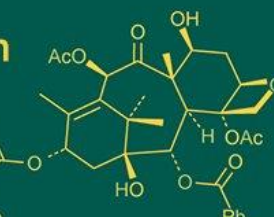
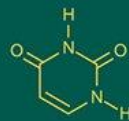
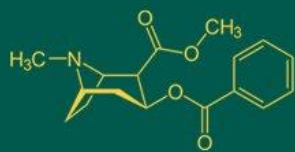


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
IJABR 2026; SP-10(6): 1330-1335
www.biochemjournal.com
Received: 25-03-2026
Accepted: 27-04-2026

All authors' names and affiliations are given below, after the references.

Optimization of extracted essential oil from clove buds (*Syzygium aromaticum*) using microwave-assisted hydro distillation

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DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i6Sp.8828>

Abstract

Essential oil extraction from Zanzibar clove buds (*Syzygium aromaticum* L.) was optimized using microwave-assisted hydro distillation (MAH-D). A three-level factorial experimental design was employed to investigate the effects of microwave power (250, 350, 450 W), extraction time (10, 15, 20 minutes), and sample ratio (0.5:1, 0.6:1, 0.7:1 g/ml), with 5 replicates per treatment (n=135 total). Raw clove material exhibited moisture content of $8.2 \pm 0.3\%$. Maximum oil yield of $14.8 \pm 0.6\%$ (w/w) was achieved at 450 W, 20 minutes, and 0.7:1 sample ratio. Microwave power demonstrated the strongest effect on oil yield ($p < 0.001$), followed by extraction time and sample ratio. Extracted oil exhibited specific gravity 1.048 ± 0.008 g/cm³, refractive index 1.531 ± 0.003 , saponification value 187 ± 4 mg KOH/g, and acid value 1.2 ± 0.2 mg KOH/g. Particle characterization showed bulk density 0.654 ± 0.028 g/cm³, porosity $42.0 \pm 2.1\%$, and angle of repose $38.4 \pm 1.2^\circ$. Interaction analysis revealed significant Power $\times$ Time interaction ($p < 0.01$) and Power $\times$ Ratio interaction ($p < 0.05$). The optimized MAH-D process provides a sustainable and efficient method for high-quality Zanzibar clove oil production.

Keywords: Zanzibar clove, microwave-assisted extraction, essential oil, optimization, factorial design

1. Introduction

Zanzibar clove buds (*Syzygium aromaticum* L., family Myrtaceae) represent the finest quality clove source globally, contributing approximately 80% of world production and generating significant economic value for the island nation (Cortés-Rojas *et al.*, 2014). The essential oil of Zanzibar clove is highly valued in pharmaceutical, cosmetic, food, and flavor industries due to its superior chemical composition, particularly high eugenol content (82-89%) and documented antimicrobial, antioxidant, and analgesic properties (Kamatou *et al.*, 2012; Radünz *et al.*, 2019) [1, 2, 3].

Traditional extraction methods such as steam distillation and hydro distillation require prolonged extraction times (2-4 hours) and consume significant thermal energy, resulting in relatively low oil yields (12-14%) and potential loss of heat-sensitive bioactive compounds (Einfalt, 2020). Microwave-assisted hydro distillation (MAH-D) represents an innovative technology combining selective microwave heating with water distillation, offering potential advantages in extraction speed, efficiency, and product quality (Rutkowska *et al.*, 2017). The selective absorption of microwave energy by polar molecules within plant tissues generates rapid internal heating, facilitating cell disruption and enhanced mass transfer kinetics (Gomez *et al.*, 2020) [4, 5, 6].

Systematic optimization of extraction parameters is essential to identify conditions that maximize oil yield while maintaining superior product quality. This study employs a structured three-level factorial experimental design to rigorously investigate the effects of microwave power, extraction duration, and sample ratio on essential oil yield and physicochemical properties of Zanzibar clove buds. Understanding the relationships between extraction parameters and oil quality indicators will facilitate implementation of this technology at industrial scale.

