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Green Extraction of Eurycomanone from *Eurycoma longifolia* Using Imidazolium–Based Ionic Liquids Combined with Microwave–Assisted Method

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Abstract

Background: Pasak Bumi (*Eurycoma longifolia* Jack) is an endemic plant that has been utilized as a medicinal agent by communities in Kalimantan and Sumatra for an extended period. The entirety of *E. longifolia*, inclusive of its leaves, stems, and roots, has been utilized as a medicinal agent. The primary compound present in *E. longifolia* is eurycomanone, which is commonly found in the roots of the plant. **Objective**: This study aims to optimize the extraction of eurycomanone from *E. longifolia* roots using imidazolium-based ionic liquids (1-butyl-3-methylimidazolium chloride [BMIM][Cl]) through microwave-assisted extraction (MAE), applying response surface methodology (RSM) for statistical meodeling. **Methods**: A Box-Behnken design was employed to evaluate the effects of three independent variable including [BMIM][Cl]concentration, extraction time, and solid-liquid ratio on eurycomanone content. Quantification was performed using a validated HPLC method, and model performance was assessed via analysis of variance (ANOVA). **Results**: The concentration of [BMIM][Cl] significantly influenced eurycomanone content ($p<0.001$), while extraction time and solid-liquid ratio showed no significant individual effects. The optimized condition yielded 0.4061 µg/mg of eurycomanone, closely matching the predicted value (0.3835 µg/mg). The model demonstreated good fit ($R^2 = 0.8135$) and non-significant Lack of Fit ($p = 0.6566$), indicating reliable predictive capability. **Conclusion**: MAE using [BMIM][Cl] is an effective and environmentally friendly approach for extracting eurycomanone. The developed model provides a robust framework for process optimization and potential scale-up in phytopharmaceutical applications.

Keywords: Eurycomanone; *Eurycoma longifolia* Jack; Ionic Liquid; Microwave-Assisted Extraction; Response Surface Methodology

1. INTRODUCTION

The growing global demand for bioactive compounds derived from medicinal plants has accelerated the search for more sustainable and efficient extraction technologies. Conventional solvent-based methods such as maceration and Soxhlet extraction are widely used but suffer from several limitations, including prolonged processing time, excessive use of organic solvents, and the degradation of thermolabile compounds [1]. These drawbacks not only reduce extraction efficiency but also raise environmental and safety concerns, particularly in large-scale applications.

The mounting demand for bioactive compounds from medicinal plants has prompted the development of more sustainable and efficient extraction technologies. Conventional solvent-based methods frequently exhibit deficiencies, including protracted extraction times, substantial solvent consumption, and the degradation of thermolabile compounds. In response to these challenges, green chemistry principles have gained increasing attention in phytochemical research. One promising approach involves the use of ionic liquids (ILs) as alternative solvents. ILs are non-volatile, thermally stable, and tunable in polarity, making them suitable for selective extraction of polar secondary metabolites. Imidazolium-based ILs, such as 1-butyl-3-methylimidazolium chloride (BMIM][Cl]), have demonstrated superior solubilizing capacity for quassinoids and other bioactive compounds due to their strong ionic interactions and hydrophilic nature [2–4].

When combined with microwave-assisted extraction (MAE), ILs offer synergistic advantages. MAE enhances mass transfer and accelerates solvent penetration into plant matrices through rapid dielectric heating, thereby reducing extraction time and minimizing thermal degradation [5,6]. The integration of ILs and MAE aligns with the principles of green chemistry by improving energy efficiency, reducing solvent consumption, and enabling cleaner processing routes [7].

Eurycoma longifolia Jack, commonly known as Tongkat Ali or Pasak Bumi, is a well-known medicinal plant native to Southeast Asia. Traditionally used for its adaptogenic, aphrodisiac, and antimalarial properties, *E. longifolia* has attracted considerable scientific interest due to its rich phytochemical profile. Among its constituents, eurycomanone—a quassinoid compound found predominantly in the roots—has emerged as a key bioactive marker with significant pharmacological activities, including anticancer, antiplasmodial, and testosterone-enhancing effects [8,9,10]. However, the efficient extraction of eurycomanone remains challenging due to its low abundance and sensitivity to processing conditions.

Although ILs and MAE have been explored independently in various extraction studies, few investigations have systematically optimized the recovery of eurycomanone using IL-MAE techniques. Moreover, the application of statistical modeling tools such as response surface methodology (RSM) remains underutilized in this context. RSM enables the evaluation of multiple variables and their interactions, providing a robust framework for process optimization and predictive modelling [11–13].

Therefore, the objective of this study is to optimize the extraction conditions of eurycomanone from *E. longifolia* roots using BMIM][Cl]-based MAE, evaluated through RSM. The study investigates the effects of IL concentration, extraction time, and solid-liquid ratio on eurycomanone yield, aiming to develop a predictive model that supports future scale-up and phytopharmaceutical formulation.

