Supplementary Data

This supplementary data is a part of paper entitled “Conformational Analysis of Diterpene Lactone Andrographolide towards Reestablishment of Its Absolute Configuration via Theoretical and Experimental ECD and VCD Methods”.

The major constituent was isolated as a pale yellowish powder (mp 231–233 °C) from *Andrographis paniculata* (whole plant) and was identified as andrographolide **1** (Fig. 1) [1-2]. The IR spectrum of **1** shows a strong peak at 1724.46 cm^{-1}, representing a lactone-type carbonyl stretch, while a weak sharp peak at 3391.91 cm^{-1} indicates the presence of the free hydroxyl group. Another characteristic peak of medium intensity observed at 1675.46 cm^{-1} shows the presence of alkenyl C=C stretch. The ^{13}C NMR spectrum of **1** (Table 1) displayed 20 carbons, attributed to a carbonyl (δ 172.6), two quaternary olefins (δ 129.8 and 148.8), olefinic methylene (δ 109.2), an olefinic methine (δ 149.3), two oxymethines (δ 66.8 and 80.9), two oxymethylene (δ 65.0 and 76.1), two quaternary aliphatics (δ 40.0 and 43.7), an aliphatic methine (δ 56.4), five aliphatic methylenes (δ 25.2, 25.7, 29.0, 38.1 and 39.0), and two methyl carbons (δ 15.5 and 23.2). Its ^{1}H NMR spectrum (Table 1) showed signals typical of olefinic methine and methylene protons at δ 6.85 (td, *J* = 6.8, 1.7 Hz, H-12) and 4.89/4.67 (s, H-17) respectively. Furthermore, the characteristic signals for two oxymethine protons were seen at δ 3.41 (dd, *J* = 9.6, 6.0 Hz, H-3) and 5.01 (d, *J* = 6.1 Hz, H-13), while signals typical for two oxymethylene protons were seen at δ 4.46 (dd, *J*=10.2, 6.1 Hz, H-15a)/4.16 (dd, *J*=10.2, 2.0 Hz, H-15b) and 4.12 (d, *J* = 11.1 Hz, H-19a)/3.37 (d, *J* = 10.8 Hz, H-19b). These ^{1}H and ^{13}C NMR spectroscopic data were consistent with those of the known andrographolide.

The relative configurations at the six stereogenic centers of **1** were determined based on its NOE spectral data. The strong NOE correlation (Fig. 2) between olefinic proton H-12 and protons H-9/H-11b/H-17b established the *cis*-orientation of H-12/H-9, and *trans*-orientation of H-12/H11b and H-12/H-17, while the correlation of protons H-11a and H-14 showed the *trans*-orientation of H-12/H-14 and *cis*-orientation of H-12/carbonyl at the lactone ring. Furthermore, the spatial correlation between proton H-5 and protons H-1b/H-3/H-7a/H-9 results in ring strain and confirmed the protons arrangement in the bicyclic ring of 1 skeleton *cis*-orientation. Next, the correlation between hydroxyl protons H-3b and H-19b established the *cis*-orientation of 19-CH_2 and 3-OH group. However, the configuration of 14-OH and 19-OH is supported only by a single NOE correlation. The ambiguity in the configuration warrants further experiments. Based on the NOE correlations, the relative configuration of the six stereogenic centers in **1** could be deduced as 3*R*, 4*R*, 5*S*, 9*R*, 10*R*, and 14*S,* as shown in Fig. 3.

The chromophoric derivatization of **1** at room temperature using 2-naphthoyl chloride or benzoyl chloride as chromophore proceeded *via* S_N2 nucleophilic substitution reaction (Scheme 1). The three hydroxyl groups in andrographolide act as active sites in this reaction. Based on its position, the allylic hydroxyl group at carbon C-14 (secondary alcohol) is more acidic compared to those at carbons C-3/C-19, consequently, it can be easily deprotonated. Trimethylamine as base at room temperature, derivatization of **1** with both benzoyl and 2-naphthoyl chlorides was only achieved at C-14 instead at C-3 or C-19 at the end of the reaction. However, by increasing the reaction temperature to 40 °C (increasing the kinetic energy), the activation energy of the reaction system has decreased and successfully introduced chromophore at both C-14 and C-19 of **1**. This fact indicated that steric hindrance around the hydroxyl group at C-3 and C-19 of **1** caused the nucleophilic attack to be less susceptible to both the chromophores. Structural characterization of all derivatives was carried out by NMR analysis in acetone-d_6. Table 1 shows the chemical structures of andrographolide derivatives (**2–5**), while Table 2 shows the ^{1}H and ^{13}C NMR spectral details of the compounds. Derivatives **2**, **3** and derivatives **4**, **5** were obtained from a single reaction of different chromophores used for derivatization.

References

[1] Hossain, M.S., Urbi, Z., Sule, A., and Rahman, K.M.H., 2014, *Andrographis paniculata* (Burm. f.) Wall. ex Nees: A review of ethnobotany, phytochemistry, and pharmacology, *Sci. World J.*, 2014, 1–28.

[2] Chen, L., Zhu, H., Wang, R., Zhou, K., Jing, Y., and Qiu, F., 2008, *ent*-Labdane diterpenoid lactone stereoisomers from *Andrographis paniculata*, *J. Nat. Prod.*, 71 (5), 852–855.

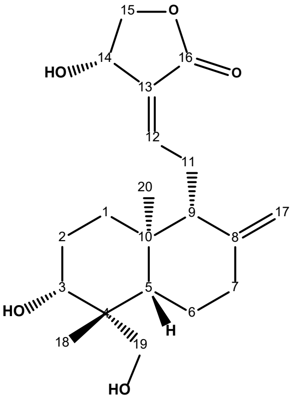


Fig 1S. Chemical structure of andrographolide with carbon numbering. The configuration was determined using single-crystal technique from the previous studies

