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ORIGINAL ARTICLE

Identification of Bioactive Diterpenoid Lactones using Nuclear Magnetic Resonance from *Andrographis paniculata*

Raghava N. Sriramaneni[1,2], Amirin Sadikun[2], Naveen Kumar Hawala Shivashekaregowda[3], Annegowda Venkatappa[4], Mohamad Zaini Asmawi[2]

[1]Department of Human Oncology, Wisconsin Institute for Medical Research, University of Wisconsin, Wisconsin, Madison, USA, [2]Department of Pharmacology, School of Pharmaceutical Sciences, Universiti Sains Malaysia, Pulau Pinang, [3]Department of Pharmacology, School of Pharmacy, Taylor's University, Kula Lumpur, Malaysia, [4]Department of Phytochemistry, Adhichunchangiri University, Mandya, Karnataka, India

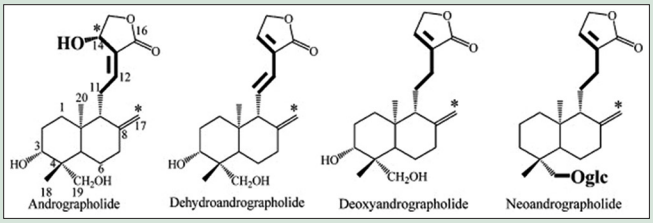
ABSTRACT

Background: *Andrographis paniculata* (AP) (Burm. F) Nees, belongs to the family *Acanthaceae*, it is also known as a highly bitter plant. In South East Asia this plant is commonly used for cold, cough and respiratory tract infections. Andrographolide (ANG), 14-deoxy-11, 12-didehydroandrographolide (DDA) are the major bioactive diterpenoid lactone compounds of the plant shown vasorelaxant effect, anticancer, anti-inflammatory, and to treat variety of diseases which is well-documented. **Materials and Methods:** The high-performance liquid chromatography (HPLC) and ^{1}H-NMR analysis of AP chloroform extract (APCE) revealed the presence of ANG, 14-deoxyandrographolide (DA), and DDA. The main advantage of ^{1}H-NMR is simple, rapid and successfully applied to quantify the active diterpenoids and an alternative to HPLC method to check the presence of various bioactive compounds and its presence in the active raw materials of AP. **Results:** The results revealed that most active bioactive compounds such as ANG, DA, and DDA are present in the APCE portion of the AP. The chemical shifts of various groups confirmed through ^{1}H-NMR that all the three ANG, DA, and DDA has potent vasorelaxant effect and majority of these compounds are present in the APCE portion and not in the methanol or aqueous fractions. The ^{1}H-NMR study confirmed that diterpenoids such as ANG, DA, and DDA from APCE has vasorelaxant effect on the animal study.

Key words: ^{1}H-NMR, *Andrographis paniculata*, andrographolide, bioactive compounds, diterpenoids, vasorelaxant

SUMMARY

- In this present study, an ^{1}H-NMR method was successfully developed to quantify four major diterpenoids (andrographolide, dehydroandrographolide, deoxyandrographolide, and neoandrographolide) in *Andrographis paniculata* chloroform extract and its commercial preparations by using a novel solvent system containing $CDCl_3$. This analytical method was proved to be of excellent accuracy, precision, and repeatability, thus it is simply affordable, rapid, and powerful tool for efficient quality assessment. For further validation, the total content and determination of two diterpenoids 14-deoxy andrographolide and 14-deoxy-11, 12-didehydroandrographolide in *Andrographis paniculata* chloroform extract were accomplished. The results were in good contrast with those determined by high-performance liquid chromatography method. Compared with high-performance liquid chromatography,^{1}H-NMR method is simple, rapid, and reliable without the creation of calibration curves, significantly much short analysis time, and thus is more suitable for the routine control of *Andrographis paniculata* chloroform extract and its viable preparations.



Abbreviations Used: ANG: Andrographolide; APCE: *Andrographis paniculata* chloroform extract; DA: 14-deoxy andrographolide; DDA: 14-deoxy-11, 12-didehydroandrographolide; AP: *Andrographis paniculata*.

