

# Effects of solvent properties on the Soxhlet extraction of diterpenoid lactones from *Andrographis paniculata* leaves

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**ABSTRACT:** A solid-liquid extraction was performed for the leaves of *Andrographis paniculata* in order to obtain two bioactive components, andrographolide and deoxyandrographolide. The problem of hydrolysable components and solvent removal difficulties in the conventional extraction led us to study further the effects of solvent properties on the conventional extraction using Soxhlet method in order to determine the best solvent or solvent mixture for high extraction yield of *A. paniculata*. This solvent selection is very important prior to alternative extraction methods since it can be used as a pre-extraction solvent, main solvent, or co-solvent. Based on the yield of extract and andrographolide and deoxyandrographolide content, methanol was found to be the best solvent.

**KEYWORDS:** andrographolide, deoxyandrographolide, Hildebrandt solubility parameter, polarity, kalmegh

## INTRODUCTION

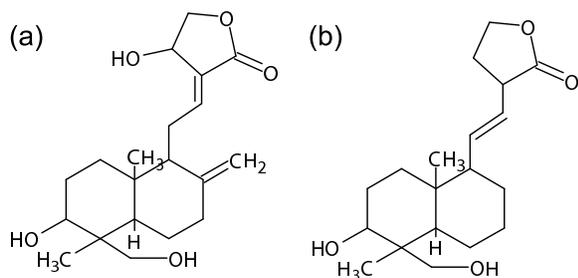
*Andrographis paniculata* NEES or (locally known as Sambiloto) grows widely in SE Asia, India, and China. In Malaysia and Indonesia this plant has been extensively used for traditional medicine and helps against fever, dysentery, diarrhoea, inflammation, and sore throat. Furthermore, it is a promising candidate for the treatment of many diseases, including HIV, AIDS, and numerous symptoms associated with immune disorders<sup>1</sup>.

Most of the bioactive components contained in the plant matrices are medium-sized molecules. Due to the presence of aromatic delocalized  $\mu$ -electrons, the molecules are highly polarizable. Their high polarizability makes the molecules liable to a variety of specific interactions with polar solvents, e.g., protonation, hydrogen bonding, and specific solvation<sup>3</sup>. However, for toxicological reasons, drug and medicine manufacturers are increasingly required to minimize the number of solvents employed in pharmaceutical processes. Certain types of solvents of known toxicity and environmental hazard (e.g., benzene, chlorocarbons) are no longer permitted to be used in the manufacture of pharmaceuticals. At the same time, the maximum content of individual solvents in drugs is

also regulated. The presence of a solvent in the extract may also affect the kinetics of crystallization and the morphology of the crystals, which is an important factor in determining the product quality<sup>3</sup>.

The three main diterpenoid lactones identified in *A. paniculata* leaves are andrographolide, neo-andrographolide, and deoxyandrographolide<sup>4-6</sup>. Andrographolide, an unsaturated trihydroxy lactone, has a molecular formula of  $C_{20}H_{30}O_5$  (Fig. 1). Andrographolide is the main component in the leaves of *A. paniculata*, which can be easily dissolved in methanol, ethanol, pyridine, acetic acid, and acetone, but slightly dissolved in ether or water. Its UV spectrum in ethanol has a  $\lambda_{max}$  at 223 nm<sup>5</sup>. Various techniques can be used for the analysis of andrographolide such as thin layer chromatography (TLC)<sup>5,7</sup>, high-performance liquid chromatography (HPLC)<sup>4,8,9</sup>, and crystallization<sup>5</sup>.

The objective of this work is to study the effect of solvent properties on the solid-liquid extraction of the *A. paniculata* diterpenoid lactones, andrographolide and deoxyandrographolide. The first part of this study deals with the physicochemical characteristic of ground dried *A. paniculata* leaves. Then, standard soxhlet extractions were carried out in order to determine the best solvent used for *A. paniculata*



**Fig. 1** Molecular structure of (a) andrographolide (b) deoxyandrographolide.

diterpenoid lactones extraction.

This work is a small part of a bigger herbal research project, which will allow us to choose the best solvent for a conventional extraction system and to recommend the best solvent to be used as co-solvent or pre-extraction solvent in supercritical carbon dioxide extraction of *A. paniculata* diterpenoid lactones. This study will also be integrated with classical pharmaceutical industry methods as an initial step to develop and implement a complete process. The focus in pharmaceutical chemistry is made on the extraction and isolation of the pure crystal of andrographolide<sup>4,5</sup>.

## MATERIALS 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 to observe their average thickness and structure. The leaves were observed as 33  $\mu\text{m}$  slabs. The density of the leaves ( $\rho$ ) was determined by helium pycnometry to be 841  $\text{kg}/\text{m}^3$ . The porosity ( $\varepsilon$ ) was calculated as the volume of pores divided by the total volume of the solid and was found to be 0.30. These values were about similar to that reported in the literature for *A. paniculata* leaves planted in Thailand<sup>4</sup>.

