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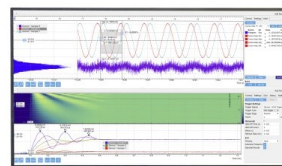
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Optimization Of Ionic Liquid-Based Microwave Extraction Of Flavonoid and Phenolic Acid From *Labisia Pumila*

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Abstract. Ionic liquids have attracted many researches in bioactive compound separation as well as carbon capture. This is due to the ability of ionic liquid to be designed based on specific needs and produced higher quantity and quality of extract product. A study showed that ionic liquid can extract a higher number of phytochemicals compared to conventional solvents and produce better yield with microwave-assisted extraction. Due to vast possible structures of ionic liquid and wide range of phytochemicals existed, a systematic approach was developed to screen ionic liquid solvents for phytochemical extraction. This study is a part of experimental validation included in the systematic approach. The main focus of this study is to optimize the process of ionic liquid-based microwave-assisted extraction of flavonoid and phenolic acid from *Labisia pumila*. A two-level, four-factor of central composite design (CCD) was employed to determine the effect of the process factors towards yield of flavonoid and phenolic acid extracted from the herb. The process factors are the temperature (Celsius), extraction time (min), power (W) and type of ionic liquid (based on their dielectric point). In this study, ionic liquid was back-extracted using organic solvents for regeneration and recycle of extraction solvent, based on Bogdanov (2015). The extracted samples were analyzed by determining the yield of flavonoid and phenolic acid through linear equation of quercetin and gallic acid. In this study, it can be observed that the recovery of flavonoid and phenolic acid from *L. pumila* using ionic liquid-based microwave-assisted extraction was about 30 – 50 percent higher compared to conventional solvent. Therefore, the study indicated that ionic liquid-based microwave-assisted extraction was an efficient and rapid method for *L. pumila* phytochemical extraction.

INTRODUCTION

Usage of herbal worldwide has been estimated around 80 percent and growing rapidly [1]. Herbal products in Malaysia consists of RM 4.6 billion value of medical and aromatic plants with an annual projected growth rate of 15 to 20 percent [2] due to an increased usage of medicinal plants endorsed by extraction and development of drugs and chemotherapeutics from plants, as well as common herbal remedies [3]. The reason for the high demand is that herbs are part of many cultures in health maintenance or treating ailments. Besides, people have the impression of any natural products are safe for consumption and the products are easily available in the market.

Herbs synthesize wide range of bioactive compounds known as phytochemicals included flavonoids, terpenes and terpenoids, alkaloids and phenolic compounds [4] that are responsible for therapeutic properties [5]. A great deal of work focused on identifying phytochemicals as they have been the source of food and medicine throughout the world for thousands of years [6]. *Labisia pumila* or famously known as Kacip Fatimah in Malaysia have been longestablished as health supplement for women among Malay tradition. Studies have shown that this herb is a good source of natural antioxidants due to its high content of anthocyanins, flavonoids, and phenolic acid [7]. It has been reported there are three different varieties of *L. pumila* in Malaysia which are var. *pumila*, var. *alata* and var. *lanceolate* [8]. These varieties can be differentiated by the leaf and petiole characteristics [9]. However, *L. pumila* var. *alata* is the most common plant found in Malaysia and used in traditional medicine [10].

Proper extraction steps play a vital role in the quality and quantity of the herbs extract [11]. The most common factors affecting extraction processes are matrix properties of the plant part, solvent, temperature, pressure and time [12]. Currently, conventional organic solvents such as ethanol and methanol are used as an extraction medium. These conventional solvents are volatile and have a slightly lower selectivity of extraction. Therefore, an alternative (sustainable) solvent that would eventually allow for the replacement of existing solvents is needed in order to yield high-end products. The ionic liquid is an alternative solvent due to having the opposite properties to those of the conventional solvents and is recognized as a good separation solvent [13] based on its capability of higher extraction efficiency [14]. Ionic liquid also is a more sustainable solvent due to the recycling capability and reduce solvent loss in extraction process.

