Process Intensification of Polyphenol and Flavonoid Extraction from Ant Nest (*Myrmecodia pendans*) via Ohmic Heating: Investigating Joule Heating and Electric Field-Time Interactions Using a Response Surface Methodology

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Abstract

Ant nest (*Myrmecodia pendans*) is a unique epiphytic plant native to the tropical forests of Eastern Indonesia, traditionally used by indigenous communities for its medicinal properties. It is characterized by its tuber-like structure, which serves as a natural habitat for ants and is believed to contribute to its rich content of bioactive compounds. This plant is particularly valued for its high levels of polyphenols and flavonoids, which are known for their antioxidant, anti-inflammatory and therapeutic effects. Ohmic heating is an innovative, energy-efficient extraction technique that utilizes electrical resistance to generate internal heat within conductive plant materials. This study applied ohmic heating as an innovative and energy-efficient extraction method to enhance the recovery of these bioactive compounds. Response Surface Methodology (RSM) with Central Composite Design (CCD) was used to optimize the extraction conditions. Two independent variables, holding time (90 - 270 s) and gradient voltage (20 - 60 V/cm), were investigated. Quadratic polynomial models were developed for total phenolic content (TPC), total flavonoid content (TFC) and extraction yield through multiple linear regression. The optimization using the desirability function indicated that the optimal extraction conditions were a holding time of 195.883 s and a gradient voltage of 52.761 V/cm, which predicted values of 256.145 mg GAE/g (TPC), 118.837 mg QE/g (TFC) and 38.154% extraction yield. Critically, the performance of the ohmic system was characterized based on electrical and thermal parameters. The initial electrical conductivity of the extraction medium ranged from 0.171 to 0.407 S/m, increasing with voltage and time. The temperature of the medium rose from ~30 °C to a peak of 87 °C during treatment, indicating efficient internal volumetric heating. Corresponding energy consumption ranged from 0.0805 to 0.3045 kWh per batch, equivalent to 28.98 - 109.62 kJ per g of dry *ant nest* powder. These results confirm that ohmic heating enables rapid and energy-efficient extraction while preserving thermal sensitivity of bioactive compounds.

Keywords: Ant nest (*Myrmecodia pendans*), Extraction, Ohmic heating, Optimization, Response Surface Methodology (RSM)

Introduction

In recent years, there has been a growing global interest in herbal medicines as part of the "back to nature" movement. This trend reflects a shift in public preference toward natural remedies perceived as safer

and more sustainable alternatives to synthetic pharmaceuticals [1,2]. The global herbal medicine market has demonstrated remarkable growth and was projected to reach a value of USD 100 billion by 2015 [3]. In Indonesia, although the herbal medicine market remains relatively small compared to the global market, it continues to show steady growth. This increased demand presents a significant opportunity to leverage Indonesia's rich biodiversity for the development and production of herbal extracts [4]. *Myrmecodia pendans*, commonly known as ant nest, is a medicinal plant indigenous to Papua, Indonesia. It has been traditionally used for its diverse therapeutic properties, such as antioxidant [5,6], anti-inflammatory [7,8] and anticancer activities [9,10]. These health benefits are attributed to its rich content of bioactive compounds, particularly phenolic and flavonoid compounds.

Empirical and scientific evidence have shown that this plant possesses xanthine oxidase inhibitory activity comparable to allopurinol, as demonstrated in *in vitro* studies using methanol extracts of its tuber. The pharmacological activities of ant nest are mainly attributed to its polyphenolic and flavonoid compounds. Engida *et al*. [11] identified several flavonoids in the ant nest, including kaempferol, luteolin, rutin, quercetin and apigenin, as well as phenolic acids. Flavonoids exhibit various biological activities, such as antibacterial, antihyperlipidemic, antiviral, antidiabetic, anti-inflammatory and anticancer properties [12,13]. Their potent antioxidant activity is associated with their ability to scavenge reactive oxygen species (ROS), such as hydroxyl radicals and superoxide anions, through hydrogen donation from their hydroxyl groups [14]. The antioxidant capacity of flavonoids is largely influenced by the position and number of hydroxyl groups attached to their core structures [15].

Extraction is a critical step in obtaining these bioactive compounds. Conventional extraction techniques are often inefficient and time-consuming, which necessitates the optimization of extraction parameters to maximize yield and quality. Electrical-assisted extraction has emerged as a promising technique due to its ability to enhance mass transfer through electroporation, thus improving extraction efficiency. Various extraction techniques have been applied to obtain bioactive compounds from medicinal plants, including maceration, batch processing, Soxhlet extraction and Microwave-Assisted Extraction (MAE). However, these extraction techniques are often limited by long extraction times, the use of volatile solvents [16,17], high energy consumption and inefficient heat transfer due to physical barriers in the extraction medium.

To overcome these limitations, emerging technologies such as ohmic heating have been developed to enhance extraction efficiency. Ohmic heating generates internal heat through the electrical resistance of the material, resulting in rapid and uniform heating [18,19]. This technology offers several advantages, including reduced solvent use [20], uniform heat distribution [19], shorter processing times, minimized degradation of heat-sensitive compounds [18] and improved mass transfer leading to higher extraction yields [21]. Furthermore, ohmic heating systems exhibit high energy efficiency, with reported efficiencies exceeding 90% [19,22]. Nevertheless, it is imperative to acknowledge the engineering limitations and operational risks associate with ohmic heating systems. Electrochemical degradation may occur due to water electrolysis, generating reactive species such as hydroxyl radicals that can compromise the stability of polyphenols and flavonoids. Moreover, prolonged operation or suboptimal system design may lead to electrode corrosion, introducing metallic contaminants and deteriorating system performance. In addition, non-uniform electric field distribution particularly in heterogeneous plant-solvent matrices can result in localized overheating ("hot spots"), thereby causing irreversible degradation of bioactive compounds. These risks necessitate rigorous process control, thermal profiling and system calibration to ensure product quality and operational safety.

Although previous studies such as that of Engida *et al*. [11] have explored the extraction of flavonoids from *M. pendans* using conventional or microwave-assisted approaches, they lack electrothermal process insights and energy-based evaluations. To the best of our knowledge, this study represents the first application of ohmic heating for *M. pendans* extraction, with a specific focus on electrothermal intensification. The novelty of this work lies in (i) the integration of ohmic heating to promote rapid and uniform extraction, (ii) the quantification of electrical conductivity, energy input and temperature evolution during treatment, (iii) the use

of Response Surface Methodology (RSM) not merely as a statistical fitting tool but as a predictive optimization platform and (iv) the establishment of empirical process models that inform scale-up, energy efficiency and bioactive compound preservation. These contributions position this study as a technically rigorous advancement in sustainable phytochemical extraction from underutilized medicinal plants.

In extraction processes where multiple independent variables influence the response factors, it is crucial to employ an optimization approach capable of evaluating these variables comprehensively. Additionally, potential interactions between the independent factors must be taken into account to accurately determine the optimal experimental conditions [23]. Given the multivariate nature of ohmic extraction processes, Response Surface Methodology (RSM) was employed as an empirical modeling and optimization tool. RSM enables the evaluation of linear, interaction and quadratic effects of multiple variables using regression-based polynomial models. It is particularly useful when mechanistic or first-principles models are either unavailable or intractable. Importantly, RSM does not infer causal relationships based on physical laws, but instead offers statistically derived predictive insights within the confines of the experimental design space [24-29]. As such, its applicability is limited to the studied parameter range and must be interpreted as a data-driven approach.

With the increasing interest in discovering new sources of bioactive compounds from medicinal plants, the utilization of the ant nest as a potential functional food and natural antioxidant has gained attention [30,31]. Developing a technically sound and scalable extraction protocol is imperative. The objectives of this study are twofold; (1) from a scientific perspective, to evaluate the effects of extraction time, gradient electric field intensity (gradient voltage) and their interaction with total phenolic content, flavonoid content and yields extract from ant nest using ohmic heating and to optimize the extraction process using Response Surface Methodology (RSM); (2) from a practical standpoint, to develop an energy-efficient and scalable extraction protocol that supports the broader utilization of ant nest as a novel antioxidant source for functional food or

herbal medicinal products. The outcomes of this research are expected to contribute to the development of an efficient extraction protocol for ant nest, enabling their broader application as a novel source of natural antioxidants with potential health benefits.