Prior to solvent extraction study,  $5.00 \pm 0.05$  g of dried and ground leaves of *A. paniculata* were placed in a Whatman cellulose thimble (25 mm  $\times$  100 mm). Samples were extracted in a Soxhlet extraction system (BÜCHI Extraction System Model B-811) using 150 ml of solvent. The heating power was set to two cycles/h so that six cycles of extraction were achieved within 3 h. Some pure and mixed solvents were used in this extraction process. For a mixture of organic-aqueous solvent, the percentage indicates the volume percentage of the organic solvent in the mixture.

The crude extract solutions obtained were then concentrated using a vacuum rotary evaporator (BÜCHI Rotavapor Model R-144) at a temperature of

60 °C or lower to remove the solvents, and completely dried in an atmospheric oven. High temperature treatment was avoided to minimize the component degradation. All extracts were then stored at room temperature before weighing gravimetrically to determine the yields.

Analyses were performed by HPLC with a system from Shimadzu. The column used for analysis was reverse phase C18-Thermo Hypersil ODS (250 mm  $\times$  4.6 mm, 5  $\mu\text{m}$  particle diameters) equipped with a UV detector. The volume injection was 20  $\mu\text{l}$ . The mobile phase was methanol/water (54/46, v/v) at constant 1 ml/min flow rate. During the analysis the wavelength of the UV detector was programmed as 250 nm from 0 to 4.5 min followed by 223 nm from 4.5 to 10 min. Chromatographic peaks were identified by comparison with the retention time of the standards. Linear calibrations of standards at an accuracy of more than 99% were carried out for the quantification of the *A. paniculata* extracts. Single injection of solvent (blank) was also made to determine the retention time of the solvent. The calibration standard curve was obtained by using pure crystals of andrographolide standard compound having 98% of purity (Sigma-Aldrich) and deoxyandrographolide standard compound (LKT Laboratories) with 99% of purity. The calibration graphs for andrographolide and deoxyandrographolide were linear from 1.1–24 and 1.05–24  $\mu\text{g}/\text{ml}$ , respectively. Recoveries of 99.1–103.2% were obtained with RSD of 1.1–1.8%.

The effect of polarity and Hildebrand solubility parameter of 16 solvents on the total extraction yield and content of bioactive andrographolide and deoxyandrographolide in *A. paniculata* leaves extracts were investigated.

## RESULTS AND DISCUSSION

### Effect of polarity

Plant matrices including leaf, stem, root, and bark contain various solute molecules with more than one functional group. Therefore, it is difficult to predict the solubility of the solutes in a particular solvent. An alternative way of considering solubility is to use the concept of polarity. Snyder<sup>10</sup> defined polarity as the relative ability of a molecule to engage in strong interactions with other polar molecules (not specifically the presence in a molecule of a large dipole moment). Polarity therefore represents the ability of a molecule to enter into interactions of all kinds and relative polarity is the sum of all possible interactions<sup>10</sup>.

The total yield and the amount of andrographolide

**Table 1** Effect of polarity and Hildebrand solubility parameter ( $\delta$ )<sup>13</sup> of the solvent on extract yield (EY) and amount (in g/100 g dried leaves) of diterpenoid lactones, andrographolide (A) and deoxyandrographolide (D), extracted from dried leaves.

Solvent	PI <sup>10</sup>	$\delta$	EY (%)	Extracted lactone	
				A	D
n-Hexane	0.1	7.24	2.50	$8.00 \times 10^{-7}$	-
Petroleum Ether	0.1	7.74	7.10	$4.58 \times 10^{-4}$	-
DCM	3.4	9.88	6.08	$1.10 \times 10^{-2}$	-
Ethyl Acetate	4.3	9.04	12.65	$5.91 \times 10^{-2}$	-
Chloroform	4.1	9.24	12.00	$5.17 \times 10^{-2}$	-
Acetone 100%	5.4	9.66	13.16	$4.29 \times 10^{-2}$	-
Acetone 70%	6.5	-	24.00	$8.91 \times 10^{-2}$	$2.72 \times 10^{-5}$
Ethanol 100%	5.2	12.90	33.15	$1.01 \times 10^{-1}$	$1.37 \times 10^{-4}$
Ethanol 75%	6.2	-	35.72	$8.67 \times 10^{-2}$	$1.75 \times 10^{-4}$
Ethanol 50%	7.1	-	40.20	$5.37 \times 10^{-2}$	$1.46 \times 10^{-3}$
Ethanol 25%	8.1	-	44.35	$2.38 \times 10^{-2}$	-
Methanol 100%	6.6	14.45	32.11	$1.14 \times 10^{-1}$	-
Methanol 75%	7.2	-	38.08	$1.04 \times 10^{-1}$	$2.11 \times 10^{-4}$
Methanol 50%	7.8	-	37.99	$8.80 \times 10^{-2}$	$2.80 \times 10^{-4}$
Methanol 25%	8.4	-	35.84	$3.66 \times 10^{-2}$	-
Water	9.0	23.40	21.54	$1.05 \times 10^{-2}$	-

PI = polarity index

and deoxyandrographolide extracted using organic solvents, water, and their mixtures are listed in Table 1. The results indicated that high extract yields of *A. paniculata* were obtained from extraction employing polar organic solvent containing hydroxyl group, namely methanol and ethanol, and aqueous acetone. Low extract yields were observed when the extraction was done using non-polar organic solvent, such as n-hexane and petroleum ether. All extracts obtained from extraction using pure organic solvents were observed as a dark green dry powder. However, extraction using water and aqueous mixed solvents produced powdery extracts with a yellowish-green colour.