Ionic liquid previously designed for various separation process such as the extraction of pharmaceutical intermediate from an aqueous phase [15], used in carbon capture and storage system [16] and extraction of metals ion and organic molecules [17] as well as azeotropic mixtures [18]. The unique characteristic of ionic liquid that able to tailor their properties based on different cations, anions, alkyl length and other substituents selection has marked them as a designer solvent and practically offer a huge potential in many types of applications [19]. However, ionic liquid screening process can be a huge challenge for researchers and engineers owing to the vast possibilities of molecules interactions.

Therefore, systematic approach and predictive thermodynamic models are crucial in ionic liquid design to select the best-suited solvent in the process system as well as to simplify and reduce experimental cost consumptions in the process. The design usually considers the structure of a single side chain attachment, an additional attachment and the double attachments to an ionic liquid. Some of the properties that have been taken into account include the solubility estimation, distribution coefficient (m), solvent loss (SL), selectivity (b), solvent power (SP), partition coefficient, thermal stability and viscosity.

This research is an extension of previous research on ionic liquid solvent design [20]. The research focuses on optimizing the flavonoid and phenolic acid extraction parameters of the final ionic liquid candidates from the previous research and developed a model of herbal phytochemical extraction process. Both of these phytochemicals were selected in this study based on the foremost compounds extracted from *L. pumila* from published researches.

METHODOLOGY

Solvent Design

This research is part of the experimental validation step of the solvent design approach using reverse phase computational approach. A framework for solvent selection was developed where initially solvent properties and constraints were listed for further solvent selections [20]. The framework was developed based on a database of imidazolium ionic liquids. Due to numerous possibilities of molecular structure of ionic liquid, many properties (such as heat capacity, solubility, melting point etc.) were unknown solved by applying property models to predict the data. Final solvents listed based on the framework designed were 15 and 31 type of ionic liquids for flavonoid and phenolic acids extraction, respectively from herbs. Three of the final solvents selected in this experimental research are based on economic and cost value.

Reagents and Equipment

The standard chemicals of quercetin and gallic acid, HPLC-grade acetonitrile and acetic acid were purchased from Merck (Germany). Ionic liquid; 1-butyl-3-methylimidazolium bromide, 1-butyl-3-methylimidazolium chloride and 1-ethyl-3-methylimidazolium tetrafluoroborate were purchased from Sigma Chemical. Ethyl acetate and methanol purchased from HmbG GmbH.

Plant Materials and Extractions

Labisia pumila or Kacip Fatimah were provided by EthnoHerbs Sdn. Bhd. The leaves and branches were oven-dried, made into coarse powder and stored in airtight container. 0.3 gram of dried powder was mixed with 3 ml of ionic liquid in a round bottom microwave flask and attached to a condenser through the hole on the top of the oven. To improve extraction yield, type of ionic liquids (based on dielectric value (A)), extraction temperature (B), microwave irradiation time (C) and microwave irradiation power (D) were optimized using Response Surface Method (RSM) with two-level, four-factor of central composite design (CCD). Details of the optimized parameters are listed in Table 1. Dielectric value for 1-butyl-3-methylimidazolium bromide, 1-butyl-3-methylimidazolium chloride and 1-ethyl-3-methylimidazolium tetrafluoroborate are 3.59, 3.85 and 13.6 each. After each extraction, the extracts were cooled and filtered to separate the herbs and extracts. The extraction yield was conveyed as milligram of target ingredient extracted per gram sample.

TABLE 1. Optimized parameters for ionic liquid microwave-assisted extraction

	Parameters	Unit	Lower values	Upper values
A	Dielectric value	Dielectric constant (ϵ)	3.59	13.6
B	Temperature	Celsius ($^{\circ}\text{C}$)	35	45
C	Irradiation time	Minutes (min)	1	10
D	Irradiation power	Watt (W)	250	450

Recovery of Ionic Liquid

Ionic liquid was considered as sustainable solvent in this matter as their ability to be recycled for next applications. Ionic liquid in this study was recovered according to [21]. A back extraction of ionic liquid was performed by phase separation using ethyl acetate. Ethyl acetate was selected as back extraction solvent due to the performance capability of flavonoid and phenolic acid solubility [22]. Filtered extracts were added with ethyl acetate in a volume of 1:2 and vortexed to mix well. The mixed solvents were separated by partitioning.