## Materials and methods
Materials and tools

The raw material used in this study was the tuber part of the ant nest plant (*Myrmecodia pendanst*) as shown in **Figure 1**, obtained from Bupul Village, Eligobel District, Merauke Regency, Papua, Indonesia. Only the tuber (swollen stem) was used, as it contains the bioactive compounds of interest. The tubers were thoroughly washed with distilled water to remove impurities, sliced into thin pieces (approximately 3 - 5 mm) and then dried in a hot air oven at 50 °C until a constant weight was achieved. The dried samples were ground using a laboratory-scale grinder and sieved to obtain a uniform particle size of 60 mesh. Distilled water (aqua destillata) served as the extraction solvent and was employed in the preparation of all solutions. Distilled water was selected as the extraction solvent due to its non-toxic, food-grade nature and compatibility with ohmic heating technology, which favors polar and conductive solvents. Moreover, water aligns with green chemistry principles, making it suitable for applications in functional foods and nutraceuticals. Although it is acknowledged that certain flavonoids may exhibit higher solubility in hydroalcoholic systems, this study focused on assessing the feasibility of ohmic-assisted extraction using a safe and environmentally sustainable solvent. A comparative analysis with other solvents is proposed as future work. Several analytical reagents of analytical grade were used, including gallic acid, which functioned as a standard in the quantification of total phenolic content and quercetin, which was utilized as a reference standard for determining total flavonoid content. Additional reagents such as sodium carbonate (Na_2CO_3) and Folin–Ciocalteu reagent were applied in the phenolic assay, while aluminium chloride ($AlCl_3$) and ethanol procured from Merck (Darmstadt, Germany), sodium nitrite ($NaNO_2$) and sodium hydroxide (NaOH) procured from SIGMA-ALDRICH were used in the flavonoid determination process.

Figure 1 The ant nest sample.

The main apparatus for extraction was a custom-built Ohmic Heating system, equipped with a voltage regulator, a control panel and stainless-steel electrodes that enabled direct current flow through the sample mixture (**Figure 2**). The system was configured to operate at voltage gradients of 20, 40 and 60 V/cm, with holding times ranging from 90 to 270 s. A temperature sensor was integrated into the system and connected to a digital control panel to monitor real-time heating up to a set point of 88 °C. Temperature was monitored in real-time using a single thermocouple placed at the center of the heating chamber. Although this setup provided consistent final temperature readings, the spatial distribution of temperature within the sample was not mapped and therefore uniform heating cannot be definitively assumed. To monitor electrical performance during extraction, a clamp meter was used to measure current flow across the circuit. A rotary vacuum evaporator was employed to remove solvents from the extracts under reduced pressure and controlled temperature, preserving thermolabile compounds. The determination of TPC (total phenolic content) and TFC (total flavonoid content) was carried out using a UV–Vis spectrophotometer, with absorbance readings measured at wavelengths specific to each assay. Additional laboratory equipment included analytical balances for precise weighing, glassware (volumetric flasks, beakers, test tubes, pipettes), filter papers, a vortex mixer to homogenize solutions before analysis, an oven for drying samples when necessary and a cold storage cabinet to preserve plant material and extracts before analysis.
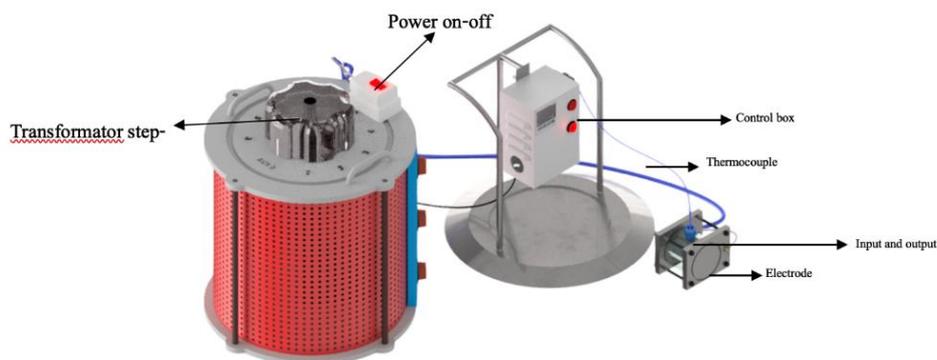
Figure 2 Design of Ohmic Heating.

Ohmic heating extraction procedure

The extraction process was performed using a laboratory-scale ohmic heating system designed specifically for this study. The system consisted of a stainless-steel extraction chamber equipped with two titanium electrodes connected to a power supply capable of delivering adjustable gradient voltages. The temperature was monitored using a digital thermometer and gradient voltage and current were measured using a multimeter. This system applied an alternating electric current to generate internal heating within the sample matrix by utilizing the electrical conductivity of the solution. The extraction process was further supported by a clamp meter to monitor and measure the electrical current during ohmic heating. The resulting crude extract was collected for subsequent concentration and analysis.

For each extraction run, 10 g of ant nest powder was mixed with 100 mL of distilled water (aquades). The solid-to-liquid ratio was maintained at 1:10 (w/v). The mixture was then subjected to ohmic heating treatment with varying holding times and gradient voltage levels as specified by the experimental design. The applied gradient voltage levels were 20, 40 and 60 V/cm, while the holding time varied from 90, 180 and 270 s. During the extraction, the temperature of the solution was maintained below 75 °C to prevent the degradation of heat-sensitive compounds.

Upon completion of the extraction process, the mixture was cooled to room temperature and filtered through Whatman No. 1 filter paper. The filtrate was collected. The filtrate (crude extract) was then concentrated using a rotary vacuum evaporator at 40 °C under reduced pressure to obtain a solvent-free, concentrated viscous extract.

An analytical balance with a precision of 0.0001 g was used for weighing samples and reagents. In addition, a laboratory oven was utilized for drying purposes, while a vortex mixer ensured homogeneous mixing of reagents during the analytical processes. Glassware such as volumetric flasks, erlenmeyer flasks, beakers, graduated cylinders and pipettes was employed for solution preparation, extraction and storage. The concentrated viscous extract was protected from light exposure using aluminium foil and stored in glass bottles at 4 °C before analysis of TPC (total phenolic content), TFC (total flavonoid content) and extraction yield.

Experimental design and optimization

A Response Surface Methodology (RSM) approach was employed to optimize the extraction parameters. A Central Composite Design (CCD) was used to investigate the effects of two independent variables (holding time (s), x_1, ranging from 90 to 270 s; Gradient voltage (volt), x_2, ranging from 220 to 330 V). The selection of voltage gradient (20 - 60 V/cm) and holding time (90 - 270 s) for the Central Composite Design (CCD) was based on preliminary single-variable experiments conducted prior to optimization (data not shown). These initial tests demonstrated that voltages below 20 V/cm yielded insufficient thermal energy for effective extraction, while voltages above 60 V/cm led to electrothermal instability and undesirable thermal side effects. Similarly, holding times under 90 s resulted in low TPC and TFC values, whereas times above 270 s showed diminishing returns and extract degradation. These experimental boundaries were also in agreement with prior literature on ohmic extraction in botanical systems [32,33]. The experimental design consisted of 29 runs, including five replications at the center point to estimate experimental error. The dependent variables (responses) analyzed were: Total Phenolic Content, y_1, expressed as mg gallic acid equivalents (GAE) per g of extract; Total Flavonoid Content, y_2, expressed as mg quercetin equivalents (QE) per g of extract; Extraction yield, y_3, expressed as a percentage (%) of dried extract weight relative to the initial sample weight. The Design-Expert® software (version XX, Stat-Ease Inc., Minneapolis, MN, USA) was used for regression analysis, development of polynomial models and optimization using the desirability function.

Determination of Total Phenolic Content (TPC) (Lee *et al.*, 2013 modified) [34]

Quantitative analysis of the total phenolic content was perfomed using a UV-Vis spectrophotometer (Shimadzu UV-1800, Japan), measuring absorbance at 765 nm and employing the Folin-Ciocalteu colorimetric method. In brief, 0.5 mL of the extract was mixed with 2.5 mL of 10% Folin-Ciocalteu reagent and incubated for 5 min at room temperature. Subsequently, 2 mL of 7.5% sodium carbonate solution was added and the

mixture was incubated in the dark at room temperature for 30 min. The total phenolic content was calculated based on a calibration curve prepared with gallic acid (0 - 200 mg/L) and expressed as mg gallic acid equivalents (GAE) per g of extract.