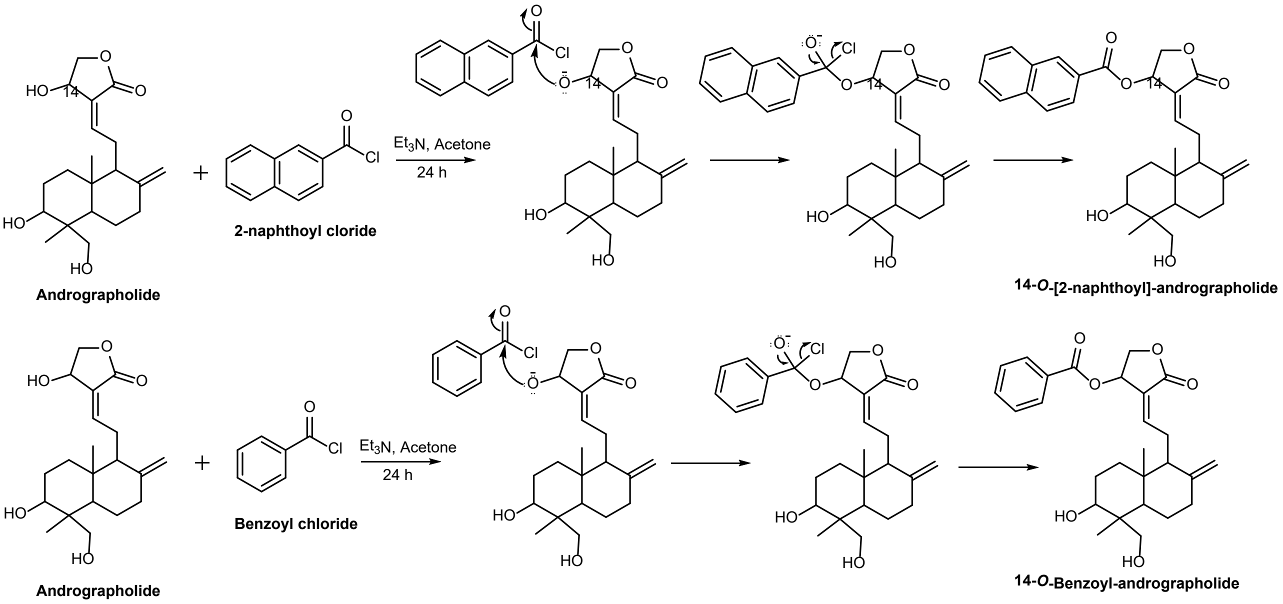
Total mass isolated: 396mg

Physical properties: pale-yellowish, powder.

Molecular mass: 350.45

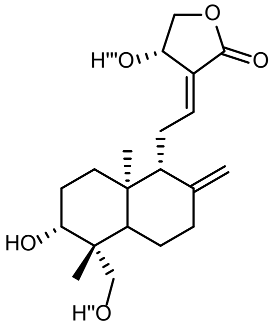
CHN Analysis:

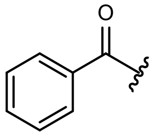
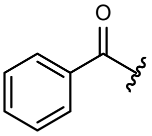
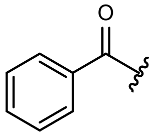
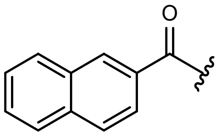
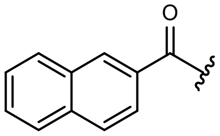
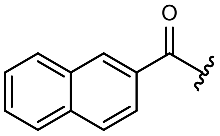
	Theoretical	Experimental
Carbon	240.20	238.66
Hydrogen	30.30	29.35
Nitrogen	0	



Scheme 1. Reaction mechanism of chromophoric derivatization of andrographolide

Table 1S. Chemical structures of andrographolide derivatives (**2–5**). Derivatives **2**, **3** and derivatives **4**, **5** were obtained from single reaction of different chromophores used for derivatization



Derivative	Yield (mg)	Molecular mass (g/mol)	Substitution	
			H”	H”’
2	5.10	454.4565	-	
3	3.12	558.6691		
4	4.68	504.6321	-	
5	7.48	658.8091		

