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Research Article

OPTIMIZATION OF MICROWAVE ASSISTED EXTRACTION OF PHENOLIC COMPOUNDS FROM *DECALEPIS HAMILTONII* ROOT USING RESPONSE SURFACE METHODOLOGY

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ABSTRACT

Polyphenolic compounds comprising flavonoids and phenolic acids are widely distributed in foods of plant origin and counted to be the most abundant antioxidant in our diet. Due to its health benefitting properties they are of great interest in recent days. *Decalepis hamiltonii* is one such plant that is rich in phytochemicals having antioxidant properties and other beneficial medicinal value. Maximum yield of these phytochemicals during extraction requires optimized conditions of process parameters. In the present study, Microwave-assisted extraction (MAE) of polyphenols from the *Decalepis hamiltonii* roots was optimized using response surface methodology. The effect of independent variables such as extraction time (1, 3 and 5 min), solid to solvent ratio (0.5, 1.75 and 3 g/25ml solvent), solvent concentration (30%, 60% and 90% ethanol) and their interaction on Total Phenolic Content(TPC) and Total Flavonoid Content (TFC) were determined by three factor- three level Box-behnken Design. The highest TPC (0.03 mg GAE/mg DHRP) and TFC (0.08 mg RE/mg DHRP) were obtained under the optimum extraction conditions of 3 min, 2.56 gm/25 ml of solvent, 53.77 % ethanol and 2.15 min, 3 gm/25 ml of solvent, 49.18 % ethanol respectively. These findings further demonstrate that extraction of bioactive phytochemicals from plant materials using MAE method consumes less extraction solvent and saves time.

Keywords: *Decalepis hamiltonii*, Polyphenols, Response Surface Methodology, Microwave Assisted Extraction.

INTRODUCTION

Antioxidants are both natural and synthetic compounds able to scavenge free radicals and inhibit oxidation processes (George and Britto, 2015). Polyphenolic compounds comprising flavonoids and phenolic acids are widely distributed in foods of plant origin and found to be the most abundant antioxidants in our diet (Hayat et al., 2009). They are a large family of natural compounds which are secondary metabolites and are derivatives of the pentose phosphate, shikimate and phenylpropanoid pathways in plants. Phenolic compounds exhibit a wide range of beneficial properties to health, such as: anti-allergenic, anti-inflammatory, anti-microbial, anti-oxidant, antithrombotic, cardio protective and vasodilatory effects. Several beneficial effects derived from phenolic compounds are mainly due to their antioxidant activity (Ajila et al., 2011).

Pressurized liquid extractor (PLE), microwave assisted extraction (MAE), ultrasound assisted extraction, soxhlet extraction, heat reflux extraction and supercritical fluid extraction are various efficient and advanced extraction techniques developed for extracting phenolic compounds from herbal medicine (Dahmoune et al., 2015). Microwave Assisted Extraction (MAE) is a relatively new method which has been increasingly used for extraction of valuable compounds from biological cells. This is an extraction technique that delivers microwave energy rapidly to a total volume of solvent and solid plant matrix. This results in subsequent heating of the solvent and solid matrix, efficiently and homogeneously (Kaufmann & Christen, 2002). There are a number of parameters that influence the microwave extraction process such as choice of solvent, solvent volume, microwave power, extraction time and matrix characteristics. Highly polar solvents interact better in a microwave environment, leading to faster heating rate, greater destruction of biological structure and higher extraction yields (Chandrasekar et al., 2015). MAE is increasingly being used as an alternative to traditional extraction method for the removal of phenolics from plant tissues as it significantly reduces extraction time and solvent consumption while generating higher extraction yields (Ballard et al., 2010).

The traditional method of optimization involves the study of one-factor-at-a-time that is laborious and time consuming. Moreover, the interactive effect of individual factors is also ignored and misleading conclusion may be drawn. Because of the above reasons it becomes cumbersome to establish the optimum conditions. It is therefore essential to optimize the procedure yielding highest quantities of phyto compounds with almost preserved functional properties. Recently, response surface methodology (RSM), a statistical experimental protocol used in mathematical modelling, has emerged as an ideal strategy for standardizing process variables of many food processes and is being extensively used. The merits of RSM include use of lesser number of experimental measurements; provide a statistical interpretation of the data and also to identify the interaction amongst variables, if any. RSM has been successfully applied in optimizing extraction condition of a range of polyphenols, antioxidants and other metabolites in plants (Ilaiyaraja et al., 2015; Alberti et al., 2014; Zhang et al., 2013).