Determination of Total Flavonoid Content (TFC) (Atanassova *et al.*, 2011 modified) [35]

Quantitative analysis of the total flavonoid content was performed using a UV-Vis spectrophotometer (Shimadzu UV-1800, Japan) and the aluminum chloride colorimetric method. An aliquot of 0.5 mL extract was mixed with 1.5 mL of 95% ethanol, followed by the addition of 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water. The mixture was incubated at room temperature for 30 min and the absorbance was recorded at 415 nm. Quercetin was used as the standard and the results were expressed as mg of quercetin equivalents (QE) per g of extract.

Determination of Extraction Yield (Ngamkhae *et al.*, 2022) [36]

The extraction yield was determined gravimetrically using the concentrated viscous extract. A precisely weighed aliquot (1.0 g) of the extract was transferred to a pre-weighed petri dish and dried in a vacuum oven at 40 °C until constant mass was achieved. The yield was expressed as a percentage of the dried extract weight relative to the initial weight of the dried ant nest powder, using the following equation:

$$Extraction\ Yield\ (\%) = \left(\frac{Weight\ of\ dried\ extract\ (g)}{Weight\ of\ initial\ dry\ sample\ (g)}\right) \times 100 \quad (1)$$

Statistical analysis

Response Surface Methodology (RSM) was applied to evaluate and optimize the extraction of bioactive compounds from ant nest using ohmic heating pretreatment. The effects of two independent variables—holding time (X_1: 90, 180 and 270 s) and gradient voltage (X_2: 20, 40 and 60 V/cm)—were investigated for their influence on three responses: Total phenolic content (TPC, mg GAE/g), total flavonoid content (TFC, mg QE/g) and extraction yield (%).

A Central Composite Design (CCD) was employed to design the experiments and develop the predictive model. The CCD consisted of 29 experimental runs, including four factorial points, four axial points and six replicates at the center point to ensure a robust estimation of pure error and to evaluate the reproducibility of the data. Each experimental condition was conducted in triplicate to ensure precision and statistical reliability of the measurements.

The experiments were randomized to minimize systematic bias and account for the potential effects of uncontrolled extraneous variables (Montgomery, 2001). The RSM analysis and statistical modeling were performed using Design-Expert® software (version XX, Stat-Ease Inc., Minneapolis, MN, USA).

The experimental data were fitted to a second-order (quadratic) polynomial regression model to describe the relationship between the independent variables and the responses. The model equation used was as follows:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_1 X_1 X_2 + \varepsilon \quad (2)$$

where:

Y = represents the predicted response (TPC, TFC or yield),

β_0 = is the intercept (constant term),

β_1 = are the linear coefficients,

β_{11} = are the quadratic coefficients,

β_{12} = is the interaction coefficient between the two variables,

ε = is the experimental error.

The adequacy and significance of the quadratic model were assessed through analysis of variance (ANOVA). The quality of the model fit was evaluated using the coefficient of determination (R^2), adjusted R^2, predicted R^2 and lack-of-fit tests. A non-significant lack-of-fit ($p > 0.05$) and high R^2 values indicated a good fit of the model to the experimental data.

Optimization of the extraction conditions was carried out using the numerical and graphical tools available in Design-Expert® software. The objective was to maximize total phenolic content, total flavonoid content and extraction yield simultaneously. The independent variables were maintained within their

experimental ranges and the desirability function approach was applied to identify the optimal combination of holding time and gradient voltage. The optimal conditions predicted by the model were validated through additional experiments performed in triplicate to verify the accuracy and reliability of the model predictions.

Results and discussion

Ohmic heating performance: Electrical thermal and energy aspects

The performance of ohmic-assisted extraction is governed by the interaction between the electrical properties of the medium, temperature development and energy efficiency. These parameters were evaluated in a batch ohmic system using *ant nest* (*Myrmecodia pendans*) powder with 100 mL of solvent under varying voltage gradients (20, 40 and 60 V/cm) and holding times. Electrical conductivity, calculated from voltage–current measurements and the geometry of the chamber,

increased with voltage gradient, indicating effective ionic mobility and dissociation within the medium. All conductivity values were within the optimal range for ohmic heating, ensuring efficient electrical-to-thermal energy conversion.

To measure the electric current in the circuit during the ohmic heating process, a clamp meter was utilized. Each treatment was conducted in three replications to ensure data accuracy. The current measurement included the initial current value before the electricity was applied and the final current value upon reaching the target temperature of 88 °C. The temperature was monitored continuously using a sensor connected to the control panel display. The initial temperature of the ant nest extract before entering the ohmic heating tube was recorded at 25 °C. The relationship between temperature and electric current across the different voltage gradients is presented in **Figure 3**.
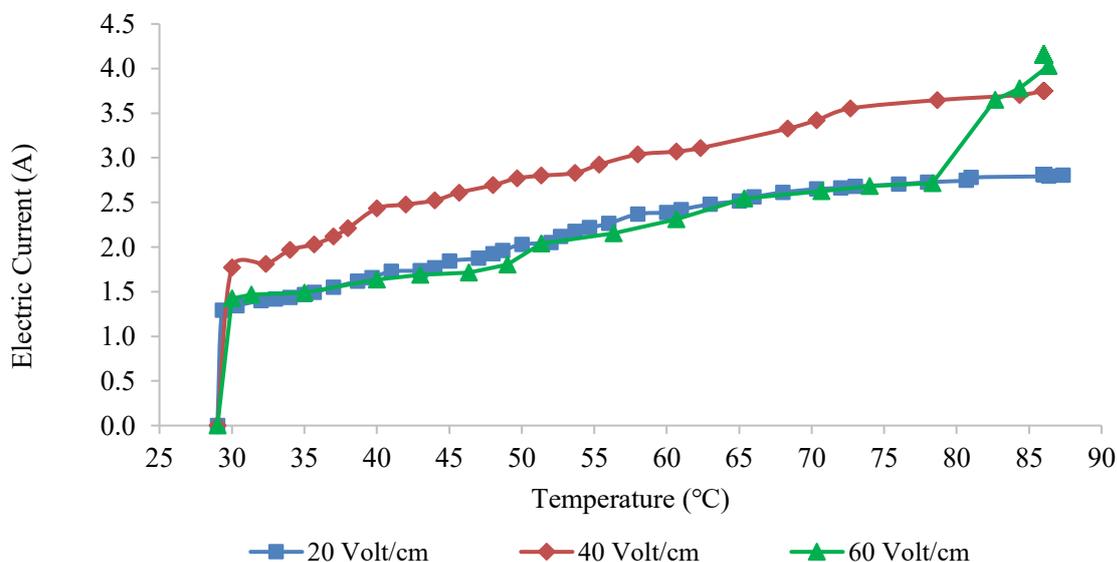
Figure 3 Relationship between temperature and electric current.

Figure 3 illustrates the relationship between temperature and electric current under varying voltage gradients during the ohmic extraction of ant nest. At a voltage gradient of 20 V/cm, the electric current increased gradually from 1.5 A at 30 °C to approximately 2.8 A at 88 °C. Higher voltage gradients (40 and 60 V/cm) resulted in a steeper increase in current, with the most pronounced jump observed at 60

V/cm. This sudden rise in current at temperatures above 80 °C indicates a significant reduction in material resistance and enhanced electro-osmosis, leading to more efficient heating. This study monitored temperature using a single central sensor. However, uniform temperature distribution across the sample was not empirically validated through multi-point thermal mapping. Future studies should incorporate

thermocouples at various radial and axial positions to confirm the spatial uniformity of ohmic heating and ensure consistent processing outcomes.

As shown in **Table 1**, an increase in voltage gradient resulted in higher average electric current, increased conductivity, elevated final temperatures and higher energy consumption. This confirms that electrical input directly influenced both the thermal behavior and the energetic demands of the process. Higher voltage gradients led to shorter heating durations to reach target temperatures, thereby improving thermal efficiency. Despite the increase in total energy consumption at higher voltages, the specific energy use

per g of sample remained within a reasonable and efficient range. These findings highlight that ohmic heating not only ensures rapid and uniform temperature elevation but also maintains acceptable energy performance for plant-based extraction applications. Electrical conductivity, calculated from voltage–current measurements and the geometry of the chamber, increased with voltage gradient, indicating effective ionic mobility and dissociation within the medium. The observed conductivity values ranged from 0.171 to 0.407 s/m, which is within the optimal range for ohmic heating operations.

