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RESEARCH ARTICLE

Optimization, chemical constituents and bioactivity of *Baeckea frutescens* L. essential oil extracted by microwave-assisted hydro-distillation

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Abstract

The objective of this study was to optimize the extraction of essential oil from *Baeckea frutescens* leaves using microwave-assisted hydro-distillation (MAHD) combined with response surface methodology (RSM). This approach aimed to improve extraction efficiency and maximize essential oil yield. The chemical composition of the extracted oil was subsequently analyzed using gas chromatography-mass spectrometry (GC-MS) to identify its principal constituents. The optimal extraction conditions were determined to be a microwave power of 550 W, an extraction time of 89 min and a liquid-to-material ratio of 9.5:1. Under these conditions, the essential oil yield was 3.96 ± 0.02 % (w/w), which represents a high extraction efficiency. GC-MS analysis revealed that the major constituents of the essential oil were α-thujene (26.81 %), α-humulene (15.35 %), trans-caryophyllene (12.29 %) and 3-carene (10.20 %). In addition, biological activity assays indicated that the essential oil exhibited notable antibacterial and antioxidant activities. It significantly inhibited the growth of *Escherichia coli* and *Salmonella typhi*, with minimum inhibitory concentrations (MICs) below 100 µg/mL. These findings suggest that *Baeckea frutescens* essential oil has strong potential as a natural antimicrobial and antioxidant agent, supporting its application in pharmaceutical and industrial products.

Keywords: antibacterial; *Baeckea frutescens*; microwave-assisted hydro-distillation; response surface methodology

Introduction

Baeckea frutescens L. is a species belonging to the Myrtaceae family. It is a small, bushy shrub ranging from 0.5 to 2 m in height. The plant typically branches at the base, with slender, soft and aromatic stems and branches. Its leaves are opposite, needle-shaped, stemless, smooth and approximately 1 cm long, featuring a single central vein (1). The species is primarily distributed across tropical regions of Asia and Oceania. The Baeckea genus is found in parts of Cambodia, Thailand, India and Hainan Island, China. In Vietnam, *B. frutescens* is commonly found in lowland and midland areas of the northern and central regions (2).

Traditionally, the plant has been used in Vietnam to alleviate headaches, rheumatism, menstrual discomfort and indigestion (2). In Malaysia, its essential oil has historically been applied in postpartum care and for rheumatic conditions (3). Previous studies have identified several important chemical constituents in *B. frutescens*, including essential oils, alkaloids, flavonoids and other polyphenols (4). Its essential oil exhibits various biological activities such as antibacterial (4), cytotoxic (5), antioxidant (6) and anticancer effects (7).

In recent years, there has been growing scientific interest in the essential oils and bioactive compounds derived from *B. frutescens*, due to their therapeutic potential. However, most existing studies have focused primarily on composition analysis and basic distillation methods, with limited attention to optimizing the efficiency of essential oil recovery. In the present study, the essential oil of *B. frutescens* was extracted using MAHD. This technique offers advantages such as reduced extraction time, lower energy consumption and environmental sustainability. To further enhance extraction efficiency, RSM combined with a Box-Behnken design (BBD) was employed to optimize the distillation parameters. The chemical profile of the essential oil was then analyzed using GC-MS.

Materials and Methods

Plant material

Leaves of *B. frutescens* were collected in October 2022, in Nghi Loc District, Nghe An Province, Vietnam. Prior to MAHD, the plant samples were air dried. The raw materials were then stored in desiccant bags and kept in a refrigerator at temperatures below 10 °C to maintain their quality until further use in the extraction process.

Chemicals and standards

The reagents and bacterial strains utilized in this study are as follows: DPPH (1,1-diphenyl-2-picrylhydrazyl) was employed to assess antioxidant activity with gallic acid serving as positive control. The bacterial strains used for the antimicrobial assay included *Salmonella thypi, Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa*. Amoxicillin was utilized as the positive control for the bacterial strains. All reagents were of analytical grade.

Methods

Microwave-assisted hydro-distillation (MAHD)

A Samsung MW71E domestic microwave oven, modified for MAHD operation, was integrated with a Clevenger apparatus. The schematic diagram in Fig. 1 illustrates the configuration of both the microwave oven and the Clevenger apparatus. Initially, 50 g of dry weight (DW**)** *B. frutescens* leaf samples were placed into the distillation flask, followed by the addition of a precise volume of deionized water. The mixture was then subjected to microwave radiation for heating. After the specified duration, the essential oil fraction was collected in a separate container.

Analysis of sample

To remove water, anhydrous sodium sulfate was added to the essential oil and the mixture was centrifuged to achieve dryness. The extracted yield was then quantified to evaluate the effectiveness of MAHD in extracting oil from *B. frutescens* leaves. The oil yield for each extraction run was calculated using the following equation:

Essential oil yield (%) = (mass of dried EO / mass of dried leaf sample) × 100

Experimental design

RSM based on the BBD was employed to optimize the distillation conditions. The factors considered included microwave power (W), liquid-to-material ratio and extraction time (min). The coded variables utilized in the BBD are presented in Table 1.

In this study, we utilized Design-Expert v11 to assess the effects of the extraction parameters. The response variable was modeled using a second order polynomial equation, as shown in equation:

$$Y_i = \beta_o + \sum^{k}_{i=1}\beta_i X_i + \sum^{k}_{i=1}\beta_{ii} X^2_i + \sum\sum_{i<j}\beta_{ij} X_i X_j$$

Where Y_i is the predicted response, β_0 is the intercept, β_i, β_{ii} and β_{ij} represent the coefficients for the linear, quadratic and interaction terms, respectively. X_i and X_j are the coded independent variables, with X_iX_j and X_i^2 representing the interaction and quadratic terms, respectively.

