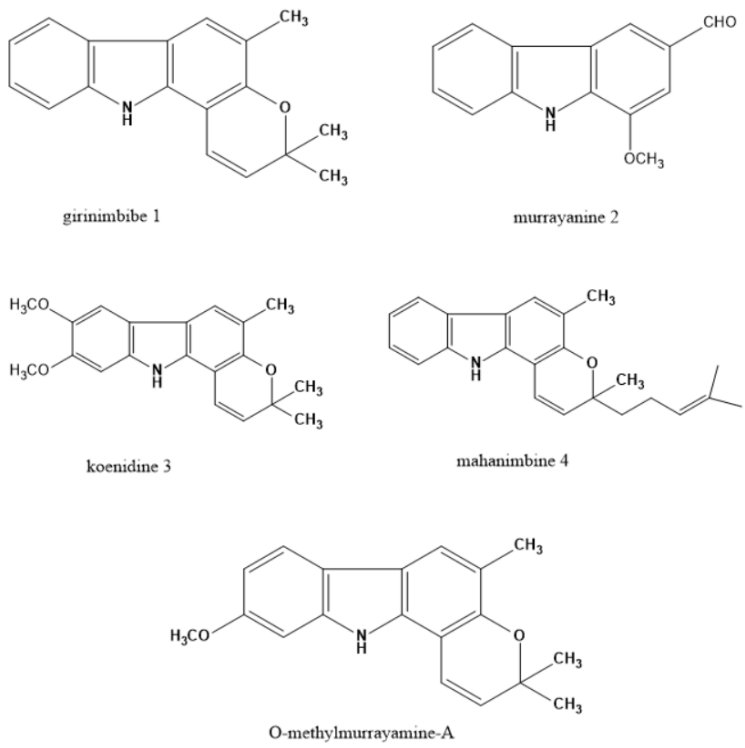


Fig. 1. Structures of the compounds isolated from *Murraya koenigii.*

Petroleum ether extract of the root bark of *Glycosmis pentaphylla* on chromatographic resolution over neutral alumina yielded glycozoline **6** [19] and glycozolidine **7** [20] (Fig. 2). Structures of these two compounds were also determined by NMR spectral data analysis (Fig. 4) and then by comparing these data with that of the reported compounds.

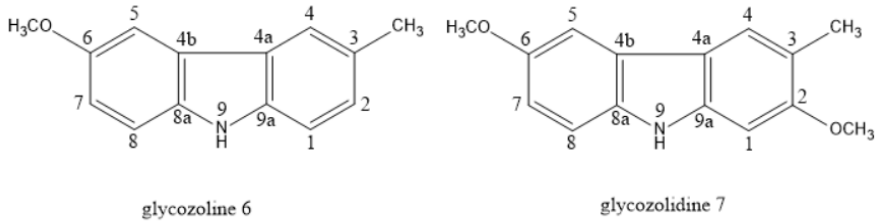


Fig. 2. . Structures of the compounds isolated from *Glycosmis pentaphylla.*

HPLC chromatogram of girinimbine **1**, murrayanine **2**, koenidine **3**, mahanimbine **4** and O-methylmurrayamine-A **5** showed retention time at 19.338 min. (conc. 0.5 mg/mL,

flow rate 0.6 mL/min), 15.579 min. (conc. 0.18 mg/mL, flow rate 0.6 mL/min), 8.662 min. (conc. 0.01 mg/mL, flow rate 1.2 mL/min), 12.993 (conc. 0.056 mg/mL, flow rate 1.2 mL/min) and 9.608 min. (conc. 0.03 mg/mL, flow rate 1.2 mL/min) respectively (Table 1, Fig. 3).

