Efficacy of ultrasonic pre-treatment on extraction of clove *(syzygium aromaticum)* essential oil

***Adya* Asad**[1]**, *Neha* Sinha**[1]**, *Sachin* Ghanghas**[1*]**, *Abhishek* Sharma**[2]**, *Nutan* Kaushik**[1,2]

[1]Amity Institute of Horticulture Studies and Research, Amity University, Noida, Uttar Pradesh, India
[2]Amity Food & Agricultural Foundation, Amity University, Noida, Uttar Pradesh, India
[*]**Corresponding Authors:** sghanghas@amity.edu

Abstract. Postharvest deterioration of horticultural crops remains a major food security problem, majorly due to fungal contamination and mycotoxin formation during storage and transportation. Clove essential oil considered a potential alternative with anti-fungal, anti- microbial properties. The extraction yield of clove essential oil (CEO) depends greatly on extraction method and operating conditions. The present study evaluated the effect of ultrasonic pre-treatment on CEO extraction during hydro-distillation using Response Surface Methodology (RSM) based on a two factor three-level Full Factorial Design. A sample were given ultrasonic pre-treatment in an ultrasonic water bath under different temperature (35–45 °C) and ultrasonication time (25–45 minutes) conditions and subjected to hydro-distillation for essential oil extraction. Experimental results showed that ultrasonic pre-treatment greatly affected CEO extraction. The maximum CEO yield of 8.62 % was obtained at 40 °C and 35 minutes, whereas the minimum yield of 4.68 % was recorded at 45 °C and 45 minutes treatment. The visualized response surface plots, contour plots, and interaction analysis validates significant combined effects of ultrasonic temperature and treatment time on extraction performance. The study showed that ultrasonic pre-treatment combined with hydro-distillation improved CEO extraction efficiency under optimized operating conditions.
Keywords: Clove (*Syzygium aromaticum*), Essential oil extraction, *Alternaria alternata*, Eugenol, Ultrasonic-assisted extraction, Hydro-distillation

1. Introduction

Post-harvest deterioration of horticultural crops remains a major food security problem, where 25–40 % of global fruit production is lost mainly due to fungal contamination and mycotoxin development during storage and transportation [1,2]. Synthetic fungicides are commonly used to control these losses, although continuous use has increased problems related to pathogen resistance, environmental contamination, and chemical residue risks to human health [3,2]. These limitations have increased interest in plant-based biological preservatives for postharvest disease management [1]. Essential oils (EOs) contain terpenoids and phenylpropanoids with strong antifungal activity [3]. Clove essential oil (CEO) obtained from *Syzygium aromaticum* contains nearly 50–90 % eugenol, which acts as a broad-spectrum antimicrobial compound by disrupting microbial cell membranes and inhibiting metabolic enzyme activity. Because of these properties, CEO is considered a potential eco-friendly alternative to synthetic fungicides [4,5]. The yield and biological activity of CEO depend greatly on extraction technology. Conventional hydro-distillation (HD) and steam distillation (SD) often produce lower yield, require longer extraction time, and may cause thermal degradation of eugenol [4-6]. Ultrasonic-assisted extraction combined with hydro-distillation has gained attention as a green extraction method. Acoustic cavitation generated during ultrasonic treatment produces microscopic bubble collapse and mechanical vibration that disrupt plant cell walls, improving solvent penetration and intracellular mass transfer [5-7]. However, optimized operational conditions for ultrasonic-assisted hydro-distillation of CEO are still not well established, and limited studies have applied statistical optimization tools such as Full Factorial Design (FFD) and Response Surface Methodology (RSM) [8,6]. Therefore, the present study aims to optimize ultrasound bath-assisted hydro-distillation conditions to maximize CEO yield and eugenol concentration for eco-friendly postharvest antifungal applications.

2. Materials and Methods

2.1 Materials and Sample Preparation

The clove buds procured from a local supermarket in Noida, Uttar Pradesh, India and were manually broken in two parts. For each experimental run, a standard weight of 50 g of broken clove buds was mixed with 500 mL of distilled water in a conical flask as shown in Fig. 1, respectively [7].