GC-MS analysis

The GC-MS system used in this study was the Trace GC Ultra - ITQ900, manufactured by Thermo Fisher Scientific (USA), equipped with a mass selective detector operating in the electron impact mode at 70 eV. The gas chromatograph used was also the Trace GC Ultra from Thermo Fisher Scientific, fitted with a TG-SQC capillary column (30 m length, 0.25 mm inner diameter and 0.25 µm film thickness). The temperature program in the oven began with a 2 min hold at 60 °C, followed by a ramp to 200 °C at a rate of 4 °C per min and then a further increase to 360 °C at 10 °C per min with a final 10 min hold for column cleaning. The injector temperature was set at 280 °C. Samples were diluted in n-hexane at a 1:50 (v/v) ratio and injected into the GC using the split mode with a split ratio of 1:15. A volume of 1.0 µL was injected and the carrier gas (helium) was set to a linear velocity of 1.0 mL/min. Chemical identification in the extracted essential oils was achieved by comparing their mass spectral fragmentation patterns with those of known compounds from the NIST17/Wiley library or published mass spectra. Quantification was based on the retention times of the compounds, consistent with both methods. The normalization technique was applied, setting the total peak area to 100 % and the proportion of each component was calculated by measuring the area under each peak (8).

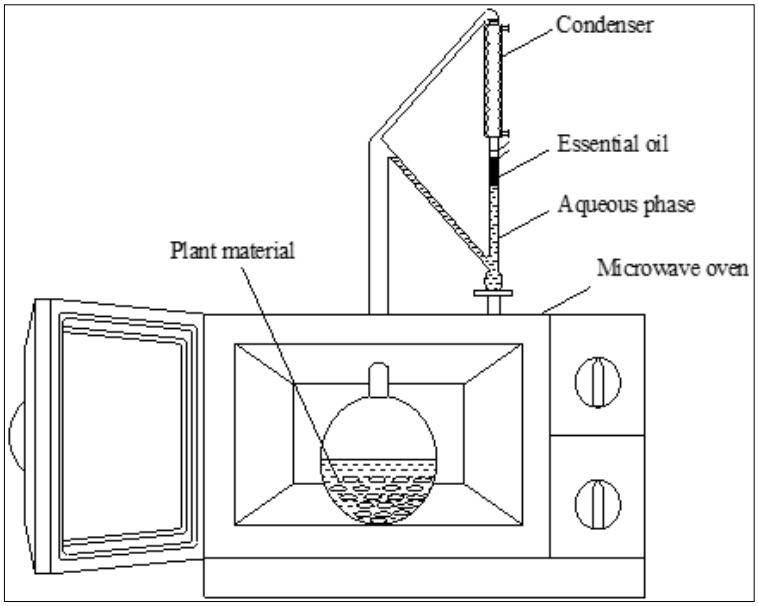


Fig. 1. Schematic diagram of MAHD method.

Table 1. The coded independent variables

Factors	Symbols	Units	Low actual	High actual	Low coded	High coded
Microwave power	X_1	W	200	600	-1	+1
Liquid-to-material ratio	X_2		6:1	10:1	-1	+1
Extraction time	X_3	Min	60	90	-1	+1

Assay for antioxidant activity

The antioxidant activity of EO can be determined through the DPPH method (9). Vitamin C was used as a reference point for the experiment. The antioxidant effect of the sample was determined using the following calculation formula:

Antioxidant activity (%) = [(AVC - AS)/AVG] × 100 %

AVG denotes the absorbance of gallic acid, while AS signifies the absorbance of the tested samples. Absorbance readings were taken at 517 nm for the DPPH assay and all tests were conducted in triplicate. The samples' ability to neutralize free radicals is measured by their IC_{50} values, indicating how concentrated a substance must be to effectively prevent a biological function or process. A substance's potency is evaluated based on its capacity to block a specific biological function or process, as indicated by its IC_{50} value. A lower IC_{50} indicates a higher antioxidant capacity.

Assay for antibacterial activity

This study examined four common gastrointestinal bacte ria (*S. thypi, S. aureus, E. coli, P. aeruginosa*) for their antibacterial activity. The MIC was determined by the two-fold culture dilution method (10). The essential oil was mixed with a solvent called dimethyl sulfoxide (DMSO) at a concentration of 2 mg/mL, making up 10 % of the solution special plastic plate called a 96-well microplate was used to combine 100 µL of the essential oil solution with 100 µL of a nutrient-rich culture medium that contains a color-changing dye called triphenyl tetrazolium chloride (TTC). After that, a bacterial solution with a very high concentration of 10^8 CFU/mL was added to the mixture, which resulted in a range of concentrations between 500 and 3.9 µg/mL in the plates. A medication called amoxicillin was used as a reference to compare the results and the blank medium without the essential oil or amoxicillin was used as a control to compare against. The microplates were then placed in a warm, controlled environment at 37 °C and left undisturbed for 24 hrs to allow the bacteria to grow. The minimum concentration of medication needed to stop bacterial growth was determined and recorded as the MIC. Cultures with no bacterial growth at or above the MIC were diluted 20 times using a sterile solution to further reduce their concentration. A small amount of the diluted culture was then transferred to a special plate called a TTC agar plate. The plates were then placed in a warm, controlled environment at 37 °C and left undisturbed for 24 hrs to allow the bacterial growth. The minimum concentration of medication needed to completely kill the bacteria in all wells was determined and recorded as the Minimum Bactericidal Concentration (MBC) (10).

Results and Discussion

The results section should provide a clear and concise summary of the key findings from the experiments, along with the logical conclusions derived from them. It is crucial to present the results objectively and transparently, ensuring that no data manipulation or falsification occurs. This section should also include relevant statistical analyses that support the findings, where applicable and be accompanied by well-organized tables or visual aids to enhance clarity and comprehension.

Optimization of MAHD

Model fitting

The design matrix, which includes 17 runs, was generated using the BBD, with three variables: Microwave power (X_1), liquid-to-material ratio (X_2) and extraction time (X_3). The design incorporated three levels for each variable and five center points. The observed and predicted outcomes are presented in Table 2.

