JOURNAL OF HORTICULTURE AND POSTHARVEST RESEARCH
2026, VOL. 9(2), 237-254

Journal of Horticulture and Postharvest Research

Journal Homepage: https://www.jhpr.birjand.ac.ir

University of Birjand

Optimization of polyphenol and flavonoids extraction from *Ocimum gratissimum* L. using response surface methodology

Tran Thien Hien[1,*], Tran Trong Kieu Ngoc[2], Nguyen Manh Ngoc Bao[1], Vo Trung Tinh[1] and Pham Nguyen Thuy Dung[1]

1, Institute of Applied Technology and Sustainable Development, Nguyen Tat Thanh University, Ho Chi Minh City, 70000 Vietnam
2, Faculty of Pharmacy, Nguyen Tat Thanh University, Ho Chi Minh City, 70000 Vietnam

ARTICLE INFO

Original Article

Article history:
Received 8 April 2025
Revised 14 July 2025
Accepted 17 July 2025

DOI: 10.22077/jhpr.2025.9175.1491
P-ISSN: 2588-4883
E-ISSN: 2588-6169

***Corresponding author:**
Institute of Applied Technology and Sustainable Development, Nguyen Tat Thanh University, Ho Chi Minh City, 70000 Vietnam.

Email: hientt@ntt.edu.vn

ABSTRACT

Purpose: The study was conducted to optimize the extraction process with high polyphenol and flavonoid content from *O. gratissimum* L. and evaluate their biological activities. **Research Method:** The extraction of *O. gratissimum* L. extract was performed by combining ultrasound-assisted and hot extraction methods. **Findings:** The results showed that extraction of *O. gratissimum* L. was performed under optimal conditions by response surface methodology (RSM), including ethanol concentration of 70%, raw material: ethanol ratio of 1: 35.71 ($g.g^{-1}$), time extraction of 69.77 min, and at a temperature of 89.82 °C, with a total polyphenol content and a total flavonoid content of 190.987 ($mgGAE.gDW^{-1}$) and 27.10 ($mgQE.gDW^{-1}$), respectively. The extract was concentrated by vacuum rotary evaporation to obtain the total extract which was subjected to evaluation for its physicochemical components and biological activities. The results showed that the total extract quality met the current standards of Vietnam without heavy metals and pesticide residues. In addition, the antibacterial, antioxidant and anti-inflammatory properties of the total extract sample from *O. gratissimum* L. also gave positive results, confirming the significant biological activity. **Research limitations:** No limitations were encountered. **Originality/Value:** *Ocimum gratissimum* L. is a medicinal herb widely used in traditional medicine across East Asia, especially in Vietnam. Due to the high content of physiochemical components, including essential oils and phenolic compounds such as polyphenols and flavonoids, *O. gratissimum* L. exhibits antioxidant, anti-inflammatory, and antibacterial properties. Therefore, studies on the biological activity of solution extracts and total extracts from *O. gratissimum* L. are essential for evaluating the potential applications in the fields of medicine, foods, and cosmetics.

Keywords:
Antibacterial, Flavonoid, *Ocimum gratissimum* l., Polyphenol, RSM

INTRODUCTION

Over the years, traditional medicine associated with natural herb has been recognized as a favorable alternative due to safety and effectiveness (Klein-Junior et al., 2021; Pathak et al., 2024). Scientists and medical experts at home and abroad are attracted to research and exploitation of herbs, medicinal herbs and natural pharmaceuticals. The process of researching and developing herbal medicine technologies with advanced improvements has made an important contribution to finding and synthesizing new compounds with broad-spectrum activity, simplicity, effectiveness, and minimizing toxic effects (Guo et al., 2023; Wang et al., 2019). Natural products will be continuously used to meet the urgent need for the development of effective drugs and will play a leading role in the discovery of drugs to treat human diseases, especially in the current period (Chopra & Dhingra, 2021).

O. gratissimum L. belongs to the Mint family, is a precious medicinal herb not only known as an important ingredient in nutritional cuisine but also plays a prominent role in traditional medicine in many countries (Azizah et al., 2023; Zahran et al., 2020). *O. gratissimum* L. is rich in secondary metabolites such as alkaloids, saponins, tannins, terpenoids, flavonoids, and polyphenols (Kpètèhoto et al., 2019; Ojewumi et al., 2024; Bhavani et al., 2019). The leaves contain a large amount of valuable chemical compounds, of which polyphenols are one of the most prominent components that help prevent many serious chronic diseases such as cardiovascular disease, diabetes, high fever, convulsions, and treat rheumatism. In addition, these phenolic compounds also have strong antioxidant effects, anti-inflammatory properties, and anti-cancer potential (Imosemi, 2020; Pandiri & Moni, 2018; Ugbogu et al., 2021). The presence of phenolic compounds in medicinal plants is important for their ability to fight bacteria that invade chronic wounds and promote wound healing. In Vietnam, *O. gratissimum* grows naturally in many places across the country, especially in the highland provinces (Dung et al., 2021; Tuan Anh et al., 2019). On average, one hectare of *O. gratissimum* planted at high density ($1m^2$/clump) can yield 12-15 tons of branches and leaves at a time of harvest, so one year can harvest three crops, each crop can yield an average of 20 tons of petals and flowers, so each year will yield about 60 tons of leaves. This shows that the source of raw materials for research and production of products from *O. gratissimum* L. is very large.

Nowadays, many researchers are conducting in-depth studies on the beneficial components of this plant that promote human health. In particular, they have focused more on discovering and identifying local plant species with bioactive compounds to develop extraction and purification processes as well as their applications in the pharmaceutical industry. Because *O. gratissimum* is a good source of phytochemicals, nutrients and valuable essential oils, bioactive isolates of this plant may be a better alternative to traditional medicine, which is used in the treatment of bacterial infections, coughs, cancers, diarrhea, anemia and inflammatory diseases (Ikpeazu et al., 2019; Olumide et al., 2019). In addition to its bioactive potential, *O. gratissimum* is also an excellent source of micronutrients (Airaodion et al., 2019). The reported clinical pharmacological activities exhibited by *O. gratissimum* are not limited to its antioxidant properties and its ability to suppress inflammatory biomarkers (Ajayi et al., 2019; Alabi et al., 2019). The presence of bioactive compounds demonstrates the diversity in the constituents of this plant. These differences arise due to variations in topography, meteorological conditions, productivity, preparation processes, and many other factors (Ebadi et al., 2017; Samadi et al., 2021). Therefore, there is a need to study the phytochemical properties of *O. gratissimum* to enhance health benefits (animal and human nutrition), as well as the environmental friendliness by using its isolated chemicals in natural weed and pest control management. In addition to these benefits, *O. gratissimum* is also

effective against diabetes, anemia, infertility, diarrhea, and inflammatory disorders (Adelakun et al., 2022; Edo et al., 2023).

For this reason, the study was conducted to optimize the extraction process and obtain the extract solution with high content of polyphenols and flavonoids from *O. gratissimum* by combining ultrasonic-assisted and hot extraction methods. Then, the antibacterial, antioxidant and anti-inflammatory activities of *O. gratissimum* total extract were also investigated.

MATERIALS AND METHODS

Plant materials

O. gratissimum L. sample was harvested in Lam Ha district, Lam Dong province (11° 55′ 26″ N 108° 11′ 31″ E) in September 2024.

Preparation of chemicals and equipment

Chemicals and solvents: Gallic acid standard, purity ≥ 98% (Sigma Aldrich Company, USA); Folin-Ciocalteu reagent (Nanjing Duly Biotech Company, China). Other solvents and chemicals in the laboratory such as ethanol, sodium carbonate was purchased from Anh San Trading and Service Company Limited (Ho Chi Minh City, Vietnam) and of analytical standards.

Research equipment: Memmert drying oven, moisture meter, V-730 UV-VIS spectrophotometer (Kern - Germany), ultrasonic bath (Daihan WUC-D22H, Korea), 1-5 mL micropipettes, volumetric flasks, beakers, triangular flasks, various types of test tubes, and other laboratory-standard instruments.