2.2 Ultrasonic Pretreated Hydro-distillation

The conical flask containing the sample mixture was placed inside an ultrasonic water bath (Make-Labman Digital Ultrasonic Cleaner, Model -2023) operated at a fixed frequency of 40 kHz. Based on observations from previous studies, the experimental range for ultrasonic pre-treatment parameters included temperature from 35°C to 45°C and treatment time from 25 min to 45 min [1]. After ultrasonic pre-treatment, the samples were subjected to hydro-distillation using a Clevenger apparatus for extraction of essential oil. The hydro-distillation process was conducted at 70–80°C for 1 h under controlled operating conditions. The treated samples placed in the round-bottom flask were heated using a heating mantle, as shown in Fig. 1. The round-bottom flask was connected to the Clevenger apparatus consisting of a condenser and a separatory funnel for collection and separation of clove essential oil (CEO). The extracted CEO was transferred into labeled glass vials and stored at 4°C in a deep freezer. The extracted CEO weight was measured using a weighing balance, and the extraction yield (%) was calculated as follows:

$$\text{Yield}(\%) = \frac{\text{Essential oil weight (g)}}{\text{Sample weight (g)}} \times 100$$

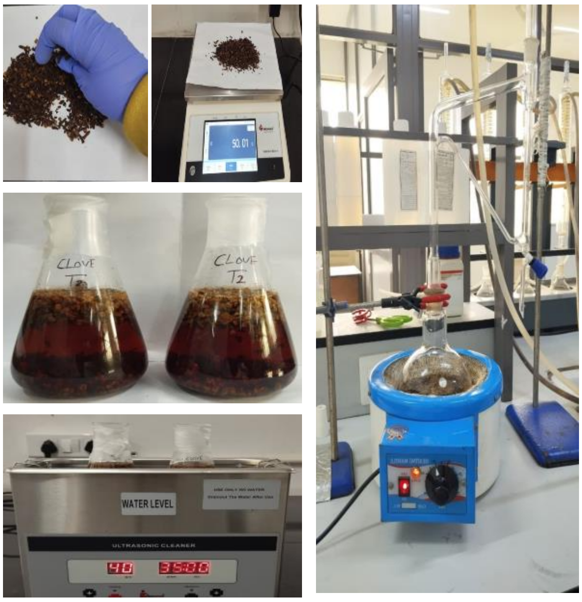

Fig. 1: Process methodology for Ultrasonic Pre-Treated Hydro-distillation system

2.3 Experimental Design

The response surface methodology (RSM) used a 3-level Full Factorial Design (FFD, 3^k) to evaluate and optimize the process parameters affecting essential oil yield. The experimental design included two independent parameters (k=2): ultrasonic temperature (°C) and ultrasonic time (minutes) with one dependent parameter (CEO (%)). Each factor was studied at three different levels (-1, 0, 1) as shown in Table 1, respectively. This resulting in a total i.e., 3^2 = 9 experimental combinations. The design was used to examine the individual and combined effects of ultrasonic temperature and treatment duration on essential oil extraction performance. The experimental combinations were made using Design Expert 13.0.5.0 software (State-Ease Inc., Minneapolis, USA) [8]. After completion of the experiments, the response data were fitted to a 2nd order polynomial equation for prediction of optimum extraction conditions as given by:

$$Y = \beta_0 + \Sigma\beta_i x_i + \Sigma\beta_{ii} x_i^2 + \Sigma\Sigma\beta_{ij} x_i x_j + \varepsilon$$

Where Y represents essential oil yield, β_0 is the constant term, and β_i, β_{ii}, and β_{ij} represent linear, quadratic, and interaction coefficients, respectively. A_i and B_j denote the independent parameters, while (ε) indicates random error. Analysis of variance (ANOVA) was used for statistical evaluation of the developed model.