Correspondence:
Dr. Raghava N. Sriramaneni,
Department of Human Oncology, Wisconsin Institute for Medical Research, Wisconsin, USA.
E-mail: sriramaneni@wisc.edu

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INTRODUCTION

Andrographolide (ANG), dehydroandrographolide, deoxyandrographolide, and neoandrographolide are the four most dominant diterpenoids, proven for a great proportion of components in *Andrographis paniculata* (AP), showing diverse activities, for example, anti-inflammatory, antiviral, immune-stimulatory, and hepatoprotective activities both *in vitro* and *in vivo* experimental studies.[1-4]

Structures of four major bioactive diterpenoids from AP, highlighted lines are the structural differences are noticed. However, various influences such as geographic region, crop time, and processing method lead to the variability in the content of these active diterpenoids.[5,6] Hence, it is very crucial to develop a practical analysis method for both the raw materials and isolated compounds of the natural plants. Various chromatographic methods such as thin layer chromatography, high-performance liquid chromatography, and column chromatography and capillary electrophoresis have already reported. In addition to that, these methods are time-consuming, not sensitive and most complicated methods when compared to ^{1}H-NMR analysis.

Nuclear magnetic resonance (NMR) spectroscopy is the study of molecules by recording the interaction of radiofrequency electromagnetic radiation with the nuclei of molecules placed in a strong magnetic field.

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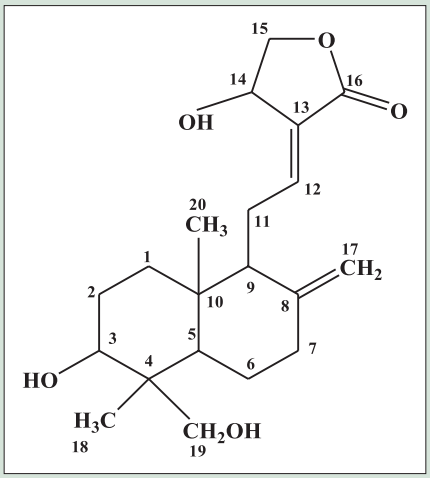

Figure 1: Chemical structure of Andrographolide. Molecular formula: $C_{20}H_{30}O_5$. Molecular weight: 350.46 g

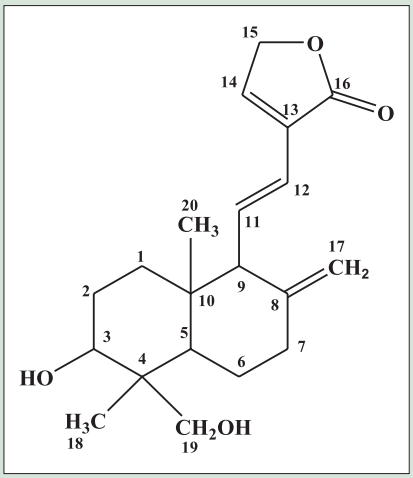

Figure 2: Chemical structure of DDA. Molecular formula: (C20H28O4). Molecular weight: 332.44 g

Like all other spectroscopic techniques, NMR spectroscopy involves the interaction of the material being examined with electromagnetic radiation.

NMR spectroscopy is a useful technique for identifying and analyzing organic compounds. This extremely important experimental technique is based on the magnetic nuclear spin of ^{1}H, ^{13}C, ^{15}N, ^{19}F, ^{31}P, and so forth. Only ^{1}H and ^{13}C will give more detailed and definitive information.

Hence, when information obtained from NMR and other analytical methods are compared, it is apparent that the most detailed and necessary information to recognize a structure is obtained from NMR spectra. However, this does not mean that NMR is always sufficient for structural analysis. For absolute analysis of structures, it is necessary to apply all spectroscopic methods. If a new compound is synthesized, the chemist who does scientific research should have all the spectroscopic data related to this chemical compound. The information provided by an NMR spectrum is useful only if it can be interpreted well. In addition to providing information related to constitutional analysis, NMR spectra provide detailed information pertinent to configurational and conformational analysis.[7]

The aerial parts of the plant AP (leaves and stems) are used to extract the active phytochemicals. Previous investigations on the chemical composition of AP showed that it is a rich source of diterpenoids and 2'-oxygenated flavonoids including ANG, neoandrographolide, 14-deoxy-11, 12-didehydroandrographolide (DDA), 14-deoxyandrographolide (DA), isoandrographolide, and DA-19 β-D-glucoside, homoandrographolide, andrographan, andrographasterin, and stigmasterol.[8]

The primary bioactive diterpenoid lactone compound of the medicinal plant AP is andrographolide (ANG).[9-11] The chemical formula is [$C_{20}H_{30}O_5$: 3-(2-{decahydro-6-hydroxy-5(hydroxymethyl)-5, 8α-dimethyl-2-methylene-1-pthalenyl} thyledene] dihydro-4-hydroxy-2 (3H)-furanone; Figure 1 is a colorless crystalline bicyclic diterpenoid lactone and has a very bitter taste.[12] Based on previous high-performance liquid chromatography (HPLC) profile (s) to further confirm the active diterpenoid lactones present AP chloroform extract (APCE), the following NMR analysis was carried out.