The process of *O. gratissimum* L. extraction

The extraction process was carried out according to (Kavyamala et al., 2023; Silva et al., 2015) with slight modifications. First, the leaves and small branches of *O. gratissimum* L. are taken, the old branches are removed and was washed many times with water, then allowed to dry at 50 °C for 24 hours. After drying, the raw materials were finely ground with a grinding device (PG2500, Vietnam) at a speed of 100 W for 1 minute. *O. gratissimum* L. powder was preliminarily extracted in ethanol solvent by ultrasonic assisted in water method with for 5 min at 70 °C. Next, the raw materials were extracted by soaking, heating and stirring with ethanol as the extraction solvent. Briefly, 1 g of *Ocimum gratissimum* L. powder was weighed and mixed with ethanol at different concentrations in a beaker and covered tightly with aluminum foil. Place the beakers on a magnetic stirrer, stir and extract for the time and temperature tested (Dlab MS-H340-S4, Dlab scientific, China). Finally, the mixture was filtered through a 0.45 µm filter paper using vacuum. The extracts were collected and evaluated the total polyphenol (TPC) and total flavonoid content (TFC). The extracted solution was then evaporated to obtain the total extract under vacuum at a temperature of 60 °C and an absolute pressure of 160-180 mBar. The obtained total extract was stored in sealed, dark bottles, in the laboratory's refrigerator for subsequent analysis.

Analytical methods

The analytical methods in this study were performed according to the descriptions of previous studies (Table 1).

Table 1. Analytical methods of extracts from *O. gratissimum* L.

Methods	Implementation process
Qualitative methods for natural compounds of extracts	Follow the regulations of the Vietnamese Pharmacopoeia and according to (Kavyamala et al., 2023).
Method for determining total polyphenol content (TPC)	Based on the method performed by (Zareiyan & Khajehsharifi, 2022) with slight modifications.
Method for determining total flavonoid content (TFC)	Based on the method performed (Zareiyan & Khajehsharifi, 2022) with slight modifications.
Antibacterial activity	Based on the method presented by (Alara et al., 2020) with slight modifications
Antioxidant activity	Based on the method presented by (Alara et al., 2020) with slight modifications
Anti-inflammatory activity	Based on the method presented by (Adekola et al., 2022) with slight modifications
Heavy metals content	According to section 9.4.11, page A-216 of the Vietnamese Pharmacopoeia, volume 2 (Vietnamese Pharmacopoeia, 2019)
Pesticide residues	According to section 12.17, page A-300 of the Vietnamese Pharmacopoeia, volume 2 (Vietnamese Pharmacopoeia, 2019)

Optimization of extraction process by response surface methodology (RSM)

The parameters affecting the extraction process after being evaluated will be optimized through the RSM model with the central matrix model design (CCD) (Belwal et al., 2016). The extract of *O. gratissimum* L. will be evaluated based on the value of total polyphenol and total flavonoid content. The evaluation factors of the model are divided into 5 levels: Central variable (0), low level (-1), high level (+1) and level (±α). Analysis of variance (ANOVA) was calculated using Design-Expert software (version 11, State Ease, Minneapolis, USA). The three independent factors evaluated are Solvent/raw material ratio (A), temperature (B), and time (C). ANOVA of the quadratic linear regression model was used to analyze the influence of input and output variables as well as the correlation of response functions and independent variables.

Data analysis

The experiments were repeated 3 times. Data values are expressed as mean ± SD (standard derivation). Apply Excel and Origin software in statistics, calculations, and determination of the best conditions.

RESULTS AND DISCUSSION

Botanical identification and preliminary assessment of medicinal quality

The sample of *Ocimum gratissimum* L. was identified morphologically and compared with the plant classification at the Ginseng and Medicinal Herbs Center of Ho Chi Minh City (Vietnam) (Fig. 1). After analyzing the 03 submitted samples, referring to existing documents, and comparing with the specimen of the species *Ocimum gratissimum* L. currently stored at the Royal Botanic Garden Museum in England, the scientific name of the sample was determined, specifically as follows:

Scientific name: *Ocimum gratissimum* L.

Synonym: *Ocimum gratissimum* var. suave (Willd) Hook.f. *Ocimum frutescens* Mill.

Botanical family: *Lamiaceae*

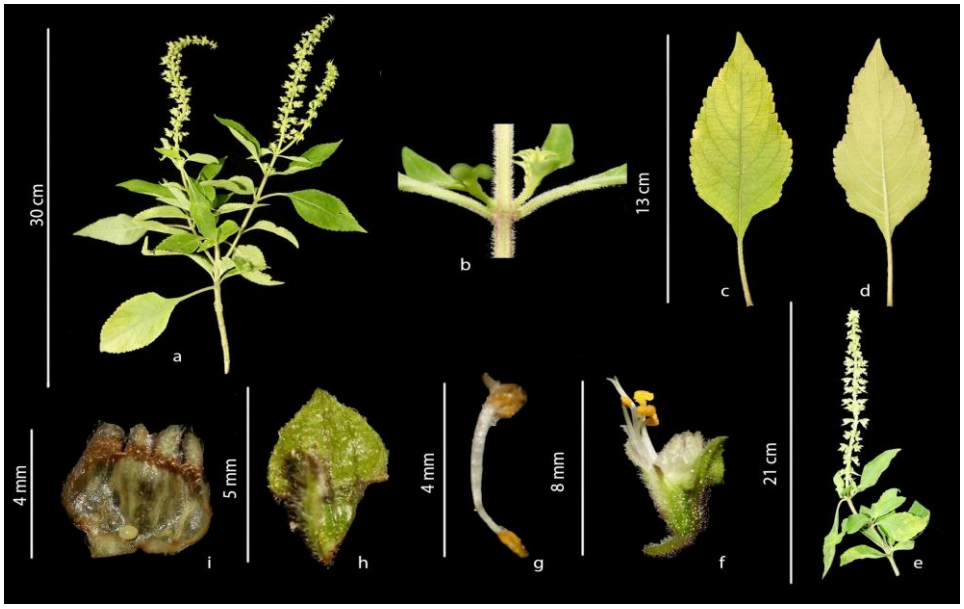


Fig. 1. *Ocimum gratissimum* L. sample for examination. a: Flowering branch; b: Stem; c, d: Front and back leaves; e: Inflorescence; f: Flower; g: Stamen; h: Calyx; i: Petal.

Ocimum gratissimum L. powder was (i) preliminarily evaluated the physical properties including: moisture content of raw materials, total ash and insoluble ash in HCl of raw materials; (ii) analyzed for qualitative chemical composition (e.g. tannin, saponin, phenolic, flavonoid, tamin, and alkaloids). The results showed that the moisture content of *O. gratissimum* L. raw material powder was quite low (< 5%), thus it was suitable for storage and use for experiments. In addition, the presence of phenolic, flavonoids, alkaloids, and tannins compounds were found in *O. gratissimum* leaves extraction with ethanol solvent (Table 2). However, saponin was not present in this extract. Similar results were reported by (Hamma et al., 2020; Kavyamala et al., 2023; Kpètèhoto et al., 2019; Okeke et al., 2023). This shows that the extract from *O. gratissimum* raw materials in this study has a complete scientific basis for subsequent experiment on quantifying TPC and TFC.

Factors affecting the extraction and recovery of polyphenols and flavonoids from *Ocimum gratissimum* L.

The present study investigated several factors affecting the extraction process, including solvent concentration, raw material:solvent ratio, extraction time, and extraction temperature. The TPC and TFC of the raw material samples were evaluated and compared based on the standard curve. Ethanol (EtOH) is a strong polar solvent that helps the extraction of polyphenol compounds to proceed smoothly, so it was chosen in this research (Ali Redha, 2021). In terms of ethanol concentration TPC tended to increase to the highest value of 103.85 ± 0.022 mgGAE.gDW^{-1} when the EtOH concentration increased from 50° to 70° (Fig. 2A). Higher EtOH concentrations (from 80° to 96°) reduced the extraction efficiency, possibly due to structural breakdown or reduced solubility of polyphenols. Similarly, the highest value of TFC (26.27 ± 1.138 mgGAE.gDW^{-1}) was obtained at the EtOH concentration of 70°. This suggests that the optimal alcohol concentration at 70° EtOH concentration is the ideal condition for extracting TPC and TFC at the highest content.

A high ratio of raw materials/EtOH helps increase the mass transfer rate due to a larger concentration difference, thereby speeding up the extraction process (Djuraev et al., 2021). The more solvent, the more active ingredients are extracted, the higher the efficiency of the

extraction process (Jha & Sit, 2022). However, in many cases, increasing the solvent ratio: raw material excessively reduces the extraction efficiency as well as increases the content of impurities. The effect of solvent volume was exhibited in Figure 2B. When increasing the solvent content, TPC and TFC gradually increased. However, at a certain stage, this content tended to decrease insignificantly. The results showed that TPC and TFC obtained the optimal value at the ratio of 1:35 ($g.g^{-1}$) (TPC = 113.34 ± 0.364 $mgGAE.gDW^{-1}$; TFC = 17.34 ± 0.208 $mgQE.gDW^{-1}$). Therefore, the ratio of materials/EtOH at 1:35 ($g.g^{-1}$) was chosen to evaluate the following factors.