Table 2: Independent process parameters and their coded levels used in experimental design

Independent Parameters	Coded levels		
	-1	**0**	**1**
US Temperature (°C)	35	40	45
US Time (minutes)	25	35	45

2.4 Statistical analysis

The experimental results were subjected to statistical analysis. The Table 2 represents the standard equation of these statistical parameters. The mean value and standard deviation were used to measure data variation among observations, while C.V. % was used to compare relative variability of the experimental data. The values of Coefficient of determination (COD) R^2, R^2adj, and R^2pred were calculated to evaluate model fitting, prediction ability, and adequacy of the developed polynomial model. Residual sum of squares (SSres), total sum of squares (SStot), prediction error sum of squares (PRESS), number of observations (n), and number of model terms (p) were used during statistical evaluation of the response data [1].

Table 1: Statistical analysis parameter with their corresponding mathematical equations

Statistical Parameters	Equations
Mean ($\bar{x}$); Standard deviation (s); Coefficient of variation (C.V)	$\bar{x} = \frac{\Sigma x_i}{n}$; $s = \sqrt{\frac{\Sigma(x_i-\bar{x})^2}{n-1}}$; C.V. % $= \left(\frac{S}{\bar{x}}\right) \times 100$
COD (R^2); Adjusted COD (R^2_{adj}); Predicted COD (R^2_{pred})	$R^2 = 1 - \frac{SS_{res}}{SS_{tot}}$; $R^2_{adj} = 1 - \left[\frac{(1-R^2)(n-1)}{n-p-1}\right]$; $R^2_{pred} = 1 - \frac{PRESS}{SS_{tot}}$

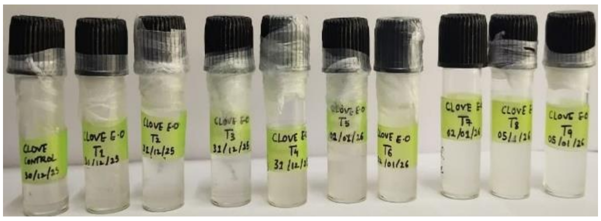

Fig. 2: Extracted CEO (%) with US pre-treatment (Control: T_0) and without US pre-treatment (Experiments: T_1- T_9)

3. Results and Discussion

3.1 RSM Modelling

Table 3 shows the experimental design results for ultrasonic pre-treatment conditions, including temperature and sonication time, and their effect on clove essential oil yield at a fixed sample-to-water ratio of 50 g:500 ml. The RSM surface plot, contour plot shows interaction effect, and individual graphs affecting clove essential oil yield given in Fig. 1, respectively. The clove essential oil yield varied from 4.68 to 8.62 %, as shown in Fig. 2, respectively. The observations are aligning with literature review studies on *Syzygium aromaticum*, which report extraction ranges between 4.5% and 8.9% under optimized acoustic and thermal condition [9,10]. The un-treated clove buds which recievies direct hydro-distillation act as a control (T_0) showed 6.9 % yield. In most treatments, ultrasonic treatment increased essential oil extraction compared with the control. A combined effect of temperature and sonication time was observed during extraction. At 35 and 40 °C, the highest yields were obtained at 35 minutes, with values of 8.52 % (T_8) and 8.62 % (T_2), respectively. Further increase in sonication time to 45 minutes reduced the yield to 7.7 %. At 45 °C, the highest essential oil yield (8.48 %, T_6) was obtained at 25 minutes. The longer duration treatment at this temperature continuously reduced the yield, and the lowest value of 4.68 % (T_4) was recorded at 45 minutes. The increase in essential oil yield during ultrasonication may be related to breakdown of plant cells and faster release of essential oil compounds into the water. Similar observations were reported in *Cinnamomum tamala* and *Plectranthus amboinicus* during ultrasonic extraction [11,12]. Longer sonication time reduced essential oil extraction in the present study. Earlier reports showed that prolonged sonication increased cell surface temperature and affected essential oil content [11]. Extended ultrasonication may also produce free radicals in the extraction system, which affects stable essential oil extraction [12]. At higher temperature, reduction in essential oil yield may be associated with loss or degradation of heat-sensitive volatile compounds during extraction.

