

# Extraction of Diterpenoid Lactones of *Andrographis paniculata* using Liquid Solvents: Effect of Solvent's Hildebrand Solubility Parameter on Extraction Efficiency

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## ABSTRACT

*Two diterpenoid lactones responsible to the multi healing properties of Andrographis paniculata have been extracted using 16 different liquid solvents. The effect of Hildebrand solubility parameter of the solvents on solid-liquid extraction yield using standard soxhlet extraction method was investigated. The Hildebrand solubility parameter concept performed well when predicting lower polar solvent effect on extraction efficiency. However, ethanol extracted less andrographolide than methanol even though ethanol has closer Hildebrand solubility parameter value to andrographolide compared to that of methanol. Methanol's shorter aliphatic chain and higher polarity, which lead to easier hydroxyl group liberation, seemed to contribute this discrepancy.*

*Keywords: extraction, diterpenoid lactones, Andrographis paniculata, solvent, Hildebrand solubility parameter.*

## 1. Introduction

*Andrographis paniculata* NEES, locally known as Hempedu Bumi grows widely in the tropical area of South East Asia, India and China with annual growth of 30 – 70 cm height. In Malaysia and Indonesia, this plant has been extensively used for traditional medicine and help against fever, dysentery, diarrhoea, inflammation, and sore throat <sup>[1]</sup>. Andrographolide and deoxy-andrographolide are the main diterpenoid lactones contained in the leaves of

this plant. With respect to their anti cancer activity, it is a promising new way for the treatment of many diseases, including HIV, AIDS, and numerous symptoms associated with immune disorders <sup>[2]</sup>.

Conventional soxhlet extraction is one of the most common methods of separating bioactive components from natural resources. The most outstanding advantages of conventional soxhlet extraction are as follows <sup>[3]</sup>: (1) the sample is repeatedly brought into contact with the fresh portions of the solvent, thereby helping to displace the transfer equilibrium, (2) the temperature of the system remains relatively high since the heat applied to the distillation flask reaches the extraction cavity to some extent, (3) no filtration is required after the leaching step, (4) sample throughput can be increased by simultaneous extraction in parallel and (5) it has the ability to extract more sample mass than other extraction methods and non-matrix dependent.

To quantitatively estimation the solubility of an analyte in a solvent, the Gidding's theory can be used. This theory relies on the differences between the Hildebrand solubility parameters for the solvent and the analyte. Examining the Gibbs-Helmholtz equation  $\Delta G = \Delta H - T \Delta S$ . Dissolution occurs when the free energy of mixing  $\Delta G$  (J/mol) is negative. Since the dissolution of some solutes is always associated with large entropy of mixing increment,  $\Delta S$  (J/mol.K), solubility will thus be determined by the heat of mixing  $\Delta H$  (J/mol). According, to Hildebrand and Scott <sup>[4]</sup>, the heat of mixing can be defined as:

$$\Delta H = v_1 v_2 (\delta_1 - \delta_2)^2 \quad (1)$$

where  $\Delta H$  is the energy change from the formation and cleavage of intermolecular bonds;  $v_1$  and  $v_2$  are the partial molar volumes ( $\text{cm}^3/\text{mol}$ ) of the solvent and solute respectively,  $\delta_1$  and  $\delta_2$  are the solubility parameters ( $(\text{J}/\text{cm}^3)^{1/2}$ ) of the solvent and solute thus determines if the solute is soluble in the solvent. The smaller the difference of the solubility parameters between the solvent and solute, the higher is the solubility. In the absence of crystal phases and hydrogen bonds, a solute will be soluble in the solvent if  $(\delta_1 - \delta_2) < 1.7\text{-}2.0$ . The solubility parameter of the dissolved analyte  $\delta_2$ , can be either be obtained from literature or calculated from group contribution method. If the structure of the analyte is known,  $\delta_2$  of many complex solutes can be calculated using Fedors' group contribution method [5]. This is done by adding up the contribution of the individual groups to the vapourisation energy ( $\text{J}/\text{mol}$ ) and the molar volume of overall structure ( $\text{cm}^3/\text{mol}$ ),

$$\sum_{i=1}^n (\Delta E_i) \text{ and } \sum_{i=1}^n (\Delta v_i).$$

The solubility parameter for the solute is calculated as the square root of the ratio of the summation of all energy contributions to the summation of the all the group volumes. The solubility parameter values estimated using the Fedors method are consistent with those obtained from other sources. Having discussed all these, it is important to remember that solubility consideration addresses only part of the extraction problem.