In addition to the concentration and ratio of EtOH, in this study, the temperature conditions of room temperature, 50 °C, 60 °C, 70 °C and 80 °C were investigated. Figure 4C showed that TPC and TFC tended to increase gradually as the extraction temperature increased. Specifically, the TPC increased from 121.33 ± 0.195 $mgGAE.gDW^{-1}$ at ambient temperature to 163.03 ± 0.273 $mgGAE.gDW^{-1}$ at 70 °C, the TFC increased from 11.34 ± 0.13 $mgQE.gDW^{-1}$ to 24.34 $mgQE.gDW^{-1}$ under the same conditions as above. However, when the temperature increased to 80 °C, the TPC and TFC decreased (141.92 ± 2.161 $mgGAE.gDW^{-1}$ and 21.17 ± 0.885 $mgQE.gDW^{-1}$, respectively). The reason may be that the increased temperature caused these compounds to decompose during the extraction process over a long period of time (Antony & Farid, 2022; Jha & Sit, 2022). Therefore, the temperature of 70 °C was chosen as the optimal temperature for the extraction of compounds in the following experiments.

The fourth factor affecting the extraction process from *O. gratissimum* L. is time. In this experiment, the following conditions were kept constant: temperature of 70 °C, and the ratio of raw materials of 1:35 ($g.g^{-1}$) with the evaluated extraction times being 15 min, 30 min, 60 min, 90 min and 120 min (Fig. 2D). The results showed that TPC and TFC increased gradually as the extraction time increased from 15 min to 90 min and tended to decrease gradually as the time increased to 120 minutes. At 90 min, TPC reached the highest value of 188.81 ± 2.356 $mgGAE.gDW^{-1}$ and TFC reached the highest with 25.22 ± 0.26 $mgQE.gDW^{-1}$. This was the value recorded as optimal for the highest TPC and TFC. Therefore, an extraction time of 90 min was chosen as the condition to conduct the optimization process for the extraction of polyphenol compounds from *O. gratissimum* L. using RSM.

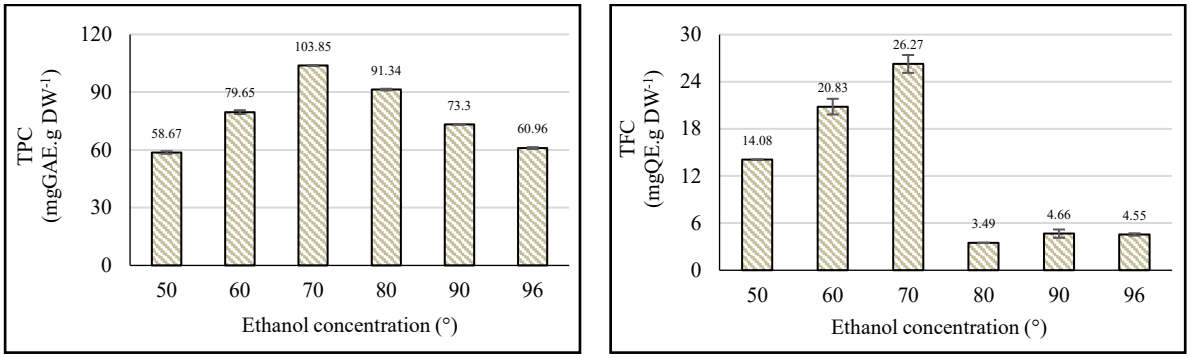
Optimization of extraction process of *Ocimum gratissimum* L. by RSM

The extraction process was optimized through the RSM model with CCD as described in Table 2. The TPC and TFC of *O. gratissimum* L. extract were investigated. ANOVA of the quadratic linear regression model was used to analyze the influence of input and output variables as well as the correlation of response functions and independent variables.

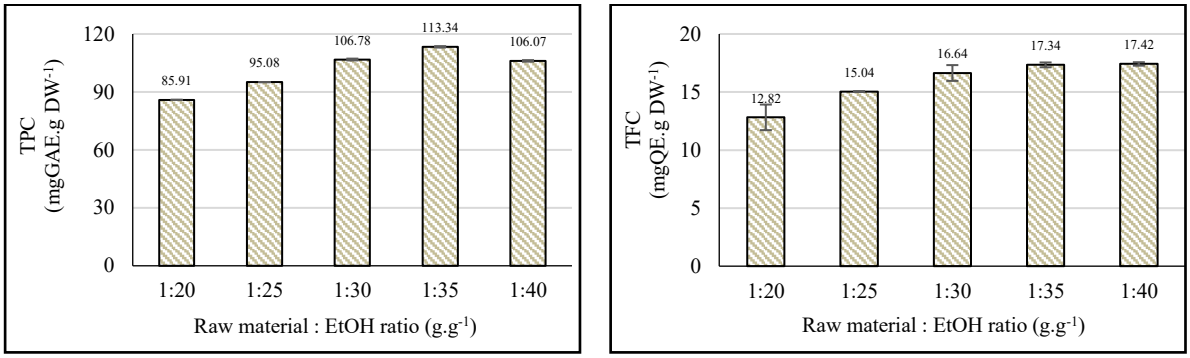
Based on the results of Table 3 and Figure 3, the two response factors (TPC and TFC) was closely dependent on the independent variables (EtOH:material ratio; extraction temperature, and extraction time). The highest TPC (191.67 $mgGAE.gDW^{-1}$) and TFC (27.42 $mgQE.gDW^{-1}$) were obtained at a ratio of 35:1 for 90 min at 70 °C. The results from DX11 software give the equation expressing the relationship between the response value and the independent variables:

$$TPC\ (mgGAE.gDW\text{-}1) = +188.96 + 13.84\times A - 9.09\times B + 6.37\times C - 2.92\times AB + 5.05\times AC + 4.30\times BC - 15.28\times A^2 - 14.62\times B^2 - 24.25\times C^2$$

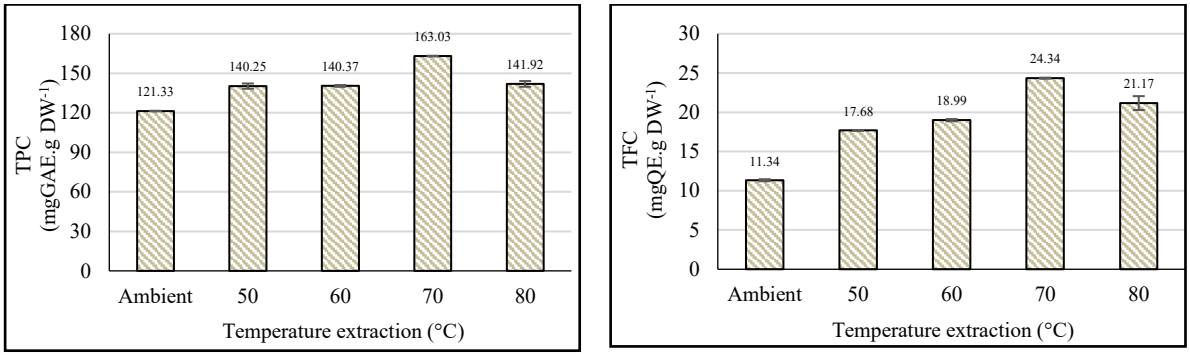
$$TFC\ (mgQE.gDW\text{-}1) = +26.40 + 1.28\times A + 0.3587\times B - 0.8770\times C + 0.3300\times AB - 0.3150\times AC + 0.2650\times BC - 4.11\times A^2 - 5.09\times B^2 - 2.55\times C^2$$



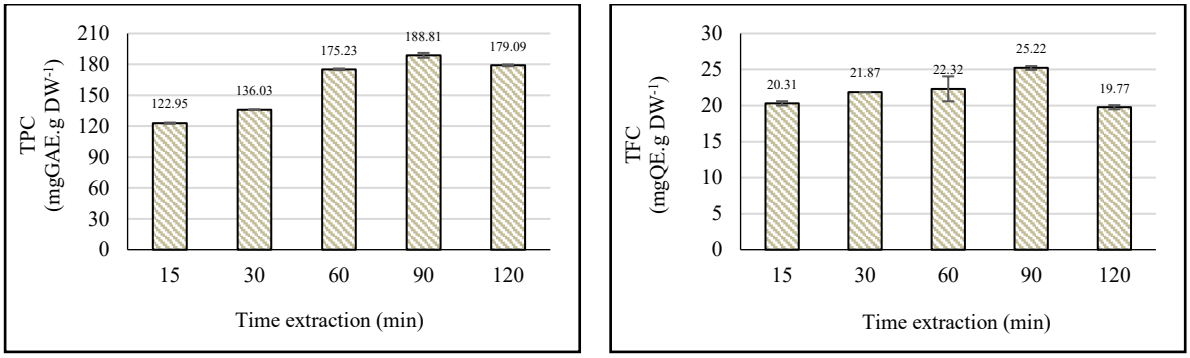
A



B



C



D

Fig. 2. Factors affecting TPC and TFC contents of the extraction process from *O. gratissimum* L. (A. Ethanol concentration factor, B. Raw materials and EtOH ratio factor, C. Temperature extraction factor, D. Time extraction factor).

