

## The Application of Response Surface Methodology in Hydrotropic Microwave Assisted Extraction of Andrographolide

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### Abstract:

Andrographolide of *Andrographis paniculata* is reported has a broad category of pharmacological activity. Various attempts have been done in order to improve the andrographolide extraction performance. Hydrotropic-microwave assisted extraction provides an effective method in andrographolide extraction. In this study, Response Surface Methodology (RSM) along with central composite design (CCD) was applied to optimize the hydrotropic-microwave assisted extraction of andrographolide. The effect of extraction time ( $X_1$ ), solid-liquid ratio ( $X_2$ ), and hydrotropes concentration ( $X_3$ ) were investigated. The result showed that the most suitable condition for extraction of andrographolide was found to be extraction at 32,5 minutes, solid liquid ratio of 0,095 and hydrotropes concentration of 4M. The maximum extraction percentage experimentally (5,45%) was found to be very close to its predicted value. It was also found that hydrotropes concentration was the most influencing variable. The mathematical model for the hydrotropic-microwave assisted extraction is :

$$Y = 4.812 + 1.66.X_1 - 2.366.X_1^2 + 0.478.X_2 - 2.473.X_2^2 + 2.216.X_3 - 0.983.X_3^2 \\ + 0.385.X_1.X_2 + 0.476.X_1.X_3 + 0.429.X_2.X_3$$

**Keyword:** andrographolide; hydrotrope; microwave asisted extraction; response surface methodology

### 1. Introduction

Hydrotrope solutions provide safe and effective media for the microwave assisted extraction of andrographolide, the major bioactive chemical constituent of plant *Andrographis paniculata*. Generally, Andrographolide was extracted from leaves and aerial parts of *A. Paniculata*. It is reported has a broad category of pharmacological activity, such as hepatoprotective, gastroprotective, antimicrobial, anticancer, antihyperglycemic, antiinflammatory, antioxidant, antidiarrheal and antimalarial activities [1,2]

Conventional methods employed in the andrographolide extraction though were reported as an effective methods, they were also reported as the cause for the thermal degradation of heat sensitive compound [3], the degradation of andrographolide [4,5] and leave traces of toxic solvents in the solute [6,7].

Combination of microwave assisted-extraction and the utilization of hydrotrop as the extraction medium is already proved as a potential alternative for the separation of andrographolide. Hydrotropes in general are water-soluble and surface active compounds which can significantly enhance the solubility of organic solutes such as esters, acids, alcohols, aldehydes, ketones, hydrocarbons and fats.

Hydrotropy has been employed in the extraction solute that sparingly soluble in water including piperine [8], curcuminoid [9,10], limonine [11], and forskolin [12]. Meanwhile microwave assisted extraction has been employed in the extraction of sylibinin [13], gossypol of cottonseed [14], poliphenol and catechin of tea [15].

Moreover, MAE is reported offer several benefit such as much lowered extraction time, much lowered temperature, enhanced product purity, and enhanced efficiency. The beneficial effects of MAE are due to the unique heating system of it. Direct interaction of microwaves with the free water molecules presents in the glands and vascular systems, causes a tremendous increase in internal pressure inside the plant cell. It is due to evaporation of the internal moisture content which result in the subsequent rupture of the plant tissue and the release of the active compounds into the organic solvent. Therefore, MAE is an interesting alternative to conventional extraction methods, especially in the case of extractions of thermolabile compounds [13, 16].

The hydrotropic-microwave assisted extraction is affected by several process parameters such as microwave power, time, solid liquid ratio, hydrotropes concentration and agitation speed. Process optimization is an urge in order to determine the most influencing variable and determine the optimum process parameters. When many factors and interactions affect the desired response, response surface methodology (RSM) is an effective tool for optimizing the process, which was originally described by Box and Wilson.

Response Surface Methodology (RSM) is widely employed for constructing and exploring estimated functional relationship between a response variable and design variables [17]. The main advantage of RSM is the reduced number of experimental trials needed to evaluate multiple parameters and their interactions. Therefore, it is less laborious and time consuming than other approaches required to optimizing a process. Response surface methodology has been successfully used to model and optimize biochemical processes including extraction processes [18].