$$\delta_2 = \left( \frac{\sum_{i=1}^n (\Delta E_i)}{\sum_{i=1}^n (\Delta v_i)} \right)^{1/2} \quad (2)$$

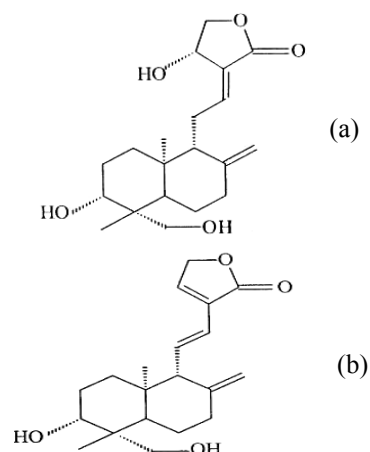
It has been well-understood that the extraction of the analyte also depends on the analyte-matrix interaction and the ability of the solvent to compete with the analytes for the sorptive sites. Hence, understanding the mechanism of the specific extraction process is crucial as it allows easier and faster optimisation of the extraction.

## 2. Material and Methods

Dried and ground leaves of *A. paniculata* were collected from Malaysian Agricultural Research and Development Institute (MARDI) and analysed using a Scanning Electronic Microscope in order to

observe their average thickness and structure. The leaves were observed as plate in shape with average thickness of 33  $\mu\text{m}$ . The density of the leaves ( $\rho$ ) was determined by helium pycnometry and the porosity ( $\epsilon$ ) was calculated as volume of pores divided by total volume of the solid. The values of both physical properties were 841  $\text{kg}/\text{m}^3$  and 0.30, respectively. Andrographolide standard compound having 98 % of purity supplied by Sigma - Aldrich (M) Sdn. Bhd. and deoxyandrographolide standard compound with 99 % of purity purchased from LKT Laboratories, Inc. (USA). Ethanol (Scharlau Chemie, HPLC grade, 99.8%), methanol (Merck, HPLC grade, 99.8%), ethyl acetate (Merck, HPLC grade, 99.5%), dichloromethane (Merck, HPLC Grade, 99%), n-hexane (Merck, HPLC Grade, 99%), petroleum ether (Labscan Asia, Analytical Grade, 99.0%), chloroform (Mallinckrodt Baker, HPLC Grade, 99.9%), acetone (J.T. Bakers, HPLC grade, 99.7%) and distilled water supplied by Analytical Laboratory, Department of Chemical Engineering, University of Malaya were employed as the liquid solvents.

Prior to solvent extraction study, 5 grams of dried and ground leaves of *A. paniculata* was placed in a cellulose thimble (25 mm  $\times$  100 mm). An amount of 150 mL of liquid solvent was used for the extraction using Soxhlet extraction system (BÜCHI Extraction System Model B-811, Switzerland) as shown in Fig. 2. The solvent extracts were then concentrated using vacuum rotary evaporator (BÜCHI Rotavapor Model R-144, Switzerland) and completely dried in an atmospheric oven. Some pure and mixed solvents were used in this extraction process. Analysis of the andrographolide and deoxy-andrographolide contents in the extract was carried out using high performance chromatography.



**Fig 1.** Molecular structure of andrographolide (a) and deoxy-andrographolide (b)



Fig. 2. The BÜCHI extraction system

### 3. Results and Discussions

The Hildebrand solubility parameter of a liquid,  $\delta$  is defined as the square root of the cohesive energy density. While, the cohesive energy density itself is defined as the ratio of the energy of vaporisation to the molar volume both referred to the same temperature<sup>[5]</sup>. Molecular size of the solute will affect its relative solubility in the corresponding solvents. The larger the molecular volume, then the greater the effect of a change in solvent polarity will be on the solubility of the solute<sup>[6]</sup>. As shown in Table 1, the values of Hildebrand solubility parameter of methanol and ethanol are 14.45 and 12.90, respectively. The Hildebrand solubility parameter of andrographolide was found as 13.25 when predicted using Fedors method<sup>[5]</sup>. Although the Hildebrand solubility parameter of andrographolide is closer to that of ethanol, it was found that this chemical is more