BBD analyzed 17 data sets and developed mathematical models to describe the results:

$$Y = 3.66 + 0.39X_1 + 0.285X_2 + 0.18X_3 - 0.12X_1X_2 - 0.06X_1X_3 + 0.09X_2X_3 - 0.27X_{12} - 0.12X_{22} - 0.12X_{32}$$

Table 2. Experimental of Box-Behnken design

Run	Factor 1 (X_1)	Factor 2 (X_2)	Factor 3 (X_3)	Response 1 (Y)
	Microwave power	Liquid-to-material ratio	Extraction time	Essential oil yield
	(W)		(min)	%
1	200	8:1	90	3.12
2	400	10:1	90	3.96
3	400	8:1	75	3.66
4	200	8:1	60	2.64
5	200	6:1	75	2.46
6	600	10:1	75	3.84
7	400	8:1	75	3.66
8	600	6:1	75	3.48
9	400	8:1	75	3.72
10	200	10:1	75	3.30
11	400	10:1	60	3.42
12	400	6:1	90	3.24
13	400	8:1	75	3.60
14	600	8:1	90	3.78
15	600	8:1	60	3.54
16	400	6:1	60	3.06
17	400	8:1	75	3.66

ANOVA for Quadratic model

The quadratic regression model for essential oil yield was analyzed using ANOVA (Y) to assess the model's significance. The results indicated statistical significance with a p-value < 0.05 and an F-value of 233.18 for Y. The coefficient of determination (R^2) for the model was 0.9967, demonstrating that 99.67 % of the variability in the response was explained by the model. The Lack of Fit F-value for Y was 0.3333, suggesting that the Lack of Fit was not significant relative to the pure error (Table 3). This indicates that the polynomial model provided a reliable fit to the data. These findings align with previous studies. For instance, a study on the optimization of essential oil extraction from Vietnamese *Citrus aurantifolia* (lemon fruit) using MAHD reported a quadratic model with an R^2 of 0.9443, indicating a strong model fit (11). The comparison highlights the effectiveness of quadratic regression models in capturing the complexities of essential oil extraction processes.

Response surface analysis

The three-dimensional response surfaces show how two factors - microwave power and liquid/material ratio - affect the results when the extraction time is 75 min. These surfaces show the relationship between microwave power and extraction time when the liquid/material ratio is 8:1. Additionally, the surfaces illustrate how the liquid/material ratio and extraction time affect the results when the microwave power is held constant at 500 W, the Z-axis on these surfaces measures the oil yield.

Response surface analysis of essential oil yield

Fig. 2 - 4 are 3D surface and contour plots that show essential oil yield. They also show how independent variables affect and interact with each other to influence the essential oil yields. Fig. 2 focuses on how the liquid-to-material ratio and microwave power influence the yield. When the microwave power increases from 200 to 600 W, the yield also increases. Additionally, the yield grows as the liquid-to-material ratio changes from 6:1 to 10:1.

In Fig. 3, the interaction between extraction time and microwave power is illustrated concerning the response (with a liquid-to-material ratio of 7:1). It was observed that the oil yield increased with the extension of extraction time from 60 to 84 min but subsequently decreased. Like Fig. 2, the yield also increased with higher microwave power. Fig. 4 explores the impact of two factors, namely liquid-to-material ratio and extraction time, using a similar analysis as presented in Fig. 2 and 3. Similarly, research on the microwave-assisted extraction of essential oils from Iranian *Rosmarinus officinalis* L. demonstrated that extraction time and microwave power significantly affected the extraction yield. The optimal conditions were determined to be an extraction time of 85 min, microwave power of 888 W and a water volume-to-mass ratio of 0.5 mL/g, yielding 0.7756 % essential oil (12).

Optimization and model verification

The optimal conditions for extracting essential oil from *B. frutescens* leaves using RSM were determined to be a microwave power of 549.03 W, a liquid-to-material ratio of 9.59:1 and an extraction time of 89.47 min. To enhance practicality, these parameters were adjusted to a microwave power of 550 W, a liquid-to-material ratio of 9.5:1 and an extraction time of 89 min. Under these modified conditions, the essential oil yield achieved was 3.96 ± 0.02 %. These results are consistent with the previous study optimized the extraction of essential oil from *Cinnamomum camphora* leaves and reported that the highest yield (3.26 %) was obtained at a microwave power of 786.27 W, a liquid-to-material ratio of 7.47:1 and an extraction time of 35.57 min (13) (Table 4).

Chemical profile of B. frutescens leaves essential oils

After distillation, the chemical composition of *B. frutescens* essential oil was analyzed using GC-MS. The results (Table 5) revealed that the major constituent was α-thujene, accounting for approximately 26.81 % of the total oil. Other prominent components included α-humulene (15.35 %), trans-caryophyllene (12.29 %), 3-carene (10.2 %) and p-cymene (6.52 %) (Fig. 5).

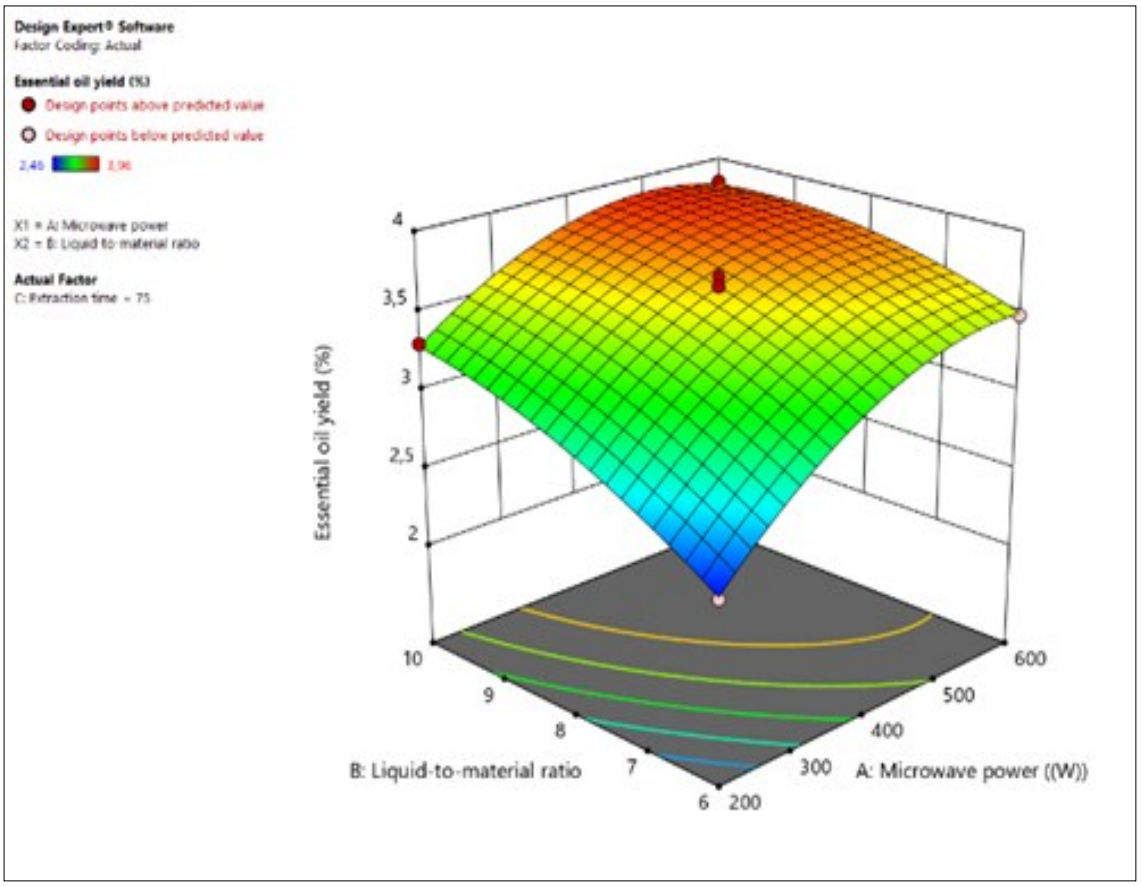
Several of these compounds, particularly α-thujene and α-humulene, are monoterpenes and sesquiterpenes known for their broad-spectrum antibacterial activities. α-Thujene, for instance, has been reported to disrupt bacterial membrane integrity, leading to cell lysis, while α-humulene exhibits anti-inflammatory and antimicrobial effects through inhibition of bacterial growth and biofilm formation. The presence of these bioactive terpenes suggests that the antibacterial activity of *B. frutescens* essential oil may be attributed to the synergistic interactions among its major monoterpene components, supporting its traditional use in treating microbial infections.

