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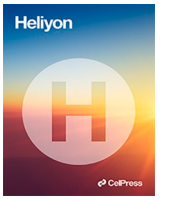

Research article

Optimized microwave-assisted azadirachtin extraction using response surface methodology

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ABSTRACT

The neem tree (*Azadirachta indica* A. Juss) is grown mainly for shade, fuel, and numerous non-timber forest products using its leaves, fruit, and bark. It produces an essential oil that is used as a source for obtaining bioinsecticides, with a broad spectrum of action in agricultural production. Its bioinsecticidal activity is due to the presence of triterpenes, such as azadirachtin, a product in continued growth of the global biopesticide market. Optimal conditions for neem oil extraction using response surface methodology (RSM) and microwave-assisted extraction (MAE) methods have been defined. However, the extraction conditions for these methods tend to consume high volumes of organic solvent and long extraction times. The aim of the present study is to determine the optimal conditions for the extraction of azadirachtin from neem seeds in a hydroalcoholic medium using MAE and RSM with a Box-Behnken design (BBD). A BBD was applied to evaluate the effects of the factors, magnetron voltage (X_1), extraction time (X_2), and pH of the extraction medium (X_3), on the yield of the azadirachtin extraction process. The effect of each variable on the extraction yield was studied independently, considering the pure coefficients (linear and quadratic) on the three levels that were studied in the experiments. Moreover, the study experiments were conducted in triplicate, data were presented as mean and standard deviation, homogeneity of variances was estimated using Levene's test, and a two-way ANOVA with Tukey's post hoc analysis was performed to identify the experimental conditions that allowed us to find the highest extraction yield and to analyze whether the response surface model adequately described our data. The most significant effects of the model correspond to quadratic and interaction effects ($p < 0.0001$); the quadratic terms voltage (X_1), extraction time (X_2), and pH (X_3); and the interaction effects between voltage-pH (X_1*X_3) and time-pH (X_2*X_3), which had a significant influence on the model. Moreover, a canonical analysis was performed. The optimal conditions were as follows: 69.22 V, 6.89 min, and a pH value of 4.35, coinciding with the zones shown in the contour plots. Furthermore, the response obtained at the optimal conditions was 37.5 μg of azadirachtin per gram of pretreated seed.

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1. Introduction

The concept of separation is broad and can be considered a mechanical operation that consists of dividing a mixture into at least two fractions of different compositions; the purpose of which is to increase the mole fraction of a component about the rest of the components of the original mixture [1]. In other contexts, separation is a physical process that often involves chemical processes [2].

The importance of separation techniques is increasingly noted in the scientific community, and the development of their application has contributed to improving production processes to achieve methodologies that are efficient, economical, and have a positive impact on the environment [3].

RSM is a statistical method that uses quantitative data from an experimental design to simultaneously determine or solve multivariate equations; the advantages of RSM are based on the ability to generate a robust mathematical model that takes into account the possible interrelationships of the variables to be evaluated and minimizes the number of experiments [4,5]. In addition, RSM can be useful in experimental designs that include more than two factors allowing a considerable reduction of running costs and operation time [6,7]. Usually, this experimental planning is implemented with a BBD owing to its high efficiency [8].

Additionally, MAE has become an efficient technique that is used to recover compounds of interest in pharmacology, materials engineering, and other research areas [9,10], integrating thermodynamic and kinetic fundamentals in the separation, and is known for its reduced use of organic solvents compared with conventional extraction methods [11]. As with any process, it is necessary to identify those conditions that allow for obtaining a higher economic and operational performance.

MAE combined with RSM has several advantages compared with other advanced extraction technologies, such as higher extraction yield, improved selectivity, better handling, and low solvent consumption [12]. It has gained significant research interest as it is considered an ecotechnological and accessible technique [13].

The neem tree (*A. indica* A. Juss) belongs to the Meliaceae family and is native to India. It is now widely distributed in most tropical and subtropical regions of the world [14,15], where it is grown mainly for shade, fuel, and numerous non-timber forest products using its leaves, fruit, and bark. Several studies have focused on the use of essential oils in the control of pests and disease vectors of public health interest [16–18]. Neem tree extract has been used as a source for obtaining bioinsecticides, with a broad spectrum of action in agricultural production. Its bioinsecticidal activity is due to the presence of triterpenes, such as azadirachtin [15,19–21].