In this study, we used hydrotropic microwave assisted extraction to extract the andrographolide of *Andrographis paniculata*. The effects of extraction time, ratio of liquid to solid, and hydrotropes concentration on extraction percentages were investigated and extraction conditions

were optimized using response surface methodology by evaluating response of the extraction percentages.

## 2. Material and Method

### 2.1. Raw Material and Chemicals

The aerial parts of *Andrographis paniculata* were collected from local plantation in Gunungpati, Semarang. Sodium benzoate (Sigma-Aldrich, 99%) were purchased from CV. Damai Sejahtera Prima.

### 2.2. Apparatus

Hydrotropic-microwave assisted extraction was conducted in a modified domestic microwave. The microwave was modified and equipped with extraction flask and a spiral condensor.

#### Response Surface Methodology

A three factor of central composite, non factorial, surface design was used in this study. Time (10-20 minutes), ratio of solid –liquid (0,05-0,1) and hydrotrope concentration (1-3 M) were input variables, the factor levels were coded as -1 (low), 0 (central point), and 1 (high). The optimization experiments were design by utilized Central Composite Design of the software of Statistica 8.0. The experimental design was comprised of 16 sequences as listed on Table 1.

The behavior of the system is explained by the following quadratic equation [1].

$$Y = \beta_0 + \sum_{j=1}^3 \beta_j X_j + \sum_{j=1}^3 \beta_{jj} X_j^2 + \sum_{i < j} \beta_{ij} X_i X_j \quad (1)$$

where, Y is the process response or output, k is the number of the patterns, i and j are the index numbers for pattern,  $\beta_0$  is the free or offset term called intercept term,  $x_1, x_2, \dots, x_k$  are the coded independent variables,  $\beta_i$  is the first-order (linear) main effect,  $\beta_{ii}$  is the quadratic effect, and  $\beta_{ij}$  is the interaction effect.

### 2.3. Extraction

Aerial parts of *Andrographis paniculata* were collected, dried and powdered. 20 grams of dried powder was subjected in 200 ml of hydrotrope solution. The mixture placed in 500 ml round bottom flask and extracted in a modified microwave extractor for several minutes at system powers of 119.7W. The mixture then was allowed to stand for 1 hour and then filtered. The residu was washed with water and double volume of demin water was added. The extract was then centrifuges for 15 minutes at 4000G, dried and weighed.

**Table 1.** THE EXPERIMENTAL SET UP

No	Time (min)	Ratio of solid:liquid	Hydrotrop Concentration (M)
1	10,00000	0,050000	1,000000
2	10,00000	0,050000	3,000000
3	10,00000	0,100000	1,000000
4	10,00000	0,100000	3,000000
5	30,00000	0,050000	1,000000
6	30,00000	0,050000	3,000000
7	30,00000	0,100000	1,000000
8	30,00000	0,100000	3,000000
9	3,18207	0,075000	2,000000
10	36,81793	0,075000	2,000000
11	20,00000	0,032955	2,000000
12	20,00000	0,117045	2,000000
13	20,00000	0,075000	0,318207
14	20,00000	0,075000	3,681793
15	20,00000	0,075000	2,000000
16	20,00000	0,075000	2,000000

### 3. Results and Discussion

Surface methodology was a good tool for optimization of extraction conditions [17]. Table 2 presents the experimental design and corresponding response data for the extraction of the andrographolid.

The regression coefficient used in the mathematical modelling of the hydrotropic microwave assisted extraction of andrographolide were achieved with the aid of Statistica 8.

