

OPTIMIZATION OF EXTRACTION METHOD AND QUALITATIVE FT-NMR ANALYSIS OF *ANDROGRAPHIS PANICULATA* LEAVES

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ABSTRACT

Three parameters; types of solvents, length of extraction time and temperature of medium were chosen in the optimization of *Andrographis paniculata* leaves extraction. Four different solvents, methanol (MeOH), dichloromethane (DCM), ethyl acetate (EtOAc) and water (H₂O) were used in a period of 1, 3, 5 or 7 hours at set water-bath temperature of 25, 30, 40 or 60°C. The extraction yield of about 200 mg ± 0.1 mg fresh cut leaves was measured based on the extract weight, and 1D-Nuclear Magnetic Resonance (NMR) profiles were employed to correlate the obtained yield to the main compound, andrographolide, percentage present.

Methanol was shown to be the best solvent with 13.75% yield in 3 hours extraction at 40°C. The NMR peak intensity analysis of the major compound (andrographolide) is also in support of these obtained parameters.

KEYWORDS: *Andrographis paniculata*, Methanol, Andrographolide, NMR

INTRODUCTION

Andrographis paniculata (Burm.f.Nees) or “HempeduBumi” belongs to the family Acanthaceae is widely grown in tropical areas of Asia like Malaysia, India, Pakistan and Sri Lanka (Vijaykumar *et al.*, 2007). It is one of the popular medicinal plants due to having a broad range of pharmacological properties including support liver function (Kapil *et al.*, 1993), and for malarial treatment (SitiNajila *et al.*, 2002). It is also claimed to help support normal body temperature, circulatory and cardiovascular function (Zhao and Fang, 1990) [4], hence used in the treatment of hypertension (Zhang and Tang, 1995) and myocardial infarction (Guo *et al.*, 1994; Guo *et al.*, 1995; Guo *et al.*, 1996).

Extensive research has revealed that extracts of *A. paniculata* have anticancer, antiinflammatory, anti-allergic, immune stimulatory, antithrombotic, hypoglycaemic and hepatoprotective activities (Gupta *et al.*, 1998; Habtemariam, 1998; Handa & Sharma, 1990; Matsuda *et al.*, 1994; Puri *et al.*, 1993; Trivedi & Rawal, 1998; Zhang & Tan, 2000).

Andrographolide is the active diterpene lactone which has been reported as the major component in *A. paniculata*. It was claimed to be responsible for various medicinal properties (Patarapanichet *et al.*, 2007; Shah *et al.*, 2007; Aromdeeet *et al.*, 2005).

Besides andrographolide, other active components identified are neoandrographolide, 14-deoxyandrographolide, 14-deoxy-11,12-didehydroandrographolide, 14-deoxy-14,15-dehydroandrographolide, homoandrographolide, andrographan, andrographon and andrographosterin (Cheung *et al.*, 2001; Reddyet *et al.*, 2003; Chaoet *et al.*, 2010; Siriponget *et al.*, 1992).

Based on the popularity of this plant traditional use and its related biological active main component of

andrographolide, the present study was done in optimizing the extraction method using different solvents in different duration of time and temperature in correlation to the 1D-NMR profiles particularly on andrographolide as further described in this paper.

EXPERIMENTAL

Materials and Method

All solvents for extraction and NMR analysis were HPLC Grade and deuterated (Darmstadt, Germany) respectively. Andrographolide used as standard in this study, was obtained as pure compound from *Andrographis paniculata* and authenticated based on spectral data comparison with reported investigation (Matsuda *et al.*, 1994).

Plant Material

Andrographis paniculata plants were bought from the Malaysian Agricultural Research and Development Institute (MARDI) Cultivation Centre, Serdang, Malaysia. The plant was identified by MARDI's in-house botanist wherein the herbarium specimen was deposited at MARDI. The plants were kept in the poly bags and watered to keep fresh. Leaves were wiped and patted dry before cut and immediately weighed of about 200 mg \pm 0.1 mg for each sample batch.

Solvent Extraction

The fresh leaves were cut into small pieces before immersed into 20 mL of selected solvent in a 50 mL conical flask. Four different solvents based on different polarity, methanol (MeOH), ethyl acetate (EtOAc), dichloromethane (DCM) and water (H₂O) were used as extraction medium. A triplicate of samples was prepared for each solvent system. In determining the best solvent to be used for the different extraction time and temperature, preliminarily all samples were placed in the water-bath shaker for three hours and the temperature was set at room temperature.