ANG is a bicyclic diterpenoid lactone and contains three hydroxyl groups at C-3, C-19, and C-14, these being secondary, primary, and allylic, respectively. It has been shown that the intact γ-butyrolactone ring, the double bonds at C-12 and C-13, C-8 and C-17, and C-14 hydroxyl group are responsible for the cytotoxic activity.[13] The stereochemistry of ANG has previously been established. The two central six-membered rings adopt a chair conformation, and the furan ring adopts an envelope conformation.[14-16] ANG forms hydrogen-bonded chains at both ends of the molecule.

DDA is another diterpenoid isolated from AP. It is a colorless needle crystal with the presence of hydroxyl, α, β,-unsaturated-γ-lactone, and exomethylene groups in its chemical structure. This is very similar to that of DA, with the exception of a double bond at C-11 and C-12.[17]

MATERIALS AND METHODS

^{1}H-NMR spectra were recorded on BRUKER-400 spectrometer, NMR facility division, University Technology Mara, Shah Alam, Kuala Lumpur, Malaysia. Using $CDCl_3$ as a solvent and tetramethylsilane as an internal standard.

RESULTS AND DISCUSSION

The ^{1}H NMR spectrum of chloroform fraction showed the presence of methyl group at H-18 (δ1.289, 3H), H-20 (δ 0.801, 3H) and methylene group at H-11 (δ2.353, 2H) and H-15 (δ4.191, 2H). The presence of two double bonds was encountered at H-12 (δ 6.899, 1H) and H-17 (δ 4.598, 2H). The presence of a primary alcoholic group showed at H-19 (δ 3.352, 2H). These structural characteristic reveals the presence of ANG in APCE [Table 1.1].

The ^{1}H NMR spectrum of chloroform fraction showed the presence of two methyl groups at H-18 (δ1.684, 3H), H-20 (δ 0.801, 3H) and one methylene group at H-15 (δ4.778, 2H). The three doubles bonds in the ring were ascertained at H-12 (δ 6.266, 1H), H-17 (δ 4.851, 2H), and H-14 (δ 7.271, 1H). The occurrence of primary alcoholic group is showed at H-19 (δ 4.533, 2H). The olefinic