Table 3: Essential oil yield under different pre-treatment experimental combination

Run	**US Temperature (°C)**	**US Time (minutes)**	**Essential oil (%)**
Control			**6.9**
T_0	35	35	8.52
T_1	40	35	8.62
T_2	40	45	7.7
T_3	40	25	8.4
T_4	45	25	8.48
T_5	35	25	8.28
T_6	35	45	7.7
T_7	45	35	6.9
T_8	45	45	4.68

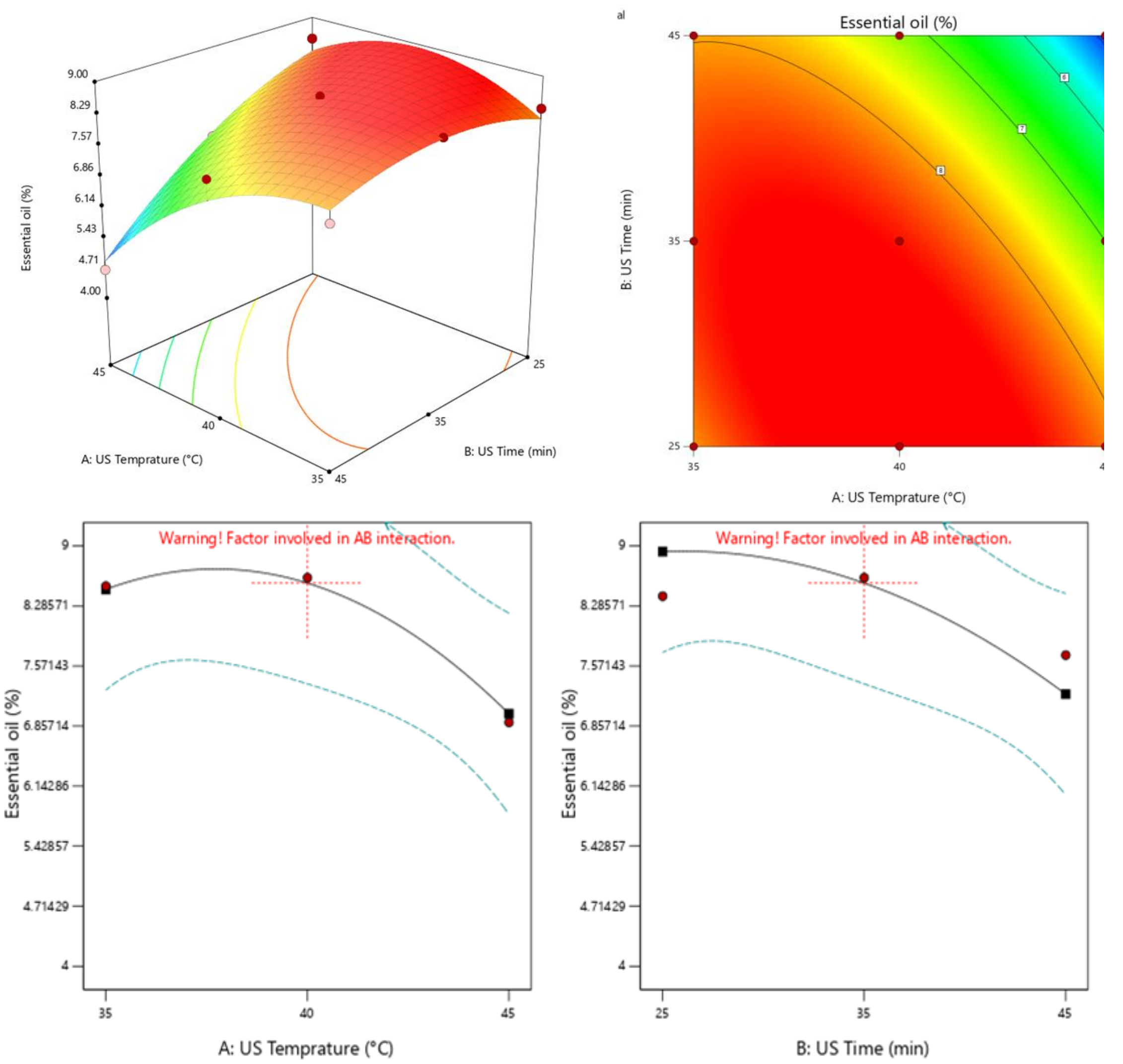

Fig. 3: Response surface plots, contour graph, and perturbation plots between independent and dependent parameters