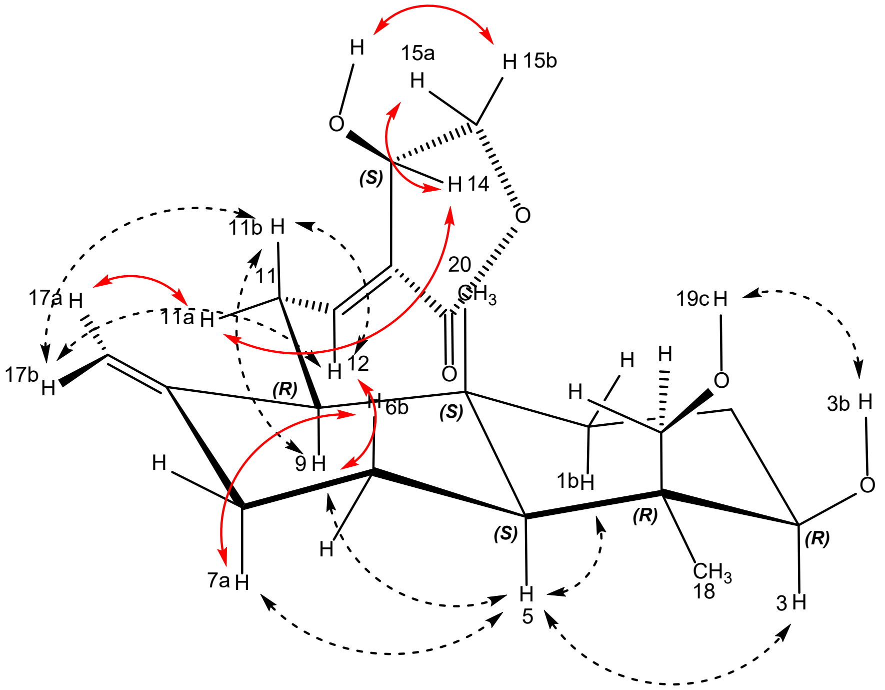


Fig 2S. Key NOESY correlations of **1**

Table 2S. The NMR spectral data of **1** and its derivatives

No	Andrographolide **1**		14-*O*-benzoyl adrographolide **2**		14,19-*O*-dibenzoyl andrographolode **3**	
	δ_H (mult., *J*=Hz)	δ_C	δ_H (mult., *J*=Hz)	δ_C	δ_H (mult., *J*=Hz)	δC
1	2.43 (ddd, *J*=12.9, 3.7, 2.5) 2.04 (td, *J*=12.6, 4.7)	39.0	2.37 (br dd, *J*=11.7, 3.3) 2.01 (m)	37.6	2.42 (br dd, *J*=11.5, 3.3) 2.07 (m)	37.8
2	1.78 (m)	29.0	1.72 (m)	28.1	1.72 (m)	27.8
3	3.41 (dd, *J*=9.6, 6.0)	80.9	3.39 (dd, *J*=9.7, 5.9)	79.6	3.42 (m)	776
4	-	43.7	-	42.6	-	42.5
5	1.32 (m)	56.4	1.31 (m)	55.0	1.41 (m)	54.8
6	1.38 (qd, *J* = 12.7, 4.1) 1.78 (m)	25.2	1.31 (m) 1.81 (m)	23.8	1.41 (m) 1.82 (m)	22.4
7	1.29 (m) 1.80 (m)	38.1	1.75 (m) 1.26 (m)	36.8	1.65 (m) 1.27 (m)	37.1
8	-	148.8	-	147.9	-	148.0
9	1.92 (dd, *J*=10.8, 2.4)	57.4	2.02 (m)	55.7	2.01 (m)	55.9
10	-	40.0	-	38.7	-	39.1
11	2.63 (ddd, *J*=16.8, 7.1, 3.8) 2.58 (ddd, *J*=17.1, 10.8, 6.8)	25.7	2.68 (ddd, *J*=16.7, 6.6, 3.7) 2.54 (ddd, *J*=17.2, 10.6, 7.1)	25.1	2.74 (dq, *J*=16.8, 3.3) 2.60 (m)	25.2
12	6.85 (td, *J*=6.8, 1.7)	149.3	6.99 (td, *J*=6.8, 1.7)-	149.6	7.02 (td, *J*=6.9, 1.6)	149.5
13	-	129.8	-	134.6		133.5
14	5.01 (d, *J*= 6.1)	66.8	6.32 (br d, J=6.1)	68.8	6.35 (br d, *J*=6.0)	68.9
15	4.46 (dd, *J*=10.2, 6.1) 4.16 (dd, *J*=10.2, 2.0)	76.1	4.72 (dd, J=11.1, 6.1) 4.45 (dd, J=11.1, 1.9)	71.3	4.53 (m) 4.47 (m)	71.3
16	-	172.6		173.8		168.6
17	4.89 (s) 4.67 (s)	109.2	4.83 (d, *J*=1.3) 4.58 (d, *J*=1.3).	107.8	4.85 (s) 4.61 (s)	107.7
18	0.75 (s)	15.5	0.63 (s)	14.4	0.73 (s)	13.8
19	4.12 (d, *J*=11.1) 3.37 (d, *J* = 10.8)	65.0	4.06 (d, *J*=10.9) 3.25 (br d, *J*=10.7)	63.4	3.94 (d, *J*=4.7) 4.74 (m)	65.7
20	1.22 (s)	23.2	1.19 (s)	22.6	1.26 (s)	22.4
1'[1"]			-	124.6		124.7
2'[2"]			8.05 (dd, *J*=8.4, 1.4)	129.4	8.01 (dd, *J*=8.3, 1.3)	129.3 [129.5]
3'[3"]			7.54 (tt, *J*=7.5, 1.5)	128.7	7.57 (m)	128.5 [128.7]
4'[4"]			7.68 (tt, *J*=7.5, 1.5)	133.6	7.64 (m)	132.8 [133.5]
5'[5"]			7.54 (tt, *J*=7.5, 1.5)	128.7	7.50 (dq, *J*=7.3, 1.5)	130.8
6'[6"]			8.05 (dd, *J*=8.4, 1.4)	129.4	8.02 (dd, *J*=8.3, 1.3)	148.0 [149.5]
7'[7"]			-	-	-	-
8'[8"]			-	-	-	-
9'[9"]			-	-	-	-
10'[10"]			-	-	-	-
C=O			-	165.5	-	165.6 [166.0]

Table 2S. The NMR spectral data of **1** and its derivatives (*Continued*)

No	14-*O*-(2-naphthoyl) andrographolide **4**		14,19-*O*-di(2-naphthoyl) andrographolide **5**	
	δ_H (mult., *J*=Hz)	δ_C	δ_H (mult., *J*=Hz)	δ_C
1	2.39 (br dd, *J*=11.4, 4.2)	38.5	2.44 (m)	38.8
	2.07 (m)		2.09 (m)	
2	1.73 (m)	29.0	1.74 (m)	28.8
3	3.41 (m)	80.9	3.44 (m)	78.5
4	-	42.3	-	43.5
5	1.32 (m)	55.9	1.44 (m)	56.9
6	1.32 (m)	25.2	2.10 (m)	26.0
	1.82 (m)		1.68 (m)	
7	1.77 (m)	37.7	1.87 (m)	38.0
	1.29 (m)		1.45 (m)	
8	-	148.9	-	149.0
9	2.08 (m)	56.7	2.13 (m)	55.7
10	-	39.7		40.0
11	2.73 (ddd, *J*=16.8, 6.3, 3.4)	26.1	2.76 (m)	26.2
	2.61 (m)		2.67 (m)	
12	7.04 (td, *J*=7.2, 1.8)	150.6	7.06 (td, *J*=7.0, 1.5)	150.6
13	-	127.7	-	127.2
14	6.41 (br d, *J*=6.1)	69.8	6.43 (br d, *J*=6.0)	69.9
15	4.78 (dd, *J*=11.2, 6.2)	72.3	4.79 (dd, *J*=11.0, 6.0)	72.3
	4.53 (dd, *J*=11.2, 1.9)		4.54 (dd, *J*=11.0, 2.0)	
16	-	183.3	-	169.6
17	4.87 (d, *J*=1.2)	108.7	4.87 (br s)	108.7
	4.66 (br s)		4.68 (br s)	
18	0.64 (s)	15.4	0.78 (s)	23.4
19	4.06 (dd, *J*=10.5, 1.6)	64.3	4.59 (d, *J*=11.5)	66.8
	3.25 (t, *J*=10.8)		4.50 (d, *J*=12.0)	
20	1.20 (s)	23.5	1.33 (s)	14.8
1'[1"]	8.71 (s)	132.1	8.70 (s) [8.62 (s)]	132.1 [131.5]
2'[2"]	-	125.6	-	125.6 [125.6]
3'[3"]	8.13 (d, *J*=7.8)	125.8	8.12-7.97 (m)	125.8 [126.0]
4'[4"]	8.06-8.03 (m)	129.4	8.12-7.97 (m)	129.2 [129.1]
5'[5"]	-	136.7	-	136.7 [136.4]
6'[6"]	7.71 (td, *J*=7.5, 1.1)	128.7	7.67 (t, *J*=8.0) [7.68 (t, *J*=8.0)]	128.7 [128.6]
7'[7"]	7.66 (td, *J*=7.5, 1.1)	129.6	7.61 (t, *J*=7.0) [7.61 (t, *J*=7.0)]	129.6 [129.4]
8'[8"]	8.06-8.03 (m)	128.0	8.12-7.97 (m)	127.9 [127.7]
9'[9"]	8.06-8.03 (m)	130.2	8.12-7.97 (m)	130.3 [130.2]
10'[10"]	-	133.5	-	133.5 [133.4]
C=O	-	169.6	-	167.0 [166.7]