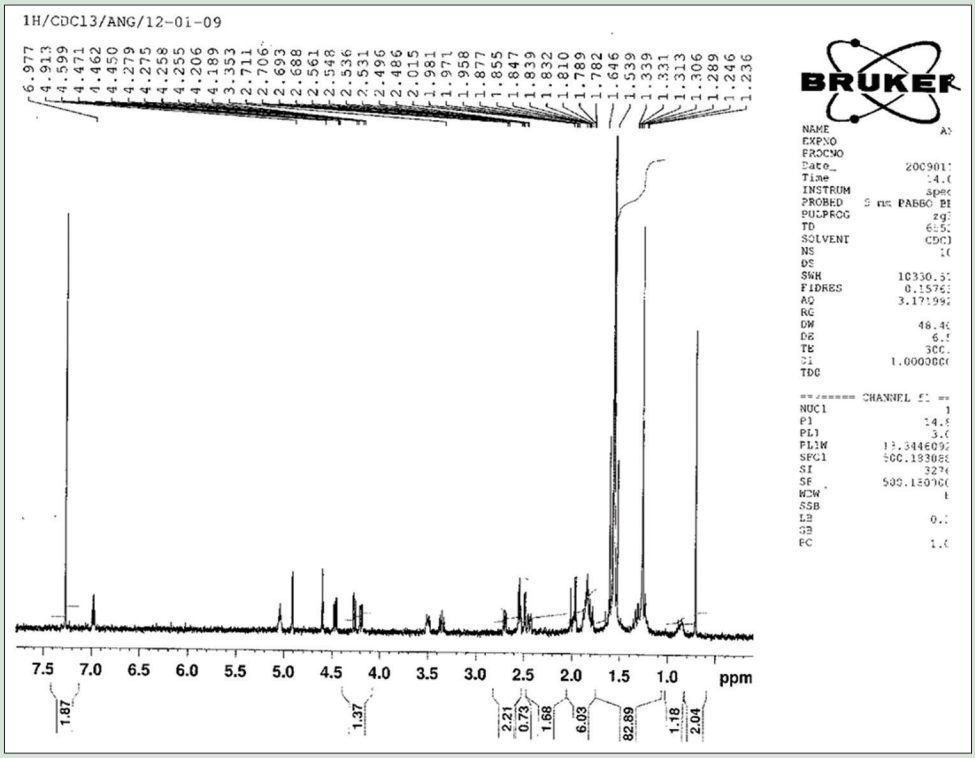

Figure 3: [1]H-NMR Spectral analysis of and andrographolide

Table 1.1: [1]H nuclear magnetic resonance chemical shifts corresponding to andrographolide in the absence and presence of *Andrographis paniculata* chloroform extract

Proton	Chemical shift ANG		APCE		Standard ANG
	δ H	J (Hz)	δ H	J (Hz)	δ H
11	2.496 (m, 2H)		2.353 (m, 2H)		2.493 m
12	6.977 (m, 1H)		6.899 (m, 1H)		6.731 m
14	4.913 (m, 1H)		4.891 (m, 1H)		4.935 m
15	4.189 (d, 2H)	20.0	4.191 (d, 2H)	12.0	4.098 d
17	4.599 (s, 2H)		4.598 (s, 2H)		4.759 s
18	1.289 (s, 3H)		1.289 (s, 3H)		0.997 s
19	3.353 (d, 2H)	20.0	3.352 (d, 2H)	8.0	3.283 d
20	0.713 (s, 3H)		0.801 (s, 3H)		0.559 s

APCE: *Andrographis paniculata* chloroform extract; ANG: Andrographolide

Table 1.2: [1]H nuclear magnetic resonance chemical shift corresponding to DDA in the absence and presence of *Andrographis paniculata* chloroform extract

Proton	Chemical shift DDA		APCE		Standard DDA
	δ H	J (Hz)	δ H	J (Hz)	δ H
3	3.510 (m, 1H)		3.795 (m, 1H)		3.76 m
11	6.90 (m, 1H)		7.172 (m, 1H)		7.23 m
12	6.141 (d, 1H)	12.0	6.266 (d, 1H)	16.0	6.20 d
14	7.271 (m, 1H)		7.271 (m, 1H)		7.27 m
15	4.795 (d, 2H)		4.778 (d, 2H)		4.77 d
17	4.824 (s, 2H)		4.851 (s, 2H)		4.85 s
18	1.568 (s, 3H)		1.684 (s, 3H)		1.62 s
19	4.540 (s, 2H)	12.0	4.533 (s, 2H), 33 3.79	12.0	4.52 s
20	0.829 (s, 3H)		0.801 (s, 3H)		0.94 s

DDA: 14-deoxy-11, 12-didehydroandrographolide; APCE: *Andrographis paniculata* chloroform extract

linkage between H-11 and H-12 can be reconfirmed by the presence of single proton at H-11 (δ 7.172, 1H) and H-12 (δ 6.266, 1H). These structural interpretations assure the presence of DDA. The [1]H-NMR analysis reveals the significant presence of ANG and DDA in APCE [Table 1.2].

CONCLUSIONS

The aerial parts of the plant AP (leaves and stems) are used to extract the active phytochemicals using various solvents. Among all the fractions only chloroform fraction of AP was found to be more active in both *in vitro* and *in vivo* experiments. Previous investigations on the chemical composition of AP showed that it is a rich source of diterpenoids such as ANG, DA and DDA are pharmacologically active in treating various ailments, these compounds are present in a higher percentage in leaves [Figures 2-6]. From the NMR analysis, the highest percentage of the active diterpenoids including ANG, DA, and DDA were found in the chloroform extract of AP.

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Nil.

Conflicts of interest

There are no conflicts of interest.