2. MATERIALS AND METHODS

2.1. Chemicals and Instruments

2.1.1 Samples and Chemicals

Root samples of *E. longifolia* obtained from the interior of West Kutai Regency, East Kalimantan, were identified and authenticated at the Dendrology Laboratory of the Faculty of Forestry, Mulawarman University, Samarinda. The materials used in this study included acetonitrile HPLC grade, demineralized water HPLC grade, and methanol HPLC grade were purchased from PT. SmartLab Indonesia, Indonesia. Standard eurycomanone was purchased from Sigma-Aldrich,

Germany (through PT Elo Karsa Indonesia). 1-Butyl-3-methylimidazolium acetate [BMIM][OAc] and 1-butyl-3-methylimidazolium chloride [BMIM][Cl] were purchased from Chen Jie Chemical Co.Ltd, Shanghai, China).

2.1.2. Instrument

The equipment used in this study, included: Grinder (Local Product, Indonesia), high performance liquid cromatograpy (HPLC) (Shimadzu, Japan), Domestik Microwave Modified (Modena, USA), Vacuum Oven (Maskot, Indonesia), Food Dehydrator (WiraTech, Indonesia), Analytical Balance, and Design Expert® software (version 13, Stat-Ease Inc., USA).

2.2. Sample Preparation

The dried roots were ground into fine powder using a mechanical grinder and sieved to obtain uniform particle size (<500 μm). Approximately 1 g of powdered sample of *E. longifolia* roots was used for each extraction trial.

2.3. Microwave-Assisted Extraction (MAE) Procedure

Procedure of MAE was performed according to our previous studies [14–16], with some modification to suit the best conditions. The extraction process was carried out using a laboratory-grade domestic microwave modified (Modena, USA) with adjustable power and time control. The sample was subjected to a series of controlled variables, including varying concentrations of ILs (0.2–0.6 mol/L), liquid-solid ratios (8–12 mL/g), and extraction times (10–30 minutes), as outlined in the experimental design. The microwave power was set at 450 W. Following extraction, the mixture was cooled, subjected to centrifugation at 4,000 rpm for 10 minutes, and the upper layer was filtered through a 0.45 μm membrane prior to HPLC analysis.

2.4. Experimental Design

A Box-Behnken design (BBD) was employed to evaluate the effects of three independent variables: ILs concentration (X_1), extraction time (X_2), and solid-liquid ratio (X_3), as showed in **Table 1**. The response variable was eurycomanone content (μg/mg). Design Expert® software (version 13, Stat-Ease Inc., USA) was used for statistical modeling and response surface analysis [17,18].

Table 1. Experimental Design using Box-Behnken RSM Design

Factor	Unit	Symbol	Levels		
			Low	Medium	High
ILs Concentration	mol/L	X_1	0.4	0.7	1
Extraction Time	Minutes	X_2	10	20	30
Liquid-Solid Ratio	mL/g	X_3	8	10	12

2.5. Quantitative Analysis of Eurycomanone

Eurycomanone was quantified using high-performance liquid chromatography (HPLC) equipped with a UV detector [19,20]. Separation was achieved using a column of Inertsil ODS-2 C18 (150 × 4.6 mm, 5 μm) with a certain condition of mobile phase at a flow rate of 1.0 mL/min. Detection was performed at 254 nm. The injection volume was 20 μL, and the column temperature was maintained at 30°C.

2.6. Validation Method for Analysis of Eurycomanone

The HPLC method was validated according to ICH guidelines [21] for specificity, linearity, accuracy, precision, limit of detection (LoD), and limit of quantification (LoQ). The linearity of the assay was confirmed over the range of 0.05–1.00 μg/mL, as indicated by a correlation coefficient (R^2) greater than 0.999. The limits of detection (LoD) and limits of quantification (LoQ) were determined to be 0.015 μg/mL and 0.045 μg/mL, respectively. The recovery tests yielded values ranging from 98.2% to 101.4%, indicating a high degree of accuracy [22].

3. RESULT AND DISCUSSION

3.1 Optimization of Mobile Phase For Eurycomanone Analysis

Optimization of the mobile phase is imperative to ascertain optimal analysis conditions; consequently, the mobile phase is selected for separation in HPLC instruments. The selection of the mobile phase for separation in HPLC analysis is a relatively straightforward process that can be accomplished through a trial-and-error approach. The composition of the mobile phase in HPLC analysis is a critical factor in the success of a study. In mixtures of components with high solubility in the stationary phase but low solubility in the mobile phase, this will result in a long retention time.

Conversely, in mixtures of components with low solubility in the stationary phase but high solubility in the mobile phase, this will result in a short retention time. This phenomenon is influenced by the polarity of the molecules involved. The initial mobile phase composition was water and acetonitrile at a ratio of 90:10. The second and third conditions were water:acetoni trile:methanol (80:10:10) and 0.1% formic acid:acetonitrile (85:10), respectively.