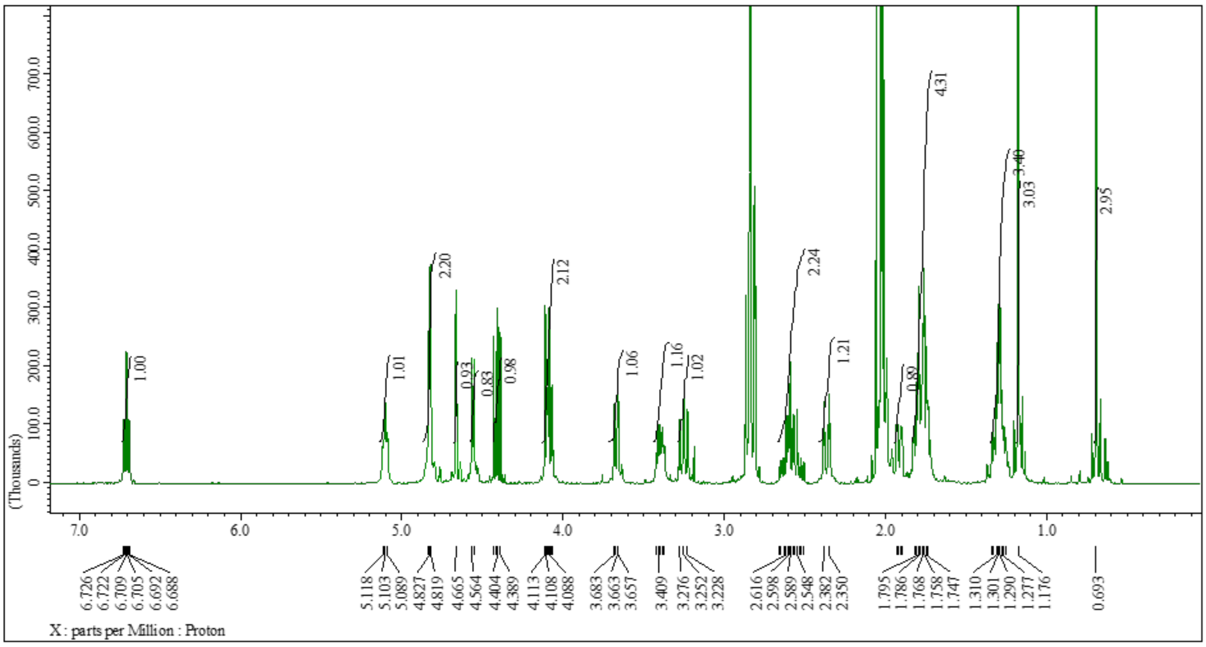


Fig 3S. ^{1}H NMR spectrum for andrographolide (**1**)

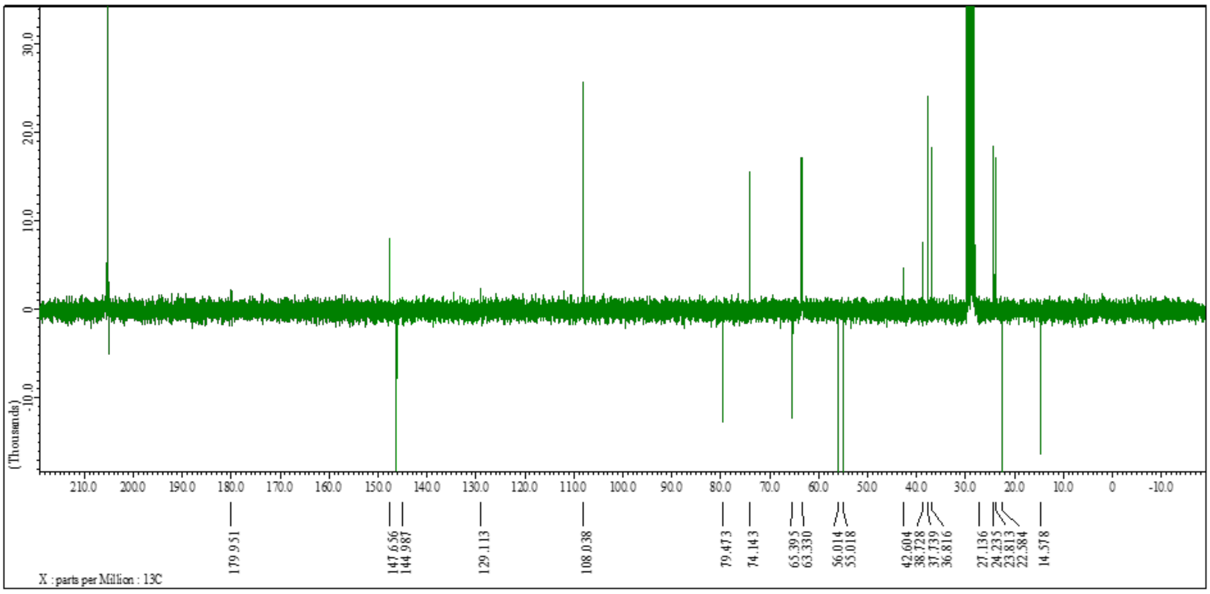


Fig 4S. DEPT ^{13}C NMR spectrum for andrographolide (**1**)

