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Experimental and Modelling Studies of Andrographolide Extraction from Andrographis Paniculata

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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 1.00 x 10^{-4} m3 of solvent for 80minutes. The standard soxhlet extraction method was conducted using methanol, ethanol, ethyl acetate and water at different extraction times to verify the mathematical model proposed in this work. 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 ***Es* = 0.8917t – 9.8114**, where ,*Es* = total extract (g) and *t* = extraction time (in minutes).

Keywords: Andrographolide, *Andrographis Paniculata,* Extraction, Modelling, Soxhlet extraction

INTRODUCTION

Andrographis paniculata (Burm. f.) Nees (*Acanthaceae*) (*AP*) is an herb originated from India and widely distributed in southern China with annual growth of 0.30 - 0.70 m height. It has been used in traditional systems of medicine to treat a number of ailments including common cold, fever, diarrhoea, liver diseases, and inflammation [18]. Recent studies have revealed some cardiovascular effects of this herb [16,19]. It is also found to be a promising new way for the treatment of HIV, AIDS [1], and numerous symptoms associated with immune disorders [4],works effectively as a immunostimulant [11,13].

Three main diterpenoid lactones identified in the *A. paniculata* leaves were andrographolide, neo-andrographolide and deoxyandrographolide. 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. [17]. The analysis of andrographolide can be done by thin layer chromatography (TLC), high – performance liquid chromatography (HPLC) and crystallisation. 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]:

Sample throughput can be increased by simultaneous extraction in parallel. It has the ability to extract more sample mass and it is non-matrix dependent.The sample is repeatedly brought into contact with the fresh portions of the solvent, thereby helping to displace the transfer equilibrium,

The temperature of the system remains relatively high due to the heat applied to the distillation flask. However, for toxicological reason, drug and medicine producers are required to minimize the number and amount of solvents employed in pharmaceutical processes [19]. The presence of a solvent in the extract may also affect the kinetics of crystallisation and the crystal morphology of the product [3]. 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.

MATERIALS AND METHODS

Materials

The leaves of *A. paniculata* were collected from chintapalli forest near Visakhapatnam. Information about its use as traditional Anticancer and other properties were collected from tribals and local Ayurvedic doctors. Various organic solvents were purchased from Qualigens; deionised water was generated in the Analytical Laboratory, Department of Chemical Engineering, Andhra University, and Visakhapatnam. The leaves are dried under sundry. Leaves were powdered using thimble and mixer. It is finely grounded to 80 mesh size (particle size 100micro meters). Prior to the solvent extraction study, 1 gram of dried - ground leaves of *A. paniculata* was placed in a cellulose thimble.

Solvent extraction

An amount of 1.00×10^{-4} m3 of solvent was used for the extraction using a standard soxhlet method for 80minutes in a soxhlet extraction system. The standard soxhlet extraction method [8]was conducted using methanol and other organic solvents at different extraction times, and different concentrations to verify the mathematical model proposed in this work[12,14]. The extracts were then concentrated using vacuum rotary evaporator and completely dried in an atmospheric oven. The crude extracts were then analysed for their andrographolide content using high performance liquid chromatography[15].

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 [6,7]. Every leaf particle is symmetrical. The mass transfer coefficient is constant.
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,
The transfer of the andrographolide is a diffusion phenomenon and independent of time,
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:

$$Es = B\,(t) - D$$

Where *Es* = total extract (g),
t = extraction time (seconds),
B, D is constants

RESULTS AND DISCUSSION

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[9,10].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. The maximum androgapholide extracted at the concentration of 60% methanol. For the model development we considered 60% methanol. The generated model equation is ***Es = 0.8917t – 9.8114***
The results are represented in Table 1, 2 and Figure 2, 3.The model showed a good agreement with the experimental data as shown in Figure 3.

CONCLUSION

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. Among the different concentrations, 60% methanol yield maximum. The final form of the proposed model is ***Es* = 0.8917t – 9.8114.**

Table-1:Effect of solvent polarity and Hildebrand solubility parameters on Extraction yield.