The regression equation showed that ultrasonic temperature, extraction time, and their interaction collectively affected clove essential oil yield during ultrasonication extraction. It is clearly seen in the mathematical model equation that individual effect shows the US temperature coefficient significantly higher than that of US time, process parameters combined effect is less significant and quadratic effect found not significant.

Clove Essential oil (%) = −62.95278 + 3.01817 × (US Temperature) + 0.890667 × (US Time) − 0.016100 × (US Temperature × US Time) − 0.032533 × (US Temperature²) − 0.004733 × (US Time²)

3.2 Effect of Ultrasonic Temperature on Extraction

As shown in the Fig. 3 and Table 3, ultrasonic temperature greatly affected clove essential oil extraction. With the increase in temperature up to 40 °C increased cavitation action and rupture of plant cells. At the same temperature with increase from 25 to 35 minutes, the minor increase in output but further increase in time to 45 minutes reduces yield to significant level (7.7 %). Similar effect has been reported by Li et al. where the decrease of yield may be due to the free radicals which could lead to the degradation of essential oils [13]. More essential oil compounds moved from plant tissue into the water under these conditions. Similar findings were reported earlier, where 30-40 °C temperature softened the plant tissue and increase the release of plant extracts during ultrasonication [14]. Earlier work also showed higher essential oil yield between 25 and 40 °C, while further rise in temperature reduced the yield [15]. Further increase to 45 °C reduced the yield in the present study. Longer exposure at higher temperature may increase evaporation and breakdown of volatile essential oil compounds during ultrasonication. Earlier reports showed that prolonged sonication increased cell surface temperature and reduced essential oil content [11]. Longer extraction periods may also increase oxidation during extraction [14]. Temperature and sonication time together affected extraction efficiency. The highest clove essential oil yield was obtained at 40 °C with 35 minutes treatment, whereas prolonged sonication at 45 °C gave the lowest yield. Similar studies reported that moderate ultrasonic conditions improved cavitation and rupture of cell walls, while excessive ultrasonication caused localized heating and lower essential oil extraction [11,16].

3.3 Effect of Ultrasonic Time on Extraction

As shown in the Fig. 3 and Table 3, essential oil yield increased with increase in ultrasonication time up to 35 minutes, followed by a reduction at longer treatment duration. The highest essential oil extraction was obtained at 35 minutes sonication time, whereas lower yield was recorded at both lesser and higher treatments. The response pattern showed a gradual increase in essential oil yield up to the optimum sonication period, followed by a decline with further ultrasonication. High-frequency ultrasound waves can induce cavitation, forming bubbles that can rupture the cell walls and release bioactive compounds. However, excessive prolonged cavitation can also degrade the compounds, resulting in a reduction in yield [1]. Similar observations were reported in *Cinnamomum tamala* during ultrasonic assisted extraction [11]. Increase in essential oil extraction at 35 minutes sonication time may be related to cavitation action, rupture of plant cells, and release of essential oil compounds into the water [16]. Another study also reported that for a constant time of sonication and increase in temperature will collapse of microbubbles during ultrasonication improved cell wall disruption and increased release of plant compounds [11]. In contrast, longer ultrasonication reduced essential oil extraction. Longer exposure may increase cell surface temperature and affect stability of volatile essential oil compounds. Similar reduction in essential oil yield after optimum sonication duration was also reported earlier [11].