The relationship of the three variables, that is, time ( $X_1$ ); ratio of solid liquid ( $X_2$ ); concentration of hydrotrope ( $X_3$ ) and the percentage of the andrographolide extraction ( $Y$ ) is shown by equation [2].

$$\begin{aligned}
 Y = & 4.812 + 1.66X_1 - 2.366X_1^2 + 0.478X_2 - 2.473X_2^2 + 2.216X_3 - 0.983X_3^2 \\
 & + 0.385X_1X_2 + 0.476X_1X_3 + 0.429X_2X_3
 \end{aligned}
 \tag{2}$$

The significant terms in the polynomial model were found by analysis of variance (ANOVA) for each response. They were considered statistically different when  $P < 0.05$ . The adequacy of model was checked by accounting for the coefficient of determination ( $R^2$ ) and adjusted- $R^2$  ( $R^2_{adj}$ ).

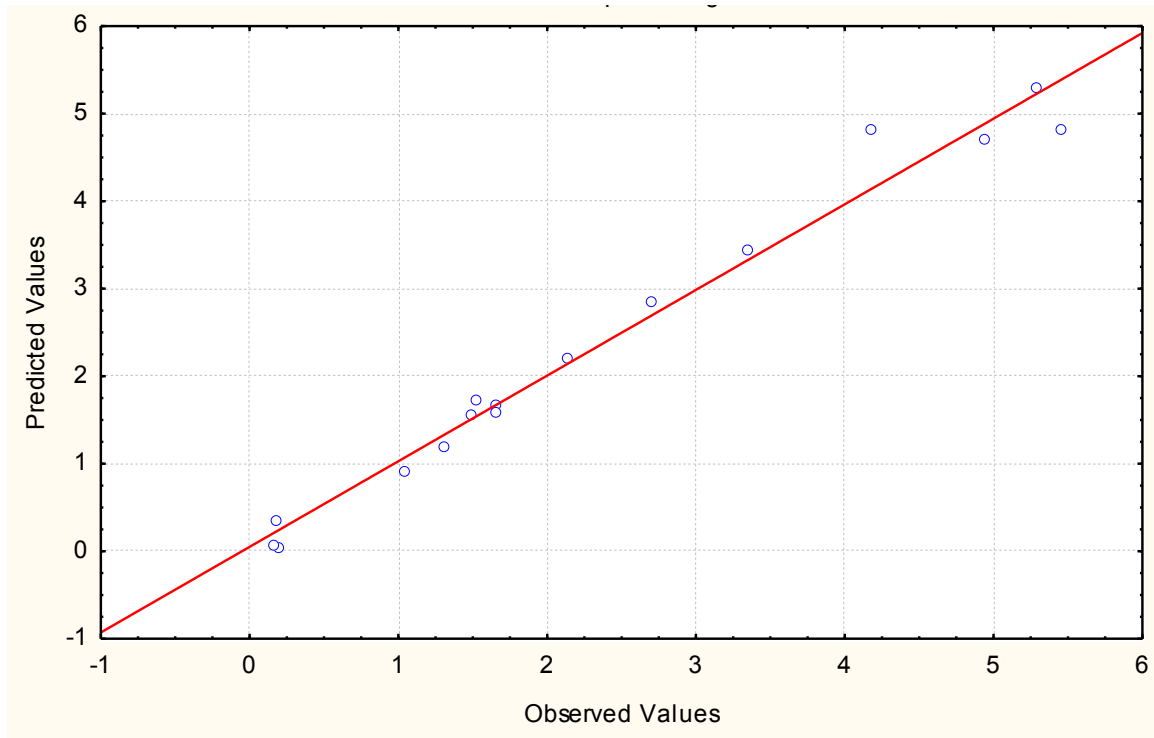
Table 3 showed the coefficient of determination coefficient,  $R^2$ , value was 0.978. This implies that the sample variation of 97.8% % could be attributed to the independent variables and the model did not explain only 2.2 % of the total variations. More over, the value of the adjusted coefficient of determination coefficient,  $R^2_{adj}$ , was 0.946, indicating a high degree of correlation between the observed and its predicted values.