Table 1 Ohmic heating parameters under different voltage gradients.

Voltage gradient (V/cm)	Avergae current (A)	Conductivity (S/m)	Final temperature (°C)	Time (s)	Power density (W/L)	Energy (kWh)	Energy (kJ/g) dry sample	Efficiency (%)
20	0.421	0.171	61	270	463.1	0.0805	28.98	45.64
40	1.013	0.325	75	270	2228.6	0.2170	78.12	14.32
60	1.527	0.407	87	270	5039.1	0.3045	109.62	5.24

As the applied voltage increased, a corresponding increase in electric current was observed. This elevated current accelerated the internal heat generation, resulting in faster and higher temperature rises. Such behavior is consistent with the principle of ohmic heating, where heat is generated volumetrically and proportionally to the current flowing through the conductive medium. Higher voltage gradients led to shorter times to reach the target temperature, improving process efficiency without exposing the bioactive compounds to prolonged heat.

Energy consumption also increased with voltage gradient due to greater power input over time. However, the specific energy requirement per g of sample remained within an efficient operational range, demonstrating that the system was energy-responsible even at higher voltage gradients. Beside that, the power density of the ohmic heating system increased proportionally with the voltage gradient. Higher power density correlates with faster heating rates but may also pose risks of non-uniform heating and thermal degradation, particularly in bioactive-rich matrices such as *Myrmecodia pendans*. These findings align with Assiry *et al*. (2010) [37], who emphasized that power

density critically affects both extraction efficiency and compound stability during ohmic processing.

The principles of ohmic heating while extracted ant nest

Ohmic Heating, also known as Joule heating or electro-heating, is a volumetric heating method where electrical current passes directly through a conductive food or plant matrix, generating heat internally. Unlike conventional thermal methods, Ohmic heating provides rapid, uniform and energy-efficient heating by converting electrical energy into thermal energy within the material itself [38]. The principle behind ohmic heating involves passing an alternating electrical current through the ant nest material, which is typically mixed with a liquid solvent such as water. Due to the natural moisture content and ionic composition of the ant nest, the material itself acts as a conductor, allowing the electric current to flow through it with minimal resistance [39]. As the current passes through the sample, electrical resistance within the material generates heat uniformly throughout its volume. This process is known as volumetric heating and differs significantly from conventional heating methods that

rely on conduction or convection from an external source [36,38].

During extraction, as the ant nest material heats up rapidly and uniformly, the internal temperature rises, leading to the softening and breakdown of cell structures. The elevated temperature not only promotes the diffusion of bioactive compounds like flavonoids, phenolics and tannins but also enhances cell membrane permeability through a phenomenon called electroporation. The electric field causes tiny pores to form in the cell membranes, facilitating the release of intracellular compounds into the surrounding solvent. Additionally, the presence of an electric field encourages electro-osmotic flow, further improving mass transfer and accelerating the extraction process. Due to the electrochemical phenomena, this leads to promote the release of intracellular compounds while maintaining their structural integrity.

The efficiency of ohmic heating in this process is influenced by the applied voltage gradient. Higher voltage gradients increase the intensity of the electric field and subsequently raise the electric current passing through the material. This results in a faster heating rate and more rapid cell disruption. As observed in the experiment based on **Figure 3**, voltage gradients of 20, 40 and 60 volts per cm resulted in varying rates of temperature increase and electric current flow. At higher gradients, the electrical resistance of the ant nest material decreases as the temperature rises, improving its conductivity and allowing for greater energy absorption. This enhances the efficiency of heat generation and leads to a more effective extraction process.

Ultimately, ohmic heating offers several advantages for the extraction of compounds from ant nests. The method reduces processing time by providing rapid and uniform heating, minimizes thermal degradation of sensitive compounds, and increases extraction yields due to improved mass transfer. Furthermore, it is an energy-efficient method since most of the electrical energy is directly converted into heat

within the material itself. These mechanisms explain why holding time and gradient voltage, the two critical factors manipulated in this study, significantly influence the extraction outcomes. By leveraging these principles, ohmic heating can significantly improve the quality and efficiency of ant nest extraction compared to conventional methods.

This phenomenon can be attributed to the decrease in the electrical resistance of the ant nest material as the temperature rises. A higher voltage gradient accelerates the electro-osmosis process due to the reduced resistance and increased thermal conductivity of the material. Consequently, electric current consumption rises in response to the need for greater energy to maintain higher temperatures. Moreover, the reduction in the material's resistance enhances its conductivity, thereby improving electrical efficiency. The higher concentration of dissolved ions in the ant nest extract also contributes to increased current flow through the material, further lowering resistance and enhancing the extraction process. These findings are consistent with previous studies, which state that materials with higher conductivity facilitate a more rapid temperature increase, thus making the heating process more effective.

Response model fitting

The second-order polynomial response surface model (Eq. (2)) was fitted to each of the response variables (Y). For the corresponding fitting of the explanatory models and the variation of the total phenolic content (TPC), total flavonoid content (TFC) and extraction yield, the sum of squares of the sequential model was analyzed. These analyses indicated that adding terms up to quadratic significantly improved the model (**Table 2**) and, therefore, could be the most appropriate model for the three response variables. The result of ANOVA and regression coefficients of the second-order polynomial model for the response variables (actual values) can be seen on **Table 3**.

Table 2 Sequential model sum of squares or Total Phenolic Content (TPC), Total Flavonoid Content (TFC) and extraction yield.

Source	df	Total phenolic content		Total flavonoid content		Yields	
		Sum of squares	Pr > F	Sum of squares	Pr > F	Sum of squares	Pr > F
Mean vs Total	1	317,623.843		109,134.902		25,272.662	
Linear vs Mean	2	110,256.335	0.0005	17,385.146	< 0.0001	752.431	< 0.0001
2FI vs Linear	1	5,433.29475	0.326	4.514	0.9317	12.201	0.503
Quadratic vs 2FI	2	128,601.091	< 0.0001	5,889.337	< 0.0001	499.586	< 0.0001
Cubic vs Quadratic	2	725.341	0.311	182.911	0.2906	18.327	0.285
Residual	21	6,164.358		139.485		144.502	
Total	29	568,804.263		5,482.186		26,699.71	

Table 3 ANOVA and regression coefficients of the second-order polynomial model for the response variables (actual values).

Source	df	Total phenolic content			Total flavonoid content			Yields		
		Coefficient	Sum of squares	*p-value*	Coefficient	Sum of squares	*p-value*	Coefficient	Sum of squares	*p-value*
Model	8	234.278	244,290.72	< 0.0001	105.434	46,553.483	< 0.0001	37.166	1,264.218	< 0.0001
Linear										
b_1		16.782	6,758.776	< 0.0001	3.374	273.218	0.181	5.016	603.787	< 0.0001
b_2		265.668	103,497.559	< 0.0001	37.912	34,497.075	< 0.0001	2.487	148.561	0.00013
Quadratic										
b_{11}	1	−102.069	128,224.207	< 0.0001	−27.489	9,300.727	< 0.0001	−6.371	498.689	< 0.0001
b_{22}	1	−54.558	36,635.893	< 0.0001	−25.783	8,182.255	< 0.0001	−2.863	101.053	0.0009
Interaction										
b_{12}	1	21.278	5,433.295	0.0003	0.613	4.514	0.861	−1.005	12.201	0.202
Residual	23		6,889.699			3,295.018			163.335	
Lack of Fit	3		973.718	0.373		839.038	0.111		42.033	0.107
Pure Error	20		5,915.98			2,455.979			121.302	
Cor Total	28		251,180.42			49,848.501			1,428.715	
R^2			0.973			0.934			0.886	
Adjusted R^2			0.966			0.919			0.861	
Predicted R^2			0.956			0.896			0.817	
Adeq Precision			28.945			19.696			16.363	
CV			6.538			9.51			9.013	

Regression analysis and ANOVA were used for fitting the model and to examine the statistical significance of the terms. The estimated regression coefficients of the quadratic polynomial models for the response variables, along with the corresponding coefficients of determination R^2 are given in **Table 3**. In addition, adj-R^2 and coefficient of variation (CV) were calculated to check the model adequacy.

The lack of fit illustrated in **Table 3** did not result in a significant *p-value* for selected variables, meaning

that these models were sufficiently accurate for predicting the relevant responses. These results, in line with the R^2 values for these response variables were higher than 0.80, indicating the regression models were suitable to explain the behavior. The R^2 values for TPC, TFC and yields were found to be 0.973, 0.934 and 0.886, respectively.