Determination of Total Flavonoid Content

The aluminum chloride colorimetric method to determine total flavonoid content is based on [23]. Quercetin was used to make the calibration curve. 10 mg of quercetin was dissolved in 10 ml of methanol and diluted to six different concentrations. 0.5 ml of diluted solution was separately mixed with 1.5 ml of 95 % ethanol, 0.1 ml of 10 % aluminum chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. The mixture was incubated in about 30 minutes and the absorbance was measured at 415 nm with a Shimadzu UV-160A spectrophotometer. The amount of 10 % aluminum chloride was substituted by the same amount of distilled water in blank. The amount of total flavonoid compounds were expressed as mg quercetin/g dry weight of the plant material. The data were presented as the average of triplicate analyses.

Determination of Total Phenolic Content

Total phenolic content measured based on the standard procedure of Folin-Ciocalteu method [24]. Gallic acid used as standard reference for total phenolic content. 1 mg of gallic acid was dissolved in 1 ml of methanol and diluted to different concentrations. 0.1 ml of the diluted solution was transferred and made up to 4.6 ml with distilled water. After addition of 0.1 ml Folin-Ciocalteu reagent and 0.3 ml of 2 % sodium carbonate (Na_2CO_3), the mixture was incubated for 45 minutes in a dark room and vortexed before measurement of absorbance at 760 nm. The amount of total phenolic content were expressed as mg gallic acid (GAE)/g dry weight of the plant material. The data were presented as the average of triplicate analyses.

RESULTS AND DISCUSSIONS Effect of Different Extraction Solvents

Different types of ionic liquids and ethyl acetate were used as extraction solvents for phenolic acid and flavonoid extraction from *Labisia pumila* herbs. The condition of extraction process was kept constant at 35 $^{\circ}\text{C}$ at 10 minutes with

250 W of microwave irradiation power. Based on Figure 1, it can be proven that ionic liquids have better solubility of flavonoid and phenolic acid compared to organic solvent (in this case, ethyl acetate was selected as model of organic solvent) and 1-butyl-3-methylimidazolium chloride ([BMIM][Cl]) was the best extraction solvent for both compounds. Ionic liquid makes a better extraction solvent due to the molecular structure of their anion and cation. The length of alkyl chain of ionic liquid cation positively influence the extraction yield but simultaneously increase the hydrophobicity of the ionic liquid with the increase of alkyl length. Both the proper hydrogen bonding and hydrophobic interactions thus resulted in stronger solvation interactions with flavonoids and phenolic acids [25].

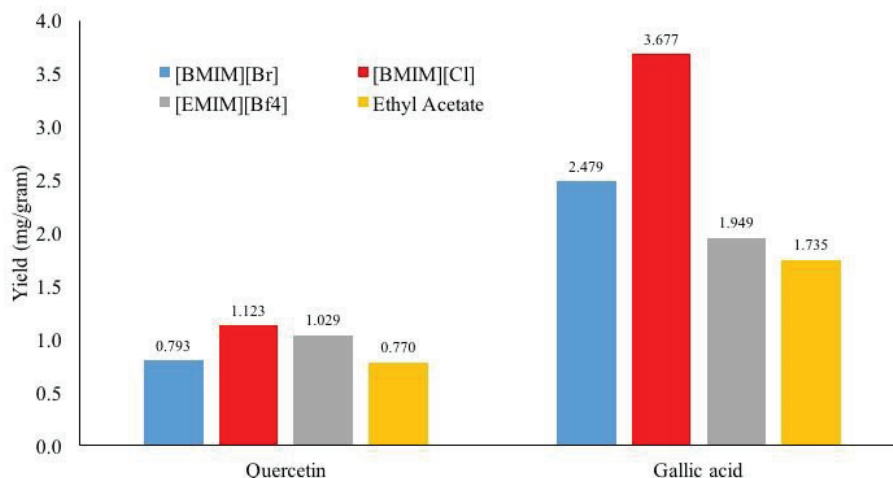


FIGURE 1. Effect of four different solvents towards the extraction of quercetin and gallic acid.