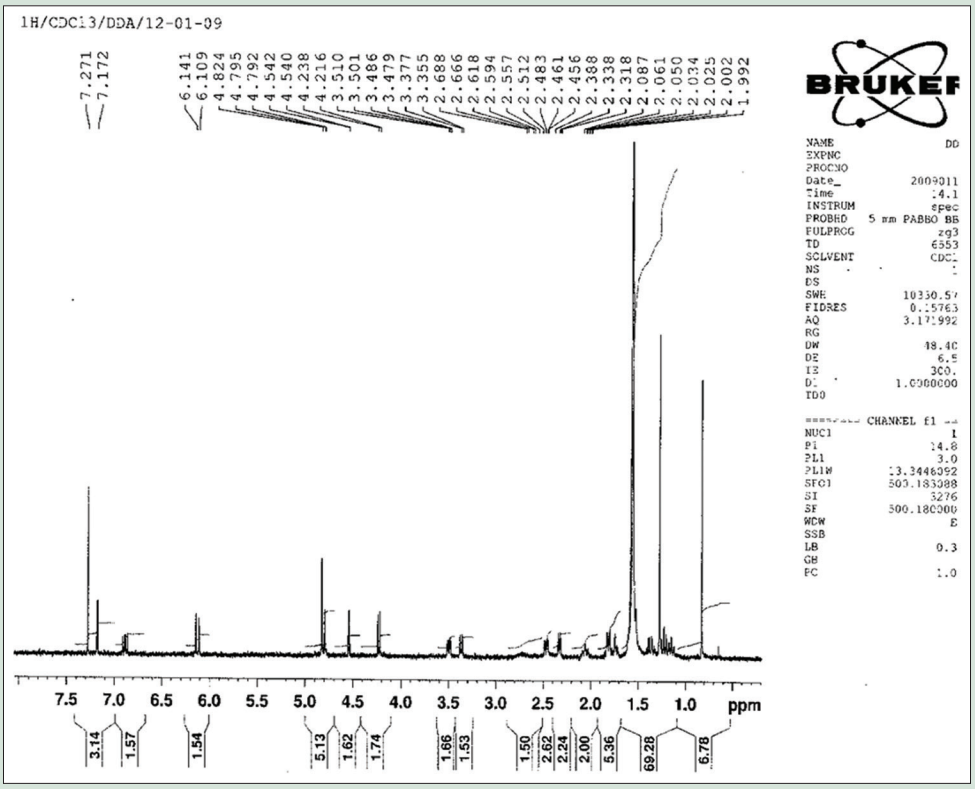

Figure 4: ^{1}H-NMR Spectral analysis of 14-deoxy-11, 12-didehydroandrographolide

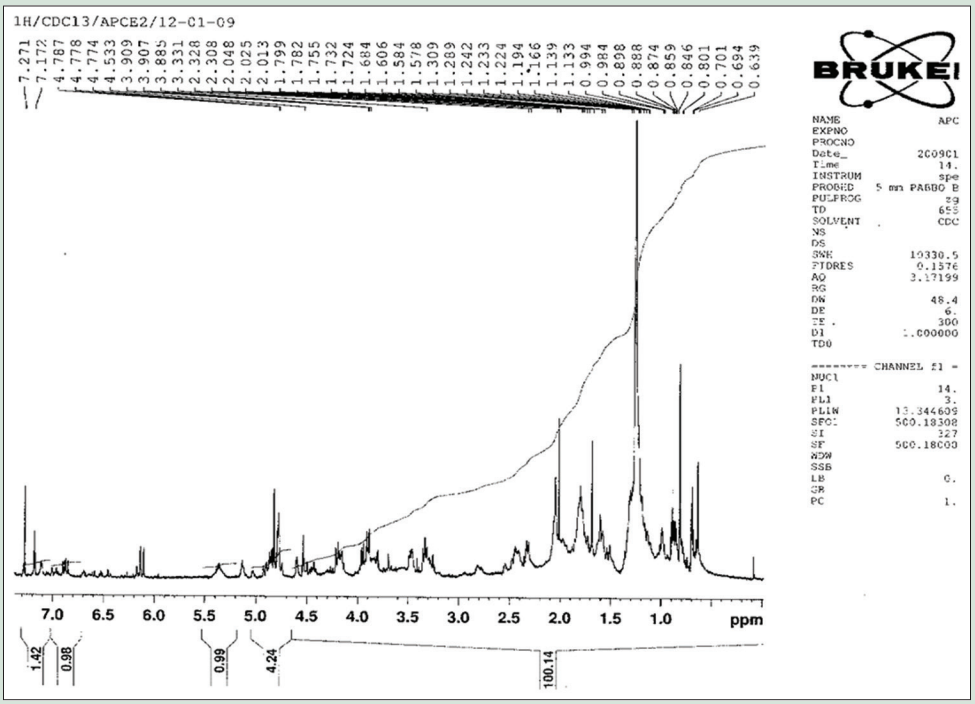

Figure 5: ^{1}H-NMR Spectral analysis of *Andrographis paniculata* chloroform extract