Decalepis hamiltonii (Wight and Arn.) belonging to the family Asclepiadaceaeis a climbing shrub that grows in the forests of peninsular India. It is widely studied as it one of the most potent antioxidant source with varieties of biological activity which could be associated with their health benefit (Nayaka et al., 2010; Srivastava et al., 2007). The roots are used in folk medicine and as a substitute for *Hemidesmus indicus* in ayurvedic preparations. The roots are also used to stimulate appetite, relieve flatulence and as a general tonic.

The roots are largely used in making pickles along with lime and also in the preparation of health drink (Nayar et al., 1978; Nayak et al., 2010)

The optimization of microwave-assisted extraction of phenolic compounds from *Decalepis hamiltonii* root was conducted in the present study with the objective of recovering maximum phenolic and flavonoids content. Response surface methodology (RSM) was used to evaluate the effect of multiple factors like extraction time, ethanol concentration and solid to solvent ratio.

MATERIALS AND METHODS

Sample Preparation

The roots *Decalepis hamiltonii* were procured from Local Market, Malleshwaram, Bangalore, India. The roots were repeatedly washed with tap water and dried in shade for about 2-3 days. The dried sample was grounded using a domestic grinder to obtain powder. The powder was then separated into different particle size fractions using a set of sieves in a laboratory sieve shaker (M/s Muhlenbau, Germany).

Chemicals and Reagents

Ethanol used for the extraction studies was analytical grade. Aluminium chloride, Sodium hydroxide and Folin-Ciocalteau were obtained from SDFCL. Gallic acid and Rutin was purchased from Sigma-Aldrich Company Ltd. Sodium nitrate and Sodium bi-carbonate were purchased from Merck specialties private limited. The water used for the extraction and analysis was triple distilled water.

Extraction and Experimental Design

Microwave assisted extraction of polyphenolic compounds was carried in domestic digital microwave oven (LG,India; Model MC-8087ABR) at constant microwave power of 360 Watts. All the experiments were conducted in 100 ml of conical flask with the solvent volume of 25 ml. The rotating plate provides equal distribution of microwave radiation energy throughout the flask. The effect of selected operating parameters on microwave assisted extraction of total phenolic content and total flavonoid content were optimized using response surface methodology and Box-behnken design was employed in this regard. Extraction of time (X_1), solid to solvent ratio (X_2) and ethanol concentration (X_3) were selected as independent variables. The experimental design is presented in Table 1 with coded and uncoded values of selected variables. The experimental design consists of 12 factorial experiments and 3 replicates of the central point.

Determination of Total Phenolic Content

Total phenolic content (TPC) assay was performed according to method of Singleton and Rossi using Folin-Ciocalteu reagent with some modifications. 0.5 ml of pre diluted extracts was mixed with 2.5 ml of 1:10 diluted Folin-Ciocalteu reagent and after 4 min 2 ml (75g/l) of sodium carbonate was added into reaction mixture. Absorbance was taken using UV- Spectrophotometer against blank at 725 nm after incubation for one hour at room temperature. Gallic acid was used as standard and total phenolic content of the extracts were expressed as milligram of gallic acid equivalent (GAE)/mg of dry *Decalepis hamiltonii* root powder (Sushma et al., 2014).

Determination of Total Flavonoid Content

Total flavonoids were determined by the aluminum chloride colorimetric assay. 0.5 ml of diluted extract was mixed with 2 ml of distilled water and subsequently with 0.15 ml of a 5% NaNO2 solution. After 6 min, 0.15 ml of a 10% $AlCl_3$ solution was added and allowed to stand for 6 min, then 2ml of 4% NaOH solution was added to the mixture. Water was added immediately to bring the final volume to 5 ml, and then the mixture was thoroughly mixed and allowed to stand for another 15 min. Absorbance of the mixture was determined at 510 nm versus a prepared blank. Rutin was used as standard compound for the quantification of total flavonoids. All values were expressed as milligrams of rutin equivalent per milligram *Decalepis hamiltonii* root powder (Mathew et al., 2013).