Azadirachtin is in a unique position to become a pivotal insecticide to expand in this market segment and considered a product of the continued growth of the global biopesticide market. According to Maximize Market Research (MMR) in 2022, pesticides are the second dominant application in the azadirachtin market. The market share of insecticide was valued at USD 14.8 billion in 2017 and is estimated to reach USD 19.5 billion with a growing CAGR of 5.9 % in the forecast period between 2022 and 2027 [22].

Given the numerous uses of neem oil and its high commercial value, it is important to develop new methods and modify the existing methods for neem oil extraction that significantly reduce the extraction time, organic solvent consumption rate, and operating costs as well as improve extraction efficiency and maintain oil quality [23]. The optimal conditions for neem oil extraction using the RSM and MAE method have been determined in several studies [24–26], but, to our knowledge, the conditions for extracting neem seed oil in hydroethanolic media by MAE-assisted RSM with a BBD have not been reported. Therefore, the present study aims to optimize the conditions for the extraction of azadirachtin from neem seeds in a hydroalcoholic medium using the MAE-assisted RSM with a BBD.

2. Materials and methods

2.1. Plant material collection and processing

The plant material was collected in the municipality of Puerto Colombia, located in the department of Atlántico, Colombia (11°00′60.0″ N and 74°50′50.2″ W). For the collection of the sample, a 1.5 km transect was established, in which 20 neem trees were identified and marked, collecting 2–3 kg of fruit in the green-yellow stage of maturity, in accordance with the protocol established by Angulo-Escalante et al. (2004) and Shidu et al. (2003), [14,27]. All plant materials were collected in October 2022 during the rainy season.

The material was transferred to the laboratory of the Universidad del Atlántico, where it was processed. In summary: (i) the fruits were washed with a 0.5 % sodium hypochlorite solution to remove residues and eliminate biological contaminants; (ii) the fruit was pulped, leaving the seeds free, which were dried in an oven with continuous air flow at 40 °C for 2 h; (iii) the seeds were subjected to a maceration grinding process to avoid the formation of fine powders that can affect the extraction efficiency; (iv) the seeds were dried in a continuous air flow oven at 40 °C for 2 h; and (v) the seeds were subjected to a maceration grinding process to avoid the formation of fine powders that can affect the extraction efficiency.

2.2. Obtaining the hydroethanolic extract

A sample of 10 g of dried and ground neem seeds was placed in a round bottom boiling flask along with 50 mL of 70 % hydroethanolic solution, maintaining a solid-liquid ratio of 0.2 g/mL. The resulting mixture was introduced into the round-bottomed balloon and heated in a microwave oven. The neem extract was collected and transferred into amber glass bottles. To vary the pH of the medium, calculated volumes of HCl at a concentration of 0.10 M were added to the solvent until the pH values (4, 5, and 6) were obtained [28].

Then, each of the mixtures was taken to the MAE equipment at different extraction times (5, 10, and 15 min). For the design of the extraction equipment, as developed by our team, a conventional microwave oven was used with modifications to manipulate the

power of the system through different voltages (Fig. 1). The oven is connected to a metal duct with a built-in condenser and in turn connected to a collector system where the light components of the sample are deposited.

Each obtained extract was washed with 50 mL of dichloromethane in a period of 6 h, maintaining a temperature of 30 °C and in constant agitation. Moreover, the mixture was deposited in a separating funnel for 15 min; subsequently, the dichloromethane phase was recovered and concentrated by removing the solvent by rotary evaporation under vacuum [29].

2.3. Quantification of azadirachtin by ultraviolet–visible spectrophotometry (UV-VIS)

The quantification of azadirachtin was performed using the UV-VIS technique in accordance with the protocol published by Haq et al. (2017), [29]. For the construction of the calibration curve, a 100 ppm solution was prepared from a 95 % azadirachtin standard (Sigma-Aldrich), and from this, four serial dilutions were made with concentrations of 6.5, 12.5, 25, and 50 ppm, respectively.