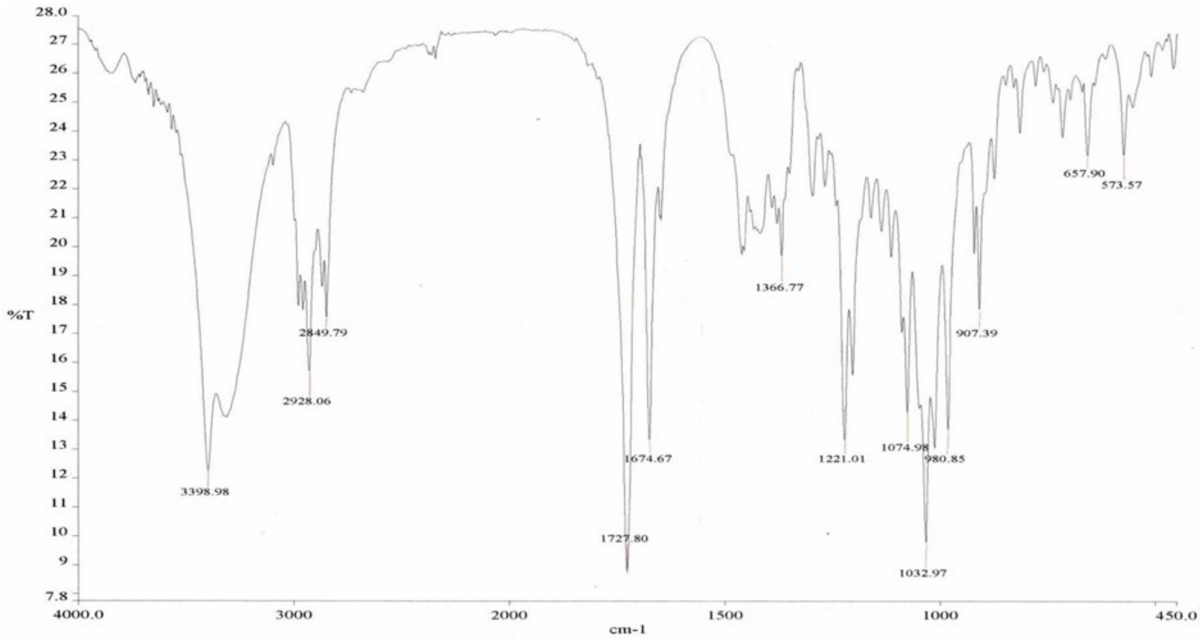


Fig 5S. IR spectrum for spectrum for andrographolide (**1**)

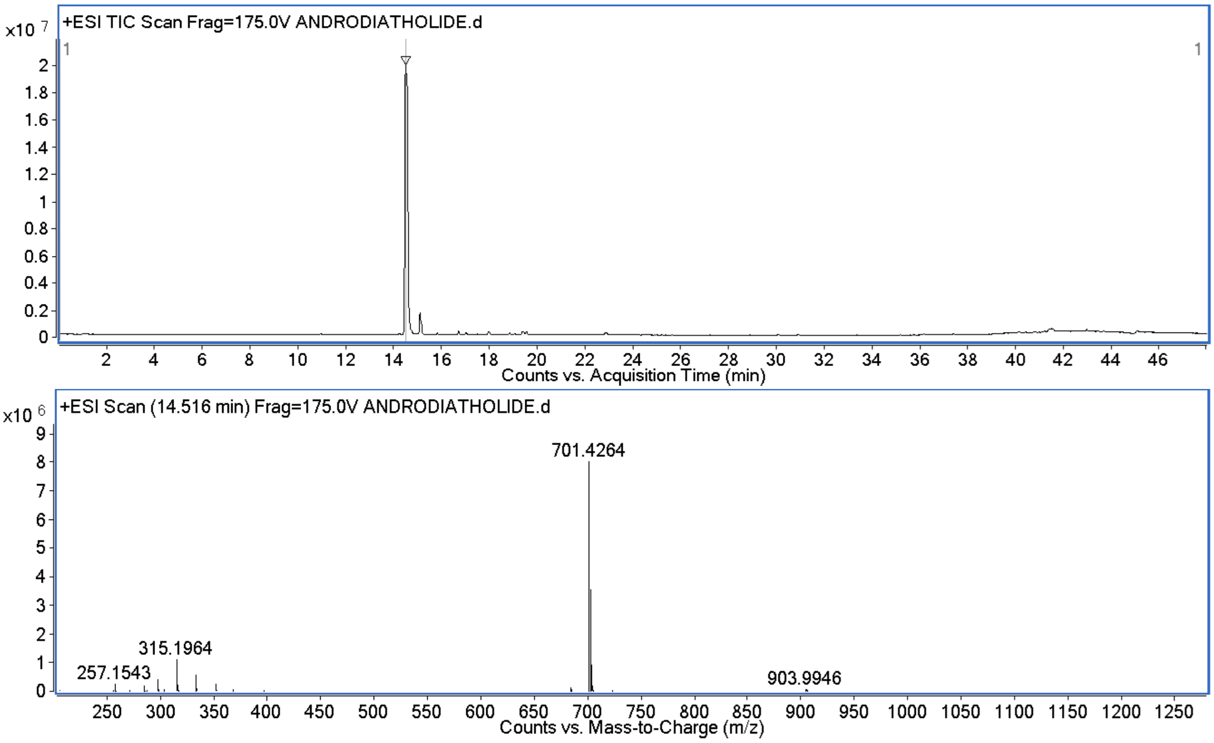


Fig 6S. MS spectra for for andrographolide (**1**)