The solubility of quercetin and gallic acid were compared with the predicted solubility value from previous research on ionic liquid solvent design [20]. The best solvents have good selectivity and capacity, high thermal stability, good availability, low cost, high surface tension and low to moderate viscosity [26]. Table 2 observed that the trend of the ionic liquid capacity to extract both compounds are similar with prediction data. Selectivity and solubility are highly dependent on composition thus it depends on the extraction temperature and microwave irradiation [27]. Although the amount of experimental flavonoid extract only does not differ much with the predicted data, the quantity of compounds in the herbs may be accounted as the maximum capacity of solubility in the solvent. The phytochemicals in herbs may also differ in quantity by different varieties of the herbs or even the age of plants during harvesting [28].

TABLE 2. Predicted and experimental value of flavonoid and phenolic acid solubility

Phytochemicals	Quercetin (mg/ml)		Gallic acid (mg/ml)	
	Predicted	Experimental	Predicted	Experimental
[BMIM][Br]	1.074	0.993	1.758	2.479
[BMIM][Cl]	1.184	1.223	1.765	3.677
[EMIM][BF ₄]	1.166	1.192	1.384	1.949
Ethyl acetate	0.965	0.770	1.078	1.735

Optimization of Ionic Liquid Microwave-Assisted Extraction Conditions

Table 3 showed the linear equations for the quercetin and gallic acid standard curve as the basis in determining the amount of both compounds in the *L. pumila* extracts. The calibration range for both compounds involved six different concentrations of 0.05, 0.1, 0.2, 0.4, 0.6 and 0.8 mg/ml. Graphs of linear standard curve were plotted to obtain the equation with high predicted R-squared. Higher R-squared denotes that less error involved in the equation.

TABLE 3. Standard curve for quercetin and gallic acid

Compound	Standard curve linear equation	R-squared	Calibration range (mg/ml)
Quercetin	$y = 5.4622x + 0.0674$	0.994	X = 0.05-0.8
Gallic acid	$y = 2.6909x - 0.1396$	0.991	X = 0.05-0.8

The objective of the study is to optimize the ionic liquid microwave assisted extraction conditions and to find the relations between the factors involved. Besides, equation models were developed by relating the extraction process parameters. Parameters involved were analyzed statistically using Design Expert 7.0 software by two-level, fourfactor of central composite design (CCD). Table 4 showed the suggested equation models by the software where both compounds and parameters involved in the extraction can be related by a quadratic model of equation.

TABLE 4. Suggested equation models for ionic liquid microwave extraction process for quercetin and gallic acid based on predicted R-squared value

Suggested type of equation	R-squared value	
	Quercetin	Gallic acid
Linear	0.4961	0.5395
2FI	0.5141	0.5566
Quadratic	0.9396	0.9508

Table 5 explained the ANOVA analysis for each of the factors of flavonoid extraction from *L. pumila*. In terms of the model's F-value of 42.60, it implies that the model is significant. There is only a 0.01 % chance that a model this large could occur due to noise. Based on the statistical value, P-value less than 0.05 indicate that the parameters are significant, however P-value between 0.05 to 0.10 may be selected as significant if the data is small and cannot be reformed. In this case, the extraction parameters of A, B, C, D and interaction parameters of AC, AD, CD and A² are significant model terms. The F-value for Lack of Fit was 4.10 implies there is a 6.26 % chance that a "Lack of Fit Fvalue" this large could occur due to noise. Although the lack of fit value is not significant (which indicates that the model can be fitted), nevertheless the value is less than 10 % showed that there is only low probability of the model to be fitted. This might occur due to the small range of parameters involved in the microwave-assisted extraction.

TABLE 5. ANOVA analysis for Response Surface Reduced Quadratic Model for Flavonoid Extraction

	Mean Square	F value	P-value
Model	0.0922	42.6011	< 0.0001
A-Dielectric value	0.2188	101.0646	< 0.0001
B-Temperature	0.0126	5.8379	0.0240
C-Irradiation Time	0.0770	35.5647	< 0.0001
D-Irradiation Power	0.3451	159.3538	< 0.0001
AC	0.0323	11.4892	0.0235
AD	0.0449	12.0720	0.0164
CD	0.0468	12.1610	0.0155
A ₂	0.3341	154.2690	< 0.0001
Lack of Fit	0.0026	4.0969	0.0626

ANOVA analysis for phenolic acid extraction factors of *L. pumila* is shown in Table 6. In terms of the model's Fvalue of 23.45, it implies that the model is significant. There is only a 0.01 % chance that a model this large could occur due to noise. In this case, many of the parameters (A, B, C and D) and the parameter interactions (AB, AC, AD, BC, BD, CD, A², B², C² and D²) are significant model terms. The F-value for Lack of Fit was 1.91 implies there is a 24.48 % chance that a "Lack of Fit F-value" this large could occur due to noise. Therefore, it can be concluded that the model constructed is suitable.