**Table 2.** RESPONSE OF THE OPTIMIZATION PROCESS

No	Time (minute)	Ratio of solid:liquid	Hydrotrop Concentration (M)	Extraction Percentage (%)
1	10,00000	0,050000	1,000000	0,17
2	10,00000	0,050000	3,000000	1,64
3	10,00000	0,100000	1,000000	0,19
4	10,00000	0,100000	3,000000	2,13
5	30,00000	0,050000	1,000000	1,30
6	30,00000	0,050000	3,000000	3,34
7	30,00000	0,100000	1,000000	1,64
8	30,00000	0,100000	3,000000	4,93
9	3,18207	0,075000	2,000000	0,16
10	36,81793	0,075000	2,000000	2,70
11	20,00000	0,032955	2,000000	1,04
12	20,00000	0,117045	2,000000	1,53
13	20,00000	0,075000	0,318207	1,49
14	20,00000	0,075000	3,681793	5,29
15	20,00000	0,075000	2,000000	4,17
16	20,00000	0,075000	2,000000	5,45

ANALYSIS OF VARIANCE FOR THE FITTED QUADRATIC POLYNOMIAL MODEL

	SS	df	MS	F	p
X <sub>1</sub>	9,44367	1	9,44367	54,13800	0,000323
X <sub>1</sub> X <sub>1</sub>	12,96849	1	12,96849	74,34482	0,000134
X <sub>2</sub>	0,78038	1	0,78038	4,47368	0,078814
X <sub>2</sub> X <sub>2</sub>	14,16543	1	14,16543	81,20654	0,000105
X <sub>3</sub>	16,77090	1	16,77090	96,14303	0,000065
X <sub>3</sub> X <sub>3</sub>	2,23901	1	2,23901	12,83561	0,011606
X <sub>1</sub> X <sub>2</sub>	0,25687	1	0,25687	1,47254	0,270543
X <sub>1</sub> X <sub>3</sub>	0,45434	1	0,45434	2,60462	0,157679
X <sub>2</sub> X <sub>3</sub>	0,36873	1	0,36873	2,11380	0,196204
Error	1,04662	6	0,17444		
Total	48,85641	15			
<b>R<sup>2</sup> =0,978; R<sup>2</sup><sub>Adj</sub>:0,946</b>					

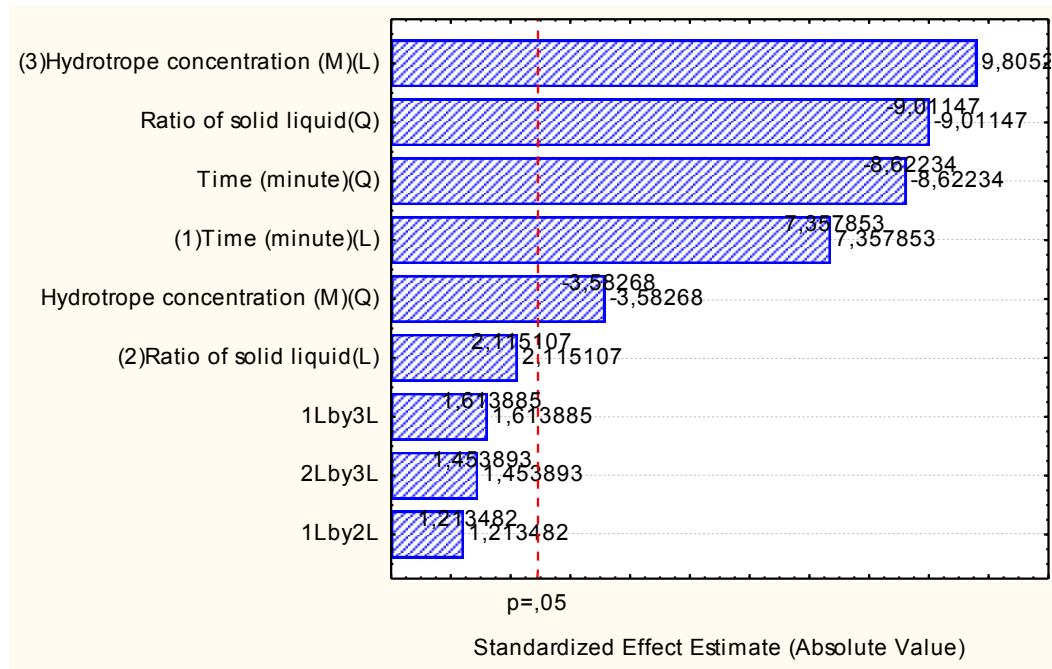


**Figure 1.** Observed versus predicted value.

The closeness of the observed and predicted value of the andrographolide extraction percentage was shown of Fig 1.