Moreover, coefficient of variation (CV) describes the extent to which the data were dispersed. As a general rule, the coefficient of variation (CV) should not be greater than 10%. Daniel (1989) [41] reported that a high CV indicates that variation in the mean value is high and does not satisfactorily develop an adequate response model. Our results showed that the coefficients of variation were less than 10% for all the responses

(**Table 2**), representing a better precision and reliability of the conducted experiments.

**Predicted and actual values of the model**

Comparison between predicted and actual values of total phenolic content, total flavonoid content and yields of ant nest extraction can be seen on **Figure 4**. The three-dimensional response surface plots illustrating the interaction effects of extraction holding time and gradient voltage on Total Phenolic Content (TPC), Total Flavonoid Content (TFC) and yield of ant nest extract are presented in **Figure 5**. The predicted optimum condition for extraction of the ant nest can be seen on **Table 3**. And overlay plot can be seen on **Figure 6**.
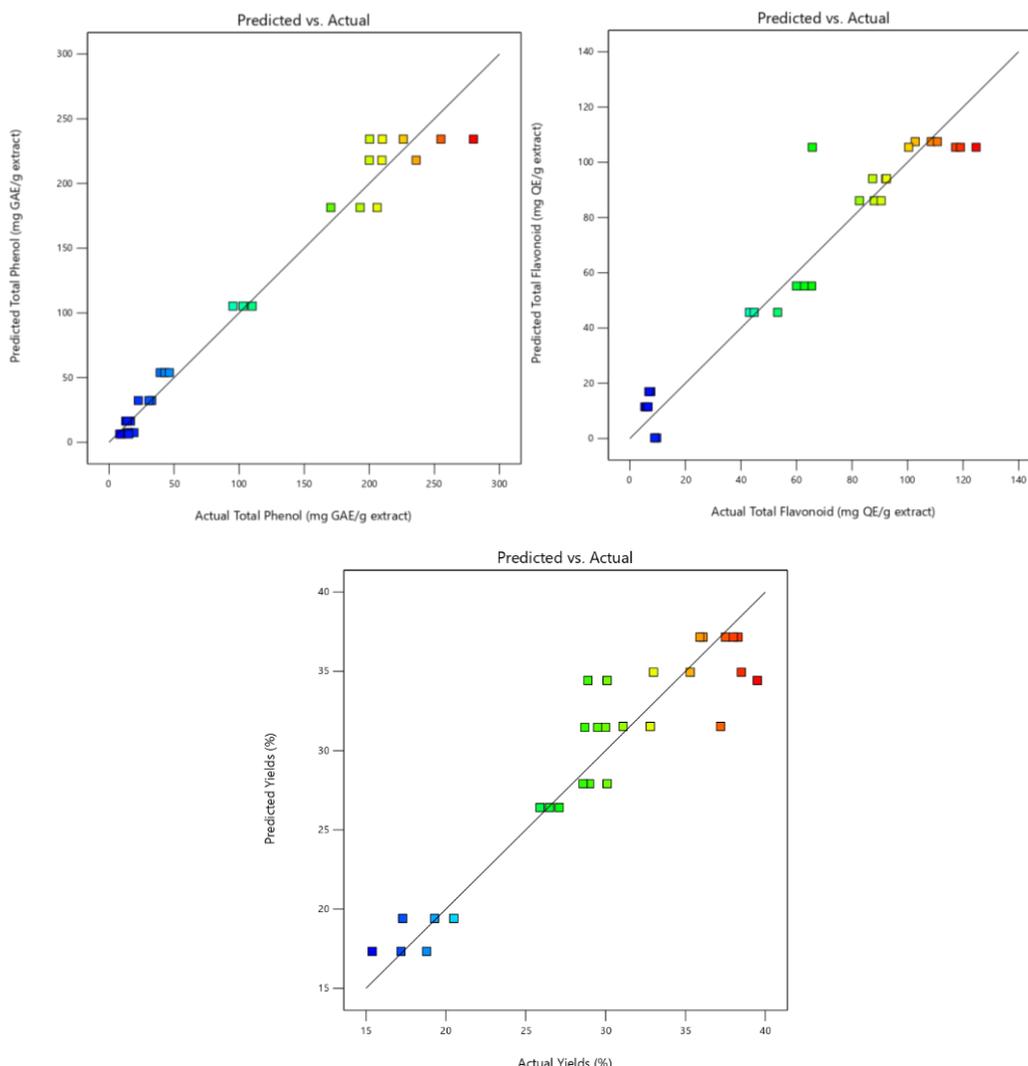
Figure 4 Comparison between predicted and actual values of total phenolic content, total flavonoid content and yields of ant nest extraction.

Figure 4 showed that the polynomial regression model was in good agreement with the experimental results. In this figure, each of the observed values is compared to the predicted value calculated from the model. The result suggests that the models used in this research were able to identify operating conditions for the selective extraction of bioactive compounds from ant nest.
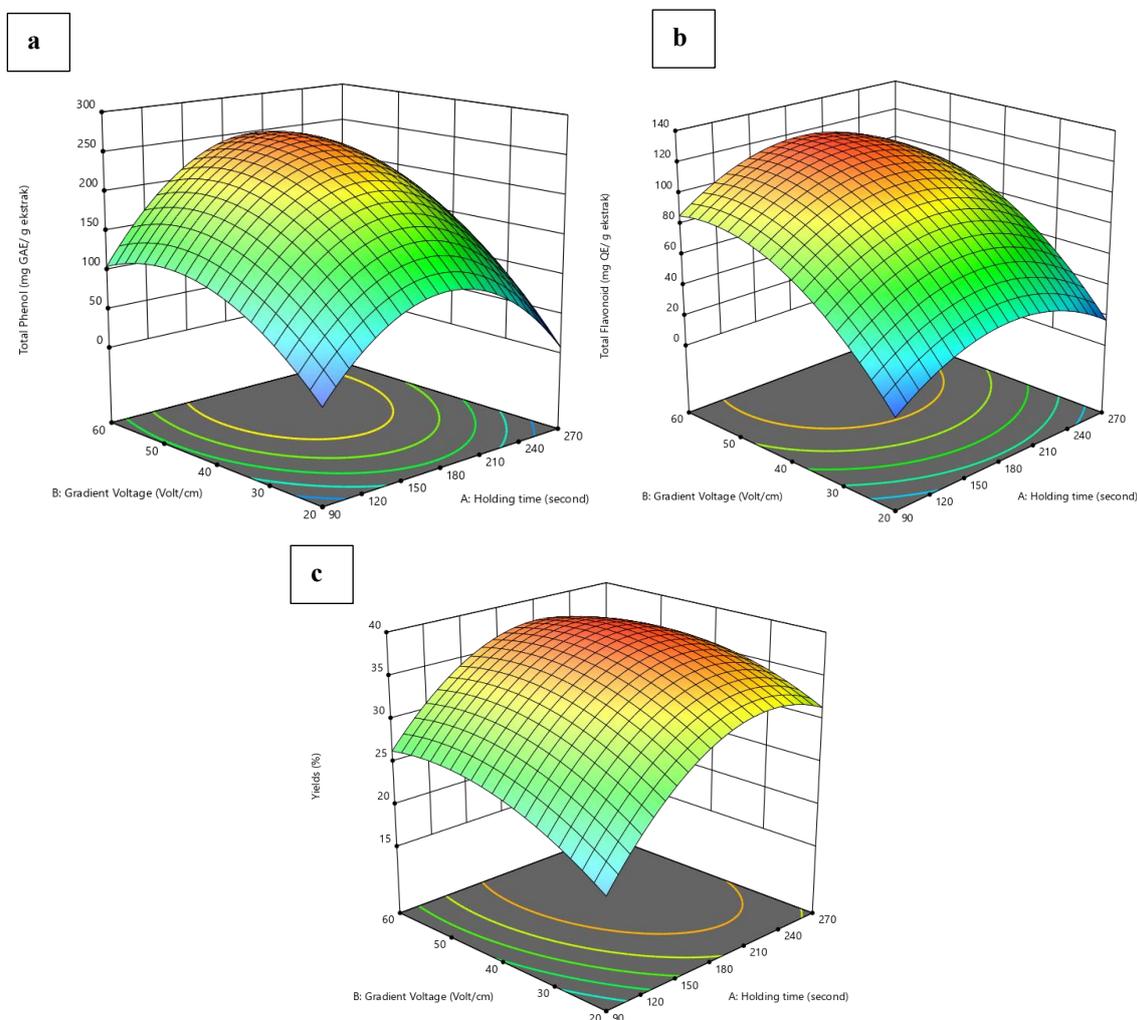
Figure 5 Response surface for the effect of extraction holding time and gradient voltage on the (a) Total Phenolic Content (TPC), (b) Total Flavonoid Content (TFC) and (c) Yields Value of ant nest.