Solvent	Polarity	Hildebrand solubility parameter	Extract Yield (%)	Extracted (g/1gm of dried leaves) andrographolide
Methanol 100%	6.6	14.45	15.518	0.131
Methanol 80%	7.08	16.242	20.91	0.18
Methanol 60%	7.56	18.034	27.068	0.266
Methanol 50%	7.8	18.93	23.75	0.531
Methanol 40%	8.04	19.826	14.619	0.29
Methanol 20%	8.28	21.618	11.92	0.0945
Ethanol 100%	5.2	12.90	18.49	0.128
Ethanol 80%	5.96	15.016	20.06	0.169
Ethanol 60%	6.72	17.132	24.34	0.342
Ethanol 50%	7.1	18.19	22.39	0.287
Ethanol 40%	7.48	19.248	18.61	0.107
Ethanol 20%	8.06	21.364	17.62	0.129
Ethyl acetate 100%	4.3	9.04	14.619	0.452
Ethyl acetate 50%	5.8	15.28	17.34	0.532
Water	9	23.40	21.068	0.43

Table-2: Effect of Yield extract with Extraction time

S. No	Time (min)	Extract Yield (gm)
1	0	0
2	5	0
3	10	0
4	15	3.554
5	20	8.011
6	25	12.534
7	30	17.658
8	35	20.639
9	40	25.989
10	45	29.677
11	50	34.988
12	55	39.548
13	60	48.334
14	65	54.137
15	70	54.137
16	75	54.137
17	80	54.137