TABLE 6. ANOVA analysis for Response Surface Reduced Quadratic Model for Phenolic Acid Extraction

	Mean Square	F value	P-value
Model	0.3709	23.4459	< 0.0001
A-Dielectric value	0.0817	5.1662	0.0363
B-Temperature	0.0626	3.9585	0.0630
C-Irradiation Time	0.1670	10.5574	0.0047
D-Irradiation Power	0.7020	44.3761	< 0.0001
AB	0.0186	11.7855	0.02928
AC	0.0319	20.1848	0.01735
AD	0.0740	46.8257	0.0450
BC	0.0006	2.6166	0.0514
BD	0.0047	3.9642	0.05932
CD	0.0023	1.4443	0.07086
A ₂	0.9157	57.8821	< 0.0001
B ₂	0.0189	11.9609	0.02894
C ₂	0.0035	22.0079	0.6449
D ₂	0.0036	22.8033	0.6391
Lack of Fit	0.0184	1.9145	0.2448

Based on the R-squared predicted by the software, both of the model fittings of quercetin and gallic acid extraction have high value reaching to the value of 1. R-squared simply explains how good is your model when compared to the baseline model. Based on the values of 0.94 and 0.95 respectively, it can be assessed that the model is a good fit. Since the value of lack of fit and the R-squared value are in a suitable range, thus it can be said that the quadratic models for gallic acid and Quercetin extraction that were developed are suitable for the use of phytochemicals extraction prediction. Equation (1) and (2) are predicted value of for the flavonoid and phenolic acid extraction from *L. pumila* using ionic liquid microwave-assisted extraction. The equations consider as model improvement of basic solubility model of equation used in previous study, with extraction parameters such as irradiation powers and dielectric point were added into the equations.

$$\text{Yield of flavonoid} = [3.459 + 0.177 (A) + 0.026 (B) + 0.063 (C) + 0.133 (D) - 0.014 (AC) - 0.017 (AD) - 0.016 (CD) - 2.954 (A^2)]^2 \quad (1)$$

$$\text{Yield of phenolic acid} = 7.329 + 0.119 (A) + 0.058 (B) + 0.094 (C) + 0.192 (D) - 0.035 (AB) - 0.045 (AC) - 0.069 (AD) + 0.006 (BC) - 0.017 (BD) + 0.012 (CD) - 6.851 (A^2) - 0.075 (B^2) + 0.036 (C^2) - 0.036 (D^2) \quad (2)$$

TABLE 7. Statistical value for quadratic model equation of ionic liquid microwaveassisted extraction process

	Quercetin		Gallic acid
Std. Dev.	0.0465	Std. Dev.	0.1258
Mean	1.0743	Mean	1.8662
R-Squared	0.9368	R-Squared	0.9508