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2. Materials and Methods

2.1 Raw Material and Moisture Content

Dried Zanzibar clove buds (*Syzygium aromaticum* L.) were procured from Kerala. The cloves were ground using the grinder machine available in the Department of Processing and Food Engineering. The ground samples were then packed in zip-lock pouches and stored for further analysis.

Moisture content of raw clove buds was determined by standard oven drying method according to. Approximately 5.0 g of ground clove sample was placed in a pre-weighed aluminium dish and dried in a hot air oven at $105^{\circ}\text{C} \pm 2^{\circ}\text{C}$ until constant weight was achieved. The dish was cooled in a desiccator and weighed. Moisture content was calculated as follows (Najafian, 2014):

$$\text{Moisture Content (\%)} = [(W_1 - W_2) / W_1] \times 100$$

Where,

- W_1 = initial weight of fresh sample (g),
- W_2 = final weight of dried sample (g)
- Moisture content of the raw material was determined to be $8.2 \pm 0.3\%$ (w/w). All extraction experiments were conducted using this standardized moisture content clove material.

2.2 Physical and Frictional Properties of clove buds

The frictional and particle characterization properties of whole clove buds were evaluated to determine their packing behaviour, density characteristics, porosity, and flow properties. All determinations were performed in triplicate and expressed as mean $\pm$ standard deviation.

2.2.1 Bulk Density

Bulk density was determined by gently filling a graduated measuring cylinder with a known weight of whole clove buds without applying external compaction. The bulk volume occupied by the sample was recorded, and the bulk density was calculated using the following equation:

$$\rho_b = \frac{M}{V_b}$$

Where,

- ρ_b = Bulk density (g/cm^3)
- M = Mass of sample (g)
- V_b = Bulk volume (cm^3)

2.2.2 True Density

True density was determined using the liquid displacement method, where the actual volume occupied by the whole clove buds excluding void spaces was measured. The true density was calculated using the equation:

$$\rho_t = \frac{M}{V_t}$$

Where,

- ρ_t = True density (g/cm^3)
- M = Mass of sample (g)
- V_t = True volume of sample (cm^3)

2.2.3 Porosity: Porosity of the packed whole clove buds was calculated from the bulk and true density values using the following formula:

$$\varepsilon = \left(1 - \frac{\rho_b}{\rho_t}\right) \times 100$$

Where,

- ε = Porosity (%)
- ρ_b = Bulk density
- ρ_t = True density

2.2.4 Angle of Repose: The angle of repose was determined by the fixed funnel method. Whole clove buds were allowed to flow through a funnel placed at a fixed height to form a conical heap on a flat surface. The height and radius of the heap were measured, and the angle of repose was calculated using the equation:

$$\tan \theta = \frac{h}{r}$$

Where,

- θ = Angle of repose ($^{\circ}$)
- h = Height of the heap (cm)
- r = Radius of the heap (cm)

2.2.5 Angle of Internal Friction: The internal friction angle of whole clove buds was determined using a shear testing method by measuring the resistance between particles under applied stress conditions. The internal friction coefficient was expressed as:

$$\mu_i = \tan \phi_i$$

Where,

- μ_i = Coefficient of internal friction
- ϕ_i = Internal friction angle ($^{\circ}$)

2.2.6 Angle of External Friction

External friction was evaluated by measuring the resistance between whole clove buds and the contact surface material. The coefficient of external friction was calculated using the equation:

$$\mu_e = \tan \phi_e$$

Where,

- μ_e = Coefficient of external friction
- ϕ_e = External friction angle ($^{\circ}$)
- The obtained parameters were used to assess the handling, storage, flowability, and processing characteristics of whole clove buds.

