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Experimental and Modeling Studies of Andrographolide Extraction from *Andrographis paniculata* in a Soxhlet Extractor

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Abstract
Andrographolide is the main diterpenoid lactone contained in the leaves of *Andrographis paniculata.* This bioactive component has multifunctional medicinal properties such as activity against fever, dysentery, diarrhoea, inflammation, and sore throat as well as immune disorder. The objectives of this work were to study the effect of polarity and Hildebrand solubility parameter of solvents in the extraction of andrographolide from *A. paniculata* and to develop a mathematical model to quantitatively describe the extraction phenomena. The extraction was carried out by employing various organic solvents and their mixtures with water as solvents using standard soxhlet method. Five grams of ground - dried *A. paniculata* leaves was extracted using 1.50×10^{-4} m^3 of solvent for 10,800 seconds. The standard soxhlet extraction method was conducted using methanol at different extraction times to verify the mathematical model proposed in this work. The crude extracts were then analysed for their andrographolide content using high performance liquid chromatography. Methanol was found to be the best solvent for the extraction of andrographolide from *A. paniculata.* The Hildebrand solubility parameter concept was not able to predict the extraction of andrographolide using polar organic solvents. The final form of the proposed model based on rapid mass transfer at the interphase of the solid-liquid surface and the introduction of volumetric mass transfer coefficient is $E_s = 0.12\times(1- e^{-(1.69E-04)t})$, where E_s = total extract, (g) and t = extraction time, (second). The model showed good agreement with the experimental data by generating *AARD* of about 0.46 %.

Keywords: *Experimental, modelling, andrographolide, extraction, Andrographis paniculata*

1.0 Introduction

Andrographis paniculata NEES grows widely in the tropical areas of South East Asia, India and China with annual growth of 0.30 - 0.70 m height. This plant has been widely used for traditional medicine and help against fever, dysentery, diarrhoea, inflammation, and sore throat. It is also found to be a promising new way for the treatment of HIV, AIDS, and numerous symptoms associated with immune disorders [1].

Three main diterpenoid lactones identified in the *A. paniculata* leaves were andrographolide, neo-andrographolide and deoxyandrographolide [2, 3]. The molecular formula of andrographolide is $C_{20}H_{30}O_5$, while its molecular structure is shown in Figure 1. Andrographolide can be easily dissolved in methanol, ethanol, pyridine, acetic acid and acetone, but slightly dissolved in ether and water. The melting point of this compound is

228^{o} – 230^{o}C and the ultraviolet spectrum in ethanol, λ_{max} is 223 nm. The analysis of andrographolide can be done by thin layer chromatography (TLC) [3], high - performance liquid chromatography (HPLC) [2, 3] and crystallisation [3].

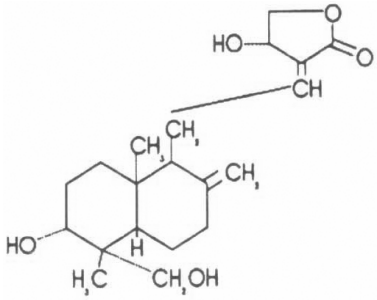

Figure 1. Molecular structure of andrographolide [3]

The liquid solvent extraction is the most common method for separating bioactive components from their natural resources. The advantages of this method over other extraction methods are as follows [4]: (i) the sample is repeatedly brought into contact with the fresh portions of the solvent, thereby helping to displace the transfer equilibrium, (ii) the temperature of the system remains relatively high due to the heat applied to the distillation flask, (iii) sample throughput can be increased by simultaneous extraction in parallel, (iv) it has the ability to extract more sample mass and it is non-matrix dependent. However, for toxicological reason, drug and medicine producers are required to minimise the number and amount of solvents employed in pharmaceutical processes. The presence of a solvent in the extract may also affect the kinetics of crystallisation and the crystal morphology of the product [5].

In order to optimise the utilisation of solvent in the extraction of bioactive components from natural resources, an estimation of the extract yield obtained is necessary. The objectives of this work were to study the effect of polarity and Hildebrand solubility parameter of solvents in the extraction of andrographolide from *A. paniculata* and to develop a mathematical model to quantitatively describe the extraction phenomena.

2.0 Materials and methods

2.1. Materials

The leaves of *A. paniculata* were collected from Malaysian Agricultural Research and Development Institute (MARDI). Andrographolide standard compound (98 % purity) was supplied by Sigma - Aldrich (M) Sdn. Bhd. and deoxyandrographolide standard compound (99 % purity) was purchased from LKT Laboratories, Inc. (USA). Various organic solvents (*Merck*, HPLC grade, 99.8%) were purchased from Bibi Saintifik Sdn. Bhd., while deionised water was generated in the Analytical Laboratory, Department of Chemical Engineering, University of Malaya

2.2 Solvent extraction

Prior to the solvent extraction study, 5 grams of dried - ground leaves of *A. paniculata* was placed in a cellulose thimble (25 mm × 100 mm). An amount of 1.50×10^{-4} m^3 of solvent was used for the extraction using a standard soxhlet method for 10,800 seconds in a soxhlet extraction system (BÜCHI Extraction System Model B-811, Switzerland). The standard soxhlet extraction method was conducted using methanol at different extraction times to verify the mathematical model proposed in this work. The extracts were then concentrated

using vacuum rotary evaporator (BÜCHI Rotavapor Model R-144, Switzerland) and completely dried in an atmospheric oven. The crude extracts were then analysed for their andrographolide content using high performance liquid chromatography.