Antioxidant activity

The antioxidant activity of *B. frutescens* essential oil was evaluated using the DPPH radical scavenging assay, with gallic acid serving as the positive control (Table 6). The results revealed a dose-dependent relationship, as higher concentrations of the essential oil led to increased free radical scavenging. However, its antioxidant efficacy was significantly lower than that of gallic acid ($P < 0.05$).

Table 3. Regression coefficients of the predicted second-order polynomial models for the essential oil yield

Source	Sum of squares	df	Mean square	F-value	p-value	
Model	2.70	9	0.2998	233.18	< 0.0001	Significant
X_1	1.22	1	1.22	946.40	< 0.0001	
X_2	0.6498	1	0.6498	505.40	< 0.0001	
X_3	0.2592	1	0.2592	201.60	< 0.0001	
X_1X_2	0.0576	1	0.0576	44.80	0.0003	
X_1X_3	0.0144	1	0.0144	11.20	0.0123	
X_2X_3	0.0324	1	0.0324	25.20	0.0015	
X_1^2	0.3069	1	0.3069	238.74	< 0.0001	
X_2^2	0.0606	1	0.0606	47.16	0.0002	
X_3^2	0.0606	1	0.0606	47.16	0.0002	
Residual	0.0090	7	0.0013			
Lack of Fit	0.0018	3	0.0006	0.3333	0.8032	not significant
R^2			0.9967			
C V %			1.05			