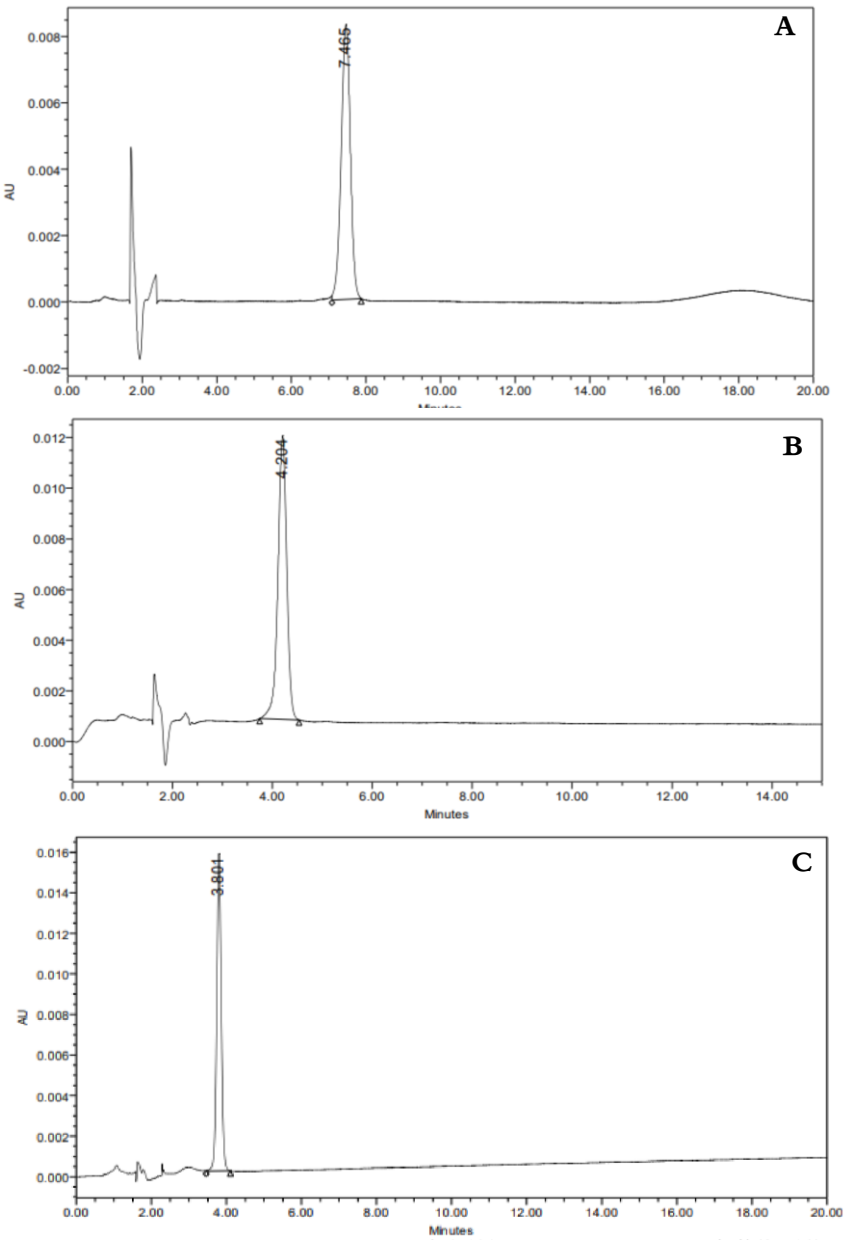

Figure 1. Chromatogram of Standard Eurycomanone based on different mobile phase, water:acetonitrile of 90:10:0 (A), water:acetonitrile:methanol of 80:10:10 (B), and 0.1% formic acid:acetonitrile of 85:10:0 (C)

The mobile phase was selected to obtain a suitable composition that would provide effective and efficient results for HPLC analysis. The first mobile phase composition tested was a combination of water:acetonitrile at a ratio of 90:10, with a flow rate of 1.0 mL/minute (**Figure 1A**). Then, the mobile phase composition was changed to a water:acetonitrile:methanol mixture with a ratio of 80:10:10, at a flow rate of 1.0 mL/minute (**Figure 1B**). Next, the third mobile phase composition (**Figure 1C**) was prepared by adding 0.1% formic acid in water, with a ratio of 0.1% formic acid:acetonitrile (85:10).
The third motion phase (**Figure 1C**) was identified as the most optimal phase due to its ability to achieve the shortest retention time while exhibiting a wider peak area. Subsequent to the acquisition of the method, a system suitability test was conducted. The system suitability test is designed to guarantee that the available HPLC operational system generates results that align with the analysis objectives. This discrepancy arises due to variations in equipment and analysis techniques. The system suitability test was performed five times.
According to USP, the method's compatibility with the available system is indicated by five parameters. The five parameters that will be examined are capacity factor, selectivity, resolution, tailing factor, and theoretical plate. The results of the system compatibility test are presented in **Table 2**.

Table 2. Results of System Suitability Test

Capacity Factor	Selectivity	Resolution	Tailing Factor	Theorytical Plate
1.7157	2.734	7.4612	1,0594	46790

System suitability testing must be conducted before the selected analysis method is used, to ensure that the system and procedures used are capable of providing acceptable data. In system suitability testing, there are several parameters, namely, the theoretical plate (N) with a requirement of >2500, and the tailing factor with a requirement of <2.5. A method is acceptable if at least two parameters meet the requirements in the system suitability test [22,23]. The research conducted yielded findings that identified the most optimal motion phase as a combination of formic acid in water and acetonitrile (85:10) with column conditions of Inertsil ODS-2 C18 (150 × 4.6 mm, 5 μm) and a flow rate of 1.0 mL/minute, as detected at a wavelength of 254 μm.