As shown in **Figure 5(a)**, both holding time and gradient voltage had a significant influence on the TPC. The TPC increased with both parameters, reaching a maximum at approximately 195.883 s of holding time and 52.761 V/cm. Similarly, **Figure 5(b)** displays the effect of the two variables on TFC. The total flavonoid content exhibited a similar trend to TPC, with an initial increase followed by a peak under conditions comparable to those observed for TPC. After this peak, a decline was observed, which may be attributed to the thermal or oxidative degradation of flavonoid compounds under excessive treatment conditions. Thermal degradation is a critical concern in the extraction of bioactive compounds such as polyphenols and flavonoids, particularly under prolonged exposure to elevated temperatures generated by ohmic heating. Although the current study was not designed to perform a complete degradation kinetics analysis, the extraction profiles of TPC and TFC across three time points (90, 180 and 270 s) suggest potential instability at extended

durations. Notably, at 40 V/cm, the TPC increased significantly from 111.7 mg GAE/g at 90 s to 233.0 mg GAE/g at 180 s, followed by a marked decline to 149.7 mg GAE/g at 270 s. A similar pattern was observed for TFC under the same conditions. These reductions are unlikely to result from diffusion limitations or saturation alone and are more plausibly explained by thermal or oxidative degradation of thermolabile compounds. This interpretation aligns with previous findings reported by Rodríguez-Roque *et al*. [32], which highlight the susceptibility of phenolic compounds to degradation at elevated temperatures beyond 60 - 70 °C. Additionally, the three-dimensional RSM response surfaces exhibit curvatures that confirm the non-linear interaction between time and voltage, with reduced TPC and TFC levels at the extreme time ranges. While the current temporal resolution limits precise kinetic modeling of degradation, these findings offer preliminary but substantive evidence that overexposure during ohmic heating may compromise extract quality. As such, the proposed optimal conditions in this study represent a balance between maximizing extraction yield and preserving compound integrity. Further studies incorporating finer time intervals and molecular-level degradation tracking (e.g., HPLC, LC-MS) are recommended to substantiate and extend these observations.

Figure 5(c) demonstrates the variation in yield with respect to holding time and gradient voltage. The extraction yield increased substantially with both factors, attaining a maximum yield of 39.5% at conditions similar to those observed for TPC and TFC. The yield plateaued and showed a slight decrease with further increases in holding time and gradient voltage, suggesting that optimal energy input facilitates efficient cell wall disruption and compound diffusion, while excessive conditions may lead to the breakdown of cellular components and reduced extractability.

Table 4 Predicted optimum condition for the extraction of ant nest.

Factor	Low	High	Optimum
Holding time (s)	90	270	195.883
Gradient voltage (Volt)	20	60	52.761
Total Phenol (mg GAE/g ectract	8.24	280	256.145
Total Flavonoid (mg GAE/ g extract)	5.38	125	118.936
Yields (%)	15.4	39.5	38.154
Desirability			0.937

The optimum extraction conditions for ant nest were determined in order to obtain the highest Total Phenolic Content (TPC), Total Flavonoid Content (TFC) and extraction yield. As shown in **Table 4**, the optimized conditions—holding time of 195.883 s and gradient voltage of 52.761 volt—resulted in a maximum TPC of 256.145 mg GAE/g extract, TFC of 118.936 mg QE/g extract and extraction yield of 38.154%. These findings indicate that under these specific parameters, the extraction process effectively enhances the recovery of phenolic and flavonoid compounds while maximizing yield efficiency.
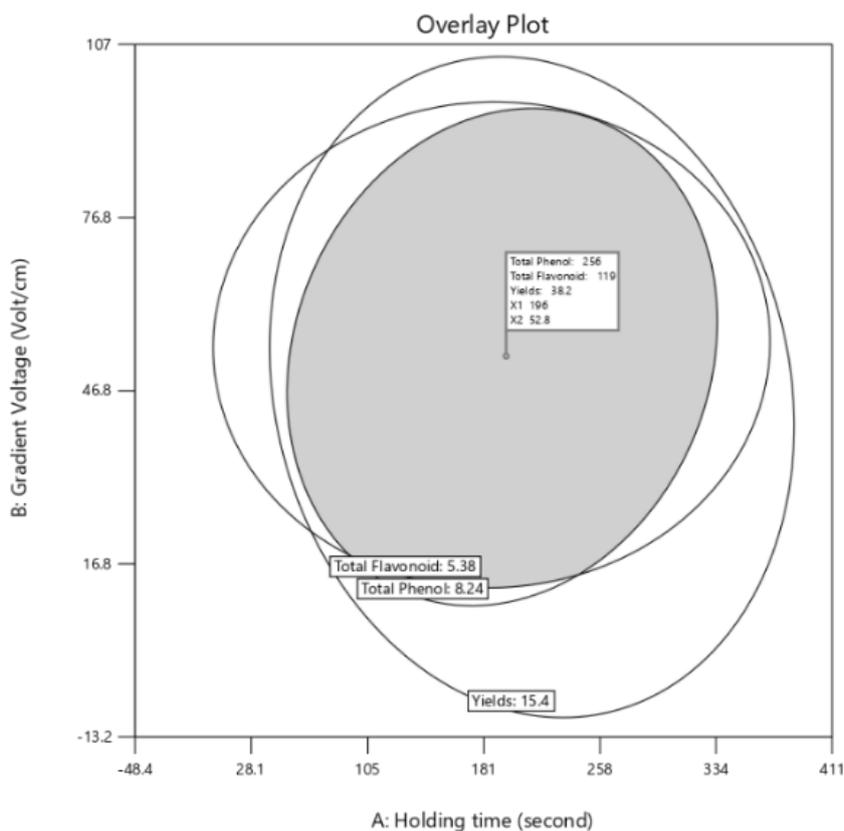
Figure 6 The optimum region by overlaying contour plots of the three responses evaluated as a function of extraction gradient voltage and holding time.

Figure 6 illustrates the overlay plot for the optimization of the extraction process of ant nest, showing the interaction between holding time (A) and gradient voltage (B). The shaded region in the plot represents the optimal area where the most desirable responses for Total Phenolic Content (TPC), Total Flavonoid Content (TFC) and yield are simultaneously achieved.

The plot demonstrates that both holding time and gradient voltage significantly influence the extraction efficiency. Lower holding times and gradient voltages tend to result in suboptimal yields and reduced recovery of phenolic and flavonoid compounds, as indicated by the boundary lines corresponding to lower response values (e.g., 10 mg GAE/g for TPC and 5.38 mg QE/g for TFC).

While these optimization results provide useful guidance for process conditions, it is important to acknowledge the potential trade-offs that arise when multiple responses are considered simultaneously. Although the present study applied a single-objective optimization approach for TPC, TFC and extraction yield individually, it is critical to recognize that these responses are not always complementary. In particular, higher extraction yields achieved through elevated voltage or prolonged extraction time may lead to thermal degradation of phenolic and flavonoid compounds, as observed in the declining TPC and TFC values at 270 s in certain conditions. These observations underscore the inherent trade-offs between maximizing extract quantity and preserving compound stability and bioactivity. Therefore, the optimal extraction condition proposed in this study should be interpreted as a practical compromise balancing efficiency and molecular integrity rather than achieving absolute maxima for each variable. While a full multi-objective optimization (e.g., Pareto front) was beyond the scope of this work, it represents an important direction for future research to more systematically account for competing objectives in extraction system design. Although the process was successfully optimized using an empirical RSM approach, the current dataset did not permit reliable kinetic or thermodynamic modeling. Further studies are recommended to develop

mechanistic models by incorporating more frequent time-point sampling and advanced thermal characterization.