Table 1

Effect of Hildebrand solubility parameter of the solvent on extract yield and diterpenoid lactones extracted from dried leaves of *Andrographis paniculata*

Solvent	Hildebrand Solubility Parameter ( $\delta$ ) <sup>[7]</sup>	Extract Yield (%)	Extracted (g/100 g dried leaves)	
			andrographolide	Deoxy andrographolide
n-Hexane	7.24	2.50	$8.00 \times 10^{-7}$	-
Petroleum Ether	7.74	7.10	$4.58 \times 10^{-4}$	-
DCM	9.88	6.08	$1.10 \times 10^{-2}$	-
Ethyl Acetate	9.04	12.65	$5.91 \times 10^{-2}$	-
Chloroform	9.24	12.00	$5.17 \times 10^{-2}$	-
Acetone 100%	9.66	13.16	$4.29 \times 10^{-2}$	-
Acetone 70%	13.78	24.00	$8.91 \times 10^{-2}$	$2.72 \times 10^{-5}$
Ethanol 100%	12.90	33.15	$1.01 \times 10^{-1}$	$1.37 \times 10^{-4}$
Ethanol 75%	15.53	35.72	$8.67 \times 10^{-2}$	$1.75 \times 10^{-4}$
Ethanol 50%	18.19	40.20	$5.37 \times 10^{-2}$	$1.46 \times 10^{-3}$
Ethanol 25%	20.78	44.35	$2.38 \times 10^{-2}$	-
Methanol 100%	14.45	32.11	$1.14 \times 10^{-1}$	-
Methanol 75%	16.69	38.08	$1.04 \times 10^{-1}$	$2.11 \times 10^{-4}$
Methanol 50%	18.93	37.99	$8.80 \times 10^{-2}$	$2.80 \times 10^{-4}$
Methanol 25%	21.16	35.84	$3.66 \times 10^{-2}$	-
Water	23.40	21.54	$1.05 \times 10^{-2}$	-

soluble in methanol compared to ethanol and other solvents. This is because, methanol has shorter hydrocarbon chain and therefore easier to liberate hydroxyl group compared to ethanol. As reported in the literature [6], solute solubility is at a maximum when the solute and solvent have the same Hildebrand solubility parameter value. Two solvents whose Hildebrand solubility parameter values are higher or lower than that of a given solute can be blended to give a mixture with a Hildebrand solubility parameter value equal to that of the solute, thus maximising solute solubility [6]. This theory agrees well with the result obtained, where addition of water in to methanol reduced the andrographolide content as the Hildebrand solubility parameter of dilute methanol will be far above of andrographolide's Hildebrand solubility parameter. The addition of water will also lead to the conversion of andrographolide into deoxy-andrographolide via hydrolysis or destruction of lactone rings [1, 8]. On the other hand, addition of water into ethanol and acetone increased their Hildebrand solubility parameter and thus, increased the extract yield and andrographolide content.

The Hildebrand solubility parameter values of other solvents studied here were far below or above the predicted Hildebrand solubility parameter of andrographolide, therefore their yield and andrographolide contents of the extracts were low. Normal hexane and petroleum ether were found as the worst solvents to be used for the extraction of andrographolide and deoxy-andrographolide. Dichloromethane and water were able to extract andrographolide at low yield, but unable to extract deoxy-andrographolide. With relative Hildebrand solubility parameters to andrographolide is close each other, chloroform, ethyl acetate, acetone and methanol 25 % had similar andrographolide yields.

#### 4. Conclusions

From the results of this study, some conclusions can be obtained as follows:

1. Non polar liquid organic solvents are almost unable to extract both andrographolide and deoxy-andrographolide.
2. Liquid organic solvents having moderate polarity are able to extract andrographolide at very low yield but still unable to extract deoxy-andrographolide.
3. Even though water is very polar, it extracts andrographolide in the same order as solvents with moderate polarity due to its strong hydrogen bonding.

4. Hildebrand solubility parameter concept explained well the effect of Hildebrand solubility parameter difference between solute and solvent on extraction yield, especially for non polar solvent.
5. Ethanol and methanol are good solvent for andrographolide and deoxy andrographolide extraction. Hildebrand solubility parameter concept failed to explain that methanol extracted more andrographolide than ethanol.

#### 5. Acknowledgement

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