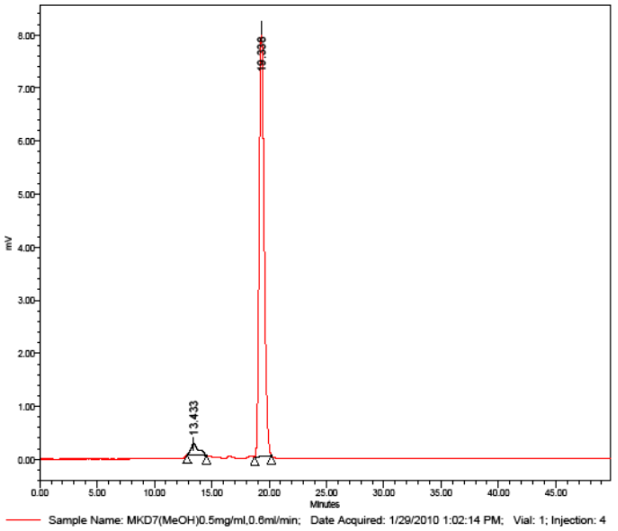
HPLC chromatogram of glycozoline **6** and glycozolidine **7** showed retention time at 16.339 min. (conc. 0.25 mg/mL, flow rate 0.6 mL/min) and 16.252 min. (conc. 0.25 mg/mL, flow rate 0.6 mL/min) (Table 2, Fig. 3).

Table 1. HPLC fingerprint data for the carbazole alkaloids isolated from *Murraya koenigii*.

Compound No.	Compound	Conc. (mg/mL)	Flow rate (mL/min)	Retention time (min
1	girinimbine	0.5	0.6	19.338
2	murrayanine	0.18	0.6	15.579
3	koenidine	0.01	1.2	8.662
4	mahanimbine	0.056	1.2	12.993
5	O-methylmurrayamine-A	0.03	1.2	9.608

Table 2. HPLC fingerprint data for the carbazole alkaloids isolated from *Glycosmis pentaphylla.*

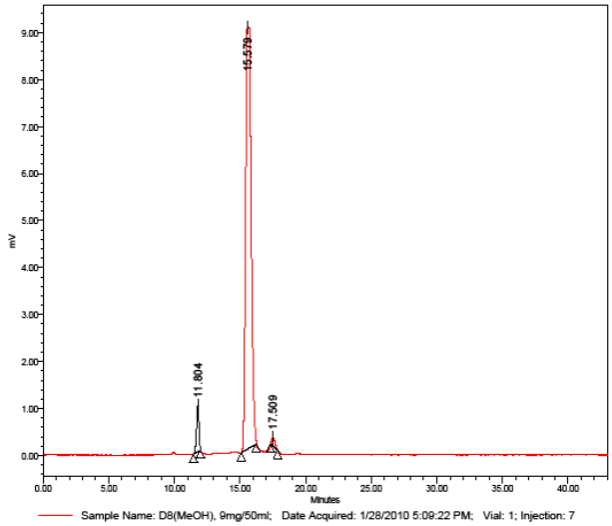
Compound No.	Compound	Conc. (mg/mL)	Flow rate (mL/min)	Retention time (min)
6	glycozoline	0.25	0.6	16.339
7	glycozolidine	0.25	0.6	16.252



Peak Summary with Statistics
Name:

	Sample Name	Vial	Inj	Retention Time (min)	Area	% Area	Height	Amount	Units
1	MKD7(MeOH)0.5mg/mL0.6ml/min	1	4	13.433	10271	4.03	215		
2	MKD7(MeOH)0.5mg/mL0.6ml/min	1	4	19.338	244359	95.97	8096		
Mean				16.386					
Std. Dev.				4.175					
% RSD				25.48					

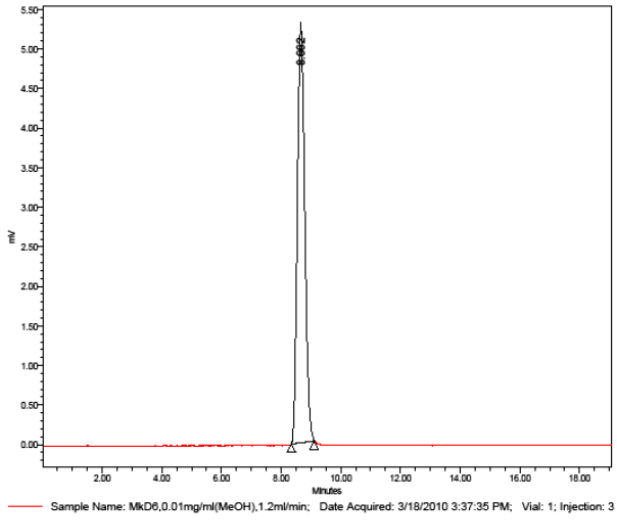
Girinimbine 1



Peak Summary with Statistics
Name:

	Sample Name	Vial	Inj	Retention Time (min)	Area	% Area	Height	Amount	Units
1	D8(MeOH), 9mg/50ml	1	7	11.804	12511	4.56	976		
2	D8(MeOH), 9mg/50ml	1	7	15.579	258612	94.18	8988		
3	D8(MeOH), 9mg/50ml	1	7	17.509	3467	1.26	183		
Mean				14.964					
Std. Dev.				2.902					
% RSD				19.39					

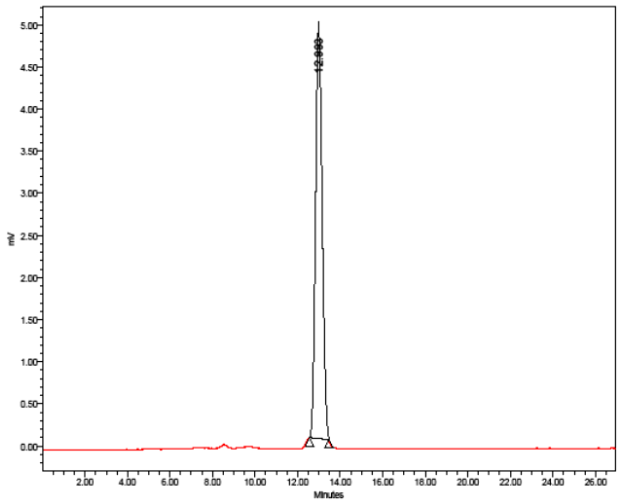
Murrayanine 2



Peak Summary with Statistics
Name:

	Sample Name	Vial	Inj	Retention Time (min)	Area	% Area	Height	Amount	Units
1	MkD6,0.01mg/ml(MeOH),1.2ml/min	1	3	8.662	91338	100.00	5251		
Mean				8.662					
Std. Dev.									
% RSD									

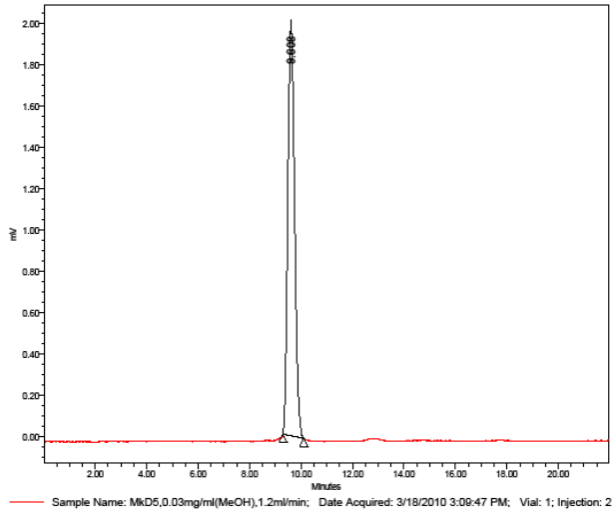
Koenidine 3



Peak Summary with Statistics
Name:

	Sample Name	Vial	Inj	Retention Time (min)	Area	% Area	Height	Amount	Units
1	MkD2,0.056mg/ml(MeOH),0.6ml/min	1	1	12.993	106623	100.00	4880		
Mean				12.993					
Std. Dev.									
% RSD									

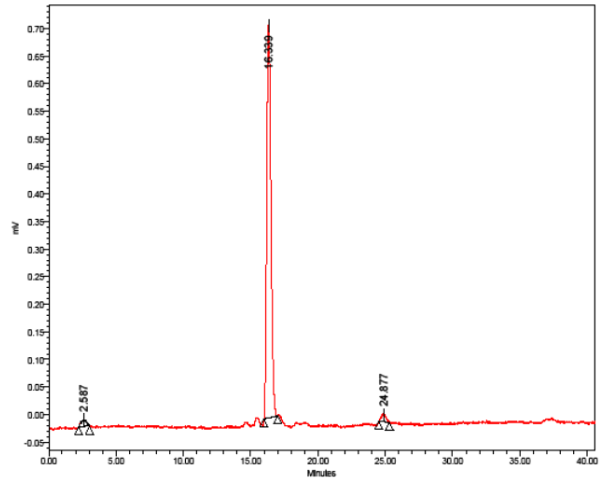
Mahanimbine 4



Peak Summary with Statistics
Name:

	Sample Name	Vial	Inj	Retention Time (min)	Area	% Area	Height	Amount	Units
1	MkD5,0.03mg/ml(MeOH),1.2ml/min	1	2	9.608	36181	100.00	1985		
Mean				9.608					
Std. Dev.									
% RSD									

O-methylmurrayamine-A 5

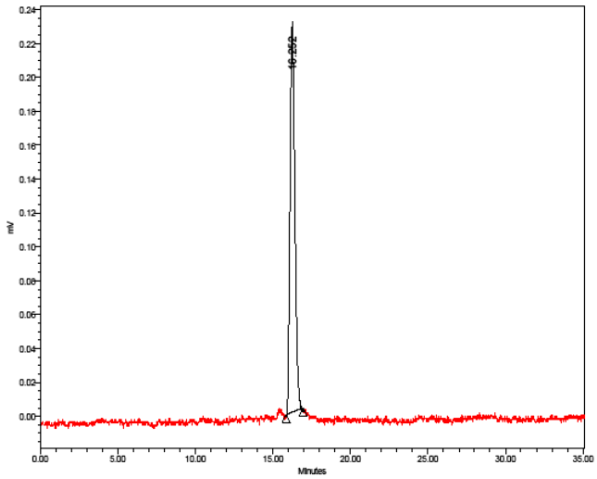


Sample Name: MC/SM-105(MeOH)0.25mg/ml,0.6ml/m; Date Acquired: 1/29/2010 11:15:24 AM; Vial: 1; Injection: 2

Peak Summary with Statistics
Name:

	Sample Name	Vial	Inj	Retention Time (min)	Area	% Area	Height	Amount	Units
1	MC/SM-105(MeOH)0.25mg/ml,0.6ml/m	1	2	2.587	321	2.02	12		
2	MC/SM-105(MeOH)0.25mg/ml,0.6ml/m	1	2	16.339	15202	95.85	712		
3	MC/SM-105(MeOH)0.25mg/ml,0.6ml/m	1	2	24.877	337	2.13	13		
Mean				14.601					
Std. Dev.				11.246					
% RSD				77.02					

Glycozoline 6



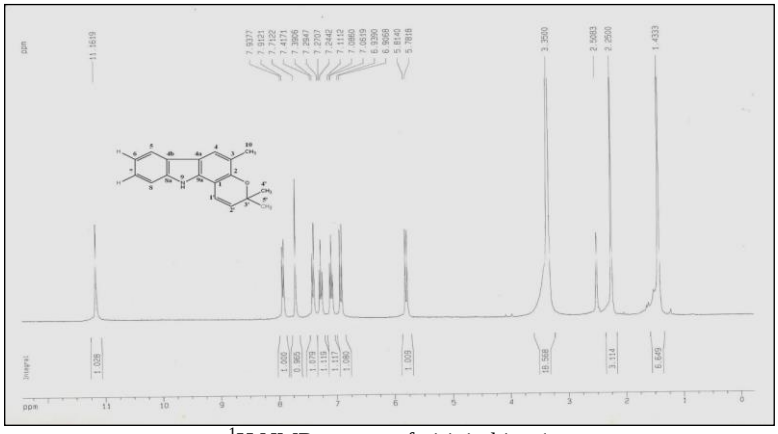
Sample Name: MC/SM108(MeOH)0.25mg/ml,0.6ml/ml; Date Acquired: 1/29/2010 12:22:37 PM; Vial: 1; Injection: 3

Peak Summary with Statistics
Name:

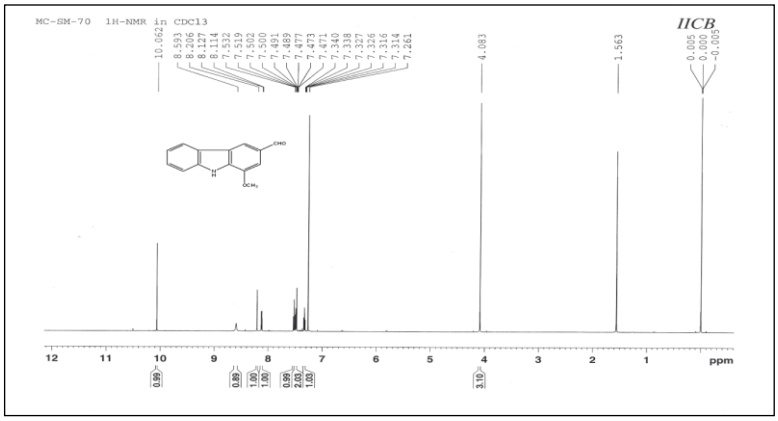
	Sample Name	Vial	Inj	Retention Time (min)	Area	% Area	Height	Amount	Units
1	MC/SM108(MeOH)0.25mg/ml,0.6ml/ml	1	3	16.252	4871	100.00	227		
Mean				16.252					
Std. Dev.									
% RSD									

Glycozolidine 7

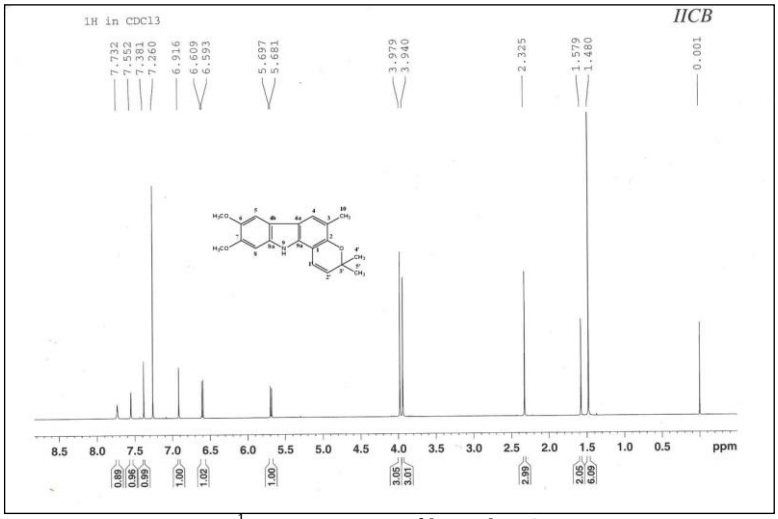
Fig. 3. HPLC chromatogram of the compounds isolated from *Murraya koenigii* and *Glycosmis pentaphylla.*