Effect of gradient voltage and holding time on yield, flavonoid and fenol of ant nest

The decrease of the response occurred directly proportionally in this study. The variation in extraction yield as a function of holding time and gradient voltage is presented in **Figure 3**. The results demonstrated that the yield increased significantly with both longer holding times and higher gradient voltage applications. At elevated gradient voltage levels, the permeability of ant nest cellular structures likely increased due to the enhanced energy input, facilitating the release of phenolic and flavonoid compounds. This improved mass transfer efficiency resulted in higher extraction yields. Additionally, the influence of holding time was more pronounced at lower gradient voltages, where extended processing allowed for a more complete diffusion of bioactive components. However, at higher gradient voltages and prolonged holding times, the yield tended to reach an equilibrium point, indicating that further extension of the extraction time did not substantially enhance the yield.

The highest yield value (39.5%) in the ant nest showed a strong dependence on the combination of gradient voltage and holding time. This result can be attributed to two main mechanisms inherent to ohmic heating, namely that moderate gradient voltage (up to 40 V/cm) triggers increased disruption of the cell structure through the phenomenon of cell membrane electroporation. This process forms pores in the membrane, facilitating solvent penetration into the cell and the release of intracellular components [42]. As a result, the diffusion of target molecules into the solvent becomes more intensive, thereby increasing the extraction yield. In addition, the unique characteristics of ohmic heating, such as rapid heating and efficient energy transfer, result in uniform heat distribution throughout the sample. This uniform heat softens the cell wall optimally and causes structural damage more efficiently than conventional methods [37]. This combination of cell wall softening and increased membrane permeability accelerates the rate of mass transfer, thereby maximizing the amounts of extracted compounds. According to [43], the use of appropriate

solvents and longer extraction times (15 - 60 min) on rambutan fruits was also shown to increase extraction yields. This is due to ohmic heating ability to produce rapid and even heating, which not only softens the cell tissue but also maintains the thermal stability of the target compounds. Controlled heat allows the solvent to penetrate deeper into the sample matrix as time increases, resulting in optimized diffusion of bioactive compounds. At higher gradient voltages (60 V/cm), the yield of the ant nest decreased. This phenomenon can be explained by excessive energy input triggering degradation of sensitive molecules, Maillard reactions, or polymerization of solutes, thus reducing extraction efficiency. Furthermore, high voltage causes an extreme temperature increase (Joule heating) due to intensive electron movement [33]. This excess heat degrades thermolabile bioactive compounds (such as phenolics and flavonoids) through oxidation or denaturation, especially in complex matrices such as ant nests. In addition, high voltage also destabilizes cell membranes, causing the release of intracellular electrolytes that increase electrical conductivity [33]. While this increase in conductivity can improve heating efficiency at moderate voltages (up to 40 V/cm), voltages above 60 V/cm create excess local heat (> 81 °C) that damages bioactive compounds through oxidation or denaturation. The holding time also indicates the optimum point. If it exceeds 180 s, the extraction yield decreases drastically, possibly due to the accumulation of heat that degrades the bioactive compounds and changes the structure of the plant matrix, thus inhibiting further release of the compounds. A similar mechanism was reported by [44], in the extraction of pomegranate juice with ohmic heating, increasing the voltage up to 55 V/cm accelerated the heating rate exponentially (4.171 °C/s) and shortened the process time by 1.5 times. However, voltages above 30 V/cm decreased the system performance coefficient (0.764 - 0.939) and risked triggering compound degradation due to overheating and electrochemical oxidation reactions.

TPC is a major health-promoting component in the ant nest, mainly through its antioxidant capacity [45]. The increase in TPC at a 40 V/cm volt gradient may occur through two mechanisms involving (1) the release of bound phenolic compounds due to cell membrane damage and (2) chemical modification through the addition of hydroxyl groups bound to the aromatic rings

of phenolic compounds, which increases their antioxidant activity and availability [46]. According to [47], the increase in the concentration of phenolic compounds can be due to the electrothermal effect of ohmic heating treatment on the permeability of plant cell walls. Electrical disruption of the cell membrane (electroporation phenomenon) or thermal effects can cause complex breakdown between phenolic compounds and other compounds such as proteins, polysaccharides and fibers, thus facilitating the release of phenolic compounds. Similar results were reported by [47,48] that ohmic heating at a voltage gradient of 40 V/cm increased the total phenolic content (TPC) in apple juice by 5.4% and manga juice by 8% which is likely due to structural changes in the tissue matrix that increase the extractability of phenolic compounds through a combination of volumetric heating and electric field effects. This synergy between electroporation and ohmic heating not only optimizes the release of phenolic compounds but also modifies their structure to enhance bioactivity, making ohmic an efficient method in the extraction of thermolabile compounds such as TPC. The decrease in TPC in the ant nest extract with a holding time of 270 s is thought to be due to the degradation of phenolic compounds during the ohmic heating process, mainly due to an increase in the voltage gradient that triggers a local temperature rise. These results indicate the optimal time of extraction pretreatment is 210 s, where thermal and electrochemical degradation can be minimized. Phenolic compounds are susceptible to degradation at extraction temperatures > 80 °C, mainly due to increased electric fields (high voltage) that accelerate the destruction of phytochemical structures through molecular collision mechanisms and electrochemical oxidation [33]. Similar results were reported by [50], where ohmic heating of pomelo juice with a voltage gradient of 30 V/cm increased TPC levels compared to 20 V/cm because high electric fields accelerate cell membrane damage, resulting in phenolic compounds being released. However, gradients > 40 V/cm cause dominant electrochemical degradation due to water electrolysis reactions (producing oxygen radicals) and electrode corrosion. In addition to electrochemical factors, the decrease in TPC can also be caused by thermal degradation due to excessive heat that breaks down phenolic structures or enhances oxidation

reactions, thereby reducing their measured concentrations [32]. Furthermore, excessive energy input can lead to polymerization that allows some phenolics to form insoluble complexes, reducing extractability [51]. Thus, the synergy between localized heat, high electric field and non-optimal exposure time is a critical factor for TPC reduction in extraction with ohmic heating.

The ant nest (*Myrmecodia pendans*) contains high flavonoid compounds, particularly quercetin, luteolin and kaempferol [52]. The results of flavonoid (TFC) values followed a similar trend to phenolics, with a maximum TFC of 116.05 mg QE/g extract under optimal ohmic heating conditions. The mechanism by which OH enhances flavonoid extraction is through (1) permeabilization of cell walls-electrical field and ohmic heating disrupt the polysaccharide matrix that binds flavonoids, thereby accelerating the release of compounds [53] and (2) enhancement of solvent-solids interactions, where rapid heating reduces solvent viscosity and improves flavonoid diffusion [54]. A similar study on grape extraction with ohmic heating (55 V/cm) showed 2 times higher increase in total phenolics compared to conventional methods through cell membrane electroporation, which forms micro pores for compound diffusion without damaging sensitive structures such as anthocyanins [55]. According to [56], a gradient of 60 V/cm results in optimal heating without damaging TPC/TFC, while gradients > 60 V/cm (90 - 120 V/cm) trigger flavonoids and phenolics degradation due to overheating (> 80 °C) and electrochemical oxidation reactions of free radicals. The synergy between cell permeabilization, diffusion enhancement and voltage gradient control makes ohmic a superior method for the extraction of thermolabile bioactive compounds. The decrease in TFC due to exposure to high gradient voltage and long holding time shows a similar pattern to TPC. This phenomenon is due to the instability of flavonoids to the combination of high temperature and electrical stress, which triggers oxidation or structural damage to the compounds, thus reducing their content in the final extract [57]. For example, a study on bignay juice by [58], proved that the degradation of flavonoids (quercetin) during ohmic heating is time-dependent-the longer the heating time, the more significant the decrease in TFC due to the sensitivity of flavonoids to heat and electrochemical

oxidation reactions. Thus, although Ohmic Heating effectively enhances initial extraction, overexposure is detrimental to bioactive compounds, confirming the importance of time and voltage optimization to minimize degradation.

Although the total phenolic and flavonoid contents were measured, the antioxidant capacity of the extracts was not evaluated. As phenolic concentration does not always correlate linearly with bioactivity, the absence of antioxidant assays such as DPPH or ABTS represents a limitation of this study. Future research should include these assays to confirm the functional integrity of bioactives following ohmic heating. One of the key limitations of the present study is the lack of compound-specific analysis. While TPC and TFC provide useful aggregate metrics for antioxidant-rich extracts, they do not capture the behavior of individual flavonoid or phenolic constituents, which may exhibit distinct extraction profiles, thermal sensitivities and degradation pathways. Future work will incorporate HPLC or LC-MS analysis to identify and quantify major bioactive compounds—such as quercetin, rutin and kaempferol—in order to gain mechanistic insight into the electrothermal effects of ohmic heating on compound integrity. This will be particularly relevant for the validation of the process in pharmacological and nutraceutical applications.