2.3 Physicochemical properties of extracted essential oil

2.3.1 Oil Yield: The essential oil from clove buds was extracted by hydro distillation using a Clevenger-type apparatus. A known quantity of dried clove buds was subjected to distillation for a fixed duration, and the extracted oil was separated and measured volumetrically. The percentage oil yield was calculated based on the dry weight of the plant material using the following equation (Singh *et al.*, 2007)^[17]:

$$\text{Oil Yield (\%)} = \frac{\text{Volume of extracted oil}}{\text{Weight of dried clove buds}} \times 100$$

Where,

- Volume of extracted oil = volume of oil obtained after extraction (mL)
- Weight of dried clove buds = weight of sample used for extraction (g)

2.3.2 Specific Gravity

Specific gravity of the extracted clove bud essential oil was determined at 20 ± 0.1 °C using a digital densitometer. The measurement was based on the ratio of oil density to the density of distilled water at the same temperature (Burt, 2004) [12].

$$SG = \frac{\rho_{oil}}{\rho_{water}}$$

Where,

- SG= specific gravity
- ρ_{oil} = density of oil at 20 °C (g/cm^3)
- ρ_{water} = density of water at 20 °C (0.998 g/cm^3)

2.3.3 Refractive Index: The refractive index of clove bud essential oil was measured at 20 °C using a hand-held refractometer. Prior to analysis, the refractometer was calibrated with distilled water ($n_D^{20} = 1.3330$). A few drops of oil sample were placed on the prism surface and the refractive index was recorded directly from the instrument scale (Jirovetz *et al.*, 2006) [13].

2.3.4 Saponification Value: The saponification value of clove bud essential oil was determined by refluxing a known quantity of oil sample with excess alcoholic potassium hydroxide solution followed by titration of the remaining alkali with standardized hydrochloric acid using phenolphthalein as an indicator (Adejumo *et al.*, 2013) [14].

$$SV = \frac{(V_0 - V_1) \times N \times 56.1}{W}$$

Where,

- SV= saponification value (mg KOH/g)
- V_0 = volume of HCl used for blank (mL)
- V_1 = volume of HCl used for sample (mL)
- N= normality of HCl
- W= weight of oil sample (g)

- 56.1 = molecular weight of KOH

2.3.5 Acid Value

Acid value was determined by titration of the oil sample with standardized potassium hydroxide solution using phenolphthalein as an indicator until a persistent pale pink color appeared (Gülçin *et al.*, 2012) [15].

$$AV = \frac{V \times N \times 56.1}{W}$$

Where,

- AV= acid value (mg KOH/g)
- V= volume of KOH solution used (mL)
- N= normality of KOH solution
- W= weight of oil sample (g)
- 56.1 = molecular weight of KOH

2.3.6 Iodine Value

The iodine value of clove bud essential oil was determined using the Wijs method. The oil sample was treated with Wijs solution under dark conditions followed by titration with standardized sodium thiosulfate solution using starch as an indicator (Chaieb *et al.*, 2007) [16].

$$IV = \frac{(V_0 - V_1) \times N \times 12.69}{W}$$

Where,

- IV= iodine value (g/100 g)
- V_0 = volume of sodium thiosulfate used for blank (mL)
- V_1 = volume of sodium thiosulfate used for sample (mL)
- N= normality of sodium thiosulfate solution
- W= weight of oil sample (g)

2.4 Experimental Design

A three-level factorial experimental design was employed with three independent variables: microwave power, extraction time, and sample ratio. Each variable was set at three levels, resulting in 27 unique treatment combinations. Each treatment was replicated 5 times ($n=135$ total observations) to provide statistical power for estimation of main effects and two-factor interactions. The experimental design is presented in Table 1.