Table 4 : ANOVA for the RSM model of clove essential oil extraction under ultrasonic-assisted extraction conditions

Source	Sum of Squares	df	Mean Square	F-value	p-value	Model
Model	11.95	5	2.39	9.38	0.0473	**Significant**
A-US Temperature	3.29	1	3.29	12.90	0.0370	
B-US Time	4.30	1	4.30	16.89	0.0261	
AB	2.59	1	2.59	10.18	0.0497	
A^2	1.32	1	1.32	5.19	0.1070	
B^2	0.4481	1	0.4481	1.76	0.2766	
Residual	0.7641	3	0.2547			
Cor Total	12.71	8				

3.4 Statistical Analysis and Numerical Optimization

As shown in Table 4, analysis of variance (ANOVA) showed that the quadratic model for clove essential oil extraction was significant with a p-value of 0.0473. Ultrasonic temperature, sonication time, and their interaction significantly affected essential oil yield, whereas quadratic terms remained non-significant under the selected extraction conditions. The response surface model used for clove essential oil extraction showed good statistical fit. The coefficient of determination (R^2) was 0.9399, while adjusted R^2 was 0.8397. The coefficient of variation (C.V.) was 6.56%, which validate experimental significance. Adequate Precision value reached 9.8327 and showed sufficient signal-to-noise ratio within the experimental range. The regression analysis observed a significant linear effect of US temperature and US time on essential oil yield, with p-values of 0.0370 and 0.0261, respectively. The interaction between US temperature and US time remained significant ($p = 0.0497$). The numerical optimization within the selected range of 30–50 °C and 20–40 minutes identified optimum conditions at 36.95 °C and 34.28 minutes. Under these conditions, the model predicted 8.727% essential oil yield with desirability value of 1. RSM optimization predicted a maximum clove essential oil yield of 8.727% at an ultrasonic temperature of 36.949°C and treatment time of 34.278 min. Validation experiments were conducted under nearly similar conditions at 37°C and 34 min using three replications (R_1, R_2, and R_3), which produced essential oil yields of 8.12%, 7.16%, and 9.1%, respectively. The experimental values remained close to the predicted response, which confirmed the adequacy and prediction ability of the developed RSM model for clove essential oil extraction. Across three replications produced maximum essential oil extraction of 9.1%. The observed yield remained close to the predicted value from the mathematical model which validated the experiment.

4. Conclusion

Ultrasonic pre-treatment affected clove essential oil extraction effectively. Essential oil yield varied from with treatment in comparison to without pre-treatment value of clove essential oil yield. Increase in ultrasonication time and temperature up to an optimum range increased essential oil because of the strong cavitation action and rupture of plant cells which promoted movement of essential oil compounds into the water. The longer ultrasonication time with higher temperature reduced the CEO yield due to evaporation and degradation of volatile compounds during extraction. Numerical optimization identified optimum conditions which validate the process parameters and response experimental yield remained close to the predicted response and the developed model predicted extraction conditions satisfactorily. The optimized ultrasonic-assisted extraction conditions improved clove essential oil yield through effective cavitation and enhanced rupture of plant tissues during extraction. These conditions also reduce extraction time and energy requirement compared with prolonged conventional extraction methods. The optimized process can be applied for industrial-scale production of clove essential oil in food preservation, pharmaceutical, and active packaging applications. The detailed quantification of active compounds such as eugenol using GC-MS and evaluation of additional parameters associated with ultrasound pre-treatment effects will be considered in future studies for deeper validation of the extraction process.