Correlation between ohmic heating parameters and bioactive compound recovery

These results confirm that holding time and gradient voltage are critical parameters in optimizing the extraction efficiency and bioactive compound recovery from ant nest. The identified optimal conditions enhance extraction yield while preserving the integrity of phenolic and flavonoid compounds. The overlay plot (**Figure 6**) clearly indicates an optimal region where the combined effects of holding time and gradient voltage maximize yield, TPC and TFC. The synergistic interaction between temperature (from internal heating) and the applied electric field enhances mass transfer, cell wall disruption and selective release of bioactive compounds. Conversely, excessively prolonged holding times and high gradient voltages may not substantially enhance the extraction outcomes and could potentially lead to degradation of bioactive compounds due to thermal and electrical stress. The presence of an optimal

zone suggests a synergistic effect between the holding time and gradient voltage parameters, where moderate to high levels of both variables enhance mass transfer and cell wall permeability, facilitating the release of phenolic and flavonoid compounds. This optimized condition ensures a balance between extraction efficiency and compound stability, providing a robust approach for maximizing the functional properties of ant nest extract.

At suboptimal conditions, these synergistic effects either do not fully manifest (low gradient voltage or short holding time) or result in compound degradation (high gradient voltage or long holding time). Based on the comprehensive analysis of these findings, the following conclusions can be drawn. In this study, we present for the first time the results of response surface methodology (RSM)-controlled experiments on the optimization of ohmic Heating-assisted extraction of ant nest, conducted to achieve high extraction yield, as well as maximize the recovery of total phenolic content (TPC) and total flavonoid content (TFC). The key findings identified optimal extraction conditions at a gradient voltage of 40 V/cm and a holding time of 195.883 s, under which the highest extraction yield (38.15%), TPC (256.15 mg GAE/g extract) and TFC (118.934 g QE/g extract) were obtained. The application of ohmic Heating in this extraction process has demonstrated significant advantages, including rapid and uniform heating, enhanced cell membrane permeability via electroporation and improved mass transfer efficiency, contributing to a higher recovery of bioactive compounds compared to conventional methods [19,20,59]. Moreover, this study has provided new data on the optimal processing conditions for the extraction of phenolic and flavonoid compounds from ant nest, a medicinal plant traditionally used for its therapeutic properties. Simultaneous optimization of the ohmic Heating parameters confirmed the feasibility of this green and energy-efficient technology in the extraction of bioactive compounds, highlighting its potential for industrial applications in the nutraceutical and functional food sectors. ant nest extracts obtained through this method may serve as a valuable source of natural antioxidants with potential health benefits, including anti-inflammatory and disease-preventive effects [60,61]. Further studies are recommended to assess the bioavailability, stability and potential

therapeutic applications of these extracts in functional formulations [62].

One of the most significant observations of this research lies in the discovery that optimal extraction does not necessarily occur at the extreme ends of the operational parameters. Rather, intermediate holding times and gradient voltages were more effective in facilitating compound recovery. This finding suggests that an excessive increase in either parameter may lead to a decline in extraction efficiency, possibly due to the degradation of thermolabile compounds or the generation of unfavorable extraction conditions. Excessive holding time or elevated gradient voltage may promote oxidation or thermal degradation of sensitive phytochemicals, reducing the overall yield and compromising the functional quality of the extract [63]. This insight is consistent with the theoretical understanding of non-thermal extraction technologies, where excessive energy input can result in undesirable reactions that negatively impact bioactive content [63,64].

The combination of moderate holding time and gradient voltage appears to promote efficient cell wall disruption, enhancing mass transfer without inducing significant degradation of the targeted compounds. This synergistic effect of time and gradient voltage reflects the potential of electrically-assisted extraction systems to create selective and efficient pathways for compound release. By applying controlled electrical energy, the integrity of phenolic and flavonoid compounds can be preserved, while improving their diffusivity from the plant matrix into the solvent system [65,66]. The electroporation mechanism, in which electric fields increase membrane permeability, may be the primary contributor to this enhanced extraction efficiency, reducing the need for harsher mechanical or thermal processing methods [67-69].

In comparison to conventional extraction techniques such as maceration or Soxhlet extraction, which often require longer processing times and higher temperatures, the optimized approach developed in this study offers several advantages [70-72]. The reduced holding time and moderate gradient voltage input not only lower energy consumption but also minimize the risk of thermal degradation, contributing to a more sustainable and environmentally friendly process. Moreover, the high yields of total phenols and

flavonoids achieved under these conditions highlight the method's potential for producing high-quality extracts suitable for use in nutraceutical, pharmaceutical, and functional food applications.

The implications of this study extend beyond simple process optimization. The demonstrated ability to recover significant concentrations of phenolics and flavonoids under energy-efficient conditions suggests broader applicability in industries seeking to implement green extraction technologies. The focus on optimizing process parameters while maintaining extract quality aligns with the growing demand for sustainable processing methods in the food and pharmaceutical industries. Furthermore, the extraction method developed here could be adapted for other bioactive compounds and plant materials, offering a versatile platform for future innovation in bioactive recovery.

While the outcomes of this research are promising, several limitations and opportunities for further study remain. The current investigation focused primarily on two extraction variables: Holding time and gradient voltage. Further research could explore the influence of additional parameters such as solvent composition, pH and temperature, as well as the integration of hybrid technologies combining electrical input with other extraction techniques, such as ultrasound or microwave-assisted methods. Additionally, although this study focused on extraction efficiency, it is important to investigate the stability and bioactivity of the extracted compounds during storage and subsequent application. Understanding the long-term stability of these extracts will be critical for ensuring their effectiveness in commercial products. Lastly, while the laboratory-scale results are compelling, the transition from lab-scale optimization to industrial-scale production presents new challenges. Factors such as process scalability, equipment design and economic feasibility need to be addressed to confirm the viability of this method for large-scale manufacturing. In particular, scaling up ohmic heating systems introduces critical issues such as non-uniform electric field distribution, skin effects and increased heat losses, which may compromise heating efficiency and extraction performance [38]. These electrothermal complexities must be carefully considered in future studies through pilot-scale trials and numerical simulations. Nonetheless, the promising

results of this study provide a strong foundation for further research and development in this field.

While the applied voltage gradients (20 - 60 V/cm) successfully enhanced extraction performance, it is important to note that high electric fields may also induce electrochemical reactions such as water electrolysis, pH shifts and free radical generation, which could affect the stability of thermolabile compounds. These electrochemical effects were not measured in the present study, representing a limitation. Future work should focus on quantifying pH changes, oxidation-reduction potential (ORP) and hydroxyl radical formation during ohmic heating to better understand and control potential compound degradation. Despite the promising results, several limitations should be acknowledged. The exclusive use of water as the extraction solvent and the absence of antioxidant activity assays or stability studies constrain the full evaluation of extract quality. Additionally, the lack of mechanistic modeling and scale-up validation limits the engineering generalizability of the findings. Future studies should address these aspects by incorporating electrochemical measurements, thermal mapping, broader kinetic analyses, and functional assays to strengthen the process understanding and applicability.

Conclusions

In conclusion, this study offers valuable insights into the role of electrical parameters in optimizing the extraction of bioactive compounds. The optimized conditions—holding time of 195.883 s and gradient voltage of 52.761 volt—resulted in a maximum TPC of 256.145 mg GAE/g extract, TFC of 118.936 mg QE/g extract and extraction yield of 38.154%. By demonstrating that controlled gradient voltage and holding time can significantly enhance phenolic and flavonoid yields while maintaining energy efficiency, the findings contribute to the growing body of research supporting sustainable and high-yield extraction technologies. This work not only advances the scientific understanding of electrically-assisted extraction processes but also provides practical applications for industries focused on the production of functional bioactive ingredients.

Declaration of Generative AI in Scientific Writing

This work has been prepared with the assistance of generative AI and AI-assisted technologies. These tools were used solely to support language refinement, grammar correction, and formatting adjustments. All core ideas, arguments, analysis and conclusions presented in this document are the original work of the authors.

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