3.1.1 Linearity Determination

Linearity is defined as the capacity of an analytical method to generate test results that correspond to the concentration of analytes present in samples within a specific concentration range. The linearity of a given measurement system can be determined by performing a series of measurements at varying concentrations. The subsequent analysis of the obtained data will entail processing to ascertain the slope, intercept, and correlation coefficient [23].
The linearity of the system can be observed through the calibration curve, which demonstrates the correlation between the response and the analyte concentration across a range of solution concentrations. The linear regression model is determined by the calibration curve, expressed as the equation $y = a + bx$, where x denotes the concentration, y represents the response, a represents the intercept, and b represents the slope. The objective of this regression is to minimize residual error, defined as the discrepancy between the observed experimental results and the values predicted by the linear regression equation. It has been established that an ideal linear relationship is achieved when the value of b is 0 and r is +1 or -1, depending on the direction of the line [22].
The acquisition of data for the linearity testing was conducted through the measurement of the chromatogram area of the eurycomanone standard solution, which demonstrated the relationship between concentration and area as depicted in the linearity curve. From the data collected, a curve was constructed to illustrate the relationship between concentration (x) and area (y). The linear regression equation was subsequently determined. The eurycomanone standard linearity curve is presented in **Figure 2**, and it is from this curve that the results of the analysis are derived [22].

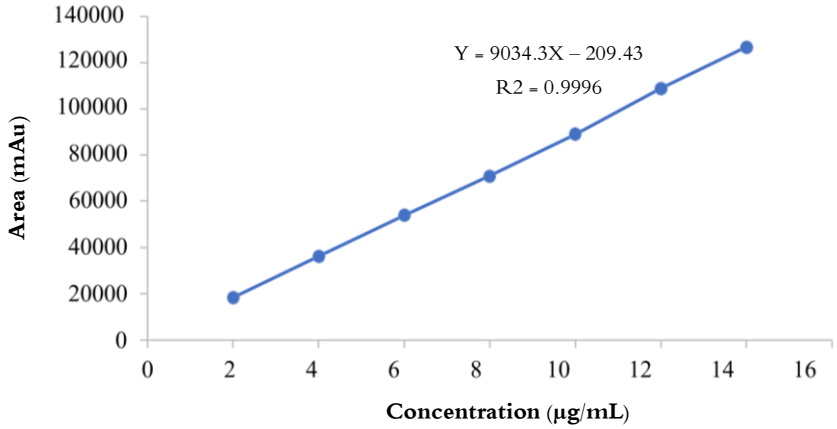

Figure 2. Linearity Curve of Eurycomanone Standard

The linearity test was obtained from the eurycomanone calibration curve. The calibration curve was developed by observing the HPLC response to eurycomanone analysis at concentrations of 2, 4, 6, 8, 10, 12, 14, and 16 ppm. The regression equation obtained was Y = 9034.3X - 209.43, with a regression value of 0.9996 (see **Figure 2**). This regression value approaches the acceptable regression value requirement, which is a value greater than 0.95 [22,23].

3.1.2 Determination of Limit of Detection (LoD) and Limit of Quantification (LoQ)

The method employed for the analysis of samples must possess high sensitivity to accurately measure the levels of compounds present within them. The detection limit is defined as the smallest concentration of a sample that can be discerned by the analysis method without necessitating quantification of an exact value. Conversely, the quantification limit is defined as the lowest concentration of a sample that can be measured quantitatively with acceptable accuracy and precision. The quantification limit is a quantitative test parameter for low analyte concentrations in complex matrices and is used to determine the presence or absence of impurities [21,22]. The analysis yielded linear correlation data for determining the LoD and LoQ, as shown in **Table 3**.

Table 3. Linear Correlation Data for Determining LoD and LoQ

Concentration	Area (mAu)	$y-y^2$	$(y-y)^2$
2	18427	-53537.7143	322502.2972
4	36217	-35847.7143	83727.55612
6	53922	-18142.7143	5502.460459
8	71012	-1052,7143	1108207.367
10	89064	16999.2857	1143295.563
12	108941	36876.2857	546437.7602
14	126870	54805.2857	359614.389
		$\sum(y-y)^2$	3569287.393

The analysis results indicate that the relationship between eurycomanone concentration and instrument response is linear, with the equation y = 9034.3x – 209.43 and a coefficient of determination (R^2) of 0.9996. An R^2 value close to 1 indicates a strong, consistent correlation between the variables and meets the linearity criteria set by the ICH Q2(R1) guideline [18], which requires $R^2 \geq 0.99$ for chromatography-based quantitative methods. Additionally, calculating the mean square deviation of the response yields a value of 3569287.399 for $\sum(y - \bar{y})^2$, which is then used to calculate the residual standard deviation (Sb). The Sb value serves as the basis for estimating the limit of detection (LoD) and limit of quantification (LoQ), which are 0.2608 ppm and 0.7904 ppm, respectively. These values indicate that the HPLC method has sufficient sensitivity to detect and measure eurycomanone at low concentrations. Similar results were reported by Khari et al. (2014) [19], who obtained LoD and LoQ values of 0.293 and 0.887 ppm, respectively, in a similar study. This linearity validation is also in

line with the findings of Yunianto et al. (2017) [20], which emphasize the importance of linear correlation in the analysis of eurycomanone as a marker compound in Eurycoma longifolia extract. In that study, the HPLC method used showed consistent linearity in the range of 0.05–1.00 ppm, with R^2>0.999 and accuracy above 98%.