2.3 Modelling of extraction using soxhlet extractor

In order to describe the andrographolide transfer from the leaf particles to the bulk of the solvent, the following hypotheses were used: (i) every leaf particle is symmetrical, (ii) the mass transfer coefficient is constant, (iii) the solvent in the extractor is perfectly mixed, while the transfer resistance in the liquid phase is negligible and the andrographolide concentration in the solvent depends only on time, (iv) the transfer of the andrographolide is a diffusion phenomenon and independent of time, (5) at the interface, the concentration of andrographolide in the solution between the internal liquid (in pores) and external to particles are equal. The final form of the equation obtained from this modelling is:

$$E_s = B.(1 - e^{-Dt}) \quad (1)$$

where E_s = total extract (g), t = extraction time (seconds), and B & D = equation constants.

3.0 Results and discussions

The results of this work are presented in Table 1 and Figure 2. In comparison to non - polar solvents, polar solvents could extract andrographolide at higher yield except water, where hydrolysis and thermal degradation might occur. Methanol was found to be the best solvent for the extraction of andrographolide. Ethanol and aqueous acetone extracted andrographolide at lower yield although their Hildebrand solubility parameters are closer to that of andrographolide. Solvents having moderate polarity extracted andrographolide much lower than ethanol did. Non - polar solvents were almost not able to extract andrographolide.

Table 1. Effect of solvent polarity and Hildebrand solubility parameter on extraction yield.

Solvent	Polarity [4]	Hildebrand Solubility Parameter [5]	Extract Yield (%)	Extracted (g/100 g dried leaves)	
				andrographolide	Deoxy andrographolide
n-Hexane	0.1	7.24	2.50	8.00×10^{-7}	-
Petroleum Ether	0.1	7.74	7.10	4.58×10^{-4}	-
DCM	3.4	9.88	6.08	1.10×10^{-2}	-
Ethyl Acetate	4.3	9.04	12.65	5.91×10^{-2}	-
Chloroform	4.1	9.24	12.00	5.17×10^{-2}	-
Acetone 100%	5.4	9.66	13.16	4.29×10^{-2}	-
Acetone 70%	6.5	13.78	24.00	8.91×10^{-2}	2.72×10^{-5}
Ethanol 100%	5.2	12.90	33.15	1.01×10^{-1}	1.37×10^{-4}
Ethanol 75%	6.2	15.53	35.72	8.67×10^{-2}	1.75×10^{-4}
Ethanol 50%	7.1	18.19	40.20	5.37×10^{-2}	1.46×10^{-3}
Ethanol 25%	8.1	20.78	44.35	2.38×10^{-2}	-
Methanol 100%	6.6	14.45	32.11	1.14×10^{-1}	-
Methanol 75%	7.2	16.69	38.08	1.04×10^{-1}	2.11×10^{-4}
Methanol 50%	7.8	18.93	37.99	8.80×10^{-2}	2.80×10^{-4}
Methanol 25%	8.4	21.16	35.84	3.66×10^{-2}	-
Water	9.0	23.40	21.54	1.05×10^{-2}	-

The model showed a good agreement with the experimental data as shown in Figure 2. Almost all of experimental data fell into the solid line representing the model and an *AARD* of about 0.46 % was obtained.

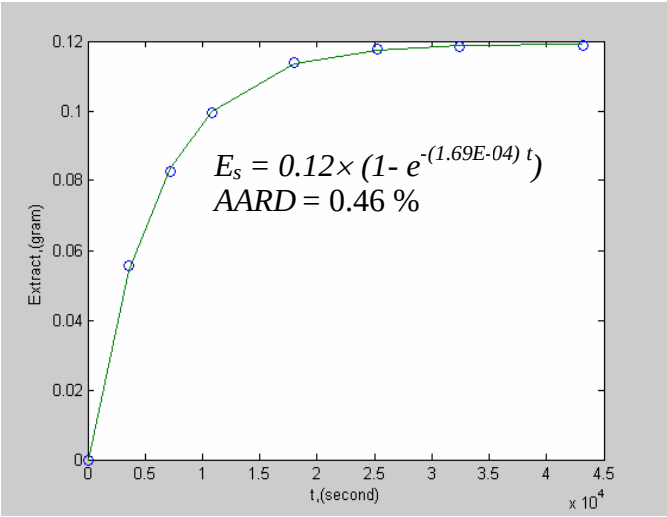

Figure 2. Comparison of extract weight calculated from the model and experimental data

4.0 Conclusions

Methanol was found to be the best solvent for the extraction of andrographolide from *Andrographis paniculata.* However, the Hildebrand solubility parameter concept was not able to predict the extraction of andrographolide using polar organic solvents. The final form of the proposed model is $E_s = 0.12\times (1- e^{-(1.69E-04)\ t})$, having an *AARD* of about 0.46 %.

5.0 Acknowledgement

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6.0 References

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