Statistical Analysis

The behavior of the system was explained by second order quadratic polynomial model. The mathematical model corresponding to the Box–behnken design was

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_1^2 + \beta_5 X_2^2 + \beta_6 X_3^2 + \beta_7 X_1 X_2 + \beta_8 X_1 X_3 + \beta_9 X_2 X_3 + \epsilon \quad \text{Eq. 1}$$

Where Y was the predicted total phenolic content and total flavoinoid content, β_0 was the intercept, β_1, β_2, β_3 were linear coefficients, β_4, β_5, β_6 were quadratic coefficients, β_7, β_8, β_9 were interaction coefficients and ε was random error. Data analysis tool in Microsoft Excel 2010® was used to analyze the experimental results of the response surface designs and KY Plot 2.0 was used to generate response surface graphs. The adequacy of the model was determined by evaluating the lack of fit, coefficient of determination (R^2) and the Fisher test value (*F-value*) obtained from the analysis of variance (ANOVA) that was generated. Differences were considered significant if $P < 0.05$. SOLVER function of the MS-Excel-2010® software was used to search for optimum yield within the range of variables. To confirm the optimum results predicted by the RSM model, experimental runs were carried out in triplicate under the predicated optimized conditions (Murthy & Manohar, 2014).

Table 1. Codes and levels of independent variables in RSM design

Independent Variable	Symbols	Coded values		
		-1	0	1
Time (min)	X_1	1	3	5
Solid: Solvent Ratio (gm/25 ml)	X_2	0.5	1.75	3
Ethanol Concentration (%)	X_3	30	60	90

Table 2. Experimental and predicted data for the TPC and TFC obtained from Box–behnken design.

Sl.No	Coded Values			Uncoded Values			TPC (mg GAE/mg DHRP)			TFC (mg RE/mg DHRP)		
	X_1	X_2	X_3	X_1	X_2	X_3	$Y_{(Exp)}$	$Y_{(Pre)}$	ε	$Y_{(EXP)}$	$Y_{(Pre)}$	ε
1	-1	1	0	1	3.00	60	0.0147	0.0146	1.28E-04	0.0745	0.0731	1.42E-03
2	-1	-1	0	1	0.50	60	0.0050	0.0057	-7.02E-04	0.0274	0.0329	-5.48E-03
3	-1	0	-1	1	1.75	30	0.0108	0.0111	-3.49E-04	0.0565	0.0554	1.03E-03
4	-1	0	1	1	1.75	90	0.0068	0.0059	9.24E-04	0.0355	0.0324	3.03E-03
5	0	1	-1	3	3.00	30	0.0266	0.0264	2.21E-04	0.0671	0.0695	-2.45E-03
6	0	-1	1	3	0.50	90	0.0161	0.0163	-2.21E-04	0.0218	0.0194	2.45E-03
7	0	-1	-1	3	0.50	30	0.0169	0.0159	1.05E-03	0.0268	0.0223	4.45E-03
8	0	0	0	3	1.75	60	0.0281	0.0281	1.39E-17	0.0607	0.0607	-2.08E-17
9	0	1	1	3	3.00	90	0.0204	0.0214	-1.05E-03	0.0510	0.0554	-4.45E-03
10	0	0	0	3	1.75	60	0.0281	0.0281	1.39E-17	0.0607	0.0607	-2.08E-17
11	0	0	0	3	1.75	60	0.0281	0.0281	1.39E-17	0.0607	0.0607	-2.08E-17
12	1	0	1	5	1.75	90	0.0104	0.0101	3.49E-04	0.0402	0.0413	-1.03E-03
13	1	1	0	5	3.00	60	0.0155	0.0148	7.02E-04	0.0743	0.0688	5.48E-03
14	1	0	0	5	0.50	60	0.0079	0.0080	-1.28E-04	0.0244	0.0258	-1.42E-03
15	1	-1	-1	5	1.75	30	0.0085	0.0095	-9.24E-04	0.0322	0.0353	-3.03E-03

X_1-Time of extraction, min; X_2-Solid to solvent ratio (gm/25ml of solvent); X_3-Ethanol Concentration (%); Y_{exp}-Experimental yield; Y_{pre}-Model predicted yield; ε-residuals (Y_{exp}-Y_{pre})

Table 3. Regression Statistics and Analysis of variance (ANOVA) for TPC and TFC

Regression Statistics	TPC	TFC
Multiple R	0.9972	0.9860
R Square	0.9945	0.9722
Adjusted R Square	0.9846	0.9221
Standard Error	0.0010	0.0052
Observations	15.0000	15.0000