The sample extracts were then dried using rotary evaporator under vacuum at the temperature set at 40°C. The weight of yield for each sample was calculated and compared based on the formulation (1) given below:

$$\text{Yield (\%)} = (\text{Weight of dried extract obtained (g)} / \text{Initial weight of leave's sample (g)}) \times 100\% \quad (1)$$

Using the best solvent which gave the highest yield, other parameters such as time and temperature were varied.

Extraction Time

The sample leaves were immersed into 20 mL of MeOH, the solvent which gave the highest yield, and put into the water-bath shaker in different time period which was 1, 3, 5 and 7 hours. For each time period, three sample mixtures were prepared.

Extraction Temperature

The leaves sample in 20 mL of MeOH in triplicates were put into the water-bath shaker set at different temperature of 25, 30, 40 and 60°C for 3 hours which is the best time duration giving the highest yield.

RESULTS AND DISCUSSIONS

Mean (2), standard deviation (SD) (3) and relative standard deviation (RSD) (4) were calculated for all sample preparations based on the formula given below:

$$X_{\text{Mean}} = (\sum_{n=1}^N x_n) / N \quad (2)$$

$$\text{Standard deviation, } \sigma = \sqrt{\left\{ \sum_{n=1}^N (x_n - X_{\text{Mean}})^2 \right\} / \{N - 1\}} \quad (3)$$

$$\text{Relative standard deviation (RSD)} = (\text{Standard deviation of a data set} / \text{Mean of a data set}) \times 100 \quad (4)$$

(Miller & Miller, 1992)

Solvent Extraction

Table 1 shows the mass of yields of four different solvent systems in which MeOH has been found to be the best solvent system to give the highest mass of yield of *A. paniculata* fresh leaves extract. MeOH is a preferred solvent as it has larger polarity range and safer as it is less toxic compared to DCM or EtOAc.

Table 1: Mass of Yield, Mean, Standard Deviation and RSD of Each Solvent System

Solvent		Mass (g)				Mean (% Yield)	Standard Deviation	RSD %
		Leaves	Round Bottom Flask	Round Bottom Flask + Yield	Yield			
MeOH	1	0.2007	46.7694	46.7972	0.0278	13.75	3.8760	28.19
	2	0.2064	39.7277	39.7640	0.0363			
	3	0.2004	36.3170	36.3367	0.0197			
DCM	1	0.2036	59.6696	59.6722	0.0076	3.85	0.1153	2.99
	2	0.2045	65.6242	65.6321	0.0079			
	3	0.2070	108.9258	108.9731	0.0082			
EA	1	0.1985	108.4162	108.4413	0.0251	10.37	2.7062	26.09
	2	0.2043	109.2360	109.2511	0.0151			
	3	0.2001	113.3964	113.4186	0.0222			
H ₂ O	1	0.2006	48.6494	48.6566	0.0072	3.60	0.1502	4.17
	2	0.2011	51.2351	51.2426	0.0075			
	3	0.2009	43.2256	43.2325	0.0069			

Extraction Time

Extraction time is crucial in minimizing energy and cost of the extraction process. Table 2 shows the mass yield of each sample based on different extraction time of 1, 3, 5 and 7 hours in MeOH.

Table 2: Mass and Mean of Yield of Each Extraction Time

Time Extraction (Hour(s))		Mass (g)				Mean
		Leaves	Round Bottom Flask	Round Bottom Flask + Yield	Yield	
1	1	0.1997	69.8838	69.9158	0.0319	0.0331
	2	0.2007	89.9638	89.9978	0.0340	
	3	0.2040	102.1756	102.2091	0.0335	
3	1	0.2004	51.3667	51.4020	0.0353	0.0412
	2	0.1990	43.2327	43.2780	0.0453	
	3	0.2043	69.3756	69.4185	0.0429	
5	1	0.2015	167.2562	167.2980	0.0418	0.0410
	2	0.2012	103.7054	103.7439	0.0385	
	3	0.2040	110.9608	111.0036	0.0428	
7	1	0.2022	54.0993	54.1219	0.0226	0.0231
	2	0.2024	50.0383	50.0561	0.0178	
	3	0.2025	39.8556	39.8845	0.0289	