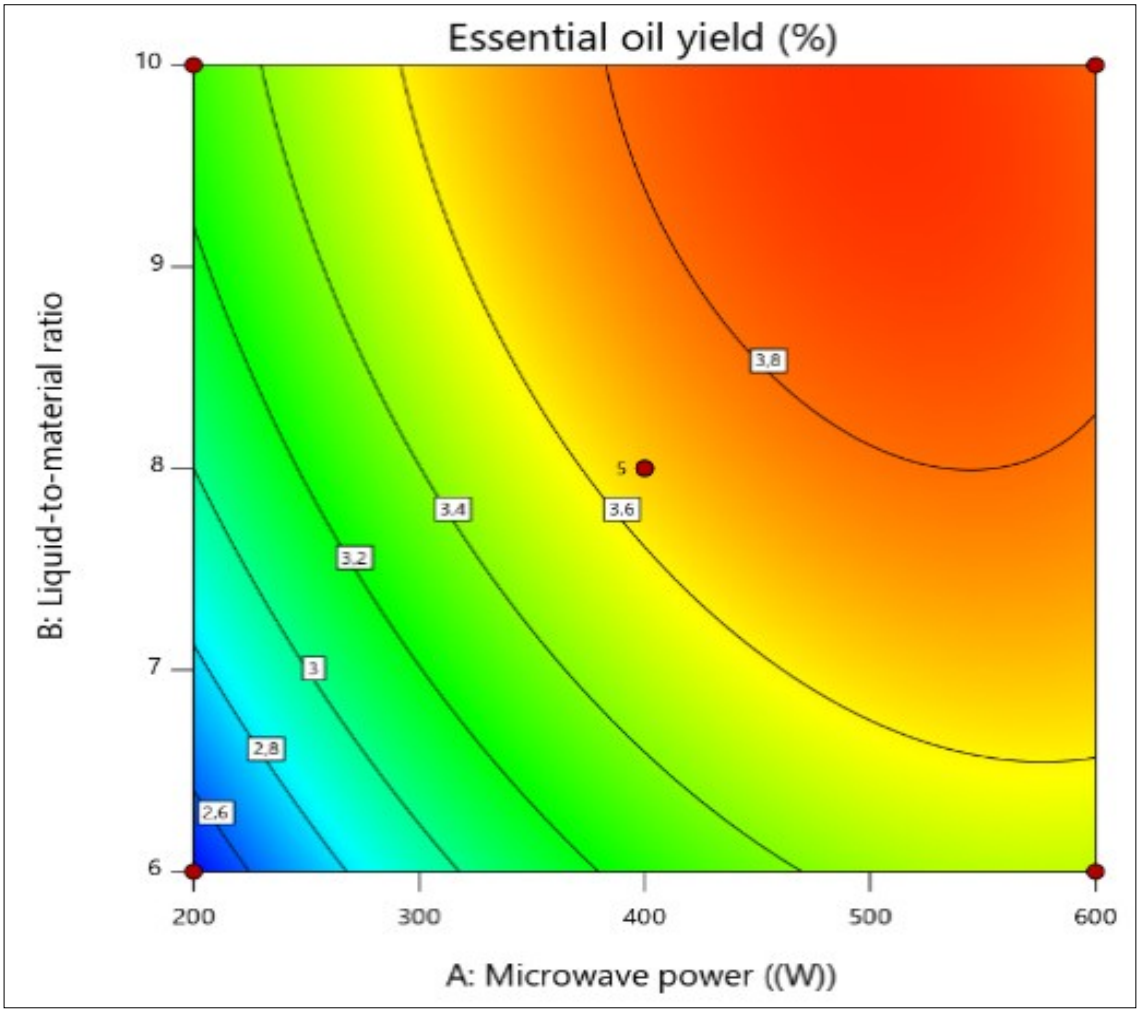
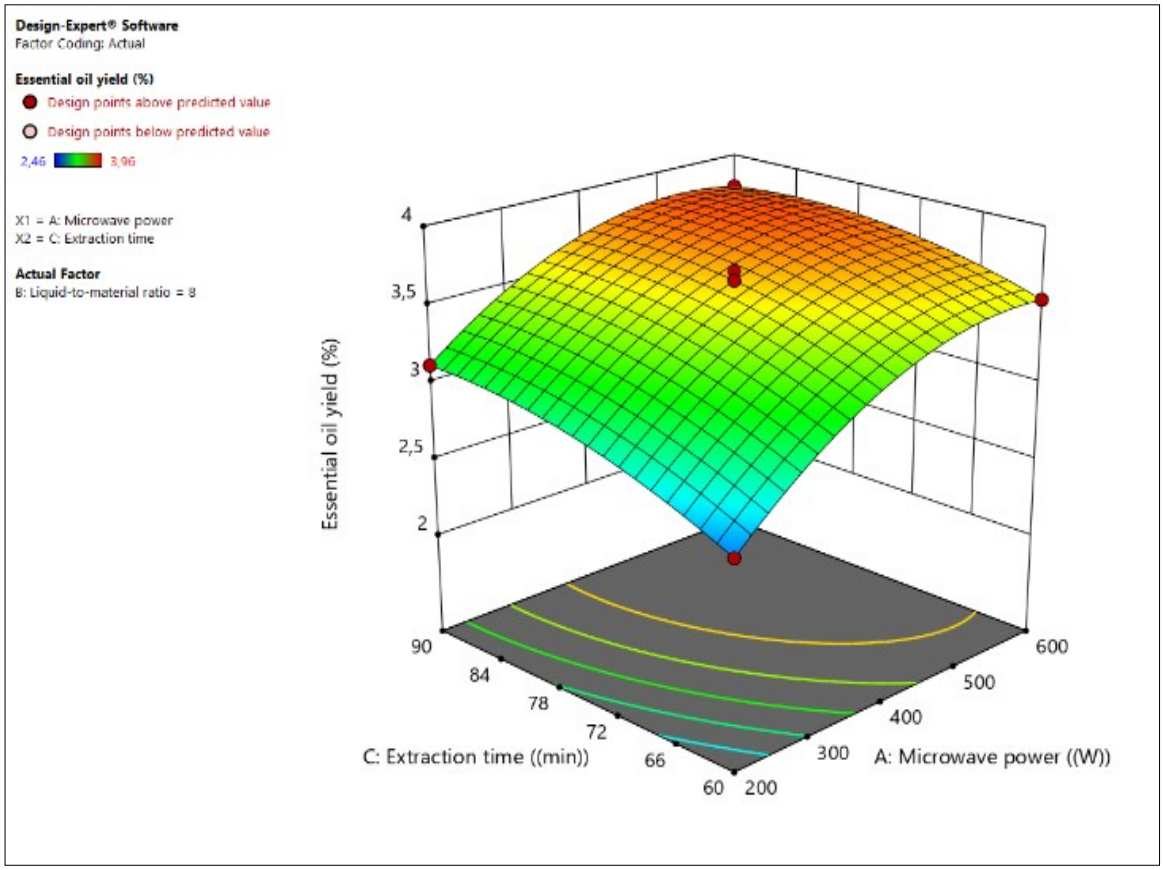


Fig. 2. Effect of microwave power and L:M ratio on essential oil (EO) yield.