ANOVA

		df	*SS*	*MS*	*F*	*Significance F*
TPC	Regression	9	0.000953	0.000106	100.3109	4.14E-05
	Residual	5	5.28E-06	1.06E-06		
	Total	14	0.000958			
		df	*SS*	*MS*	*F*	*Significance F*
TFC	Regression	9	0.004767	0.00053	19.41931	0.00224
	Residual	5	0.000136	2.73E-05		
	Total	14	0.004903			

Table 4. Regression coefficient of polynomial functions of response surface of total phenolic content and total flavonoid content.

	TPC				TFC			
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Coefficients*	*Standard Error*	*t Stat*	*P-value*
A0	-0.03407	0.003493	-9.75168	0.000193	-0.01866	0.01776	-1.05085	0.341441
A1	0.020373	0.001034	19.70405	6.22E-06	-0.00083	0.005256	-0.15817	0.880511
A2	0.013136	0.001604	8.188509	0.000442	0.031423	0.008156	3.852921	0.011966
A3	0.000601	8.04E-05	7.476337	0.000676	0.001497	0.000409	3.66423	0.014531
A4	-0.00352	0.000134	-26.3626	1.47E-06	-0.00139	0.000679	-2.04357	0.09643
A5	-0.00206	0.000342	-6.02902	0.001807	-0.00319	0.001739	-1.83647	0.125717
A6	-5.4E-06	5.94E-07	-9.10275	0.000268	-1.6E-05	3.02E-06	-5.16505	0.00357
A7	-0.00021	0.000205	-1.02031	0.354385	0.000285	0.001044	0.273298	0.795553
A8	2.42E-05	8.56E-06	2.828402	0.036744	0.000121	4.35E-05	2.774896	0.039138
A9	-3.6E-05	1.37E-05	-2.62394	0.046875	-7.4E-05	6.96E-05	-1.06561	0.335329

Table 5. Experimental and predicted values of TPC and TFC under optimum conditions.

	Time	Solid: Solvent Ratio	Ethanol Concentration (%)	Experimental	Predicted	% Error
TPC	3.00	2.56	53.77	0.0302	0.0295	2.28
TFC	2.15	3.00	49.18	0.0813	0.0782	3.80