Table 2. Matrix of independent variables and survey levels by RSM.

No.	Independent factor	Symbol	Survey level				
			-α	-1	0	+1	+α
1	EtOH:RM ($g.g^{-1}$)	A	29.95:1	32:1	35:1	38:1	40.04:1
2	Temperature (°C)	B	62	65	70	75	78
3	Time (min)	A	73	80	90	100	107

Table 3. Matrix table of experimental and predicted values (TPC & TFC) of the extract *Ocimum gratissimum* L.

No.	A ($g.g^{-1}$)	B (°C)	C (min)	TPC ($mgGAE.gDW^{-1}$)		TFC ($mgQE.gDW^{-1}$)	
				Actual	Prediction	Actual	Prediction
1	32:1	65	80	130.19	130.12	14.23	14.18
2	38:1	65	80	153.81	153.58	16.75	16.70
3	32:1	75	80	109.58	109.17	13.73	13.71
4	38:1	75	80	120.54	120.93	17.58	17.55
5	32:1	65	100	124.70	124.17	12.55	12.52
6	38:1	65	100	167.53	167.79	13.82	13.79
7	32:1	75	100	120.33	120.44	13.12	13.11
8	38:1	75	100	152.45	152.38	15.70	15.70
9	29.95:1	70	90	121.99	122.46	12.60	12.64
10	40.04:1	70	90	169.29	169.03	16.89	16.93
11	35:1	62	90	162.62	162.91	11.34	11.41
12	35:1	78	90	132.41	132.33	12.60	12.61
13	35:1	70	73	109.53	109.66	20.60	20.66
14	35:1	70	107	131.03	131.10	17.70	17.71
15	35:1	70	90	190.33	188.96	26.40	26.40
16	35:1	70	90	191.17	188.96	27.38	26.40
17	35:1	70	90	187.89	188.96	26.41	26.40
18	35:1	70	90	186.03	188.96	27.42	26.40
19	35:1	70	90	191.67	188.96	25.39	26.40
20	35:1	70	90	186.72	188.96	25.40	26.40

ANOVA analysis was used to evaluate the model and the results are shown in Table 4. The regression equations of TPC and TFC are quadratic and achieve a good correlation with the statistically significant variance analysis ($p<0.05$). This shows that this model is suitable and can accurately predict the research objective function (TPC and TFC). The TPC and TFC equations also show that the parameters can explain the changes in the TPC ($R^2 = 0.9983$) and TFC ($R^2 = 0.9937$) objective functions with a fairly high correlation coefficient. This shows the close correlation between the predicted TPC, TFC and the TPC, TFC obtained in practice. If only considering the correlation coefficient R^2, a multivariate regression model by the response surface method is considered good when the minimum R^2 coefficient is 0.8; This value is much lower than the R^2 of the TPC and TFC objective functions in this study. Furthermore, the values of the coefficient of variation (CV) of the TPC and TFC objective functions are 1.14% and 3.46%, respectively, for the proposed model, which is less than 10%, with high accuracy and reliability of the testing process. The coefficients in the regression equations of TPC and TFC all have statistically significant differences ($p<0.05$), except for the value of the coefficient showing the interaction between the ethanol: raw material ratio and the extraction temperature (AB) ($p>0.05$) for TPC; and for TPC, the values are B, AB, AC, BC, respectively. The models of TPC and TFC have a quadratic function form that is

completely dependent on independent factors such as the solvent: raw material ratio, temperature and extraction time.

Figure 3 demonstrates that the optimal conditions for solvent:material ratio, temperature, and extraction time after applying DX11 optimization software were achieved. Accordingly, the optimal point is the solvent:material ratio of 35.71:1 ($g.g^{-1}$), extraction temperature at 69.77 (°C), and extraction time of 89.82 min to achieve the highest desirability of 0.983. To confirm the optimal conditions, this study was conducted an extraction experiment of *O. gratissimum* L. powder under those conditions and the results are shown the actual TPC and TFC contents obtained were 190.987 ($mgGAE.gDW^{-1}$) and 27.10 ($mgQE.gDW^{-1}$), respectively.

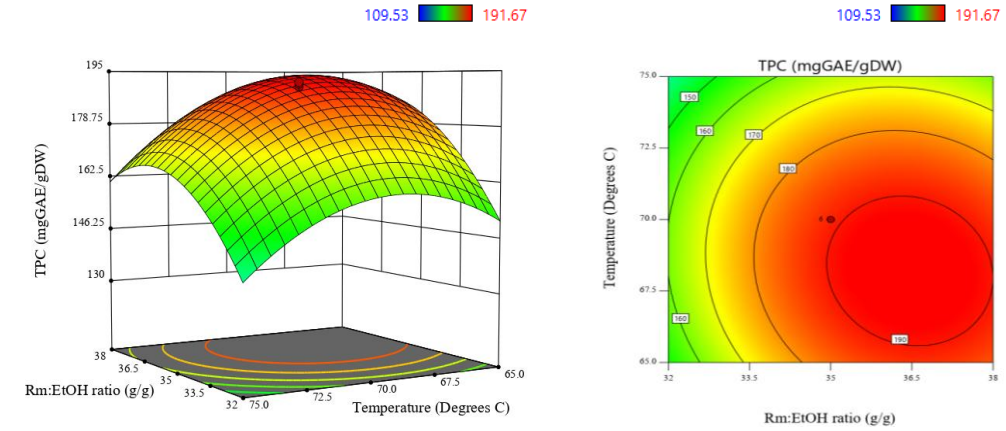
Evaluation of heavy metals content and pesticide residues on the extract from *Ocimum gratissimum* L.

This study also analyzes the heavy metals content and pesticide residues of the total extract sample at The Ky Moi Science and Technology Service Joint Stock Company (HCMC, Vietnam). The results shown that no mercury (Hg) content was detected in the extract sample, lead (Pb = 0.06 mg.kg-1) and arsenic (As = 0.15 mg.kg-1) content detected was within the allowable detection limit according to Vietnamese standards on National technical regulation on the limits of heavy metals contamination in food with the allowed limit of Pb ≤ 1.0 (mg.kg-1) and As ≤ 0.3 (mg.kg-1) (QCVN 8-2:2011/BYT, 2011). Pesticide groups such as Diazinon, Fenvalerate and Dichlovos are commonly used in spraying pesticides to kill leaf-eating pests on vegetable crops. This study also analyzed the residue indicators of pesticides belonging to the above 3 groups (Taghizadeh et al., 2022). The results showed that the test samples did not contain excessive toxic substances. This is a positive result of the test samples meeting safety standards for heavy metals and pesticide residues.

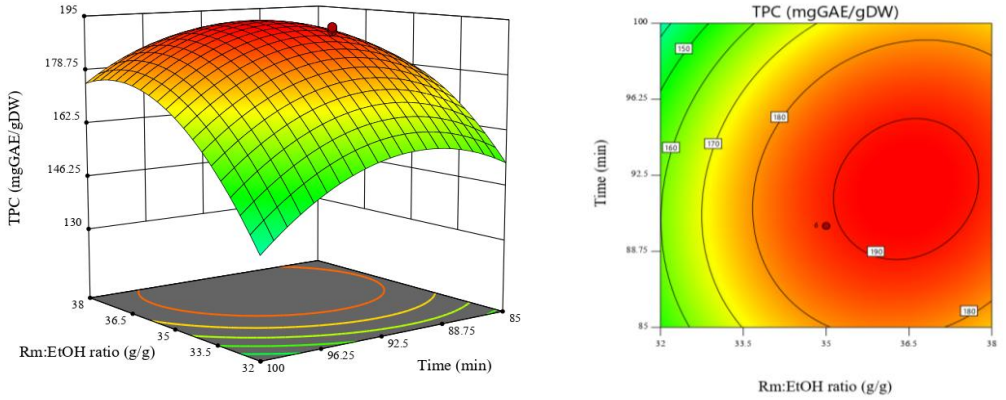
Table 4. ANOVA analysis for quadratic regression model.