References

1. A.H. Abdul Aziz, D.N. Rizkiyah, L. Qomariyah, I. Irianto, M.A. Che Yunus, N.R. Putra, Unlocking the Full Potential of Clove (*Syzygium aromaticum*) Spice: An Overview of Extraction Techniques, Bioactivity, and Future Opportunities in the Food and Beverage Industry. Processes **11**, 2453 (2023)
2. R. Kaur, S. Kaushal, V. Kaur, V. Kumar, A. Kalia, Optimization and comparison of microwave and ultrasound assisted extraction of clove bud oleoresins in terms of chemical composition and biological potential. J. Food Meas. Charact. **18**, 9999–10017 (2024)
3. P. Ramudingana, N. Makhado, C.N. Kamutando, M.S. Thantsha, T.P. Mamphogoro, Fungal biocontrol agents in the management of postharvest losses of fresh produce-A comprehensive review. J. Fungi **11**, 82 (2025)
4. Q.A. Al-Maqtari, A. Rehman, A.A. Mahdi, W. Al-Ansi, M. Wei, Z. Yanyu et al., Application of essential oils as preservatives in food systems: challenges and future prospectives-a review. Phytochem. Rev. **21**, 1209–1246 (2022)
5. C. Chilev, D. Peshev, Hydro-distillation versus steam distillation for clove oil production. J. Chem. Technol. Metall. **60**, 311–318 (2025)
6. J.N. Haro-González, G.A. Castillo-Herrera, M. Martínez-Velázquez, H. Espinosa-Andrews, Clove essential oil (*Syzygium aromaticum* L. Myrtaceae): Extraction, chemical composition, food applications, and essential bioactivity for human health. Molecules **26**, 6387 (2021)
7. P. Suttiarporn, T. Seangwattana, T. Srisurat, K. Kongitthinon, N. Chumnanvej, S. Luangkamin, Enhanced extraction of clove essential oil by ultrasound and microwave assisted hydrodistillation and their comparison in antioxidant activity. Curr. Res. Green Sustain. Chem. **8**, 100411 (2024)
8. D. Mohanty, G. Dalei, S. Das, D. Jena, B.R. Das, Essential oils of *Tagetes patula*: a study on ultrasound-assisted extraction, characterization, and in vitro biological profiles. Biomass Convers. Biorefin. **15**, 18013–18024 (2025)
9. P.L. Jain, S. Srivastav, S.R. Patel, M.A. Desai, Synergetic effect of ultrasound and hydrodistillation for extraction of patchouli oil: Screening, optimization and comparison. Chem. Eng. Process. **179**, 109079 (2022)
10. P. Suttiarporn, T. Taithaisong, S. Namkhot, S. Luangkamin, Enhanced eugenol composition in clove essential oil by deep eutectic solvent-based ultrasonic extraction and microwave-assisted hydrodistillation. Molecules **30**, 504 (2025)
11. P.H. Jon, J.H. Shourove, M.K. Ali, O. Rahman, M.U. Thakur, G.R. Islam, Optimizing sonication-assisted hydrodistillation of *Cinnamomum tamala* essential oil using response surface methodology and artificial neural network modeling. Sci. Rep. (2026)
12. N.A.A.R. Zahari, G.H. Chong, L.C. Abdullah, B.L. Chua, Ultrasonic-assisted extraction (UAE) process on thymol concentration from *Plectranthus amboinicus* leaves: Kinetic modeling and optimization. Processes **8**, 322 (2020)
13. Y. Li, Y. Zhang, X. Sui, Y. Zhang, H. Feng, L. Jiang, Ultrasound-assisted aqueous enzymatic extraction of oil from perilla (*Perilla frutescens* L.) seeds. CyTA J. Food **12**, 16–21 (2014)
14. P. Stržincová, I. Šurina, M. Jablonský, V. Majová, A. Ház, K. Hroboňová, A. Špačková, Analyzing the Effect of Extraction Parameters on Phenolic Composition and Selected Compounds in Clove Buds Using Choline Chloride and Lactic Acid as Extraction Agents. Processes **12**, 653 (2024)

15. S. Mollaei, S. Hazrati, Z. Mamizadeh, H. Hashempour, The effect of ultrasonic pre-treatment on the yield, chemical composition and biological activity of essential oil in *Oliveria decumbens* flowers. J. Appl. Res. Med. Aromat. Plants **24**, 100313 (2021)
16. D. Belhachat, L. Mekimene, M. Belhachat, A. Ferradji, F. Aid, Application of response surface methodology to optimize the extraction of essential oil from ripe berries of *Pistacia lentiscus* using ultrasonic pre-treatment. J. Appl. Res. Med. Aromat. Plants **9**, 132–140 (2018)