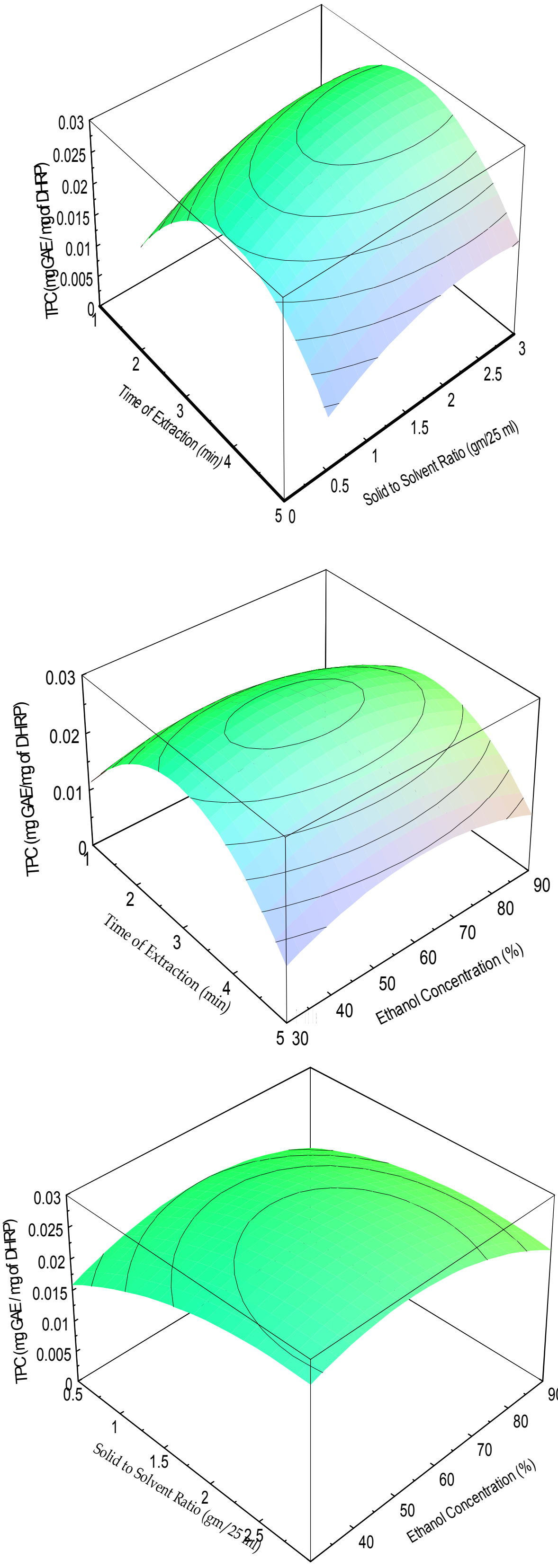

Figure 1. Response surface graphs illustrating the effect of (a) time of extraction and solid to solid ratio (b) time of extraction and ethanol concentration and (c) solid to solvent ratio and ethanol concentration on total phenolic content (TPC).

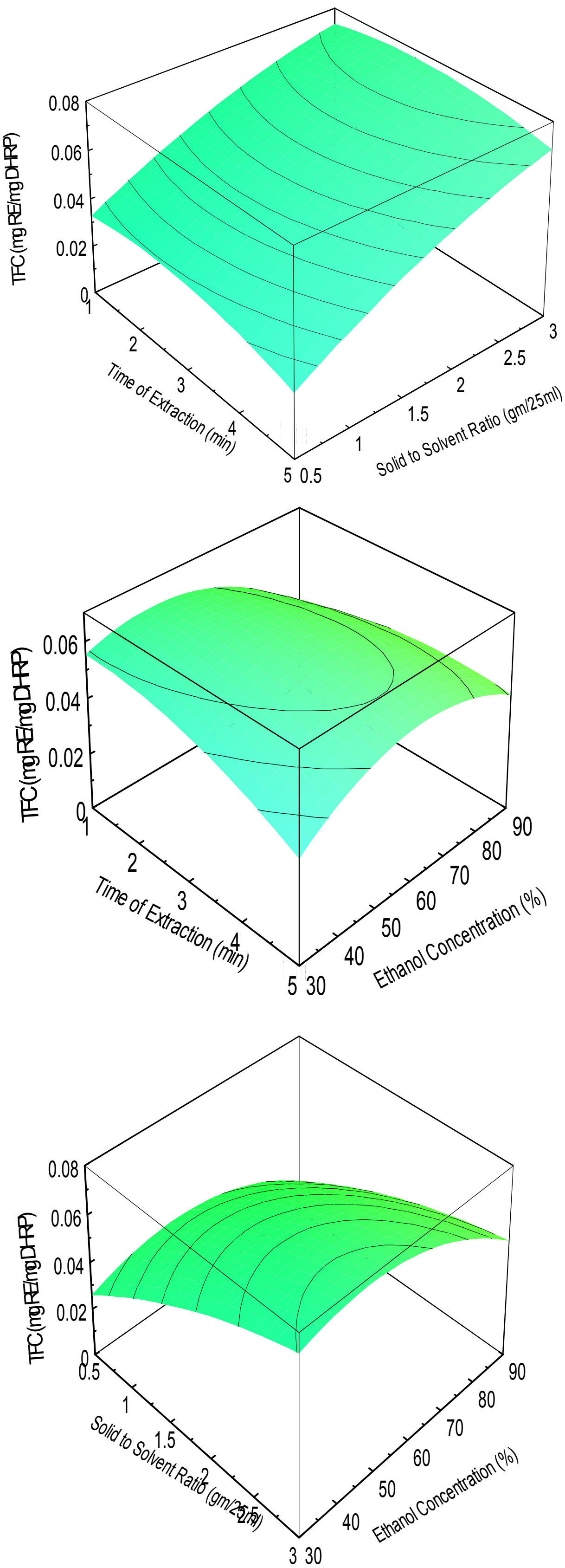

Figure 2. Response surface graphs illustrating the effect of (a) time of extraction and solid to solid ratio (b) time of extraction and ethanol concentration and (c) solid to solvent ratio and ethanol concentration on total flavonoid content (TFC)