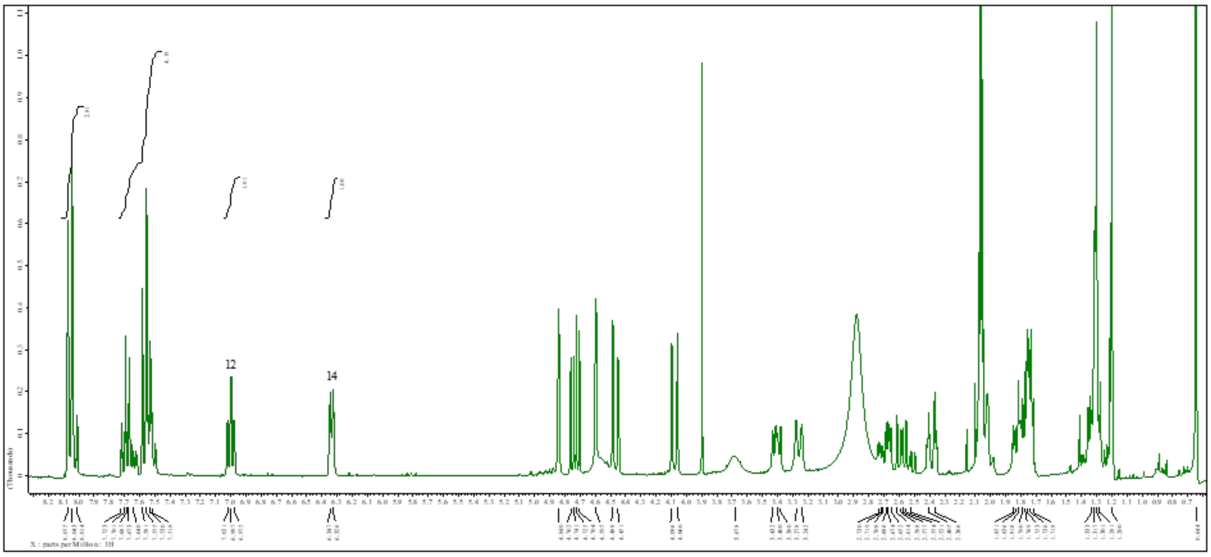


Fig 7S. ^{1}H NMR spectrum for 14-O-benzoyl adrographolide (**2**)

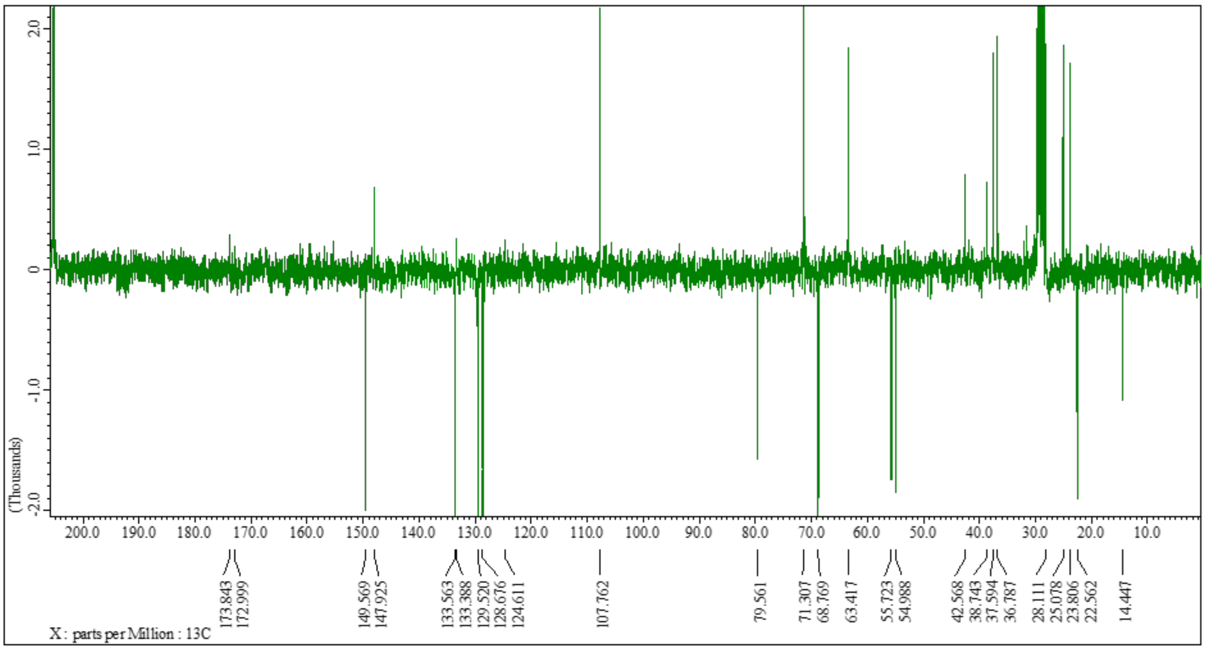


Fig 8S. DEPT ^{13}C NMR spectrum for 14-O-benzoyl adrographolide (**2**)

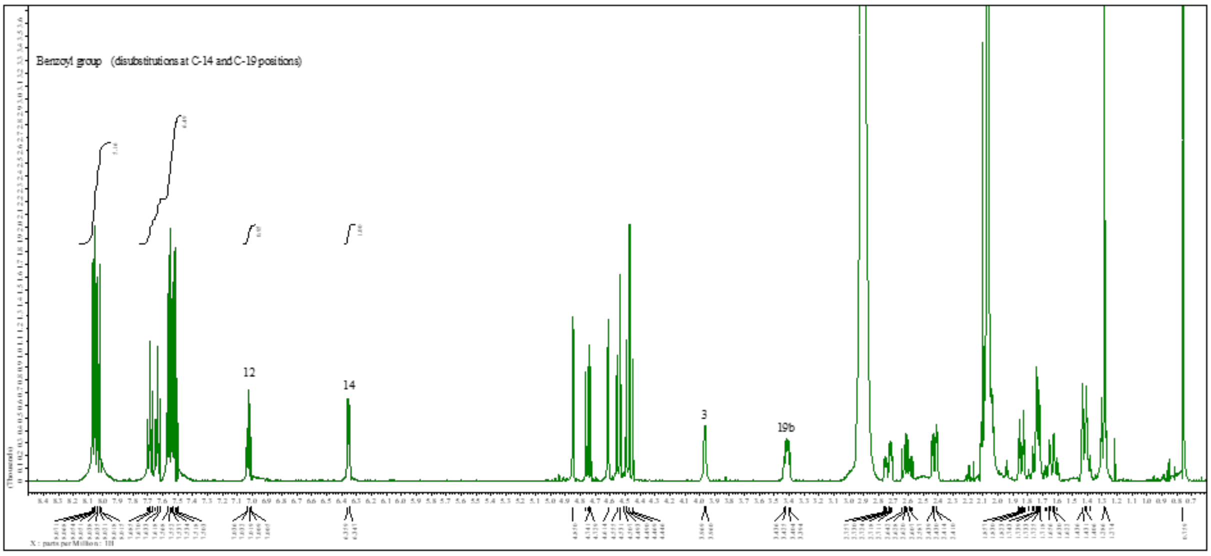


Fig 9S. ^{1}H NMR spectrum for 14,19-O-dibenzoyl andrographolode (**3**)