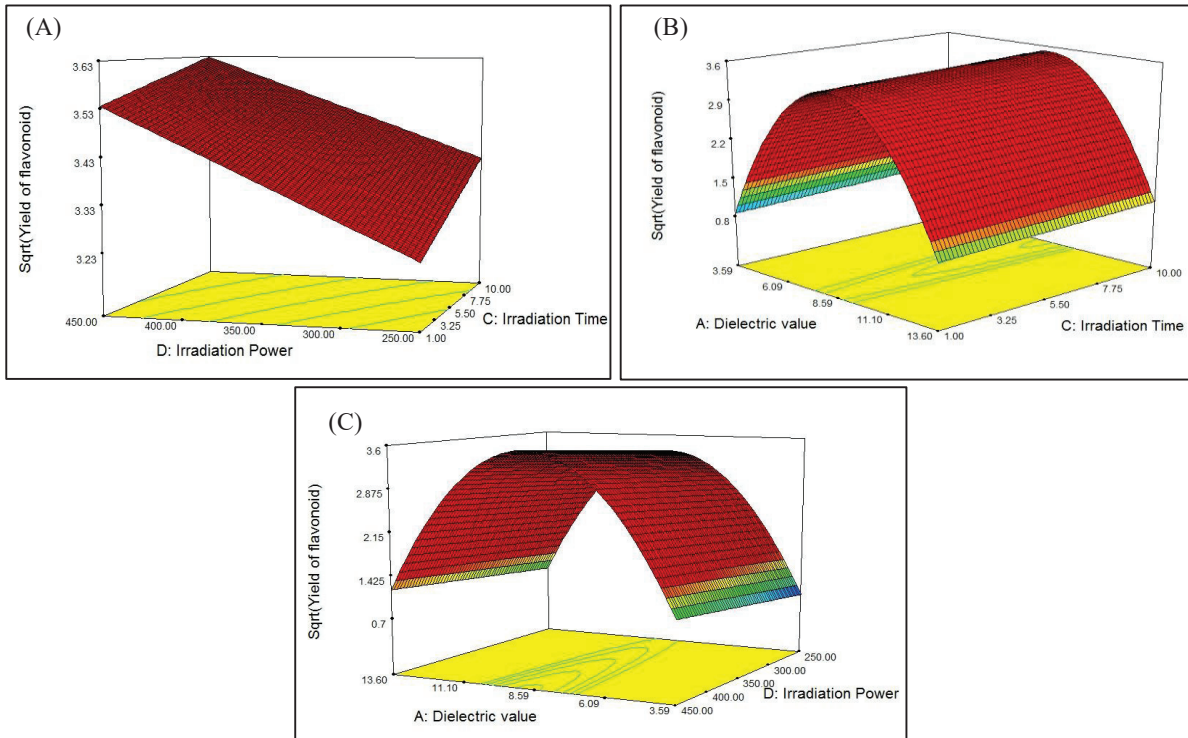
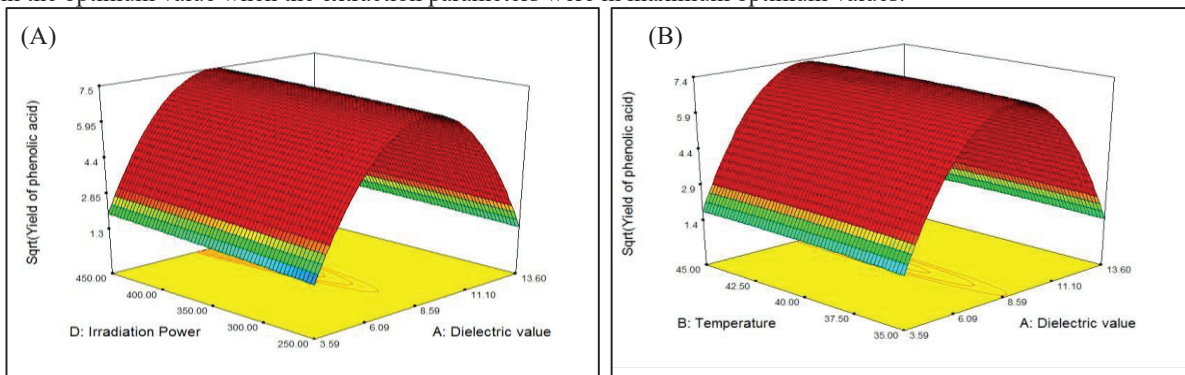


FIGURE 2. Response surfaces (3D representation) effect of: irradiation power and time (A), dielectric value and irradiation time (B), dielectric value and irradiation power (C) for flavonoid extraction from *L. pumila* using ionic liquid.

The interaction parameters of flavonoid and gallic acid extraction by microwave extraction were represented by Figure 2 and Figure 3. The interaction parameters showed the significant parameters that relate to the extraction process model. Figure 2 (B) and Figure 2 (C) showed that the yield of flavonoid increase when dielectric value increase while extraction time reduced and irradiation power increased. Yield of flavonoid increase with irradiation time and power proportionally. Based on Figure 3 (A), (B) and (C), the yield of phenolic acid increase when dielectric value increase while extraction time, irradiation power and time increased. The same can be said on the relation of irradiation time, irradiation power, temperature and time. Therefore, it can be concluded that yield of phenolic acid and flavonoid will be in the optimum value when the extraction parameters were in maximum optimum values.