2.4. Experimental design and variables

A BBD [30,31] was applied to evaluate the significant effects of the factors, magnetron voltage (X1), extraction time (X2), and pH of the extraction medium (X3), on the yield of azadirachtin obtained. Table 1 shows the range of the independent variables and their levels.

The experimental design contained 15 combinations of variables (Table 2), the data analysis and the construction of the model were obtained through the R Studio program using the "rms" package, which allowed us to know the optimal conditions to obtain greater performance of the azadirachtin extraction. The study experiments were conducted in triplicate, data were presented as mean and standard deviation, homogeneity of variances was estimated with Levene's test, and a two-way ANOVA with Tukey's pot hoc analysis was performed to identify the experimental conditions that allowed us to find the highest extraction yield and to analyze whether the response surface model adequately describes our data. Statistical significance of the model and model variables will be determined at a 5 % probability level ($\alpha = 0.05$). The data were modeled under a second-order regression model (Equation (1)):

$$y = \beta_o + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{i=1}^{k} \sum_{j=1}^{k} \beta_{ij} x_i x_j + \varepsilon \quad (1)$$

where y is the response variable (dependent variable); β_o are the regression coefficients; βi, βii, and βij are the linear, quadratic, and interactive coefficients of the model; x_i and x_j represent the levels of the independent variables; and ε represents the error.

Most studies conducted on metabolite and oil extraction processes conform to second-order models [26,32,33].

2.5. Methodology for the effect of the variable

The effect of the independent variables of the azadirachtin extraction process was analyzed through the second-order optimization model (Equation (1)), as obtained by the RStudio program. The effect of each variable on the extraction yield was studied independently, considering the pure coefficients (linear and quadratic) on the three levels studied in the experiment (Table 1).

3. Results and discussion

Table 3 presents the measured absorbance obtained for each sample at the end of the processing and purification step. The relationship between absorbance and concentration was determined using the Lambert-Beer law (Equation (2)):

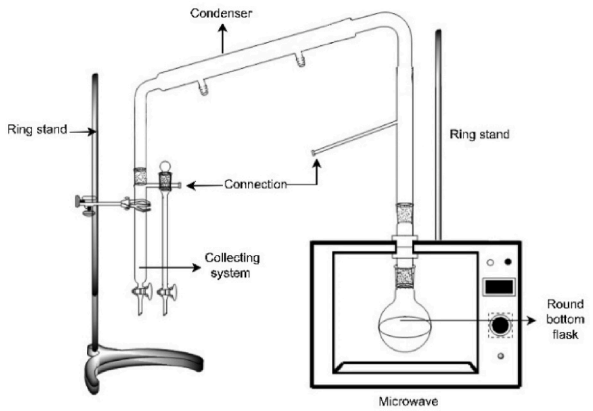

Fig. 1. Schematic diagram of the microwave-assisted equipment.

Table 1
Factors and levels used for the experimental design.

Factor	Low (−1)	Medium (0)	High (1)
Voltage (V)	60	70	80
Time (min)	5	10	15
pH	4	5	6

Table 2
Box-Behnken experimental design.

Experiments	Independent variables					
	Voltage (V)		Time (min)		pH	
	Cod	Exp	Cod	Exp	Cod	Exp
1	−1	60	−1	5	0	5
2	1	80	−1	5	0	5
3	−1	60	1	15	0	5
4	1	80	1	15	0	5
5	0	70	−1	5	−1	4
6	0	70	1	15	−1	4
7	0	70	−1	5	1	6
8	0	70	1	15	1	6
9	−1	60	0	10	−1	4
10	1	80	0	10	−1	4
11	−1	60	0	10	1	6
12	1	80	0	10	1	6
13	0	70	0	10	0	5
14	0	70	0	10	0	5
15	0	70	0	10	0	5

Cod, coded variables. Exp, experimental variables.