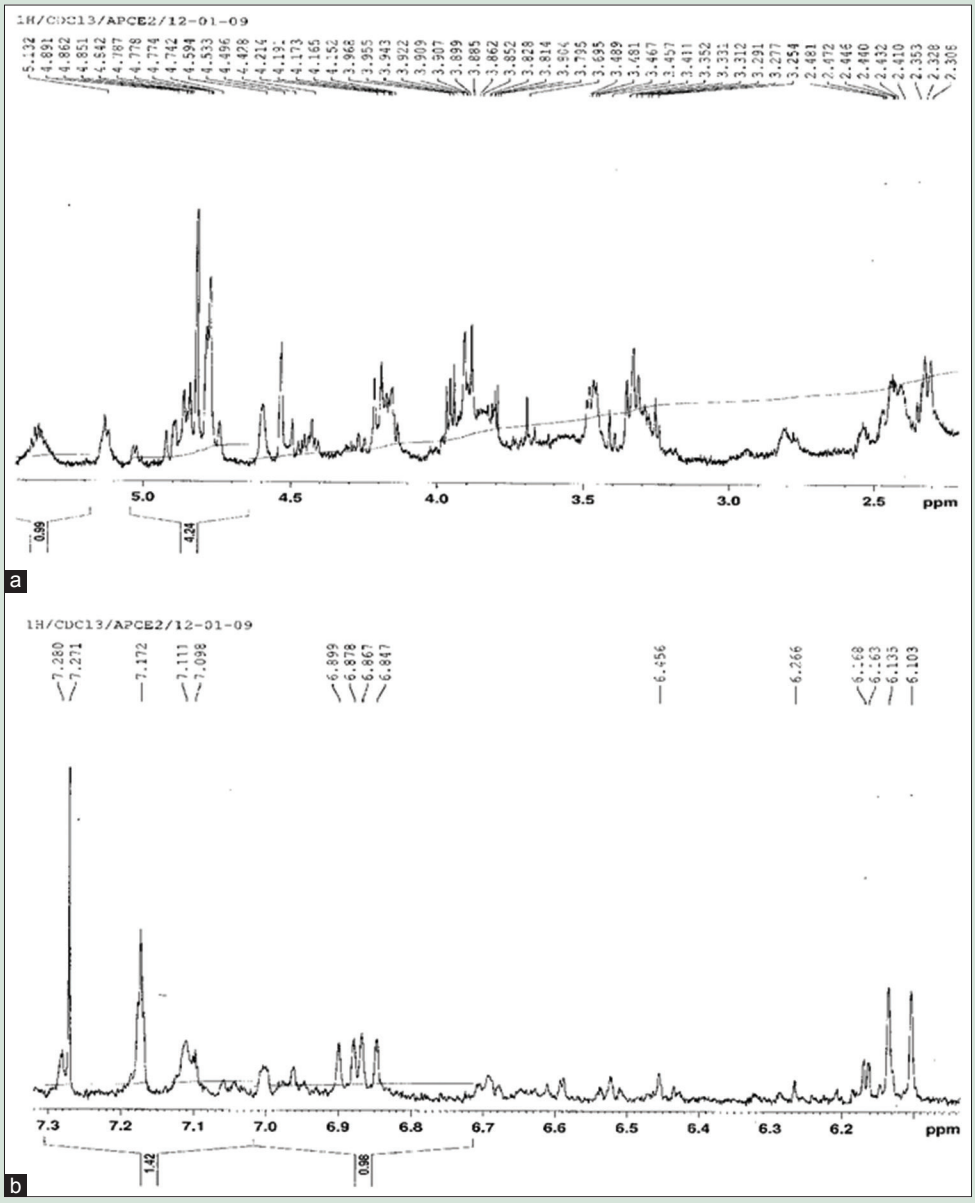

Figure 6: (a and b) ^{1}H-NMR Spectral analysis of *Andrographis paniculata* chloroform extract

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