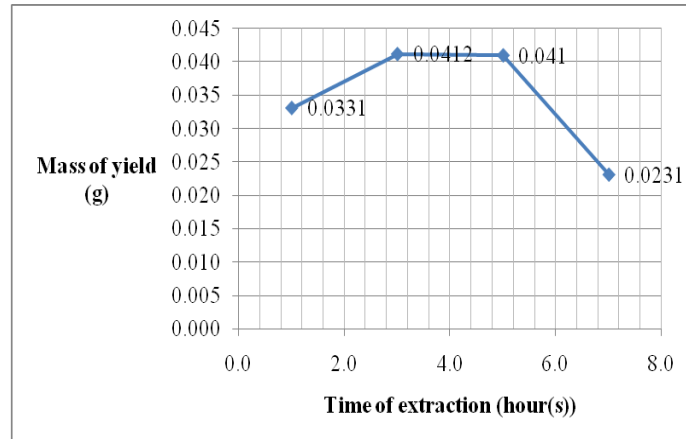


Figure 1: Graph Plot on the Yield Due to Different Extraction Times

Obtained yields as listed in Figure 1 exhibited that 3 and 5 hours of extraction time has resulted in a very close weight of yields. In order to minimize the extraction time while obtaining a high sample yield, 3 hours of time extraction was chosen to be the suitable extraction period.

It was reported that prolonged extraction time might lead to longer exposure to oxygen, thus increase the chances for occurrence of oxidation particularly on phenolic compounds (Nacz & Shahidi, 2004; Chirinos *et al.*, 2007).

Extraction Temperature

By choosing MeOH as the solvent and time of extraction of 3 hours, the temperature of the samples was varied as shown in Table 3. The sample yield was high when the temperature of the water bath was set at 40°C and a slight decrease of 12.2% yield when set at 30°C.

Based on the percentage yields of all samples, it could be observed that the suitable parameters for *A. Paniculata* extraction are MeOH as solvent, time extraction of 3 hours and water bath temperature set at 40°C.

Table 3: Mass, Percentage Yield, Standard Deviation and RSD of Different Temperatures

Temperature (°C)		Mass (g)				Mean (% Yield)	Standard Deviation	RSD %
		Leaves	Round Bottom Flask	Round Bottom Flask + Yield	Yield			
25	1	0.2004	49.3868	49.4025	0.0157	7.7991	0.1197	1.5354
	2	0.2064	52.2048	52.2211	0.0163			
	3	0.2022	48.4640	48.4795	0.0155			
30	1	0.2002	51.4030	51.4259	0.0229	11.8863	1.0204	8.5850
	2	0.2053	50.9886	51.0154	0.0268			
	3	0.2015	52.4161	52.4386	0.0225			
40	1	0.2045	52.2096	52.2378	0.0282	13.4109	0.3573	2.6641
	2	0.2026	52.1551	52.1816	0.0265			
	3	0.2028	46.6076	46.6347	0.0271			
60	1	0.2044	52.2143	52.2254	0.0111	6.0391	0.5484	9.0807
	2	0.2051	48.4654	48.4781	0.0127			
	3	0.2017	39.7287	39.7418	0.0131			

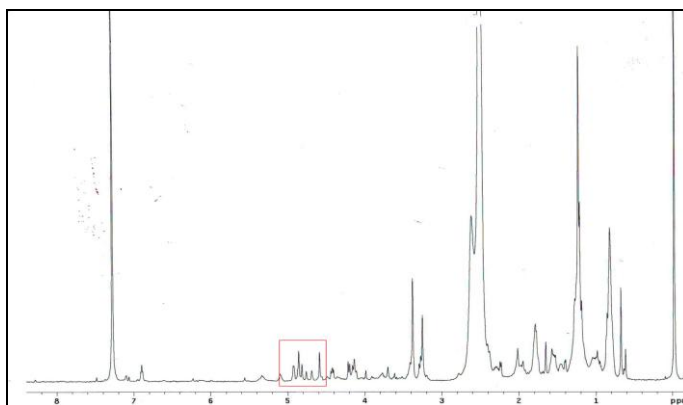
Qualitative Analysis of *A. paniculata* Extracts by FT-NMR

Figure 2: *A. paniculata* Extract in MeOH (NMR Solvent: CDCl_3 + TMS)

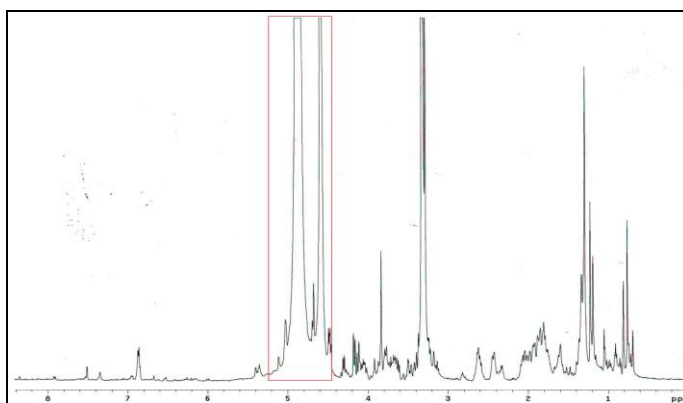


Figure 3: *A. paniculata* Extract in MeOH (NMR Solvent: CD_3OD + TSP)