Source	ANOVA Analysis of TPC					ANOVA Analysis of TFC				
	Sum of Squares	df	Mean Square	F-value	p-value	Sum of Squares	df	Mean Square	F-value	p-value
Model	17396.13	9	1932.90	644.16	< 0.0001	641.67	9	71.30	176.41	< 0.0001
A-Ratio	2617.79	1	2617.79	872.40	< 0.0001	22.26	1	22.26	55.07	< 0.0001
B-Temp	1128.37	1	1128.37	376.04	< 0.0001	1.76	1	1.76	4.35	0.0636
C-Time	554.85	1	554.85	184.91	< 0.0001	10.50	1	10.50	25.99	0.0005
AB	68.27	1	68.27	22.75	0.0008	0.8712	1	0.8712	2.16	0.1728
AC	203.72	1	203.72	67.89	< 0.0001	0.7938	1	0.7938	1.96	0.1913
BC	148.18	1	148.18	49.38	< 0.0001	0.5618	1	0.5618	1.39	0.2657
A^2	3364.86	1	3364.86	1121.37	< 0.0001	242.93	1	242.93	601.08	< 0.0001
B^2	3079.24	1	3079.24	1026.18	< 0.0001	372.91	1	372.91	922.68	< 0.0001
C^2	8472.24	1	8472.24	2823.45	< 0.0001	93.59	1	93.59	231.56	< 0.0001
Residual	30.01	10	3.00			4.04	10	0.4042		
Lack of Fit	1.15	5	0.2309	0.0400	0.9985	0.0206	5	0.0041	0.0051	1.0000
Pure Error	28.85	5	5.77			4.02	5	0.8042		
Cor Total	17426.14	19				645.71	19			

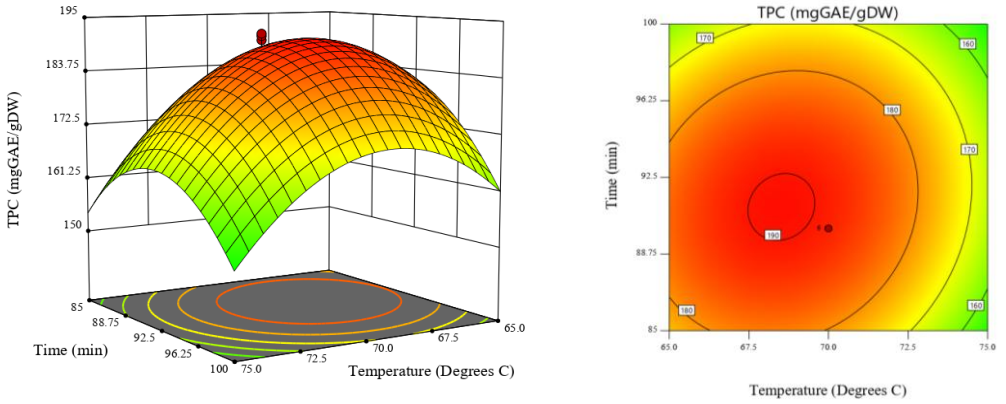
	TPC	TFC	TPC		TFC	
Std. Dev.	1.73	0.6357	R^2	0.9983	R^2	0.9937
Mean	151.99	18.38	Adjusted R^2	0.9967	Adjusted R^2	0.9881
C.V. %	1.14	3.46	Predicted R^2	0.9971	Predicted R^2	0.9908
			Adeq Precision	65.1402	Adeq Precision	33.3482


109.53
191.67
TPC (mgGAE/gDW)
195
178.75
162.5
146.25
130
38
36.5
35
33.5
32
Rm:EtOH ratio (g/g)
75.0
72.5
70.0
67.5
65.0
Temperature (Degrees C)
109.53
191.67
TPC (mgGAE/gDW)
Temperature (Degrees C)
Rm:EtOH ratio (g/g)

A1


TPC (mgGAE/gDW)
195
178.75
162.5
146.25
130
38
36.5
35
33.5
32
Rm:EtOH ratio (g/g)
100
96.25
92.5
88.75
85
Time (min)
TPC (mgGAE/gDW)
Time (min)
Rm:EtOH ratio (g/g)

A2


TPC (mgGAE/gDW)
195
183.75
172.5
161.25
150
85
88.75
92.5
96.25
100
Time (min)
75.0
72.5
70.0
67.5
65.0
Temperature (Degrees C)
TPC (mgGAE/gDW)
Time (min)
Temperature (Degrees C)

A3

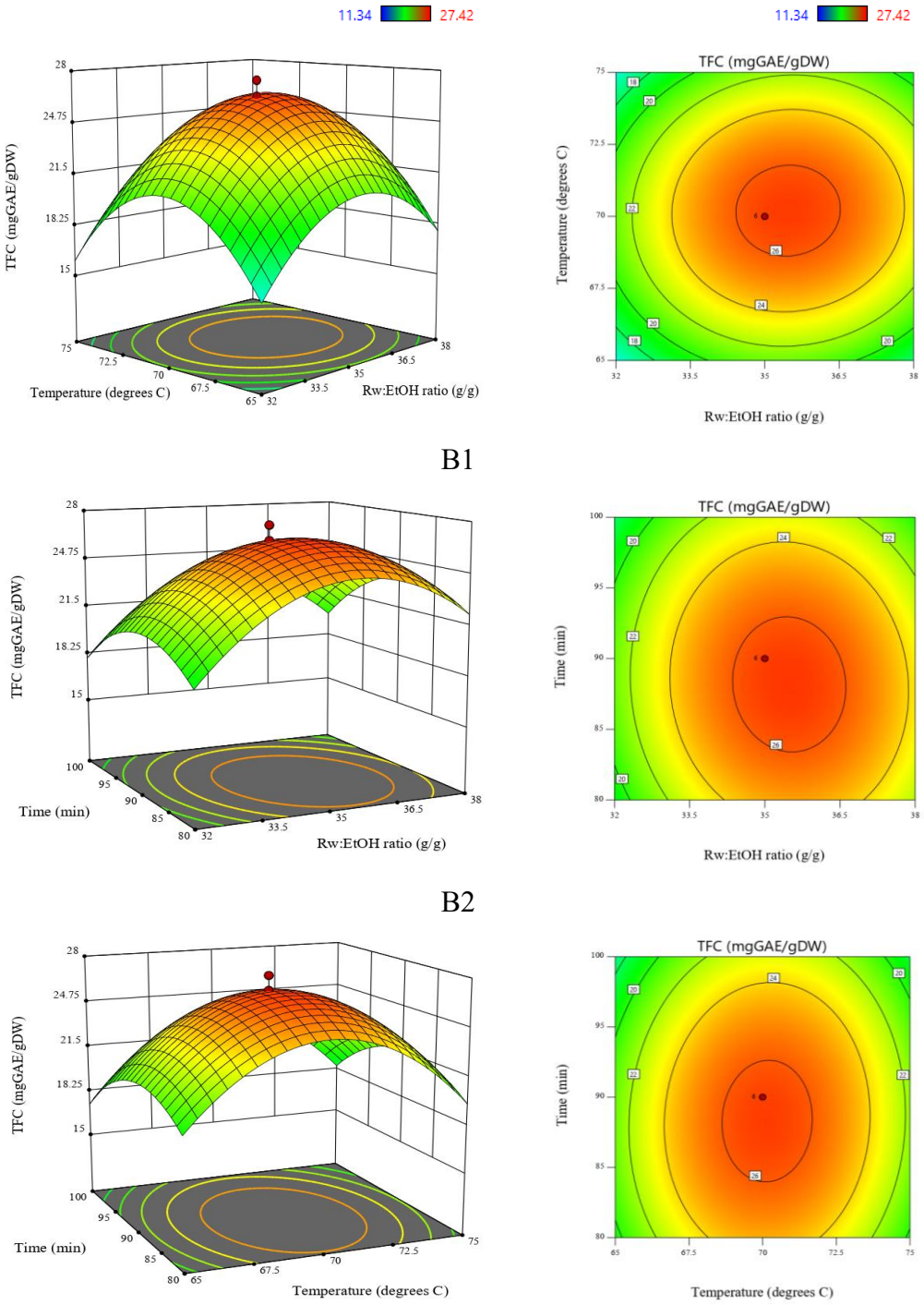


Fig. 3. Three-dimensional plot for optimal surface response of TPC and TFC. Interaction between raw materials: EtOH ratio and extraction temperature on TPC (A1), on TFC (B1); interaction between raw materials: EtOH ratio and extraction time on TPC (A2), on TFC (B2); interaction between extraction temperature and extraction time on TPC (A3), on TFC (B3).