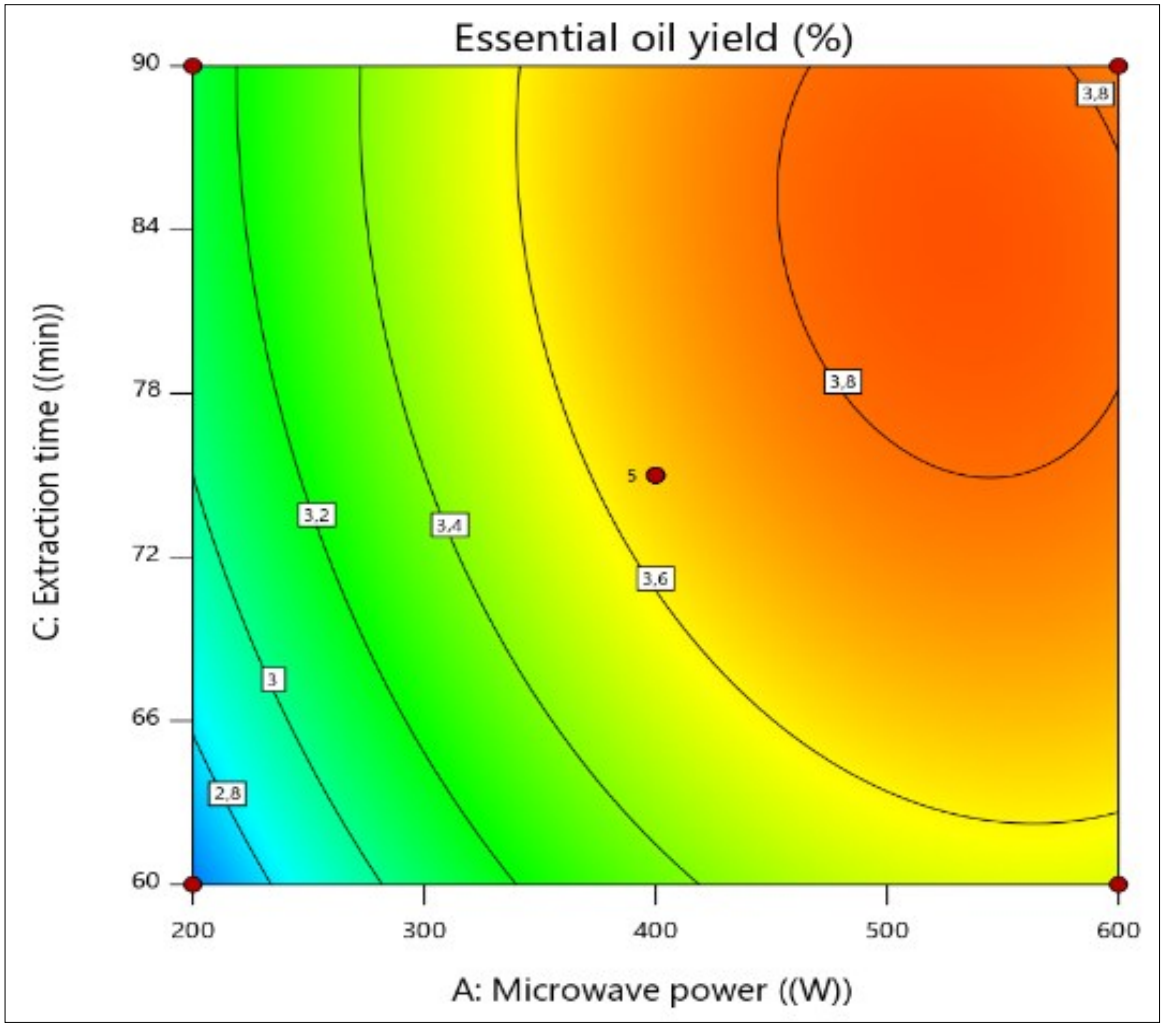
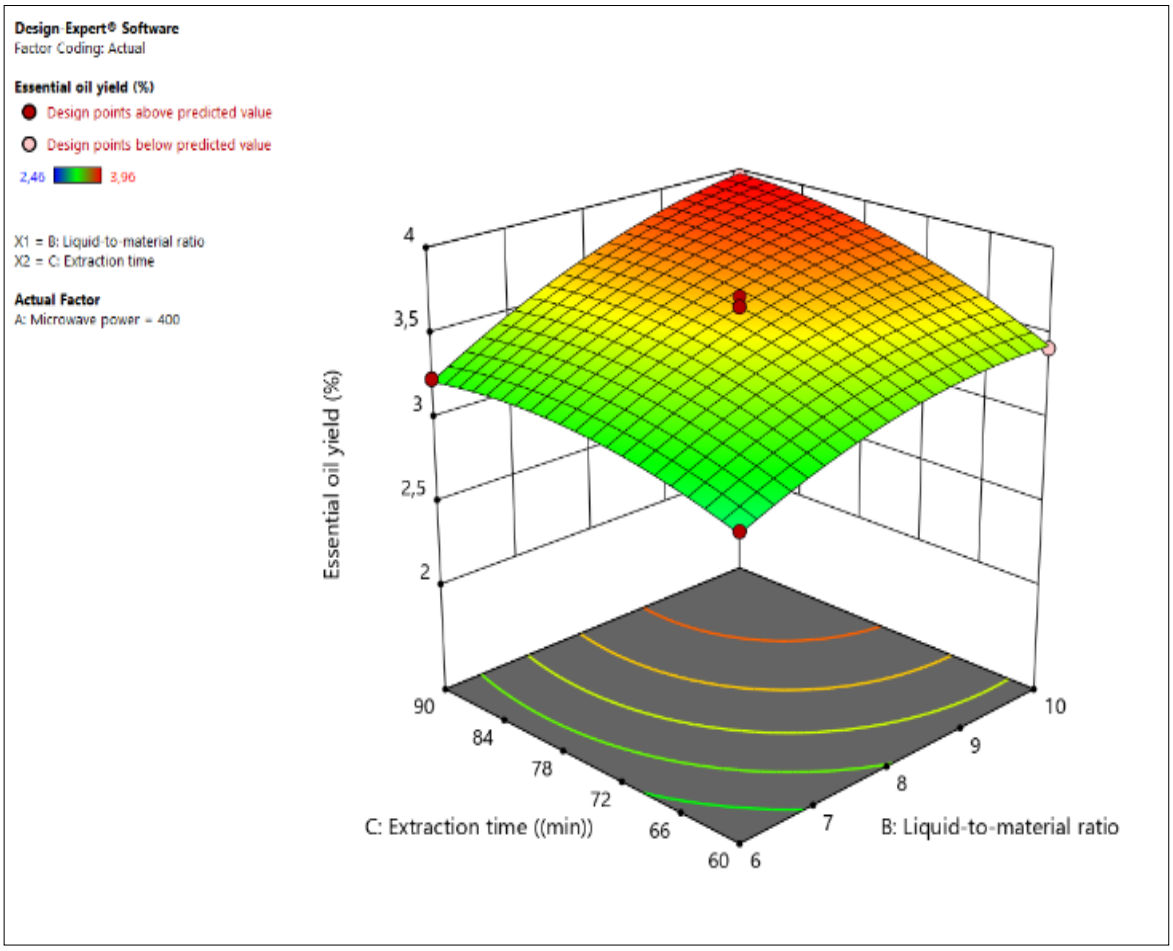


Fig. 3. 3D surface and contour plots of microwave power and extraction time of oil yield.