Figures 2 and 3 show the NMR spectra of *A. Paniculata* MeOH extract analyzed in methanol- d_4 (CD_3OD) and chloroform- d (CDCl_3) respectively. Despite less resolution for the peaks in CDCl_3 compared to CD_3OD , CDCl_3 was chosen as the NMR solvent as there were no solvent peaks such those observed in CD_3OD in the regions of δ 3.0 - 3.3 and 4.5 - 5.1, which overlapping with a few important peaks of the plant extract.

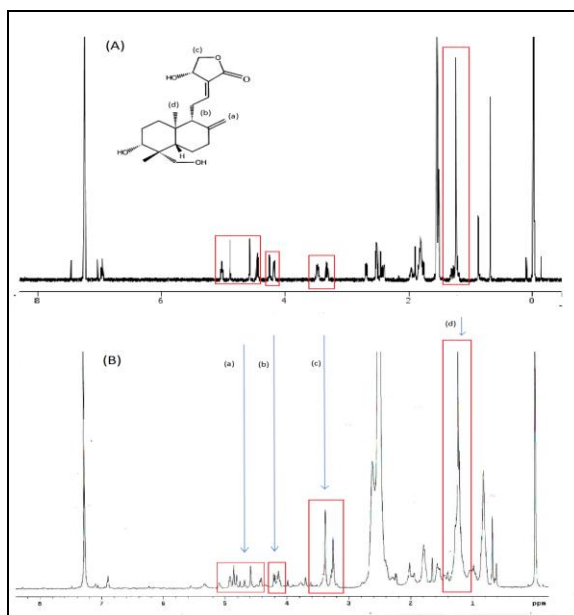


Figure 4: (A) NMR Spectrum of Standard Andrographolide (B) NMR Spectrum of *A. paniculata* Extract in MEOH (NMR Solvent: CDCl_3 + TMS)

Figure 4(A) shows the standard NMR spectrum of andrographolide and Figure 4(B) is the spectrum of one of the obtained samples. In comparing both spectra, the peaks were identified to be those of (a) =CH₂, (b) –CH, (c) –CH₂ and (d) –CH₃.

Optimization method based on the chosen parameters was correlated to the NMR spectra of the samples. The peaks intensities of each NMR spectrum indicated the degree of extraction wherein higher intensity of a particular compound such as andrographolide suggesting better extraction.

NMR Analysis on Solvent Extraction

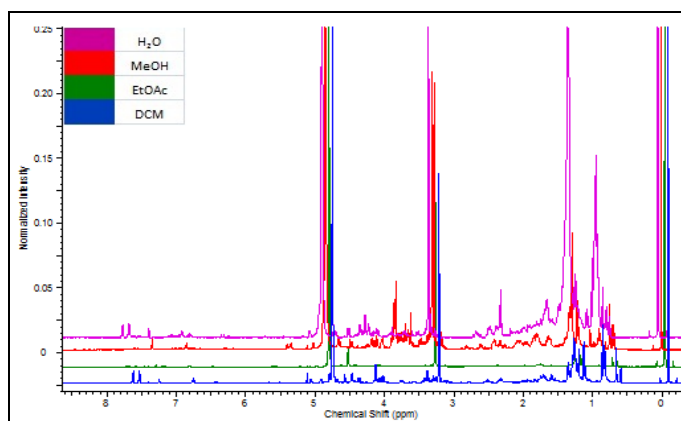


Figure 5: NMR Overlay Spectra of *A. paniculata* Extracts in Chloroform (CHCl₃), Methanol (MeOH), Ethyl Acetate (EA), Dichloromethane (DCM) and Water (H₂O)

Figure 5 shows the overlay of NMR spectra based on the solvent extraction. The X- and Y-offset of the overlay spectra were set to the 0.05 ppm and 0.05 Rel, respectively. The best peak intensity can be observed in the 3 hours extraction in H₂O and MeOH followed by DCM and EtOAc. Extraction in H₂O shows the highest peak intensity at chemical shift range from 0.8 to 2.4 ppm, whereas extraction in MeOH shows the highest peak intensity range from 3.5 to 4.9 ppm. Avani-gadda & Vangalapati, (2010) reported that methanol was the best solvent for the extraction of andrographolide in comparison to non-polar solvents or more polar solvents. This present study also showed the same pattern of methanol being the most suitable solvent for andrographolide extraction.