Evaluation of antioxidant activity, antibacterial ability; anti-inflammatory ability of the extract *O. gratissimum* L.

Evaluation of antioxidant activity of total extract

DPPH and ABTS scavenging methods are commonly used for evaluating the antioxidant activity of biological compounds (Wołosiak et al., 2021). The DPPH method is based on the color change from deep purple to light yellow when the DPPH free radical is reduced by substances capable of donating electrons or hydrogen atoms (Gulcin & Alwasel, 2023). This is a simple, rapid method and is often used to determine the ability to reduce free radicals in organic solvents. In contrast, the ABTS method generates $ABTS^+$ free radicals through oxidation by strong oxidants such as persulfate, and then measures the green color reduction of $ABTS^+$ when reduced by antioxidants (Lang et al., 2024). This method is suitable for both aqueous and organic solvents, so it has a wider range of applications. Both methods provide quantitative data on antioxidant capacity through the IC_{50} value, which represents the concentration required to inhibit 50% of free radicals, thereby helping to evaluate the effectiveness of antioxidant compounds in the study.

The IC_{50} values of both DPPH (IC_{50} = 544.432 µg.ml^{-1}) and ABTS (IC_{50} = 98.388 µg.ml^{-1}) of *O. gratissimum* total extract were significantly higher than the IC_{50} values of the Vitamin C sample (IC_{50} = 27.463 µg.ml^{-1} and 13.087 µg.ml^{-1}). A high IC_{50} value reflect that the TPC and TFC in the extract is not high, and the antioxidant activity not strong. In addition, when comparing the DPPH and ABTS methods with the same *O. gratissimum* L. extract sample, it was found that the $IC_{50\ DPPH}$ value was much larger than the $IC_{50\ ABTS}$ value, which can be understood that the free radical scavenging ability of the total extract by the ABTS method is better DPPH method. The reason may be due to the different reaction mechanisms between ABTS and DPPH, or the chemical composition of *O. gratissimum* has stronger activity towards $ABTS^{+\bullet}$ radicals. DPPH is active in hydrophobic environments (mainly ethanol, methanol) and reacts with non-polar antioxidant compounds. ABTS can be active in both hydrophobic and aqueous environments, so it can scavenge free radicals with a wider range of compounds, including polar compounds. At a concentration of 2000 µg.ml^{-1}, the DPPH and ABTS radical scavenging activities of the total extract from *O. gratissimum* were 74.623% and 87.970%, respectively, which confirms that the ABTS scavenging ability was higher than DPPH. Future investigations should consider optimizing the solvent-to-plant ratio to evaluate its influence on the antioxidant capacity and extraction efficiency of *O. gratissimum* L.

Antibacterial activity of the total extract

The antibacterial activity of *O. gratissimum* L. total extract was evaluated against *E. coli* and *S. aureus* by the agar disc diffusion method. Many studies have shown that the content of polyphenols and flavonoids in plants is associated with antibacterial ability. *O. gratissimum* is known for many different uses in traditional medicine, gastrointestinal infections (diarrhea, dysentery), skin infections (dermatitis, eczema, scabies), upper respiratory tract infections, cough, asthma, bronchitis, wounds and ulcers, insect bites, nosebleeds, stroke, and anemia. Results from the present study have shown that all crude extracts of *O. gratissimum* L.exhibited inhibitory activity against both Gram-positive and Gram-negative bacteria, which is similar to (Ugbogu et al., 2021).

The results presented that the *O. gratissimum* extract using ethanol 70° solvent has antibacterial ability against both *E. coli* and *S. aureus* strains, as shown by the inhibition zone diameter of 10.15 ± 0.03 mm and 11.80 ± 0.07 mm, respectively. This proves that the total extract from *O. gratissimum* has antibacterial ability against gram-negative and gram-positive bacteria, which is also consistent with the studies of (Bhavani et al., 2019).

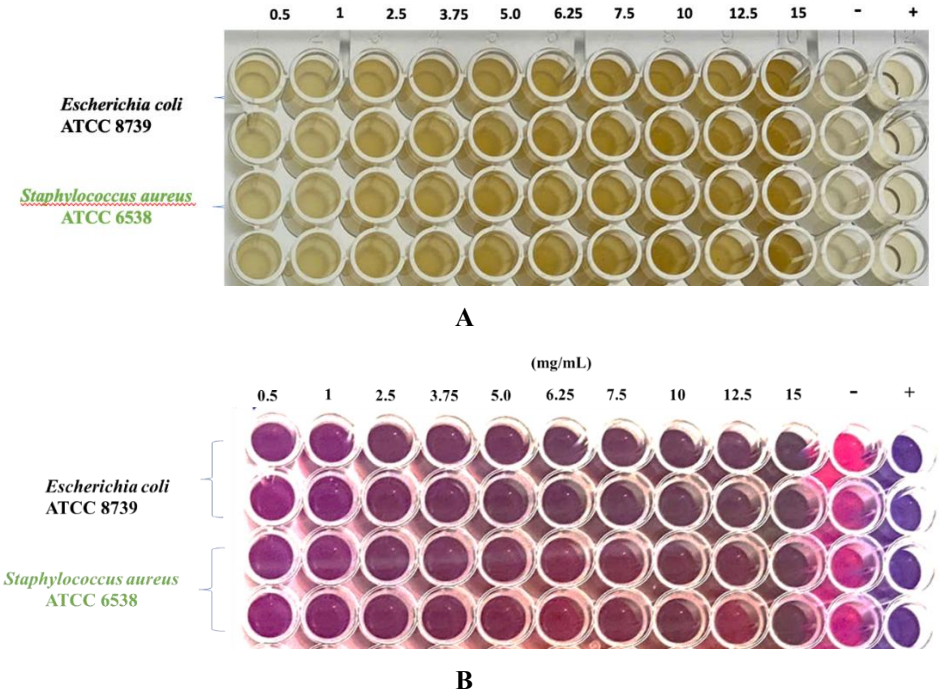


Fig. 4. MIC of total *O. gratissimum* extract at different concentrations on *E. coli* and *S. aureus strains* (A: before reagent preparation; B: after reagent preparation)

In addition, this study also combines the agar disc diffusion method with MIC method to evaluate the antibacterial activity of the total extract from *O. gratissimum*. The sample was diluted to a concentration range from 0.5 to 15 mg.ml^{-1}. As the concentration of total extract decreased, the MIC of the total extract from *O. gratissimum* was 2.5 mg.ml^{-1} and 2.5-3.75 mg.ml^{-1} for two strains of *E. coli* and *S. aureus*, respectively. Compared with standard antibiotics, this concentration may not be strong enough to effectively treat infections caused by *E. coli* and *S. aureus*. At the concentration of 1.0 mg.ml^{-1}, the solution began to turn to light pink, indicating that the anti-bacterial activity was slightly exhibited. The total extract at a concentration of 5 mg.ml^{-1} completely inhibited *E. coli* bacteria. The total extract at a concentration of 6.25 mg.ml^{-1} gradually decreased for *S. aureus* bacteria.

Anti-inflammatory activity of O. gratissimum total extract

Protein denaturation is the process by which native proteins lose their tertiary and secondary structures due to disruption of hydrogen, hydrophobic, and disulfide bonds (Sridevi et al., 2015). Protein denaturation is a well-established cause of tissue inflammation, associated with symptoms such as redness, pain, heat, and swelling (Kumar et al., 2018). Therefore, the compounds that inhibit heat-induced protein denaturation have the potential for the use as therapeutic anti-inflammatory drugs (Dharsana & Mathew, 2014). In this study, the potential of *O. gratissimum* total extract to inhibit protein denaturation was investigated by using the heat-induced inhibition of bovine serum albumin (BSA) denaturation method. The results from Figure 10 showed that at the highest concentration of 5000 µg/mL, the inhibition percentage reached 71.66%. The IC_{50} value at 1765.246 µg.ml^{-1} indicated that the protein denaturation inhibitory activity of *O. gratissimum* was weaker than diclofenac.