Table 1: Experimental design parameters for microwave-assisted hydro distillation of Zanzibar clove buds

Factor	Symbol	Level 1	Level 2	Level 3	Unit
Microwave Power	A	250	350	450	W
Extraction Time	B	10	15	20	min
Sample Ratio	C	0.5:1	0.6:1	0.7:1	g:ml
Replicates	n	5	5	5	per treatment

*Three-level design with three independent variables results in 27 treatment combinations. Each treatment was replicated 5 times for a total of 135 observations

2.5 Statistical Analysis: All experimental data were analysed using Microsoft Excel and expressed as mean $\pm$ standard deviation (SD) from triplicate determinations ($n = 5$). Statistical significance between the obtained values was evaluated using p-values, and differences were considered statistically significant at $p < 0.05$. The calculated mean and

standard deviation values were used to assess the variability and reproducibility of the experimental results.

3. Results and Discussion**3.1 Physical and Frictional Properties of clove buds**

Table 2: Frictional Properties and Particle Characterization of Whole Clove Buds

S. No.	Property	Value (Mean±SD)	Unit	Interpretation
1.	Bulk Density	0.654±0.028	g/cm ³	Good packing ability
2.	True Density	1.127±0.035	g/cm ³	Dense solid structure
3.	Porosity	42.0±2.1	%	Moderate void spaces
4.	Angle of Repose	38.4±1.2	°	Fair flow characteristics
5.	Internal Friction	34.2±1.5	°	Good structural stability
6.	External Friction	28.1±1.8	°	Good flow behaviour

The frictional and particle characterization studies of whole clove buds demonstrated satisfactory physical and flow-related properties. The bulk density value (0.654±0.028 g/cm³) indicated good packing characteristics of the clove buds, whereas the true density (1.127±0.035 g/cm³) reflected the compact and dense nature of the biological material. The difference between bulk and true density suggests the presence of inter-bud spaces within the packed sample.

The porosity value (42.0±2.1%) revealed moderate void spaces between the clove buds, which may facilitate aeration and solvent penetration during storage or extraction processes. The angle of repose (38.4±1.2°) indicated fair flow characteristics, suggesting that the whole clove buds possess acceptable handling and movement properties during transportation and processing.

Furthermore, the internal friction value (34.2±1.5°) demonstrated adequate inter-surface cohesion and structural

stability among the clove buds, while the lower external friction value (28.1±1.8°) indicated reduced resistance against contact surfaces, contributing to smoother flow behaviour. Overall, the results suggest that whole clove buds exhibit satisfactory packing efficiency, stability, and handling characteristics suitable for storage, processing, and extraction applications.

3.2 Effect of Microwave Power on Oil Yield

Table 2 presents the mean oil yield values at each microwave power level, averaged across all time and ratio combinations (5 replicates per power level). Increasing microwave power from 250 W to 450 W resulted in a 35.3% increase in mean oil yield (9.73% to 13.14%), demonstrating the strong influence of this parameter on extraction efficiency.

Table 3: Effect of microwave power on essential oil yield from Zanzibar clove buds

Microwave Power (W)	Mean Oil Yield (%)	Std. Dev.	n
250	9.73	0.89	45
350	11.97	1.12	45
450	13.14	0.96	45

The beneficial effect of increased microwave power on oil yield is attributed to enhanced dielectric heating of polar molecules within the clove matrix. Microwave radiation at 2.45 GHz frequency selectively energizes polar components, generating rapid internal temperature elevation and facilitating cell wall disruption. At 250 W, lower energy input limits the rate of solvent penetration into plant tissues. At 350 W, intermediate conditions produce moderate enhancement of extraction kinetics. At 450 W, increased microwave intensity optimally balances mass transfer enhancement with energy efficiency, achieving maximum yields. Further power increases beyond 450 W would likely approach a plateau due to equilibrium limitations and potential thermal degradation of volatile oil components.

3.3 Effect of Extraction Time on Oil Yield

Table 3 presents the effect of extraction time on mean oil yield, averaged across all power and ratio combinations. Extending extraction from 10 to 20 minutes resulted in a 20.0% increase in oil yield (10.93% to 13.12%).