$$A = 0.03657 * c - 0.03830 \tag{2}$$

where the azadirachtin extraction yields (μg/g) were calculated using Equation (3):

$$Y_{AZA} = \frac{Azadirachtin\ mass\ (\mu g)}{Treated\ seed\ mass\ (g)} \tag{3}$$

In general, the extraction efficiency of secondary metabolites depends on multiple factors, the most relevant being the nature of the plant material, polarity of the solvent, temperature, particle size generated during grinding, and extraction technique used, among others. These effects can be manifested independently or in combination [26,34–36]. For MAE-assisted extractions in hydroethanolic systems, it is essential to evaluate the effect of pH, extraction time, and voltage of the equipment used during extraction [36,37], since to date they have not been reported for RSM optimization processes using a BBD. In our study, to achieve the highest azadirachtin content, the BBD model was used to further optimize the MAE parameters, including the solid/liquid ratio (0.2 g/mL), extraction time (5, 10, and 15 min), microwave voltage (60, 70, and 80 V), and pH of system (4, 5 and 6).

In this study, the BBD was applied because it requires a smaller number of trials and provides reliable information in a shorter

Table 3
Azadirachtin absorbances and concentration.

No.	Absorbance	Concentration (μg/mL)	Yield (μg/g)
1	1.131 ± 0.029	31.974 ± 0.786	29.170 ± 0.781
2	1.101 ± 0.034	31.145 ± 0.925	27.979 ± 0.837
3	1.206 ± 0.008	34.025 ± 0.219	30.815 ± 0.361
4	1.121 ± 0.029	31.692 ± 0.798	28.829 ± 0.547
5	1.197 ± 0.018	33.770 ± 0.505	37.454 ± 1.640
6	1.121 ± 0.030	31.710 ± 0.812	28.435 ± 0.731
7	1.134 ± 0.032	32.056 ± 0.877	22.140 ± 0.802
8	1.132 ± 0.026	32.002 ± 0.713	30.670 ± 0.229
9	1.079 ± 0.029	30.543 ± 0.800	29.090 ± 0.881
10	1.131 ± 0.030	31.965 ± 0.812	28.536 ± 0.679
11	1.149 ± 0.021	32.467 ± 0.573	21.940 ± 0.419
12	1.138 ± 0.028	32.157 ± 0.773	29.171 ± 0.860
13	1.069 ± 0.034	30.270 ± 0.922	36.032 ± 1.311
14	1.086 ± 0.003	30.753 ± 0.078	34.533 ± 2.141
15	1.136 ± 0.018	32.120 ± 0.481	36.964 ± 1.469

operation time [8]. In this order of ideas, 15 experiments were projected and performed as shown in Tables 2 and 3 The predictive regression analysis of azadirachtin was used to fit the second-order polynomial model, including the linear (X_1, X_2, and X_3) and quadratic (X_1^2, X_2^2, and X_2^3) coefficients, as well as the interactions (X_1*X_2, X_1*X_3, and X_2*X_3) with a significance level of 5 %.

Table 4 shows the results obtained from the two-way ANOVA for the regression equation. Additionally, the most significant effects of the model correspond to the quadratic and interaction effects ($p < 0.0001$). The lack of fit, presenting a value of $p = 0.032$, indicates that the mathematical model can be used to predict the responses [38]. The R^2 and R^2 adj obtained (0.836 and 0.794, respectively) support the acceptability of the model and allow its use for optimization purposes since the mathematical model fits above 70 % of the response behavior in terms of the R^2 adj as established by Cheok et al. (2012), [6]. Moreover, the quadratic terms voltage (X_1), extraction time (X_2), and pH (X_3) and the interaction effects between voltage-pH (X_1*X_3) and time-pH (X_2*X_3) had a significant influence on the model (Table 4), suggesting that the interrelation between azadirachtin extraction efficiency and the MAE variable was not linear. Thus, the polynomial describing the relationship between azadirachtin extraction efficiency and the variables is presented in Equation (4):

$$Y_{AZA} = 37.842 - 2.458x_3 + 1.927x_1x_3 + 4.386x_2x_3 \quad - 4.564{x_1}^2 - 2.092{x_2}^2 - 4.075{x_3}^2 \tag{4}$$

where Y_{AZA} is the yield of azadirachtin (μg/g), x_1 is the voltage, x_2 is the extraction time, and x_3 is the pH of the medium.