3.1.3 Determination of Accuracy and Precision

Accuracy is one of the critical parameters in the validation of quantitative analytical methods, reflecting the extent to which the measurement results approximate the true value. In this study, the accuracy of the HPLC method for quantifying eurycomanone from *E. longifolia* roots was tested at three concentration levels: low (2 ppm), medium (8 ppm), and high (16 ppm), as can be seen in **Table 4**. The assessment was carried out through a recovery test, which is the re-measurement of compounds that have been added to the sample matrix.

Table 4. Results of Eurycomanone Standard Accuracy Determination

Replication	Area Size (AUC)	Concentration	Average	Deviation Standard	Recovery Percentage (%)
2 ppm (1)	17269	1.9347			
2 ppm (2)	17789	1.9922	1.9463	0.0413	97.32
2 ppm (3)	17065	1.9121			
8 ppm (1)	70231	7.7970			
8 ppm (2)	72832	8.0849	8.0154	0.1932	100.19
8 ppm (3)	73548	8.1642			
16 ppm (1)	145415	16.1191			
16 ppm (2)	143356	15.8912	16.1247	0.2365	100.78
16 ppm (3)	147628	16.3640			

The test results show that the recovery percentage ranges from 97.32% to 100.78%, with low standard deviation at each concentration level. These values are within the acceptable range according to ICH Q2(R1) guidelines [21], which state that the ideal recovery for chromatography methods is between 98–102% for high concentrations and 95–105% for low to medium concentrations. These findings indicate that the method used is highly accurate and reliable for the analysis of eurycomanone in various concentrations.

A study by Khari et al. (2014) [19] also reported similar results, where the HPLC method for eurycomanone showed a recovery between 94.2% and 99.8%, with LoD and LoQ of 0.293 and 0.887 ppm, respectively. Meanwhile, Mingmuang et al. (2018) [24] developed an HPLC method for *E. longifolia* roots with recoveries between 96% and 105%, demonstrating consistency between studies in terms of method accuracy and sensitivity. Yunianto et al. (2017) [20] emphasize that accuracy validation is very important in determining the levels of marker compounds such as eurycomanone, especially since this compound is used as an indicator of the quality of *E. longifolia* roots extract.

Table 5. Results of Eurycomanone Standard Precision Determination

Replication (8 ppm)	Area Size (AUC)	Concentration	Average	Deviation Standard	Coefficient of variation Percentage (%)
1	69593	7.7264			
2	76234	8.4615			
3	77312	8.5808	8.1310	0.3445	0.0423
4	74079	8.2229			
5	71239	7.9086			
6	71032	7.8857			

Meanwhile, precision is an essential component in the validation of analytical methods, serving as an indicator of the degree of consistency exhibited by measurement outputs when executed multiple times under uniform conditions. In this study, the precision of the HPLC method for eurycomanone analysis was evaluated through six replicates at a standard concentration of 8 ppm. The measurement results show (in **Table 5**) that the detected concentration of eurycomanone

ranged from 7.7264 to 8.5808 ppm, with an average of 8.1310 ppm. The standard deviation of the six replicates was 0.3445 ppm, and the coefficient of variation (CV) was 0.0423%. This CV value is well below the recommended maximum limit of <2% for quantitative analytical methods [22], indicating that this method has excellent precision.

High precision means the HPLC method can produce consistent and reproducible results, even when performed by the same operator in quick succession. This is important for quality control and routine testing because it ensures that the results of analyses are not affected by technical fluctuations or systemic errors. According to a study by Carr (1990) [25], good precision indicates that the analytical method has been well standardized and can be used for repeated testing without significant variation. These findings align with research by Aisha et al. (2012) [23], which shows that the HPLC method for eurycomanone has a CV of less than 1% in both intraday and interday precision testing. Furthermore, studies by Mingmuang et al. (2018) [24] and Khari et al. (2014) [19] reported similar low CV values (<0.5%) in eurycomanone precision validation, confirming that this compound can be consistently analyzed using reverse-phase liquid chromatography methods.

3.2 Experimental Design Using Box-Behnken RSM Design

3.2.1 Effect of ILs types

In the realm of natural material extraction processes, imidazolium-type IL solvents have emerged as the prevailing solution due to their inherent capacity to disrupt cell walls and facilitate the dissolution of target compounds. In this study, the IL solvents employed were 1-butyl-3-methylimidazolium chloride ([BMIM][Cl]) and 1-butyl-3-methylimidazolium acetate ([BMIM][OAc]).