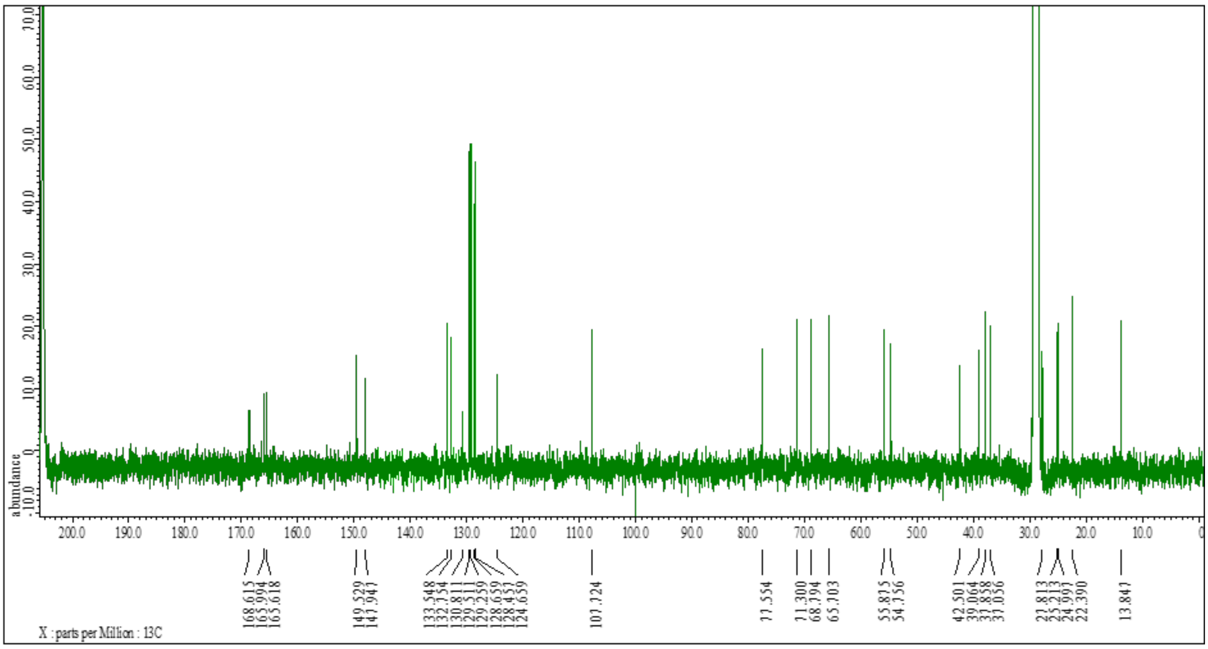


Fig 10S. ^{13}C NMR spectrum for 14,19-O-dibenzoyl andrographolode (**3**)

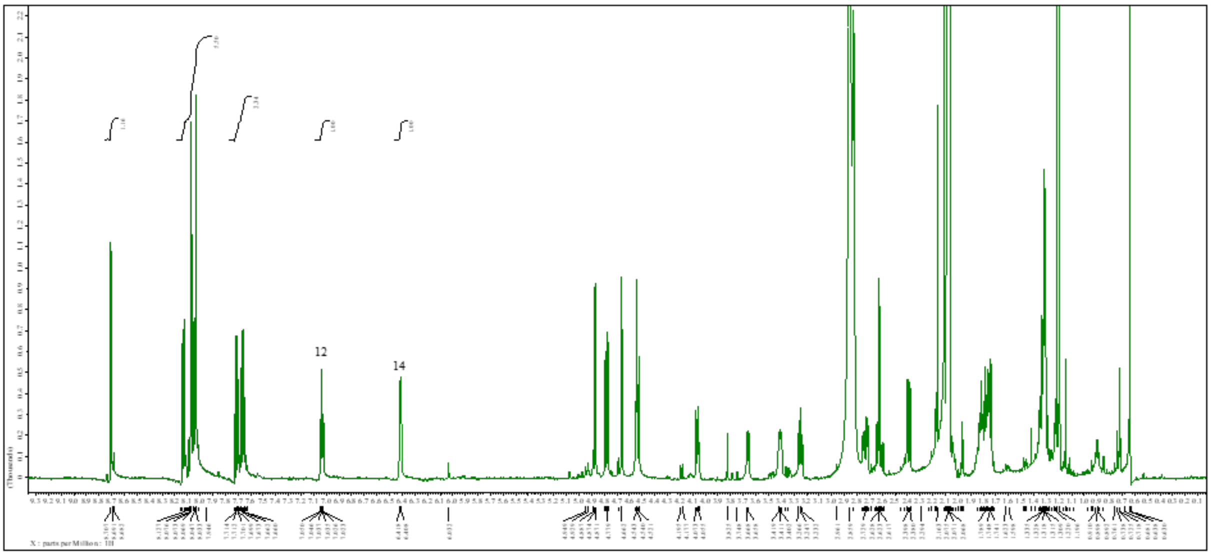


Fig 11S. ^{1}H NMR spectrum for 14-O-(2-naphthoyl) andrographolide (**4**)