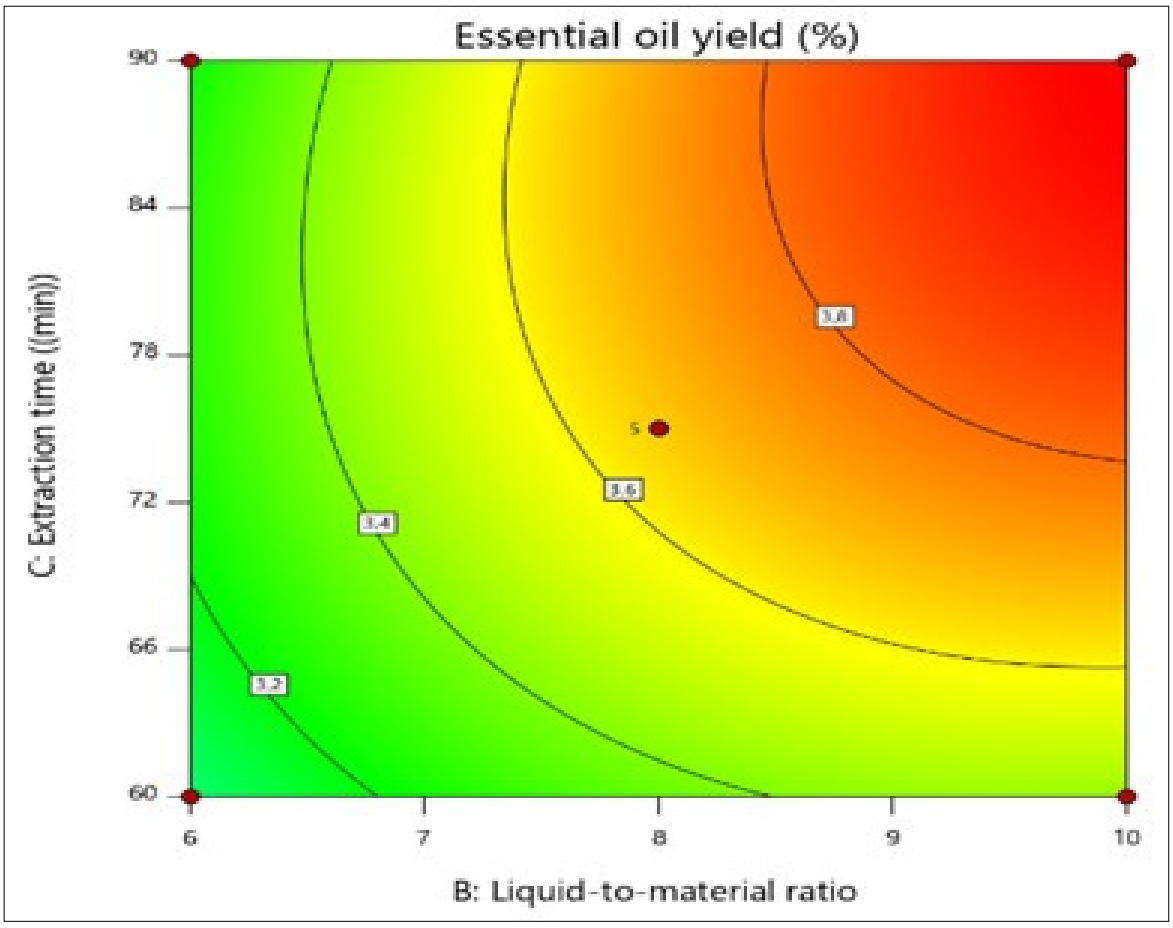


Fig. 4. 3D surface and contour plots of liquid-to-material ratio and extraction time of oil yield.

Table 4. Optimal conditions, along with the predicted and experimental response values for the extraction of B

Independent variables			Dependent variables (Response)	Optimum value	
X_1	X_2	X_3		Experimental (*)	Predicted
550 (W)	9.5:1	89 (min)	Y (%)	3.96±0.02	4.00

X1: Microwave power (W); X2: Liquid-to-material ratio; X3: Extraction time (min); Y: Essential oil yield (%).

(*) Results are presented as mean ± standard deviation (SD) based on three measurements (n = 3) from three different extraction processes

Table 5. Chemical composition of *B. frutescens* leaf essential oil

No	Name	Apex RT	Percent (%) Area
1	α-Thujene	5.21	26.81
2	α-Pinene	5.40	3.84
3	Sabinene	6.48	0.21
4	Santolina triene	6.59	0.50
5	Franklinone	6.96	0.23
6	α-Phellandrene	7.39	0.43
7	γ-Terpinene	7.77	3.33
8	p-Cymene	8.02	6.52
9	trans-β-Ocimene	8.16	1.19
10	Eucalyptol	8.26	2.74
11	3-Carene	9.15	10.20
12	α-Terpinene	10.15	1.44
13	2-β-Pinene	10.53	1.73
14	trans-Sabinene hydrate	13.29	2.05
15	α-Terpineol	13.77	0.29
16	Kryptogenin dioxime	18.56	0.15
17	Androst-4-en-3.11.17-trione, 9-methylthio-	19.95	0.21
18	Germacrene D	20.21	0.17
19	trans-Caryophyllene	21.68	12.29
20	α-Humulene	22.79	15.35
21	β-Chamigrene	23.84	0.11
22	β-Maaliene	24.14	0.25
23	α-Muurolene	24.27	0.12
24	γ-Cadinene	24.71	0.21
25	δ-Cadinene	25	1.39
26	Ylangene	25.28	0.11
27	(-)-Spathulenol	26.89	0.66
28	Cembrene	27.36	0.19
29	Ledene oxide-(II)	27.68	0.80
30	β-Guaiene	28.21	0.22
31	Junipene	28.34	0.46