The Pareto chart (Fig.2) illustrates the standardized effects of the independent variables and their interactions on the dependent variable. In the Pareto chart, the effect of each factor as well as the level of its effects on responses is expressed by the length of its bar in the graph.

Fig. 2 shows a significant positive effect of linear factors of hydrotrope concentration. It is indicating the increase of the percentage of the andrographolide extraction with increasing value of hydrotrope concentration. From its effect value, can be concluded that hydrotropes concentration was the most influencing variable. On the other side, factor time shows a significant negative linear effect on the percentage of the andrographolide extraction.



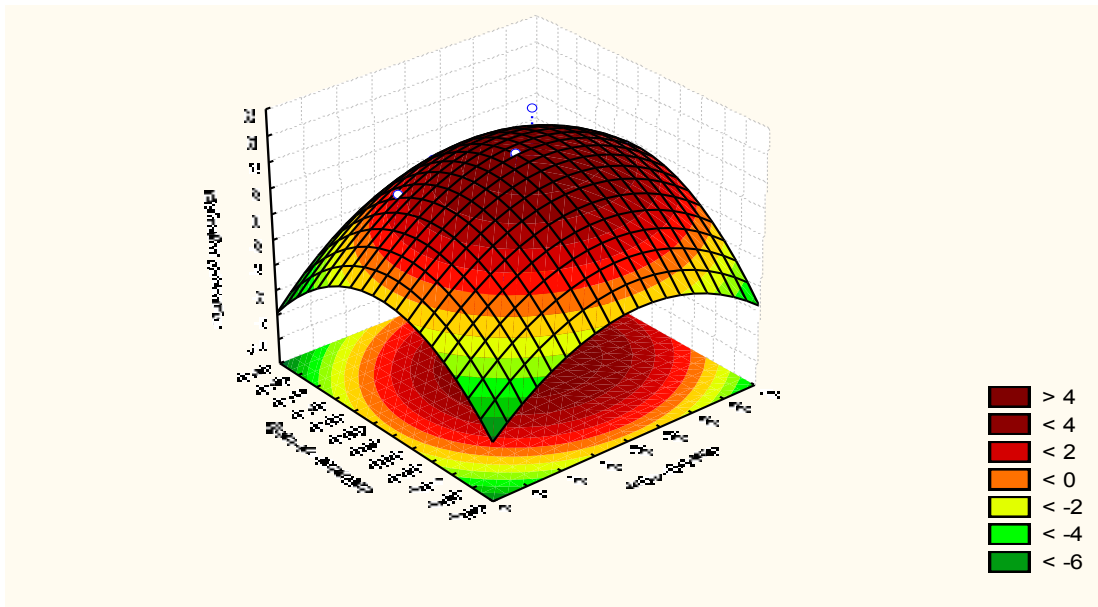
**Figure 2.** Pareto chart showing the effects of observed factors and their combined impact on the extraction percentage of andrographolide.

The interpretation of the interactions simplifies using of three-dimensional plots for the regression model. Such three-dimensional surfaces can provide useful information about the behaviour of the system within the experimental design, facilitate an examination of the effects of the experimental factors on the responses and contour plots between the factors.