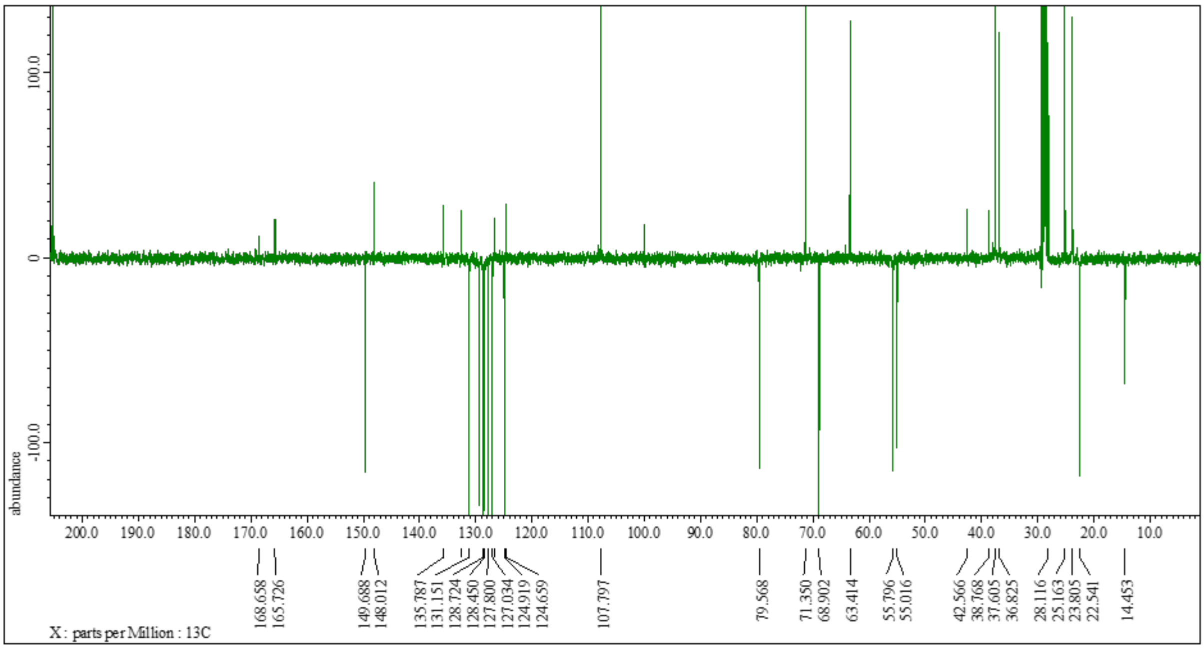


Fig 12S. ^{13}C NMR spectrum for 14-O-(2-naphthoyl) andrographolide (**4**)

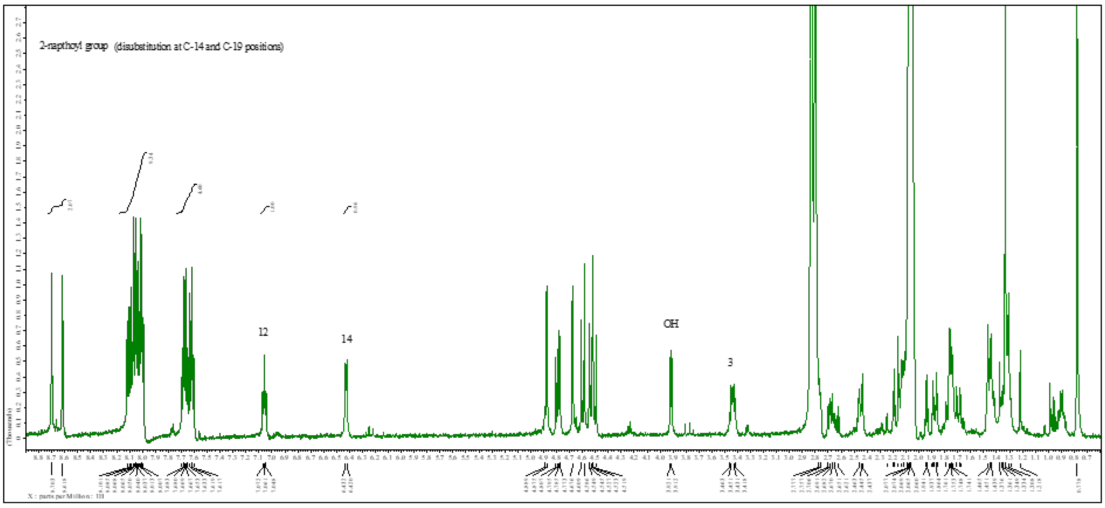


Fig 13S: ^{1}H NMR spectrum for 14,19-O-di(2-naphthoyl) andrographolide (**5**)

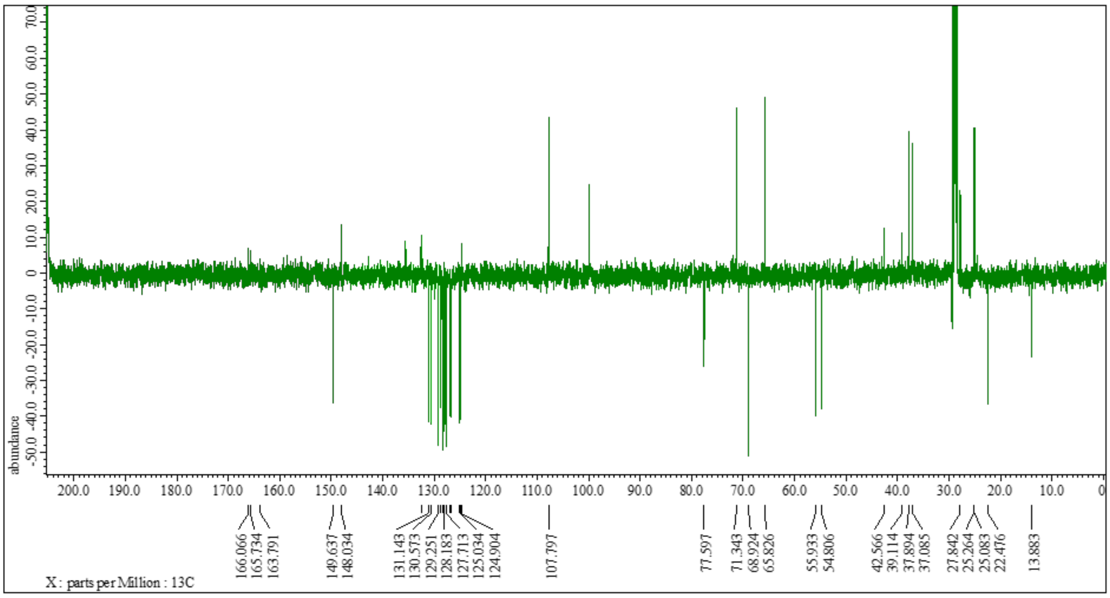


Fig 14S. DEPT ^{13}C NMR spectrum for 14,19-O-di(2-naphthoyl) andrographolide (**5**)

Table 3S. Key peaks for the experimental and calculated VA and VCD spectra of andrographolide (**1**)

Peak	VA		VCD	
	Experimental	Calculated	Experimental	Calculated
1850	x	x		
1820			x	x
1720	x	x		
1700			x	x
1570		x		
1470			x	x
1420			x	x
1270			x	x
1220			x	x
1200	x	x		