3.1. Influence and optimization of MAE parameters on the azadirachtin extraction yield

Fig. 2 shows the behavior of the variables studied on the extraction yield of azadirachtin in the MAE process. In these graphs, the presence of a maximum point over the experimental region can be observed. Moreover, through the interaction between the study variables, it was shown that the existing interactions of Fig. 2 (B and E), and Fig. 2 (C and F) were more significant than that observed in Fig. 2 (A and D). The trend of the surface plots is like the optimization performed by Suttiarporn and Choommongkol (2020) [26], on the MAE of nimbolide, a secondary metabolite belonging to the limonoids (the same group to which azadirachtin belongs). This same trend of surface curves can also be observed in the studies of Nde et al. (2015) on the optimization of neem oil extraction using microwaves.

3.2. Determination of the independent effect of pH, extraction time and voltage on yield

3.2.1. Effect of voltage on azadirachtin performance

Based on the behavior presented in Fig. 3, it can be stated that the magnetron voltage has a significant effect on the recovery of azadirachtin; a tendency to that of a parabola can be observed due to the significance of the quadratic effect in the model that was observed in Table 4. Increasing azadirachtin yields of approximately 31–37.05 μg/g were observed at voltage ranges between 60 and 70 V. The yield began to decrease at higher voltages from 70 to 80 V until a minimum value was found (30.9 μg/g).

The trend described above can be interpreted in such a way that the temperature of the system starts to increase until the appropriate extraction temperature is reached to achieve higher extraction efficiency. Moreover, it can be observed that, at voltages above 70 V, the extraction yield starts to decrease. Therefore, it is expected that under these conditions, the extraction temperature is high enough and the metabolite is thermally degraded, since azadirachtin is a thermolabile chemical compound [26,38,39].

During the MAE process, the magnetron is responsible for generating the microwaves, which are directed toward the solvent in the equipment cabinet. The solvent will respond to the microwave emission based on its dielectric properties. The heat generation is produced by the solvent's resistance to ionic migration causing friction between the molecules in the extraction medium [40,41]. The transmission of microwaves to the solvent represents the energy transferred from the magnetron and is controlled by the regulation in

Table 4
Regression analysis of the mathematical model and ANOVA for the response surface model of the azadirachtin content.

Source	Degrees of freedom	Sum of squares	Mean square	F-value	p-value
Linear	3	150.08	50.02	10.769	3.69×10^{-5}
X_1	1	4.59	4.59	0.989	0.327
X_2	1	1.51	1.51	0.325	0.572
X_3	1	143.97	143.97	30.996	2.87×10^{-6}
Quadratic	3	410.78	136.92	29.479	1.25×10^{-9}
X_1^2	1	190.78	190.78	41.072	3.26×10^{-8}
X_2^2	1	34.61	34.61	7.452	0.003
X_3^2	1	185.39	185.39	39.913	2.95×10^{-7}
2-way					
Interaction	3	276.86	92.28	19.868	1.23×10^{-7}
X_1*X_2	1	0.47	0.47	0.031	0.751
X_1*X_3	1	45.45	45.45	3.012	0.003
X_2*X_3	1	230.94	230.94	15.306	3.28×10^{-8}
Lack of fit	3	55.91	18.63	5.6141	0.032
Pure error	32	106.66	3.333		
Total	44	1000.29			
R^2	0.8375		R^2 (adj)	0.7957	