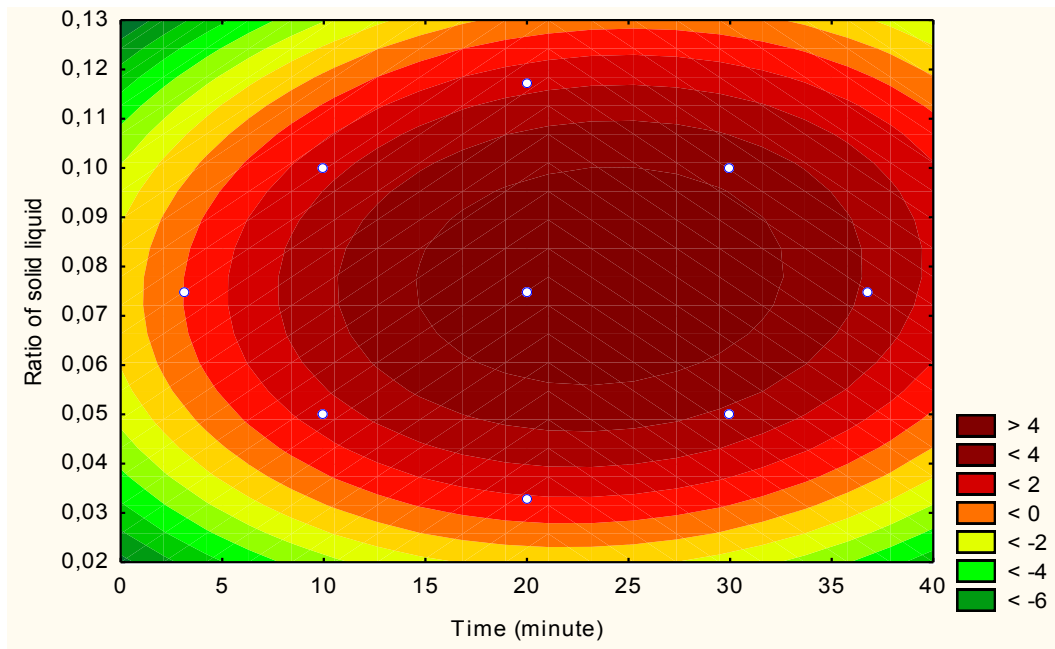
When two variables within the experimental range were displayed in three-dimensional surface plots, the third variable was kept constant at the intermediate level.

As shown in Fig. 3 and Fig. 4, when the hydrotrope concentration ( $X_3$ ) was fixed at 2 level, the extraction percentage increased as the extraction time ( $X_1$ ) and ratio of solid liquid ( $X_2$ ) increased.

The extraction percentage was positively correlated with the extraction time and ratio of solid liquid when extraction time was lower than 32.5 minute and ratio of solid liquid was lower than 0,095. Moreover, it was negatively correlated when extraction time was higher than 32.5 minute and ratio of solid liquid was higher than 0,095.



**Figure 3.** Response surface plot of extraction time versus ratio of solid liquid, and their mutual interactions on andrographolide extraction percentage.