Table 6. Experimental Design (Box-Behnken RSM Design) on Eurycomanone Conteng using [BMIM][Cl] and [BMIM][Ac] as an ILs solvent

Run	[BMIM][Cl] Conc. (mol/mL)	Extraction Time (Minutes)	Solid-Liquid Ratio (g/mL)	Eurycomanone Content (µg/mg)	
	X_1	X_2	X_3	[BMIM][Cl]	[BMIM][Ac]
1	0.7	30	12	0.2001	0.0623
2	0.4	10	10	0.1673	0.1587
3	0.4	30	10	0.1803	0.1550
4	0.7	20	10	0.2297	n.d.
5	0.7	20	10	0.2568	n.d
6	0.4	20	8	0.4061	0.0537
7	0.7	20	10	0.2052	0.0581
8	0.7	30	8	0.2007	n.d.
9	0.4	20	12	0.2130	0.0414
10	1	30	10	0.2060	0.0132
11	0.7	20	10	0.2305	n.d.
12	1	10	10	0.1458	0.2024
13	1	20	8	0.1182	0.0805
14	0.7	20	10	0.1479	n.d.
15	0.7	10	8	0.2048	n.d.
16	0.7	10	12	0.1930	n.d.
17	1	20	12	0.1198	0.1680

In this study (**Table 6**), the IL [BMIM][Cl] solvent produced a concentration of 0.4061 µg/mg, the highest concentration, while the IL [BMIM][Ac] solvent produced a concentration of 0.2024 µg/mg, the second highest concentration. The lowest concentration was not detected (n.d.). Extracting eurycomanone from *E. longifolia* roots produced different concentrations with the two IL solvents. The [BMIM][Cl] solvent produced a higher concentration than the [BMIM][OAc] solvent. This difference is due to the variation in IL cations and anions. Imidazolium salts with chloride

anions mix well with water because chloride-based ILs are hydrophilic. However, acetate anions are only slightly soluble in water [26]. Based on the results in **Table 6**, only the use of [BMIM][Cl] was continued for optimization of the extraction conditions to obtain the relationship between the response and each variable, which is expected to serve as a guide in designing the IL-MAE method to achieve optimal and efficient eurycomanone extraction conditions.

Previous research by Malik et al. (2023) [27] also showed that the type of solvent has a dominant effect on the yield of eurycomanone produced, where polar solvents such as 70% ethanol produce the highest yield compared to non-polar solvents such as n-hexane or ethyl acetate. This supports the findings in this study that ionic solvents with polar characteristics such as [BMIM][Cl] are more effective in extracting polar compounds from plant matrices.

3.2.2 Effect of Extraction Condition

Extraction condition variables are factors that, when measured, provide varying values or quantities of an independent variable that affect a dependent variable. The study incorporated several condition factors, including IL concentration, which is the concentration of the solvent utilized in the extraction process; extraction time, which is the duration of the extraction process employing a microwave; and solid-liquid ratio, which is the ratio between the solvent and the sample.

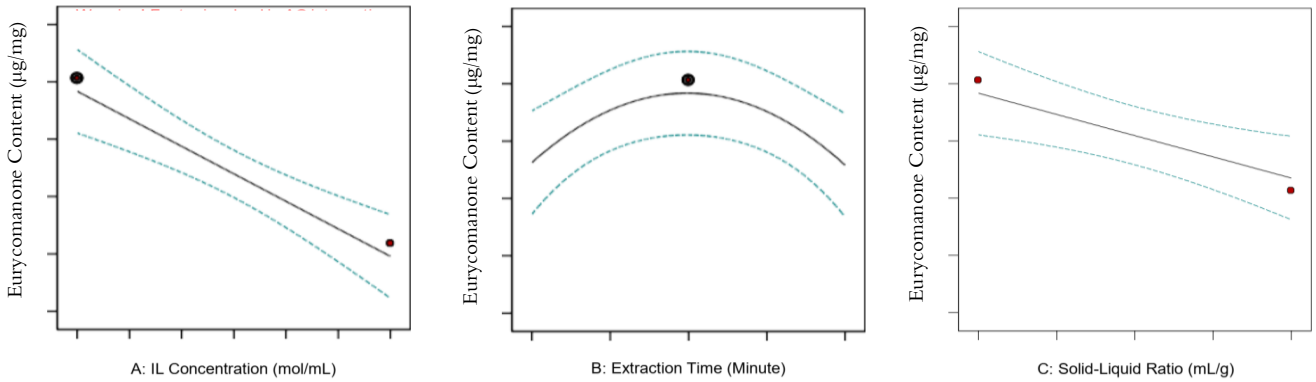

Figure 3. Single Factor Plot of [BMIM][Cl]

As illustrated in **Figure 3**, the effect of condition variables on eurycomanone content demonstrates a concentration-dependent relationship, where (A) indicates the highest eurycomanone content at a concentration of 0.4 mol/L, which decreases with increasing concentration, (B) shows that the optimal extraction time is 20 minutes, as longer extraction times result in lower eurycomanone content, and (C) highlights the impact of the solid-liquid ratio on eurycomanone content yield, demonstrating an increase in content as the solid-liquid ratio decreases.

As illustrated in **Figure 3A**, an augmentation in the concentration of the ionic solvent [BMIM][Cl] results in a substantial escalation in the Eurycomanone content. This finding aligns with the outcomes of the previous analysis of variance (ANOVA) experiment, wherein factor X_1 exhibited the highest F-value and the most significant p-value. This finding suggests that ionic solvents function as highly effective extraction media, capable of effectively dissolving bioactive compounds from plant matrices. A study by Yunianto et al. (2017) [20] also confirms that the selection of an appropriate solvent is crucial for the successful isolation of marker compounds, include eurycomanone.