NMR Analysis on Extraction Time

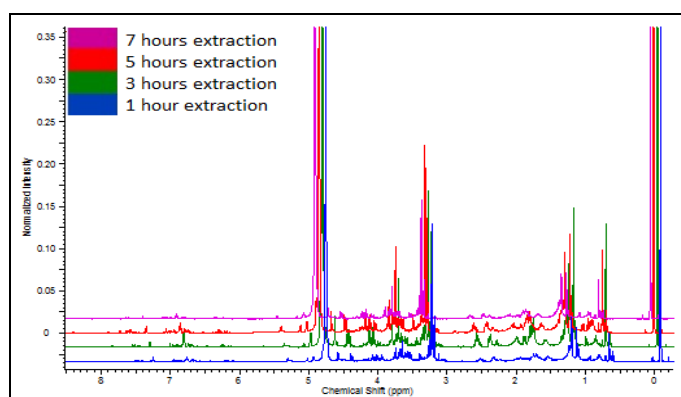


Figure 6: NMR Overlay Spectra of *A. Paniculata* Extracts in MeOH for 1, 3, 5 and 7 Hour(s)

The best peak intensity can be observed in the 3 hours extraction in MeOH as shown in Figure 6, in agreement to the result of the mass percentage yield. The X-offset and Y-offset of the overlay spectra were set to the 0.05 ppm and 0.05 Rel, respectively. The extraction time can either be as short as few minutes or very long up to 24 hours (Lee *et al.*, 2005;

Hismath *et al.*, 2011). The range of time was determined based on the practical and economical aspects besides the desired high extraction yield. Due to the slight difference in the yields of extraction in 7, 5 and 3 hours, the shortest extraction time which is 3 hours was chosen as the parameter.

NMR Analysis on Extraction Temperature

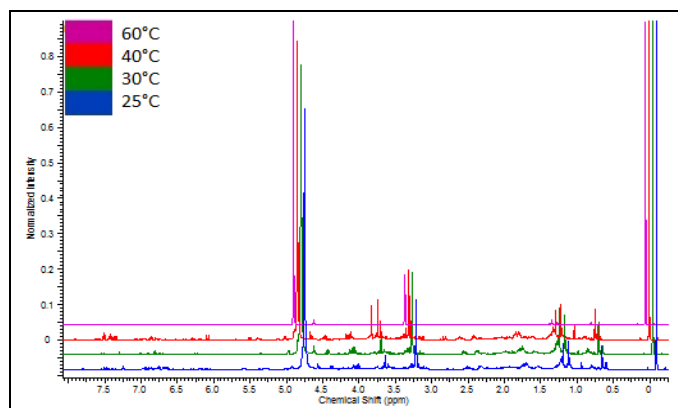


Figure 7: NMR Overlay Spectra of *A. paniculata* Extracts in MeOH at 25, 30, 40 and 60°C

The NMR results based on the temperature set for extraction showed that the intensity of the peaks in 25°C, 30°C and 40°C were more pronounced compared to 60°C as shown in Figure 7. The best peak intensity can be observed in 40°C extraction. The X-offset and Y-offset of the overlay spectra were 0.05 ppm and 0.05 Rel, respectively. Hismath *et al.* (2011) reported that, moderate extraction temperature of 25, 35 and 45°C were chosen as the lower, middle and upper levels, respectively, to be the extraction temperature. In general, increasing the temperature beyond certain values may encourage possible concurrent decomposition of the compounds which were already mobilized at lower temperature or even the breakdown of the compounds that are still remained in the plant matrix. High temperature may encourage solvent loss through vaporization and increase the cost for extraction process from the industrialization point of view (Hismath *et al.*, 2011). Hence, 40°C is a suitable extraction temperature which has shown to give a pronounced peaks intensity in NMR analysis.

CONCLUSIONS

Based on the mass percentage yield of all samples, it can be concluded that the suitable parameters for *A. paniculata* extraction are methanol solvent, extraction time of 3 hours at 40°C. From the NMR analysis, the highest percentage of the major compound, andrographolide, could also be observed in methanol extract, extraction time of 3 hours and extraction temperature of 40°C.

ACKNOWLEDGEMENTS

The authors would like to express their gratitude to the Universiti Putra Malaysia for the financial support through RUGS Project no. 91968. Our appreciation goes to Associate Professor Dr. Johnson Stanslas, Pharmacotherapeutics Unit, Department of Medicine, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia for providing the andrographolide.

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