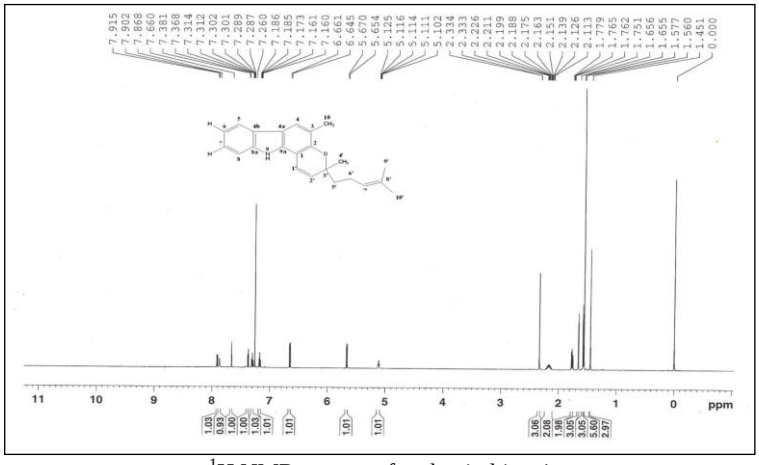
^{1}H NMR spectra of girinimbine 1



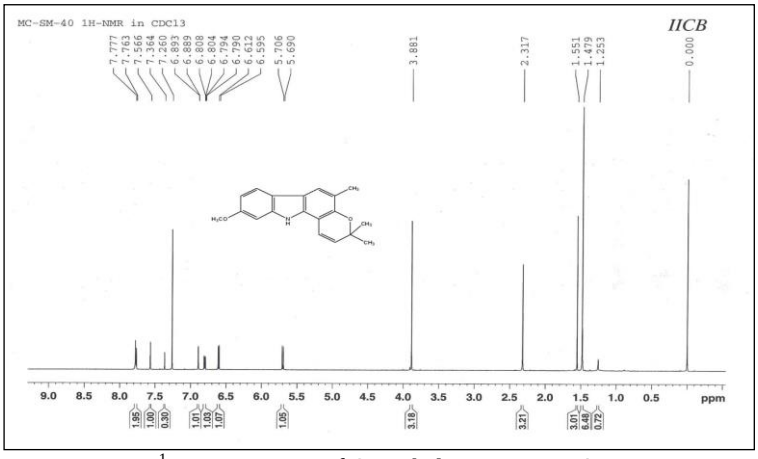
^{1}H NMR spectra pf murrayanine 2



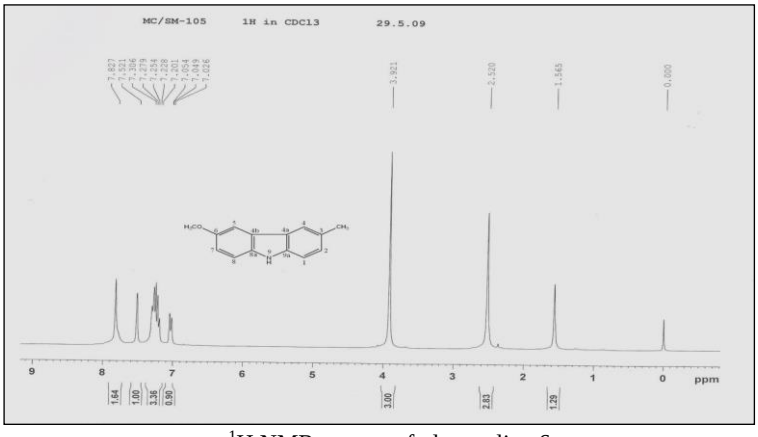
^{1}H NMR spectra of koenidine 3



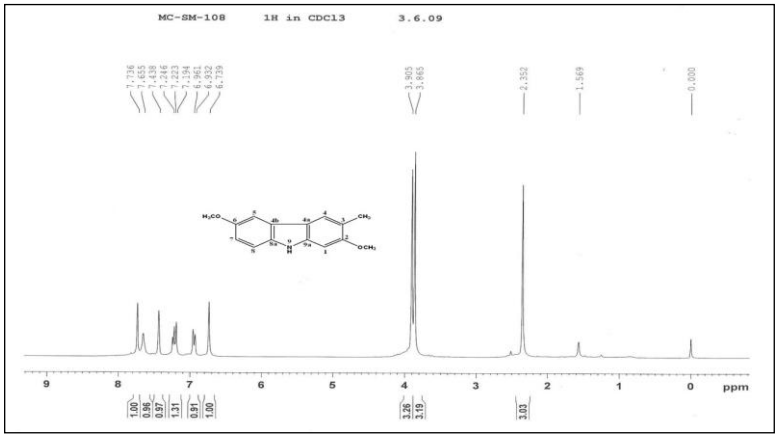
^{1}H NMR spectra of mahanimbine 4



^{1}H NMR spectra of O-methylmurrayamine-A 5



^{1}H NMR spectra of glycozoline 6



^{1}H NMR spectra of glycozolidine 7

Fig. 4. ^{1}H NMR spectra of the compounds isolated from *Murraya koenigii* and *Glycosmis pentaphylla.*

4. Conclusion

Carbazole alkaloids are a very important group of natural products which possess various therapeutic activities. They are found in various medicinal plants belonging to the family Rutaceae. Scientists from different countries have isolated an innumerable number of carbazole alkaloids, and their structures have been determined by detailed spectral data analysis. However, it is not possible to go through the detailed spectral data analysis in some cases, as it is costly, time-consuming, and needs a high level of expertise. The HPLC fingerprint region data discussed in this paper will help the researchers in quick and unambiguous identification of some known carbazole alkaloids without taking the hazards of spectral data analysis. These HPLC data can also be taken as a confirmatory measure for the identification of these alkaloids.

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