Figure 3B shows that the eurycomanone content tends to increase over time, but not significantly. The curve is relatively flat, and the confidence interval is quite wide, indicating high variability and a weak contribution to the response. These findings are consistent with the ANOVA results, which show that extraction time (X_2) is not statistically significant (P-value = 0.1692). Research by Malik et al. (2023) [27] also shows that, although extraction time affects total yield, its effect on eurycomanone content is not always consistent.

Figure 3C shows that the solid–liquid ratio has minimal effect on the eurycomanone content. The curve tends to be flat and does not show a clear trend, with a wide confidence interval. This indicates that, within the tested range, changes in the ratio do not have a significant impact on extraction efficiency. A study by Istiqamah, et al., (2021) [28] also shows that

the ratio of solvent to material mass is not always directly proportional to the active compound content, especially if the solvent used is already chemically strong enough.

3.2.3 Optimization of Extraction Condition Using Box-Behnken RSM Design

Three parameters were used to obtain the eurycomanone content from *E. longifolia* roots: ILs concentration (0.4, 0.7, and 1 mol/L), extraction time (10, 20, and 30 minutes), and liquid-solid ratio (8, 10, and 12 mL/g). Design Expert software version 10 was then used with the Box-Behnken response surface design method to optimize the effect of each interacting parameter on the eurycomanone content. After obtaining the results from the experimental data using the Box-Behnken design, the response variables were analyzed using analysis of variance (ANOVA). ANOVA was used to evaluate differences among the available factors and to measure interacting parameters. If the "prob > F" value is lower than 0.05, the relationship between the variables and the response is more significant. Conversely, if the "prob > F" value is higher than 0.1, the relationship is insignificant [29].

Table 7. ANOVA results From Box-Behnken RSM Design on Eurycomanone Conteng using [BMIM][Cl] and [BMIM][Ac] as an ILs solvent

Source	Sum of Squares	df	Mean Squares	F-Value	p-value Prob>F
Model	0.055	7	7.904E-003	5.61	0.0100
X_1-ILs Concentration	0.036	1	0.036	25.76	0.0007
X_2-Extraction Time	0.036E-004	1	7.258E-004	0.51	0.4912
X_3-Solid-Liquid Ratio	5.197E-003	1	5.197E-003	3.69	0.0870
X_1X_2	5.570E-004	1	5.570E-003	0.40	0.5452
X_1X_3	9.477E-003	1	9.477E-003	6.72	0.0291
X_2^2	3.061E-003	1	3.06E-003	2.17	0.1747
$X_1X_2^2$	0.019	1	0.019	13.17	0.0055
Residual	0.013	9	1.409E-003		
Lack of Fit	5.887E-003	5	1.177E-003	0.69	0.6566
Pure Error	6.797E-003	4	1.699E-003		
R^2 = 0.8135					

Based on **Table 7**, the results of the analysis of variance (ANOVA) demonstrate that the constructed regression model is statistically significant, with an F-value of 5.61 and a p-value of 0.0100. This finding suggests that, in general, the model demonstrates a satisfactory ability to explain the variation observed in the response data. This finding is further substantiated by the coefficient of determination (R^2) value of 0.8135, which indicates that 81.35% of the observed variability in eurycomanone content can be attributed to the model. Among all the factors that were tested, the ILs concentration (X_1) emerged as the most influential variable, with an F-value of 25.76 and a p-value of 0.0007. This high level of significance indicates that changes in the concentration of the ionic solvent directly and strongly affect the extraction efficiency of the target compound. In contrast, extraction time (X_2) and liquid-solid ratio (X_3) did not demonstrate a significant individual effect, with p-values of 0.1692 and 0.5600, respectively. This finding suggests that within the range of values that were examined, these two factors did not play a substantial role in the variation observed in response.

Among all the factors that were tested, the ILs concentration (X_1) emerged as the most influential variable, with an F-value of 25.76 and a p-value of 0.0007. This high significance indicates that changes in the concentration of the ionic solvent directly and strongly affect the extraction efficiency of the target compound. In contrast, extraction time (X_2) and liquid-solid ratio (X_3) did not demonstrate a significant individual effect, with p-values of 0.1692 and 0.5600, respectively. This finding suggests that within the range of values that were examined, these two factors did not play a substantial role in the variation observed in response. The interactions between factors (X_1X_2, X_1X_3, X_2X_3) and the quadratic effects (X_1^2, X_2^2, X_3^2) also did not show statistical significance, with p-values all above the threshold of 0.05. This finding suggests that the extraction system under investigation did not exhibit dominant synergistic or non-linear effects.

The absence of a significant correlation between the variables was evidenced by the F-value of 0.69 and the p-value of 0.6566, which fell below the conventional threshold for statistical significance. This finding indicates that there is an absence of evidence to suggest that the model is incapable of capturing the patterns present in the data. Consequently, the model can be regarded as being consistent with the experimental data.