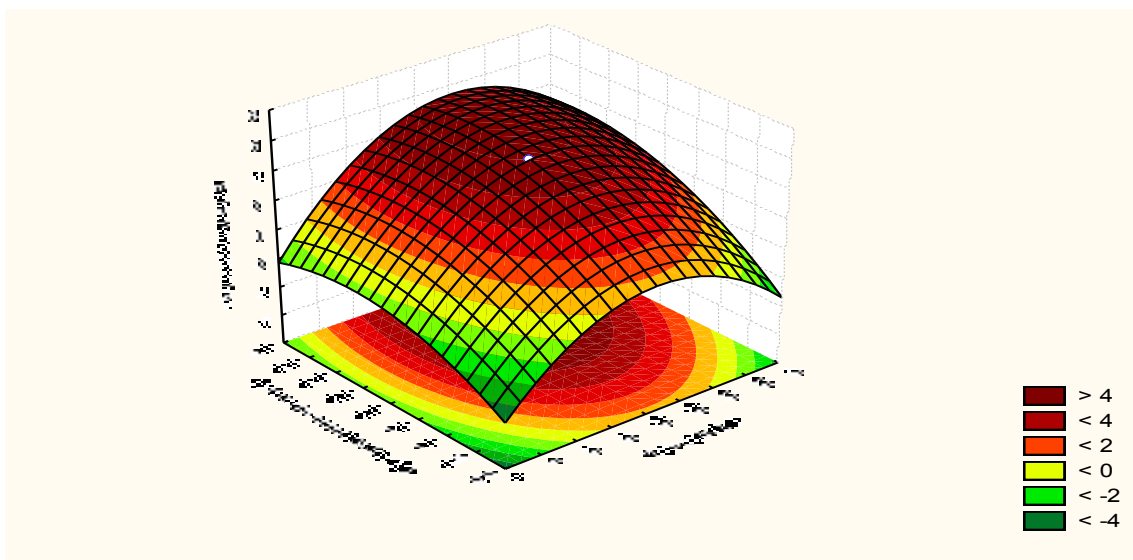


**Figure 4.** Fitted surface profile of extraction time and ratio of solid liquid, and their mutual interactions on andrographolide extraction percentage.

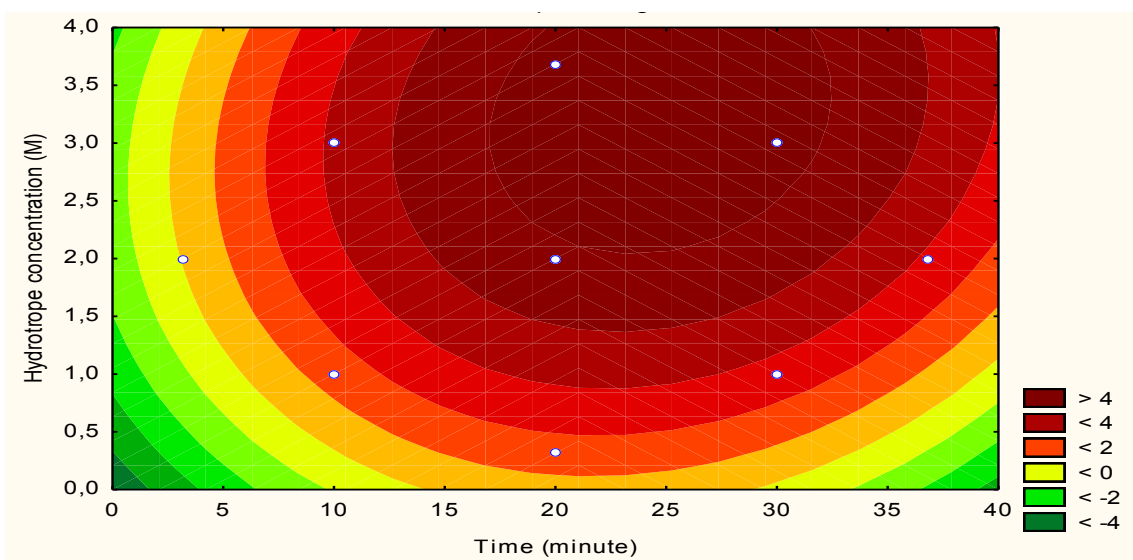
Fig. 5 and Fig. 6, showed that when the ratio of solid liquid ( $X_2$ ) was fixed at 0.75 level the extraction percentage increased as the extraction time ( $X_1$ ) and hydrotrope concentration ( $X_3$ ) increased.



The extraction percentage was positively correlated with the extraction time and hydrotrope concentration when extraction time was lower than 32.5 minute and ratio hydrotrope concentration was lower than 4 M. Moreover, it was negatively correlated when extraction time was higher than 32.5 minute.



**Figure 5.** Response surface plot of extraction time versus hydrotrope concentration, and their mutual interactions on andrographolide extraction percentage.



**Figure 6.** Fitted surface profile of extraction time versus hydrotrope concentration, and their mutual interactions on andrographolide extraction percentage.

The optimum condition for extraction of andrographolide was found to be extraction at 32.5 minutes, solid liquid ratio of 0.095 and hydrotropes concentration of 4M.

#### 4. Conclusion

The optimum condition for extraction of andrographolide was found to be extraction at 32.5 minutes, solid liquid ratio of 0.095 and hydrotropes concentration of 4M. The maximum extraction percentage experimentally (5.45%) was found to be very close to its predicted value. It was also found that hydrotropes concentration was the most influencing variable. The mathematical model for the hydrotropic-microwave assisted extraction is :

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#### 5. Acknowledgement

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