CONCLUSION

In this study, the total extract from *O. gratissimum* L. was prepared by using 70% ethanol solvent with the assistance of ultrasound. The preliminary evaluation indicated the presence of natural compounds such as phenolics, alkaloids, saponins, flavonoids, and terpenoids in the total extract of *O. gratissimum* L. After using RSM with the optimal values such as: the ratio of raw materials: ethanol of 1: 35.71 ($g.g^{-1}$), the time of 69.77 minu and the temperature of 89.82 °C, the TPC and the TFC was obtained as 190.987 ($mgGAE.gDW^{-1}$) and 27.10 ($mgQE.gDW^{-1}$), respectively. Then, the total extract was analyzed for heavy metal content and pesticide residues. Results have shown that no mercury (Hg) was detected in the extract sample, while lead (Pb) and arsenic (As) content was detected at the allowable detection limit according to QCVN 8-2:2011/BYT. Pesticide groups such as Diazinon, Fenvalerate and Dichlovos were not detected in the total extract sample. Finally, the total extract sample also exhibited lower antibacterial, antioxidant and anti-inflammatory properties than the standard substances. Findings from the present study have shown that *O. gratissimum* can be potential for application in the fields of medicine, nutrition and aesthetics.

Conflict of interest

The authors at this moment hereby declare that there is no conflict of interest.

Acknowledgments

This work was supported by Nguyen Tat Thanh University; Ho Chi Minh City, Vietnam provides the required facilities to carry out this work

REFERENCES

Adekola, M. B., Areola, J. O., Fagbohun, O. F., Asaolu, F. T., Ogundepo, G. E., Fajobi, A. O., & Babalola, O. O. (2022). In-vitro antioxidant and anti-inflammatory activities of ethanol stem-bark extract of Blighia sapida K.D. Koenig. *Journal of Pharmaceutical Analysis, 12*(2), 350–354. https://doi.org/10.1016/j.jpha.2021.04.002

Adelakun, S. A., Ogunlade, B., Akintunde, O. W., & Omilachi, V. O. (2022). Long-term exposure to cimetidine induced gonado-toxicity in male rats: modulating role of *Ocimum gratissimum*. *Revista Internacional de Andrología, 20*, S2–S16. https://doi.org/10.1016/j.androl.2020.10.012

Airaodion, A. I., Ibrahim, A. H., Ogbuagu, U., Ogbuagu, E. O., Awosanya, O. O., Akinmolayan, J. D., Njoku, O. C., Obajimi, O. O., Adeniji, A. R., & Adekale, O. A. (2019). Evaluation of phytochemical content and antioxidant potential of *Ocimum gratissimum* and *Telfairia occidentalis* leaves. *Asian Journal of Research in Medical and Pharmaceutical Sciences*, 1–11. https://doi.org/10.9734/ajrimps/2019/v7i130110

Ajayi, A., Ben-Azu, B., Onasanwo, S., Adeoluwa, O., Eduviere, A., & Ademowo, O. (2019). Flavonoid-Rich fraction of *Ocimum gratissim*um attenuates lipopolysaccharide-induced sickness behavior, inflammatory and oxidative stress in mice. *Drug Research, 69*(03), 151–158. https://doi.org/10.1055/a-0654-5042

Alabi, A. O., Ajayi, A. M., Omorogbe, O., & Umukoro, S. (2019). Anti-nociceptive and anti-inflammatory effects of an aqueous extract of blended leaves of Ocimum gratissimum and Psidium guajava. *Clinical Phytoscience, 5*(1), 34. https://doi.org/10.1186/s40816-019-0130-2

Alara, O. R., Abdurahman, N. H., Ukaegbu, C. I., & Alara, J. A. (2020). Optimization of microwave-assisted extraction of phenolic compounds from Ocimum gratissimum leaves

and its LC–ESI–MS/MS profiling, antioxidant and antimicrobial activities. *Journal of Food Measurement and Characterization, 14*(6), 3590–3604. https://doi.org/10.1007/s11694-020-00602-1

Ali Redha, A. (2021). Review on extraction of phenolic compounds from natural sources using green deep eutectic solvents. *Journal of Agricultural and Food Chemistry, 69*(3), 878–912. https://doi.org/10.1021/acs.jafc.0c06641

Antony, A., & Farid, M. (2022). Effect of temperatures on polyphenols during extraction. *Applied Sciences*, 12(4), 2107. https://doi.org/10.3390/app12042107

Azizah, N. S., Irawan, B., Kusmoro, J., Safriansyah, W., Farabi, K., Oktavia, D., Doni, F., & Miranti, M. (2023). Sweet basil (*Ocimum basilicum* L.)—a review of its botany, phytochemistry, pharmacological activities, and biotechnological development. *Plants, 12*(24), 4148. https://doi.org/10.3390/plants12244148

Belwal, T., Dhyani, P., Bhatt, I. D., Rawal, R. S., & Pande, V. (2016). Optimization extraction conditions for improving phenolic content and antioxidant activity in Berberis asiatica fruits using response surface methodology (RSM). *Food Chemistry*, 207, 115–124. https://doi.org/10.1016/j.foodchem.2016.03.081

Bhavani, T., Mohan, R. R., Mounica, C., Nyamisha, J., Krishna, A. G., Prabhavathi, P., Raja, D. R. R., & Baba, D. K. H. (2019). Phytochemical screening & antimicrobial activity of Ocimum gratissimum review. *Journal of Pharmacognosy and Phytochemistry, 8*(2), 77–79.

Chopra, B., & Dhingra, A. K. (2021). Natural products: A lead for drug discovery and development. *Phytotherapy Research, 35*(9), 4660–4702. https://doi.org/10.1002/ptr.7099

Dharsana, J. N., & Mathew, S. M. (2014). Preliminary sceening of Anti inflammatory and Antioxidant activity of Morinda umbellata. *International Journal of Pharmacy & Life Sciences, 5*(8), 3774–3779.

Djuraev, K., Yodgorova, M., Usmonov, A., & Mizomov, M. (2021). Experimental study of the extraction process of coniferous plants. *IOP Conference Series: Earth and Environmental Science, 839*(4), 042019. https://doi.org/10.1088/1755-1315/839/4/042019

Dung, P. N. T., Dao, T. P., Le, T. T., Tran, H. T., Dinh, T. T. T., Pham, Q. L., Tran, Q. T., & Pham, M. Q. (2021). Extraction and analysis of chemical composition of Ocimum gratissimum L essential oil in the North of Vietnam. *IOP Conference Series: Materials Science and Engineering, 1092*(1), 012092. https://doi.org/10.1088/1757-899X/1092/1/012092

Ebadi, M. T., Sefidkon, F., Azizi, M., & Ahmadi, N. (2017). Packaging methods and storage duration affect essential oil content and composition of lemon verbena (*Lippia citriodora* Kunth.). *Food Science and Nutrition, 5*(3), 588–595. https://doi.org/10.1002/fsn3.434

Edo, G. I., Samuel, P. O., Ossai, S., Nwachukwu, S. C., Okolie, M. C., Oghenegueke, O., Asaah, E. U., Akpoghelie, P. O., Ugbune, U., Owheruo, J. O., Ezekiel, G. O., Onoharigho, F. O., & Agbo, J. J. (2023). Phytochemistry and pharmacological compounds present in scent leaf: A review. *Food Chemistry Advances, 3*, 100300. https://doi.org/10.1016/j.focha.2023.100300

Gulcin, İ., & Alwasel, S. H. (2023). DPPH Radical Scavenging Assay. *Processes, 11*(8), 2248. https://doi.org/10.3390/pr11082248

Guo, M., Qin, S., Wang, S., Sun, M., Yang, H., Wang, X., Fan, P., & Jin, Z. (2023). Herbal medicine nanocrystals: a potential novel therapeutic strategy. *Molecules, 28*(17), 6370. https://doi.org/10.3390/molecules28176370

Hamma, I. I., Tafinta, I. Y., Abdulmalik, A., Theophilus, J., & Abubakar, M. (2020). Phytochemical screening and antibacterial activity of the crude extract of scent leaf

(*Ocimum gratissimum*) on *Escherichia coli* and *Staphylococcus aureus*. *Asian Plant Research Journal*, 1–7. https://doi.org/10.9734/aprj/2020/v5i230101

Ikpeazu, V. O., Emmanuel, O., Ekweogu, C. N., Akara, E. U., & Ugbogu, E. A. (2019). A comparative nutritional assessments of leaf extracts of *Ocimum gratissimum* and *Solanum aethiopicum*. *American Journal of Biomedical Research, 7*(2), 27–31. https://doi.org/10.12691/ajbr-7-2-1

Imosemi, I. O. (2020). a Review of the Medicinal Values , Pharmacological Actions , Morphological Effects and Toxicity of Ocimum Gratissimum Linn. *European Journal of Pharmaceutical and Medical Research, 7*(7), 29–40.