RESULT AND DISCUSSION

Extraction Model and Statistical Analysis

The microwave assisted extraction efficiency of polyphenols from plant materials may get affected by parameters such as microwave power, extraction time, solvent type, composition of solvent, solvent to solid ratio, soaking time, particle size of sample and extraction cycles. In this present investigation, the relationship between yield of polyphenols extraction and the process variables (extraction time, solid to solvent ratio and ethanol concentration) were identified by three factors Box-behnken design and further process variables were optimized. There were a total of 15 experimental runs for optimizing the three selected variables. The range of extraction parameters chosen in this study were based on preliminary experiments. The influence the extraction include extraction time (X1; 1–5 min), Solid: Solvent Ratio (X2; 0.5-3 gm/25 ml), Ethanol Concentration (X3; 30-90). Since the variables have different units and ranges, each of the variables must first be normalised and forced to arrange from-1 to +1 in order to obtain a more even response (Bas & Boyac, 2007; Setyaningsih et al., 2015). The different combinations of the independent variables and their experimentally measured predicted response in terms of Total Phenolic Content and Total Flavonoid Content are presented in Table 2.

The analysis of variance (ANOVA) of yield of TPC and TFC extraction yield and regression statistics showed that experimental data had correlation coefficient (R^2) of 0.9945 and 0.9722 for TPC and TFC respectively with the calculated model suggesting that the model was well fitted and could be used to predict the total phenols from microwave-assisted DHRP extract. Table 3 indicates that the model is highly significant, which is evident from the high Fisher (F) ratio value.

Effect of Extraction on Total Phenolic content and Total Flavonoid Content

The coefficients of the response surface model as provided by Eq. 1 were evaluated for TPC and TFC. The final predictive equation constants obtained for TPC and TFC were given in Table 4.The effect of extraction time, solid to solvent ratio and ethanol concentration on TPC were also shown in response plots (Figure 1). In the present study the effects of linear terms and quadratic terms on extraction of TPC were highly significant as $p < 0.001$. There was no significant effect of interaction between extraction time and extraction temperature but interaction between extraction time and ethanol concentration and that of ethanol concentration and solid to solvent ratio were found to be significant ($p < 0.05$).

The effect of extraction conditions on total flavonoid content is shown in Figure 2. The effect of linear terms, quadratic and interaction terms are highly significant on the extraction of total flavonoid content at given experimental conditions. A positive sign of the coefficient means a synergistic effect, while a negative sign represents an antagonistic effect (Karimi et al., 2012).

Optimization of Microwave Assisted Extraction Parameters and Validation of Model

Optimization of process variables is one the important steps in design and analysis of experiments. The main objective of the experimentation is to find the levels of factors which optimize response. The process parameters selected in this study was optimized using the SOLVER function of MS-Excel-2010®software. The predicted optimum time, solid to solvent ratio and ethanol concentration was found to be 3 min, 2.56 g/25 ml and 53.77 %respectively. The predicted yield to be 0.03 mg GAE/mg DHRP of total phenolic content. For extraction of Total Flavonoid Content the Optimum conditions of time, solid to solvent ratio and ethanol concentration to be were found to be 2.15 min, 3 g/25 ml and 49.18% respectively. The predicted yield was 0.079 mg RE/ mg DHRP. Three trials of the experiment were conducted at optimum conditions and average values are presented in Table 5. The experimental results were showing 2.28 % and 3.8 % error predicted yield for TPC and TFC respectively. This shows that optimization is reliable in the present study.

CONCLUSION

In the present study microwave assisted extraction process was optimized for the extraction of polyphenols from *Decalepis hamiltonii* powder using three levels of Box-behnken design. MAE was found to be a viable alternative to traditional solvent extraction techniques for the recovery of polyphenols from *Decalepis hamiltonii*. The high correlation of the mathematical model indicated that a quadratic polynomial model could be employed to optimize the microwave assisted extraction of polyphenols. Under the optimum conditions, total phenolic content and total flavonid content obtained was 0.03mg GAE/mg DHRP and0.079 mg RE/ mg DHRP respectively. The optimum conditions of time, solvent to solid ratio and ethanol concentration for TFC and TPC was found to be 3 min, 2.56g/25 ml, 53.77 % and 2.15 min, 3 g/25 ml, 49.18% The experimental results were showing 2.28 % and 3.8 % error predicted yield for TPC and TFC respectively.

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