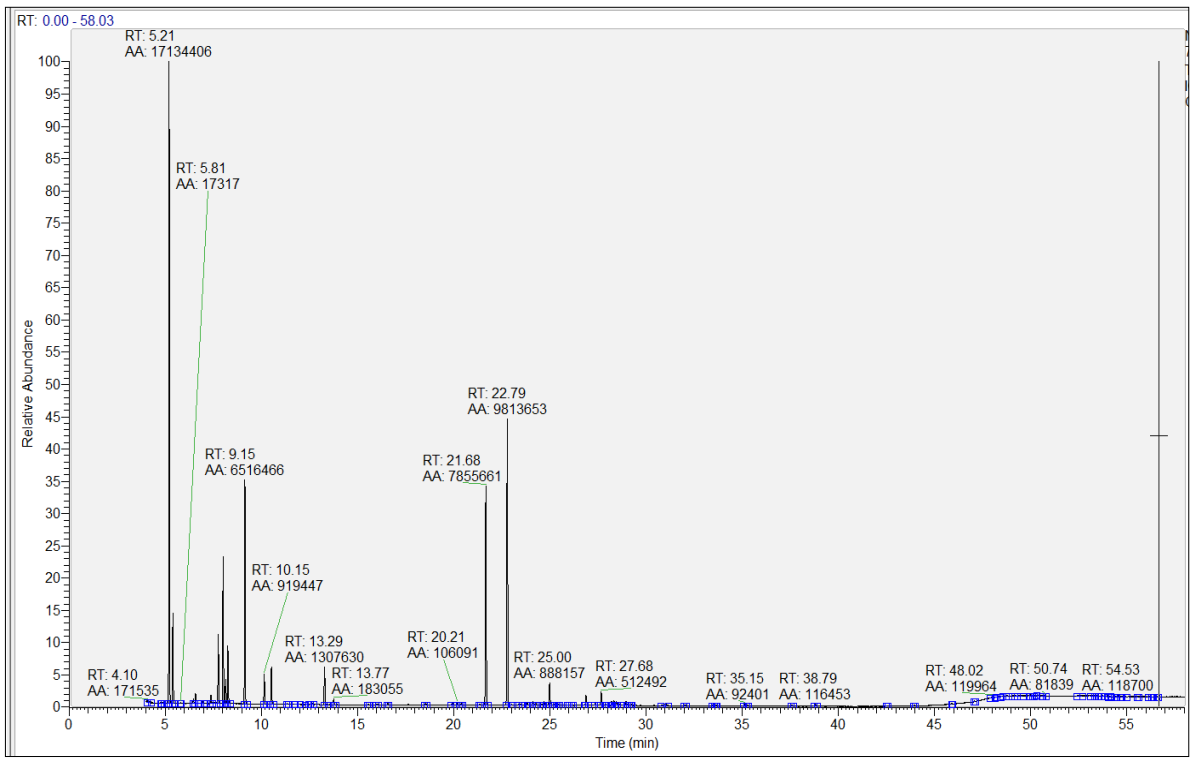


Fig. 5. A representative gas chromatogram of EO.

The IC_{50} value for the essential oil was 85.68 ± 0.06 mg/mL, indicating the concentration required to inhibit 50 % of DPPH radicals. In comparison, gallic acid demonstrated a much stronger antioxidant capacity, with an IC_{50} of 3.81 ± 0.18 mg/mL. This marked difference underscores the relatively moderate antioxidant potential of the essential oil.

The observed activity may be attributed to the presence of unsaturated hydrocarbon compounds containing double and triple bonds, which can stabilize free radicals by electron donation (14, 15). Although less potent than synthetic antioxidants, *B. frutescens* essential oil still exhibits measurable antioxidant properties, suggesting its potential as a natural antioxidant source in functional food or pharmaceutical formulations.

Antibacterial activity

The antibacterial activity of the essential oil obtained via MAHD was evaluated using the microdilution method and the results are presented in Table 6. The essential oil exhibited notable inhibitory effects against several gastrointestinal bacterial strains, with the lowest MIC observed against *E. coli* (32.3 µg/mL) and *S. typhi* (33.4 µg/mL). These values were comparable to those of the standard antibiotic Ofloxacin (31.2 and 32.7 µg/mL, respectively), indicating that the essential oil demonstrates moderate to strong antibacterial potential.

Among the tested microorganisms, Pseudomonas aeruginosa showed the highest resistance (MIC = 131 µg/mL), while *E. coli* and *S. aureus* were more sensitive. These results suggest that the essential oil could serve as a natural antimicrobial agent, particularly effective against certain gram-negative and gram-positive bacteria.

The antibacterial efficacy of *B. frutescens* essential oil may be attributed to its high content of monoterpenes such as α-thujene and α-humulene. These compounds are known to disrupt bacterial membranes by compromising the integrity of polysaccharide, fatty acid and phospholipid layers, ultimately leading to leakage of intracellular contents and cell death (16). Additionally, minor constituents containing nitrogen and sulfur may enhance these effects by interfering with microbial metabolism and enzyme function.

These findings are consistent with previous research showing that monoterpenes like thymol and carvacrol exert broad-spectrum antibacterial activity by targeting membrane permeability (17). Similarly, oxygenated monoterpenes such as 1,8-cineole and α-terpinyl acetate found in *Vitex agnus-castus* essential oil have demonstrated comparable mechanisms of antibacterial action (18).

Conclusion

The volatile compounds present in *B. frutescens* leaves were extracted using the MAHD technique. The experimental parameters, including microwave power, liquid-to-material ratio and extraction time, were optimized using the BBD within the framework of the RSM. The study determined the optimal extraction conditions to be a microwave power of 550 W, a liquid-to-material ratio of 9.5:1 and an extraction time of 89 min. Under these conditions, the essential oil yield was 3.96 ± 0.02 % (w/w). Furthermore, the essential oil derived from *B. frutescens* demonstrated promising natural antioxidant and antibacterial properties. Ongoing investigations will further assess the pharmacological effects of the essential oil. The findings of this study enhance the understanding of the chemical composition of *B. frutescens* essential oil in Vietnam, supporting the development of medicinal herb cultivation to boost agricultural productivity. Moreover, the study provides a scientific foundation for the industrial application of *B. frutescens* essential oil in natural product-based pharmaceuticals, cosmetics and food preservation.

Acknowledgements

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Authors' contributions

The study was conceived and coordinated by NTHT, TDT and NNT, who also contributed to the design. NKK and NTT participated in the study's design and coordination. Essential oil extraction from *Baeckea frutescens* leaves was carried out by DMD, NTHT, NKK and NNT. Bioactivity assays were conducted by NNT, NTHT and NKK. Statistical analysis was performed by NTN and NTHT. Data interpretation and manuscript drafting were completed by NNT, NTHT and NKK. All authors have read and approved the final manuscript.

Table 6. Antioxidant and antibacterial properties of the EO from *B. frutescens* leaves extracted using MAHD

Bioactivity	Microorganism/Test	MAHD	Positive control
Antioxidant activity	IC_{50} (mg/mL, DPPH)	85.68 ± 0.06	3.81 ± 0.18
Anti-bacterial activity	*S. typhi* - MIC (µg/mL)	33.4	32.7
	S. typhi - MBC (µg/mL)	65.5	65.5
	S. aureus - MIC (µg/mL)	63.8	62.5
	S. aureus - MBC (µg/mL)	125	125
	E. coli - MIC (µg/mL)	32.3	31.2
	E. coli - MBC (µg/mL)	125	125
	P. aeruginosa - MIC (µg/mL)	131	125
	P. aeruginosa - MBC (µg/mL)	250	250

Gallic acid served as the positive control for assessing antioxidant activity, while amoxicillin served as the positive control for antibacterial activity. Means in a row that are followed by different letters indicate statistically significant differences (P < 0.05).

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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