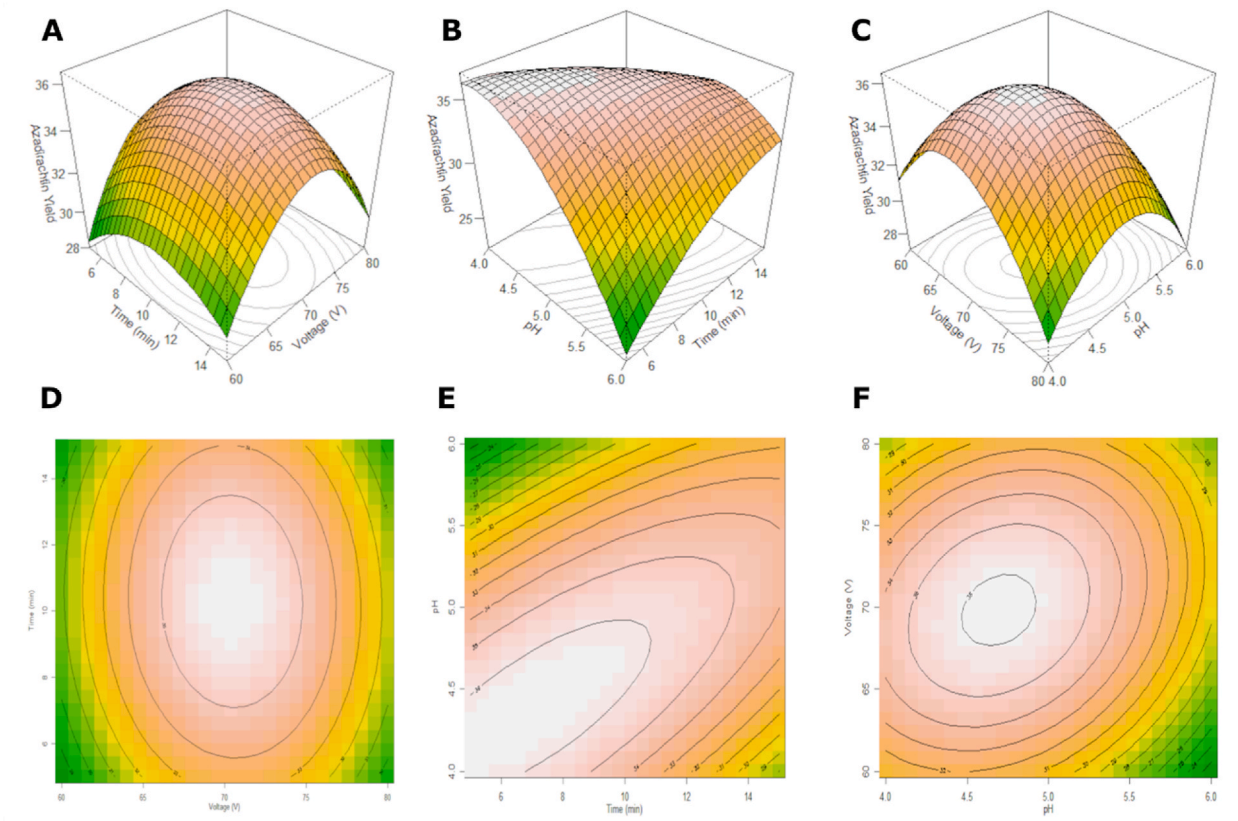

Fig. 2. Response surface plots (**A**, **B**, and **C**) and contour plots (**D**, **E**, and **F**) showing the effects of the factors on azadirachtin content.

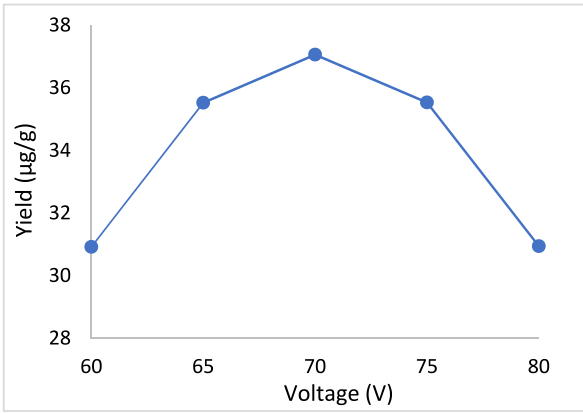

Fig. 3. Effect of voltage on azadirachtin performance.

the power supply in the microwave [42]. The energy supplied, due to the processes of ion migration and dipolar rotation, is transformed into thermal energy. The application of a higher voltage will raise the temperature of the system because of the higher microwave transmission, thus accelerating the heating process [38,39].