The analysis of extracts from diterpenoid lactones in *A. paniculata* leaves indicated a large yield of andrographolide when the extractions were carried out using methanol and ethanol as solvents compared to water (Table 1). Methanol, ethanol, and water have similar solubility properties because they contain a hydroxyl group which is hydrophilic. Andrographolide, which is a large and polar hydrocarbon molecule, dissolves better in methanol and ethanol although it has more polar function<sup>11</sup>.

However, when water was added to methanol or ethanol, the andrographolide content in the extract decreased (Table 1). On the other hand, the content of deoxyandrographolide in aqueous methanol and aqueous ethanol extracts increased after adding water. This is because andrographolide might also

be hydrolysed into deoxyandrographolide due to the extraction conditions applied. Addition of water into methanol or ethanol will increase their boiling points. The problem with increasing the temperature is that all reactions are accelerated, including all the unwanted side reactions. Since andrographolide is a lactone, the opening of the lactone ring would be likely to initiate a destruction mechanism. However, the lactone ring would react in a different manner with water, methanol, or ethanol. Water will open the ring by hydrolysis, whereas alcohols will do it by transesterification. Hydrolysis is expected to be much faster reaction than transesterification. Therefore, the rate of destruction should depend on both the temperature and the composition of the solvents. This fact is in good agreement with the previous work where destruction of andrographolide occurred at a higher temperature in the presence of excess water<sup>4</sup>. Further addition of water into methanol and ethanol caused the methanol and ethanol to be diluted and unable to extract deoxyandrographolide since water itself is unable to extract it. The addition of water into polar solvents such as acetone and ethanol increases the solvent polarity and thus increases the yield of *A. paniculata* extracts. In the case of the addition of water to acetone, the increase of hydrogen bonding helped the aqueous acetone to extract both andrographolide and deoxyandrographolide. However, this phenomenon was not applicable to methanol, as the extract yields decreased with the increase of aqueous methanol polarity. Low polarity solvents such as n-hexane and petroleum ether, and intermediate polarity solvents such as ethyl acetate, dichloromethane and chloroform, were not able to significantly extract the diterpenoid lactones studied. This is because andrographolide is a lactone with strong polarity due to hydroxyl and carbonyl groups attached to the rings. Therefore, these solvents might be preferably used in the pre-extraction step, where the removal of undesirable components might be necessary. However, there is a requirement from environmental and toxicology considerations to reduce the quantity of organic solvents used, and chlorinated solvents in particular (including those such as dichloromethane and trichloromethane), even though both chlorinated solvents are not covered by the Montreal Protocol<sup>3,10</sup>.

#### Effect of Hildebrand solubility parameter

The Hildebrand solubility parameter of a liquid,  $\delta$  is defined as the square root of the cohesive energy density. 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>12</sup>. Molecu-

lar size of the solute will affect its relative solubility in the corresponding solvents. The larger the molecular volume, the greater the effect of a change in solvent polarity will be on the solubility of the solute<sup>10</sup>. As shown in Table 1, the values of  $\delta$  for methanol and ethanol are 14.45 and 12.90, respectively. The Hildebrand solubility parameter of andrographolide was found to be 14.80 when predicted using Fedors' method<sup>12</sup>. This solubility property supports the fact that andrographolide is more soluble in methanol than in ethanol and other solvents. Solute solubility is at a maximum when the solute and solvent have the same  $\delta$ <sup>10</sup>. Two solvents whose  $\delta$  values are higher or lower than that of a given solute can be blended to give a mixture with a  $\delta$  value equal to that of the solute, thus maximizing solute solubility<sup>10</sup>. This theory agrees well with the result obtained, where addition of water to methanol reduced the andrographolide content as  $\delta$  for dilute methanol will be far above that of andrographolide. On the other hand, addition of water into ethanol and acetone increased their  $\delta$  values 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  $\delta$  values of andrographolide. Hence their yield and the andrographolide contents of the extracts were low.

## CONCLUSIONS

This study showed that methanol was the best solvent for the extraction of diterpenoid lactones of *A. paniculata*, in terms of high extract and component yield. Ethanol and aqueous acetone were also found to be able to extract andrographolide, but at lower yield. The polarity and Hildebrand solubility parameter concepts explained the phenomena observed well. Petroleum ether, n-hexane, ethyl acetate, acetone and chloroform might be the best solvents to remove the unwanted components. However, the use of large amounts of unsafe organic solvents may not be a good choice for the processing of high-value phytochemicals, especially for the use in the pharmaceutical industry. Further work is being carried out to look into the potential of high-pressure extraction using carbon dioxide and methanol as co-solvent.

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