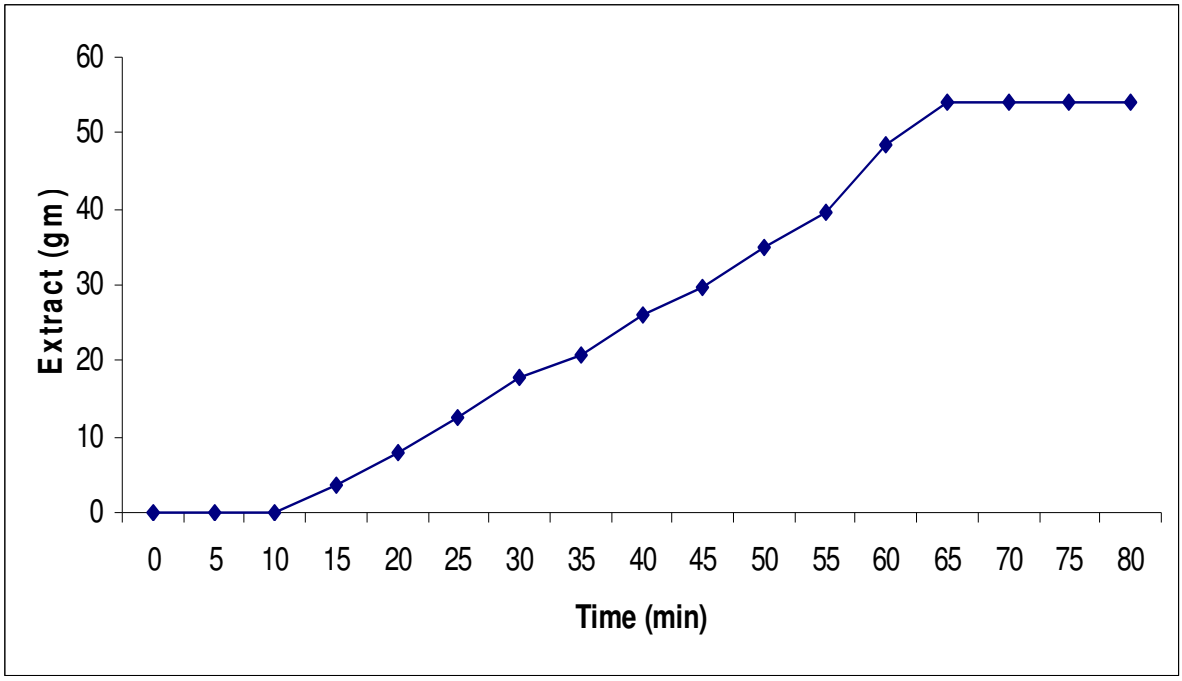

Fig.-1: Effect of Yield extract with extraction time

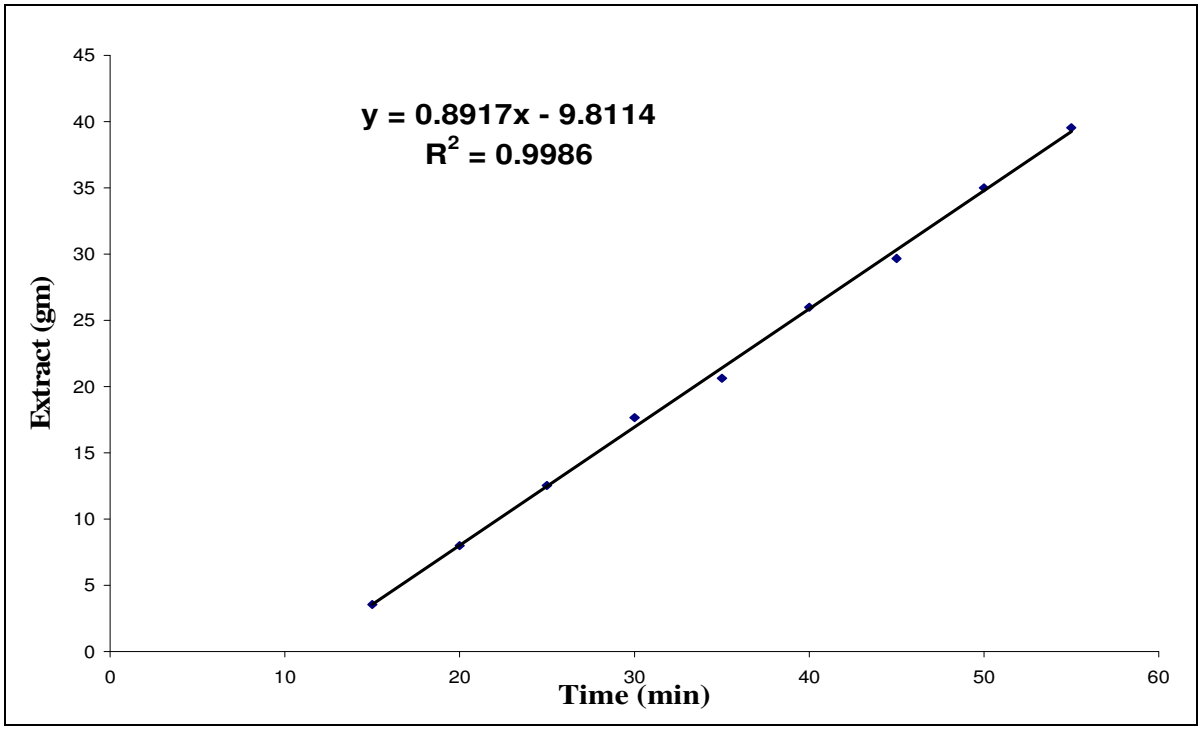

Fig.-2: Comparison of extract weight calculated from the model and experimental data

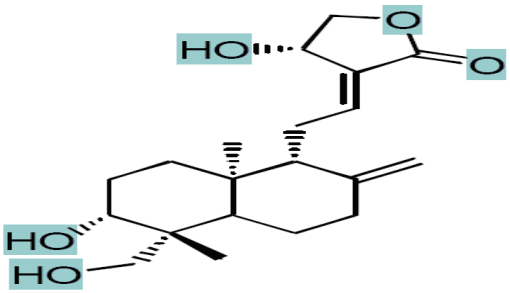

Fig.-3: Molecular structure of Andrographolide

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