3.2.2. Effect of extraction time on azadirachtin yields

Extraction time is an important factor affecting microwave-assisted extraction. This parameter has become an advantageous aspect compared with conventional extraction techniques; the usual times in MAE are between a few minutes to half an hour depending on the plant matrix to avoid thermal degradation and oxidation during extraction [43]. The irradiation time can be affected by the dielectric properties of the solvent used. Since solvents with high dielectric constants can heat up quickly, it was convenient to use short extraction times [44], as it was in this case when using a 70 % hydroethanolic solution as the extraction medium.

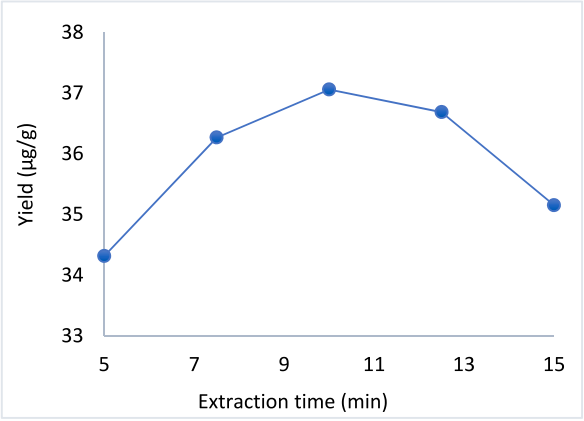

Fig. 4. Effect of extraction time on azadirachtin yield.

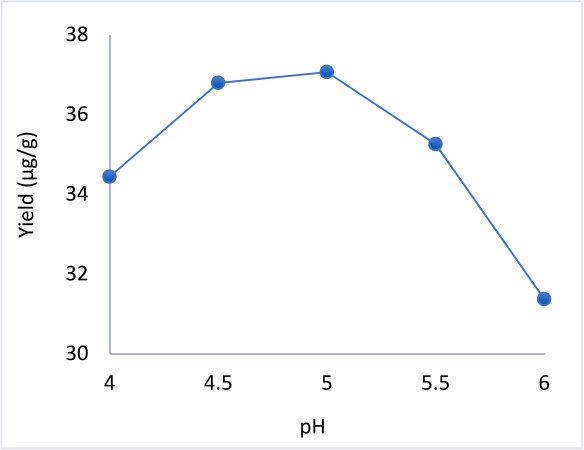

Fig. 5. Effect of pH on azadirachtin yield.

It is expected that the longer the irradiation time, the higher the extraction of the essential oil and/or secondary metabolite and thus the higher the yield; however, in some situations, the yield may decrease when having prolonged extraction times [45]. This occurs up to the point where the compounds of interest start to degrade due to exposure to microwave radiation. Based on the analysis of the linear and quadratic effect of the extraction time as shown in Fig. 4, the results revealed that the recovery of azadirachtin increases when the extraction time increases until it reaches a maximum value (10 min) and then subsequently decreases. A similar pattern was observed in the studies conducted by Dai (1999), [46]. His research on the optimization of the microwave-assisted extraction of azadirachtin found a growth trend from minute 3 to minute 10 and a subsequent decrease.

3.2.3. Effect of the pH of the extraction medium on azadirachtin yield

Depending on the nature of the components, pH is a critical factor influencing the solubility of the compounds of interest [47]. When changes in the pH of the extraction medium occur, the charge state of the solute can be affected, causing differences in solubility [48]. Azadirachtin is unstable in slightly alkaline and strongly acidic solutions; its laboratory half-life in slightly acidic solutions (pH 4–6) is 50–100 days at room temperature [49]. In our model the pH of the extraction medium was found to be significant for azadirachtin extraction as can be seen in Fig. 5, wherein it was observed that at a pH above 5, the extraction yield of azadirachtin decreases.