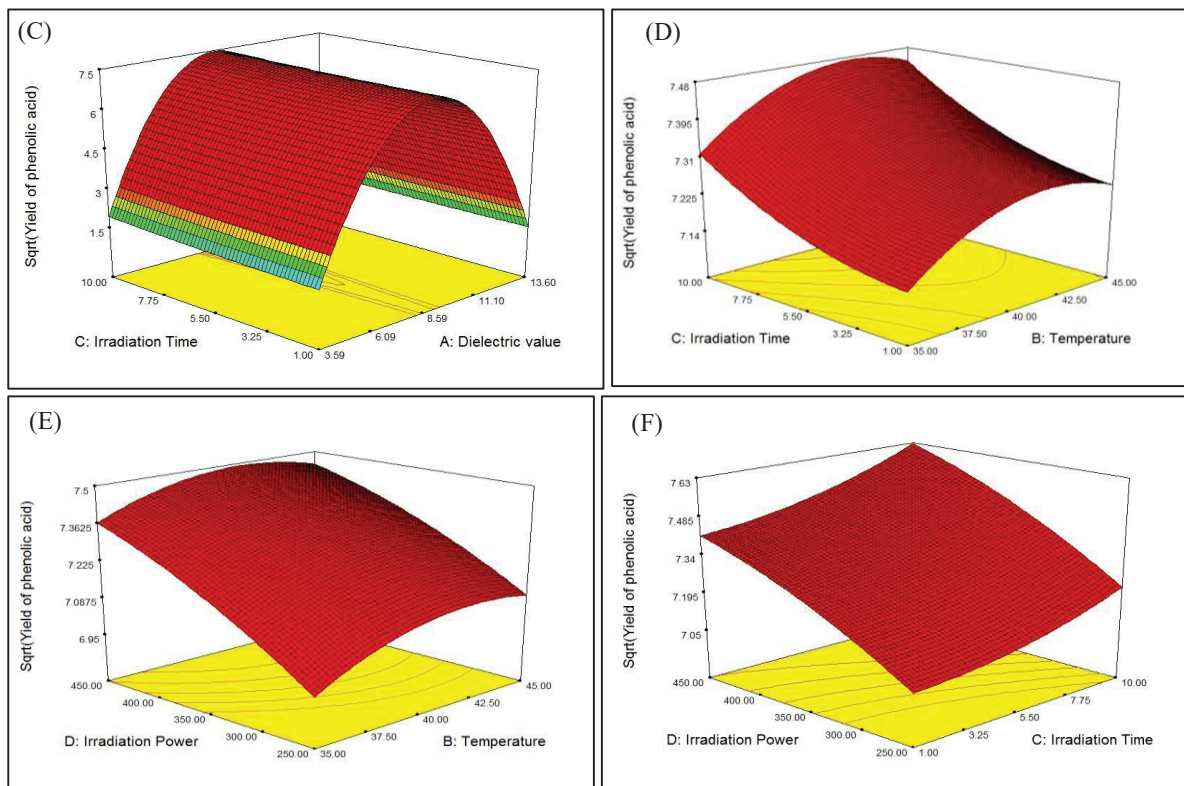


FIGURE 3. Response surfaces (3D representation) effect of: dielectric value and irradiation power (A), dielectric value and temperature (B), dielectric value and irradiation time (C), irradiation time and temperature (D), irradiation power and temperature (E) and irradiation power and time (F) for phenolic acid extraction from *L. pumila* using ionic liquid.

CONCLUSIONS

It can be concluded from this study that ionic liquid is an alternative solvent because ionic liquid can extract higher yield of flavonoid and phenolic acid. Besides, ionic liquid can be recycled and reused multiple times for the extraction process, therefore it can be cost effective as ionic liquid can extract 50 % higher yield than conventional solvent. In this study, two model of equations were developed for flavonoid and phenolic acid extraction using ionic liquid by microwave-assisted extraction. The models developed are significant with a good fit and high coefficient of determination (R-squared value).

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