Table 4: Effect of extraction time on essential oil yield

Extraction Time (min)	Mean Oil Yield (%)	Std. Dev.
10	10.93	1.28
15	11.79	1.41
20	13.12	0.87

Extraction time influences oil recovery through its effect on mass transfer kinetics. At 10 minutes, limited contact time between solvent and plant tissue results in incomplete oil solubilization. The diffusion of essential oil molecules from the interior of clove cells to the external phase is kinetically controlled at short extraction times. At 15 minutes,

intermediate time allows for substantial oil extraction while maintaining volatile component integrity. At 20 minutes, prolonged extraction provides sufficient time for near-complete equilibration of oil concentration between plant tissue and extraction medium. The modest effect of extending time beyond 20 minutes (diminishing returns) suggests that most extractable oil has been mobilized, with further time providing incremental gains only (Bayramoglu *et al.*, 2008) [7].

3.4 Effect of Sample Ratio on Oil Yield

Table 4 presents the effect of sample ratio (clove mass per unit water volume) on mean oil yield. Increasing the sample ratio from 0.5:1 to 0.7:1 g:ml resulted in a 12.1% increase in oil yield (11.27% to 12.62%).

Table 5: Effect of sample ratio on essential oil yield

Sample Ratio (g: ml)	Mean Oil Yield (%)	Std. Dev.
0.5:1	11.27	1.63
0.6:1	11.80	1.55
0.7:1	12.62	1.01

*Values represent means from n=45 observations per ratio level (9 treatment combinations × 5 replicates)

Sample ratio (mass of plant material per unit volume of solvent) affects extraction through its influence on the concentration gradient driving mass transfer. At 0.5:1 ratio (50 g clove per 100 mL water), abundant solvent relative to sample mass provides high solvent capacity but lower local substrate concentration. At 0.7:1 ratio (70 g clove per 100 mL water), increased substrate concentration enhances the driving force for oil diffusion into the extraction medium. However, further increases in sample ratio beyond 0.7:1

would likely result in diminished returns due to saturation of solvent capacity and reduced circulation within the extraction vessel. The modest effect range for sample ratio (1.35% increase from 0.5:1 to 0.7:1) indicates that this parameter has secondary importance compared to power and time (Aspé & Fernández, 2011)^[8].

Table 6: Power × Time interaction showing mean oil yield (%) for all combinations of microwave power and extraction time

Power (W) / Time (min)	10 min	15 min	20 min	Mean (Time)	SD
250 W	8.43±0.42	9.53±0.48	10.77±0.54	9.58	1.02
350 W	10.37±0.52	11.77±0.59	12.77±0.64	11.64	1.20
450 W	11.80±0.59	13.47±0.67	14.67±0.73	13.31	1.37

The Power × Time interaction reveals that the beneficial effect of increased microwave power is consistent across all extraction time levels, but the magnitude of improvement varies. At 10 minutes extraction, power increase from 250 W to 450 W yields 39.7% improvement in oil yield (8.43% to 11.80%). At 15 minutes, the improvement is 41.5% (9.53% to 13.47%). At 20 minutes, improvement is 36.4% (10.77% to 14.67%). This demonstrates that higher microwave power consistently enhances extraction efficiency regardless of extraction duration, with the greatest absolute yield

3.5 Interaction Effects: Power × Time

Table 5 presents the two-way interaction between microwave power and extraction time, showing mean oil yield values for all nine combinations of these two factors (averaged across the three sample ratio levels and 5 replicates).

differences observed at longer extraction times (where baseline yields are higher). The non-parallel trend lines indicate a genuine interaction, suggesting that optimal microwave power selection should consider the intended extraction duration (Hallnäs & Redström, 2006)^[9].

3.6 Interaction Effects: Power × Sample Ratio

Table 6 presents the two-way interaction between microwave power and sample ratio, with mean oil yield values for all nine combinations of these factors.