In summary, the findings of this study demonstrate that the concentration of the ionic solvent plays a pivotal role in the optimization of eurycomanone extraction. While the other factors are not statistically significant, their inclusion in the model remains pertinent for maintaining the experimental structure and potential minor contributions that may arise under different operating conditions. The high validity of the model and its suitability for the data provide a robust foundation for the implementation of this method in production scale or further development.

Table 8. The best conditions for IL-MAE are based on the results of the Box-Behnken RSM design analysis.

Factor	Name	Level	Low Level	High Level	Std. Dev.
X_1	ILs Concentration	0.4	0.4	1	0.000
X_2	Extraction Time	20	10	30	0.000
X_3	Liquid-Solid Ratio	8	8	12	0.000

Response	Predicted	Observed	Std. Dev.	CI for Mean		99% Population	
				95% CI Low	95% CI Hight	95% TI Low	95% TI High
Eurycomanone Content	0.3835	0.4061	0.0375	0.3107	0.4564	0.1654	0.6017

The equation obtained for the eurycomanone content was $Y = 0.21 - 0.095X_1 + 0.009525X_2 - 0.025X_3 + 0.012X_1X_2 + 0.049X_1X_3 - 0.027X_2^2 + 0.096X_1X_2^2$, where in this equation Y was the eurycomanone content (μg/mg), X_1 was the ILs concentration (mol/L), X_2 was extraction time (minutes), and X_3 was liquid-solid ratio (mL/g). This equation will serve as a guide for designing efficient extraction conditions to obtain extracts with optimal eurycomanone content from Pasak Bumi roots. **Table 8** presents the optimum conditions recommended by the Box-Behnken RSM design analysis of the eurycomanone content of Pasak Bumi root using the IL-MAE method with [BMIM][Cl] as the ILs solvent.

Table 8 shows the predicted points from the Box-Behnken RSM design analysis to determine the optimal conditions. The system recommends the following optimal condition parameters for the IL-MAE method: an ILs concentration of 0.4 mol/L, an extraction time of 20 minutes, and a liquid-solid ratio of 8 mL/g, resulting in an eurycomanone yield of 0.4061 μg/mg. These results fall within the confidence interval (CI) range of 0.317 to 0.4564 μg/mg. A confidence interval is a range of values used to estimate population parameters based on sample data with a certain level of confidence. Meanwhile, the tolerance interval is the limit of the range of values within which a certain proportion of the population is estimated to fall, with a certain level of confidence.

In this study, researchers evaluated the influence of three main factors on the effectiveness of extracting the Eurycomanone compound: the ILs concentration (X_1), extraction time (X_2), and the solid-liquid ratio (X_3). Each factor was tested at three levels: low, medium, and high. The ILs concentration varied from 0.2 to 0.6 mol/L, the extraction time varied from 10 to 30 minutes, and the liquid-solid ratio varied from 8 to 12 mL/g. All levels were set consistently without deviation, as indicated by a zero standard deviation for each factor.

The experimental results show that the observed eurycomanone content is 0.4061 μg/mg, which is slightly higher than the predicted value of 0.3835 μg/mg. Considering the standard deviation of the response is 0.0375 μg/mg, this difference is still within reasonable limits. To assess the reliability of this estimate, the researchers calculated the confidence and tolerance intervals. The 95% confidence interval for the average eurycomanone content is 0.3107 to 0.4564 μg/mg. This indicates that with a 95% confidence level, the average eurycomanone content of the tested population is estimated to fall within this range. However, to ensure that most individuals in the population meet the standard, a more stringent approach, the

tolerance interval, is used. A 95% tolerance interval that covers 99% of the population indicates that almost all samples have an estimated eurycomanone content between 0.1652 and 0.6017 μg/mg. This range is wider because it considers interindividual variation, not just the average.

The use of BMIM][Cl] in combination with MAE offers a greener alternative to conventional solvent systems. ILs are non-volatile, recyclable, and customizable, reducing environmental impact and operator exposure. MAE further enhances sustainability by minimizing energy consumption and extraction time. Together, these innovations align with several principles of green chemistry, including energy efficiency, safer solvents, and waste reduction

Overall, these results suggest that the extraction method is effective and reliable in terms of both average estimation and assurance of population variation. The statistical approach provides a strong foundation for method validation and potential application in production-scale processes or further research.

4. CONCLUSION

The present study successfully demonstrated the effectiveness of microwave-assisted extraction (MAE) using imidazolium-based ionic liquid [BMIM][Cl] for the recovery of eurycomanone from *E. longifolia* roots. Among the variables that were tested, ILs concentration was identified as the most influential factor, significantly enhancing extraction yield. The developed response surface model demonstrated robust predictive capability ($R^2 = 0.8135$) and statistical validity, exhibiting no substantial lack of fit. The optimized extraction conditions yielded eurycomanone content that closely matched the model prediction, thereby confirming the reliability of the approach. Moreover, the utilization of [BMIM][Cl] aligns with the principles of green chemistry, thereby providing a sustainable alternative to conventional solvents. These findings provide a robust foundation for scale-up and formulation of eurycomanone-rich extracts for phytopharmaceutical applications. Future research may explore the recyclability of ionic liquids, bioactivity profiling of the extract, and integration of this method into continuous extraction systems.

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