Furthermore, the degradation rate of azadirachtin at different pH conditions does not change significantly at slightly acidic conditions (between 4 and 6). The data reported by Lynn et al. (2015) [50], revealed that the azadirachtin concentration during the first 14 days of the stability test was approximately 55.9 % at pH 4 and 54.3 % at pH 6. Under alkaline conditions (pH = 9.0), the degradation of azadirachtin occurred rapidly.

3.3. Optimization of azadirachtin extraction

The design and analysis of the experiments are crucial in the field of engineering, since they help to improve the performance of a

process or system [51], especially when seeking to establish the conditions that allow obtaining a higher economic and operational performance, being the response surface a set of techniques whose purpose is to optimize the response surface that is influenced by different variables that can affect a process [52].

The objective of the optimization was to determine under which experimental conditions have a higher yield of extracted azadirachtin using the MAE method. As an optimization technique, it was assessed through canonical analysis using Equation (4). The optimal conditions found were as follows: 69.22 V, 6.89 min, and pH value of 4.35. These values coincide with the zones shown in the contour plots (Fig. 3). Thus, it could be concluded that the effect of the interaction of the variables played an important role in the extraction of azadirachtin compared with the linear and quadratic effects and their tendency to the central point of the design as observed in Section 3.1. Since the optimal conditions found were within the region proposed by the experimental design, it was not necessary to perform a range analysis. The response obtained at the optimal conditions was 37.5 μg of azadirachtin per gram of pretreated seed.

3.4. Comparison with other extraction methods

The yields recorded for azadirachtin varied, which is even more noticeable when describing alternative isolation procedures. Moreover, these can vary from less than 0.01–0.9 % by weight of seeds [53]. In comparison with the results obtained experimentally, the amount of azadirachtin in the percentage of seed weight is equivalent to an approximate value of 0.04 %. The yields obtained were higher than those reported by Uebel et al. (as cited in Morgan, 2009), which were 0.015 % (preparative chromatography) and 0.035 % (maceration), respectively.

One of the reasons why a good recovery was not obtained is the sample size used for the experiments. Thus, the probability of obtaining better yields is through the use of representative sample sizes. Large sample sizes were not taken because the study wanted to be carried out in the same tree, since the variability, composition of essential oils and their metabolites changes in each tree and the area where the tree is located [54].

Conditions external to a plant (exogenous), such as climate and soil type, affect the concentration and yield of essential oils. Since the fruits were harvested during the rainy season, it was presumed that the conditions affected the composition of neem metabolites and therefore yields were not found [19,55,56]. Significant differences in yields were found in dry and rainy seasons, so it is necessary to conduct seasonal studies to find better harvesting seasons and thus achieve greater efficiency. Furthermore, these findings are consistent with those of the present study and strengthen the hypothesis of low yields.

4. Conclusion

The aim of this study is to find the optimal conditions for the extraction of azadirachtin from neem seeds using the MAE method and the surface response methodology with a BBD. The effects of voltage, extraction time, and pH value were analyzed, and it was determined that the predicted optimal extraction conditions were 69.22 V, 6.89 min, and a pH of 4.35, obtaining a maximum azadirachtin production of 37.5 μg. However, given that in this study the model was not validated, it is necessary to conduct further studies to understand the behavior of the system based on the variables to improve and optimize azadirachtin extraction efficiency and its possible application at an industrial level. Furthermore, the azadirachtin extraction process can be made more efficient by improving the conditions prior to its operation (e.g., analyzing the best period for harvesting neem fruits and increasing the amount of raw material (prepared seeds)).

Data availability statement

The authors confirm that the data supporting the findings of this study are available within the article.

CRediT authorship contribution statement

Robinson Martínez-Castro: Writing – review & editing, Writing – original draft, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Jiress Flórez-Santiago:** Writing – review & editing, Validation, Supervision, Investigation, Data curation, Conceptualization. **Roger Valle-Molinares:** Writing – review & editing, Validation, Supervision, Investigation, Conceptualization. **Julián Cabrera-Barraza:** Writing – review & editing, Investigation, Data curation. **Fabián Espitia-Almeida:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization, Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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