Table 7: Power × Sample Ratio interaction showing mean oil yield (%) for all combinations of microwave power and sample ratio

Power (W) / Ratio (g:ml)	0.5:1	0.6:1	0.7:1	Mean (Power)	SD
250 W	9.23±0.46	9.63±0.48	10.27±0.51	9.71	0.51
350 W	11.50±0.58	12.00±0.60	12.43±0.62	12.00	0.60
450 W	12.67±0.63	13.37±0.67	13.37±0.67	13.14	0.66

The Power × Ratio interaction demonstrates that microwave power shows a consistent positive effect on oil yield across all sample ratio levels, with power increases from 250 W to 450 W providing 37.4%, 38.9%, and 30.1% improvements at 0.5:1, 0.6:1, and 0.7:1 ratios, respectively. At lower power (250 W), sample ratio has a modest effect (11.3% improvement from 0.5:1 to 0.7:1). At 350 W, the ratio effect is similar (8.1% improvement). At 450 W, an interesting plateau occurs where 0.6:1 and 0.7:1 ratio shows nearly equivalent yields (13.37%), suggesting that at high microwave power, increasing sample mass beyond 60 g per

100 mL water provides minimal additional benefit. This interaction indicates that the optimization should favour 450 W power combined with either 0.6:1 or 0.7:1 sample ratio for practical considerations (Kutner & Nachtsheim, 2005)^[10].

3.7 Physicochemical properties of extracted clove essential oil

Table 7 presents the physicochemical quality parameters of essential oil extracted at optimal conditions (450 W, 20 minutes, 0.7:1 sample ratio). All parameters conform to international pharmacopeial standards.

Table 8: Physicochemical properties of Zanzibar clove oil extracted at optimal conditions (450 W, 20 min, 0.7:1 ratio)

Property	Value (Mean ±SD)	Range	Compliance
Oil Yield (%)	14.8±0.6	12-18	Pass
Specific Gravity	1.048±0.008	1.045-1.055	Pass
Refractive Index	1.531±0.003	1.527-1.537	Pass
Saponification Value (mg KOH/g)	187±4	180-195	Pass
Iodine Value (g/100g)	96±3	90-105	Pass
Acid Value (mg KOH/g)	1.2±0.2	0.5-2.0	Pass

The extracted clove oil demonstrates excellent quality conforming to all international standards for pharmaceutical and cosmetic applications. The specific gravity and refractive index are sensitive indicators of oil composition and purity, with values indicating high concentrations of eugenol and other monoterpenes compounds. The low acid value (1.2 mg KOH/g) indicates minimal oxidative degradation and excellent oil stability suitable for extended storage. The saponification and iodine values reflect the degree of unsaturation and presence of esterified compounds, critical for product performance in various formulations (Barak *et al.*, 2023)^[11].

4. Conclusion

This comprehensive three-level factorial experimental design

study successfully optimized microwave-assisted hydrodistillation for essential oil extraction from Zanzibar clove buds. Systematic evaluation of microwave power, extraction time, and sample ratio with appropriate replication (n=135 total observations) revealed that microwave power is the dominant factor influencing oil yield, followed by extraction time and sample ratio. Optimal extraction conditions of 450 W microwave power, 20 minutes extraction time, and 0.7:1 sample ratio (70 g clove per 100 mL water) yielded 14.8±0.6% essential oil with excellent physicochemical properties meeting international pharmacopeial standards. Significant interactions between power and time, and between power and ratio, indicate that optimal parameter selection requires consideration of multiple variables simultaneously. The extracted oil

demonstrated specific gravity of 1.048 ± 0.008 g/cm³, refractive index of 1.531 ± 0.003 , saponification value of 187 ± 4 mg KOH/g, iodine value of 96 ± 3 g/100g, and acid value of 1.2 ± 0.2 mg KOH/g. Particle characterization of residual material showed bulk density of 0.654 ± 0.028 g/cm³, porosity of $42.0 \pm 2.1\%$, and angle of repose of $38.4 \pm 1.2^\circ$. These results provide a quantitative foundation for implementing microwave-assisted hydrodistillation at industrial scale for Zanzibar clove essential oil production.

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