Jha, A. K., & Sit, N. (2022). Extraction of bioactive compounds from plant materials using combination of various novel methods: A review. *Trends in Food Science & Technology, 119*, 579–591. https://doi.org/10.1016/j.tifs.2021.11.019

Kavyamala, D., Jayamma, P., Rani, N. U., & Babu, M. M. (2023). Phytochemical screening of aqueous, acetone, ethanol and methanol leaf extracts of *Ocimum teniflorum, Ocimum gratissimum and Ocimum sanctum*. *International Journal of Economic Plants, 10*(May, 2), 109–113. https://doi.org/10.23910/2/2023.0515b

Klein-Junior, L. C., de Souza, M. R., Viaene, J., Bresolin, T. M. B., de Gasper, A. L., Henriques, A. T., & Heyden, Y. Vander. (2021). Quality control of herbal medicines: from traditional techniques to state-of-the-art approaches. *Planta Medica, 87*(12/13), 964–988. https://doi.org/10.1055/a-1529-8339

Kpètèhoto, H. W., Amoussa, A. M. O., , Roch Christian Johnson, E. E. M., Houéto, Mignanwandé, F. M. Z., Yédomonhan, H., Loko4, F., Bankolé, H., Latifou, & Lagnika. (2019). Phytochemical analysis and antioxidant potential of *Ocimum gratissimum* Linn (Lamiaceae) commonly consumed in the Republic of Benin. *Journal of Applied Biology & Biotechnology, 7*(4), 75–83. https://doi.org/10.7324/JABB.2019.70412

Kumar, L., Verma, S., Singh, M., Chalotra, T., & Utreja, P. (2018). Advanced drug delivery systems for transdermal delivery of non-steroidal anti-inflammatory drugs: a review. *Current Drug Delivery, 15*(8), 1087–1099. https://doi.org/10.2174/1567201815666180605114131

Lang, Y., Gao, N., Zang, Z., Meng, X., Lin, Y., Yang, S., Yang, Y., Jin, Z., & Li, B. (2024). Classification and antioxidant assays of polyphenols: a review. *Journal of Future Foods, 4*(3), 193–204. https://doi.org/10.1016/j.jfutfo.2023.07.002

Ojewumi, M. E., Babatunde, D. E., Orjiakor, T. G., Ojewumi, E. O., & Olawale-Success, O. O. (2024). Exploring the therapeutic potential of *Ocimum gratissimum* extracts against microbial infections: A Comprehensive review. *Medicine India, 3*, 45. https://doi.org/10.25259/MEDINDIA_11_2024

Okeke, L. A., Anie, C. O., Nwobodo, D. C., & Vitus Kasie Okolo. (2023). Phytochemical analysis and antibacterial activities of various leaf extracts of *Ocimum gratissimum* and Newbouldia laevis. *GSC Biological and Pharmaceutical Sciences, 22*(3), 144–152. https://doi.org/10.30574/gscbps.2023.22.3.0104

Olumide, M. D., Ajayi, O. A., & E. O. Akinboye. (2019). Comparative study of proximate, mineral and phytochemical analysis of the leaves of *Ocimum gratissimum, Vernonia amygdalina and Moringa oleifera*. *Journal of Medicinal Plants Research, 13*(15), 351–356. https://doi.org/10.5897/JMPR2019.6775

Pandiri, I., & Moni, A. (2018). Ocimum herb species: a potential treatment strategy for diabetic kidney disease. *Journal of Advanced Biotechnology and Experimental Therapeutics, 1*(3), 88. https://doi.org/10.5455/jabet.2018.d16

Pathak, A., Gupta, A. P., & Pandey, P. (2024). *Herbal Medicine and Sustainable Development Challenges and Opportunities* (pp. 1–26). https://doi.org/10.1007/978-3-031-21973-3_48-1

QCVN 8-2:2011/BYT. (2011). *National technical regulation on the limits of heavy metals contamination in food*.

Samadi, S., Saharkhiz, M. J., Azizi, M., Samiei, L., Karami, A., & Ghorbanpour, M. (2021). Single-wall carbon nano tubes (SWCNTs) penetrate *Thymus daenensis* Celak. plant cells and increase secondary metabolite accumulation in vitro. *Industrial Crops and Products, 165*(March), 113424. https://doi.org/10.1016/j.indcrop.2021.113424

Silva, L. de L., Heldwein, C. G., Reetz, L. G. B., Hörner, R., Moraes, D. P. de, Duarte, F. A., Flores, É. M. de M., Zanella, R., Pereira, A. M. S., & Heinzmann, B. M. (2015). Influence of extraction method on antibacterial activity of ethanolic extracts of Ocimum gratissimum L. *Journal of Medicinal Plants Research, 9*(7), 199–206. https://doi.org/10.5897/JMPR2014.5387

Sridevi, G., Ibrahim, M., Sembulingam, K., Srividya, S., & Prema, S. (2015). Evaluation of in vitro anti-inflammatory activity of *Pergularia Daemia*. *World Journal of Pharmaceutical Research SJIF Impact Factor 5*, 990(6), 1100–1108. www.wjpr.net

Taghizadeh, S. F., Karimi, G., Tzatzarakis, M., Tsakiris, I., Ahmadpourmir, H., Azizi, M., Afshari, A., Ghorani, V., Yarmohammadi, F., Tsatsakis, A., & Rezaee, R. (2022). Probabilistic risk assessment of exposure to multiple metals and pesticides through consumption of fruit juice samples collected from Iranian market. *Food and Chemical Toxicology, 170*(October), 113493. https://doi.org/10.1016/j.fct.2022.113493

Tuan Anh, T., Thi Duyen, L., My Hang, L., Duc Lam, T., Giang Bach, L., Chinh Nguyen, D., & Quoc Toan, T. (2019). Effect of drying temperature and storage time on Ocimum gratissimum Linn. leaf essential oil from Central Highlands, Vietnam. *Materials Today: Proceedings, 18*, 4648–4658. https://doi.org/10.1016/j.matpr.2019.07.449

Ugbogu, O. C., Emmanuel, O., Agi, G. O., Ibe, C., Ekweogu, C. N., Ude, V. C., Uche, M. E., Nnanna, R. O., & Ugbogu, E. A. (2021). A review on the traditional uses, phytochemistry, and pharmacological activities of clove basil (*Ocimum gratissimum* L.). *Heliyon, 7*(11), e08404. https://doi.org/10.1016/j.heliyon.2021.e08404

Vietnamese Pharmacopoeia. (2019). Vietnamese Pharmacopoeia (V). *Medical publishing house*.

Wang, S., Dong, G., & Sheng, C. (2019). Structural simplification of natural products. *Chemical Reviews, 119*(6), 4180–4220. https://doi.org/10.1021/acs.chemrev.8b00504

Wołosiak, R., Drużyńska, B., Derewiaka, D., Piecyk, M., Majewska, E., Ciecierska, M., Worobiej, E., & Pakosz, P. (2021). Verification of the conditions for determination of antioxidant activity by ABTS and DPPH Assays—a practical approach. *Molecules, 27*(1), 50. https://doi.org/10.3390/molecules27010050

Zahran, E. M., Abdelmohsen, U. R., Khalil, H. E., Desoukey, S. Y., Fouad, M. A., & Kamel, M. S. (2020). Diversity, phytochemical and medicinal potential of the genus Ocimum L. (Lamiaceae). *Phytochemistry Reviews, 19*(4), 907–953. https://doi.org/10.1007/s11101-020-09690-9

Zareiyan, F., & Khajehsharifi, H. (2022). In-vitro phytochemical analysis of essential oil and methanolic and hydromethanolic extracts of *Ocimum gratissimum*. *Journal of Plant Biochemistry and Biotechnology, 31*(4), 894–906. https://doi.org/10.1007/s13562-022-00768-3