# Extraction of Essential Oil from Leaves of Lemongrass Using Microwave Radiation: Optimization, Comparative, Kinetic, and Biological Studies

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ABSTRACT: Use of microwave radiation in extraction of natural products is increasing in an exponential manner because of its inherent greener features. The extraction of essential oil from the leaves of lemongrass was carried out using microwave radiation and compared with hydrodistillation. The process was optimized using the Taguchi method, and the highest yield was obtained at a 200 mL volume of water, 30 min of rehydration time, and 45 min of extraction time. The extraction time was found to be the most influential parameter affecting the process. A data fit for kinetic desorption models was carried out. The biological activities of the lemongrass oil were also assessed. A microscopic study was performed to understand the extraction mechanism. A comparison showed that microwave-assisted extraction is a better alternative to hydrodistillation in terms of yield, extraction time, biological activity, energy efficiency and environmental friendliness.

KEYWORDS: *Microwave-assisted extraction, Taguchi method, Kinetic model, Biological assessment, Microscopy*
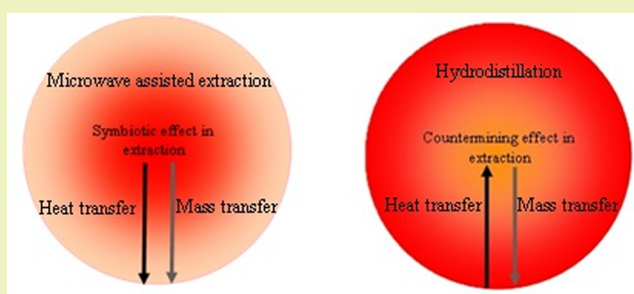
INTRODUCTION

A current trend of modern society is "green consumerism", which is desiring fewer synthetic ingredients in food, flavor, and perfume. Plant-derived chemicals are considered as "generally recognized as safe" (GRAS), and hence, they are widely being used in cosmeceuticals, food, flavor, and fragrance.[1−3] In addition, application of phytochemicals in the medicinal field is increasing because of the broad spectrum of structural diversity, undoubted effectiveness as phytomedicines, and usage as intermediate compounds for the production of synthetic drugs.[4,5] Essential oil, one of the important phytochemicals, is a mixture of various bioactive compounds, and because of this, it demonstrates various biological activities.[6] Lemongrass, a perennial *Cymbopogon* grass, is the rich source of lemon-scented essential oil.[7] Essential oil, extracted from the leaves of lemongrass, possesses a wide range of biological activities like anticancer, antimicrobial, antifungal, and antioxidant properties,[8−12] which have resulted in the application of lemongrass oil in orthopedics, muscular and skin problems,[9] food preservation,[3,13] herbicides,[14] and much more. The oil is also regarded as an antidepressant agent.[15] The exotic citrus odor of the oil has contributed to its application as a flavoring agent in food, perfume and toiletries.[16] Citral, a mixture of two isomers, neral and geranial, is the major compound present in lemongrass oil, and it is also responsible for the bioactivities of the oil.[17,18] The quality of the oil is judged by the amount of citral present in the oil.[17,18] Higher citral content is therefore desirable in the oil obtained from the leaves of lemongrass.

Conventionally, lemongrass oil was extracted using solvent extraction, steam distillation, and hydrodistillation.[19−22] How-ever, the conventional techniques suffer from severe drawbacks like the inability to resolve solvent residual problems in the finished product, longer extraction time, and energy inefficiency of the process. The usual techniques are further hindered the stringent regulatory norms for the quality of the final product isolated from the plant as well as for the protection of the environment. Supercritical fluid extraction was applied for the extraction of lemongrass oil.[19,20,23] Although this technique has proved its significance as a green route for the extraction of natural products, the higher cost of equipment and the high operating pressure make the process viable only for high-value products.

In recent years, use of microwave radiation in extraction of phytochemicals has gained significant exposure because it provides higher extraction efficiency with reduced time and less solvent consumption.[24−27] Energy competence and environmental friendliness are the key features of microwave-assisted extraction (MAE).[25−31] In addition, the use of MAE, which can be employed on water-containing biomass, provides an alternative to extraction with organic solvents.[25,31] A higher rate of extraction and yield of the natural product could be obtained in the case of MAE compared to conventional as well as other novel techniques.[24,25,32]

Authors have conducted a parametric study for the extraction of essential oil from the leaves of lemongrass using MAE[33] and obtained promising results to further carry out the work. In the

present study, optimization of the extraction of the essential oil from the leaves of lemongrass using microwave radiation was conducted using the Taguchi method.[34−39] An analysis of variance (ANOVA) was applied to find the most influential parameter affecting the process. The chemical composition of the lemongrass oil extracted by MAE was studied and compared with the oil obtained by hydrodistillation (HD). A kinetic study involving kinetic desorption models was also carried out to verify the extraction efficiency. Because it was advisable to assess the biological activities of the essential oil for strengthening the method employed for the extraction, antimicrobial and antioxidant activities of the oils extracted by both HD and MAE were investigated. A scanning electron microscopic study was performed to have an insight into the extraction process. The effect of heat and mass transfer processes on the extraction of essential oil from lemongrass leaves by MAE and HD was investigated. A comparison of MAE and HD was done in terms of extraction time, yield, energy consumption, and environment impact. This work provides an exhaustive study on MAE starting from optimization to testing of the lemongrass oil, which is required for a technique to be considered at a higher scale.

■ MATERIALS AND METHODS

Materials. Leaves of lemongrass were collected in the month of September from Navsari Agriculture University (20° 57′ N latitude, 72° 54′ E longitude), Navsari, Gujarat, India. The harvesting time of leaves was 35 days. The leaves were kept in a moisture-free environment after drying them under a shed for 48 h at a room temperature. The moisture content of the dried leaves was found to be 6%. In our previous study,[22] size reduction showed a negative effect on yield of essential oil; hence, leaf size was reduced to only 25 mm and not further.

Methods. *Microwave-Assisted Extraction.* An extraction of essential oil from the leaves of lemongrass was performed in the MAE unit (M/s Falcon Microwave Technology, Mumbai, India). The water soaked plant material (100 g on dry basis) was kept in a flask of 2 L capacity along with the different volumes of water. Upon application of microwaves, the water molecules residing in the cells of leaves try to realign themselves with the electromagnetic field very rapidly.[40,41] The excited molecules generate a high amount of heat and are vaporized. The increased vapor content inside the cells leads to an increase in internal pressure toward the cell wall, subsequently leading to the rupture of the cell walls. Essential oil, being volatile in nature, is vaporized and carried along with water vapor toward the condenser, kept above the microwave cavity. The condensed essential oil and water are then decanted and separated. The oil was dehydrated and stored at 2 °C. The MAE unit was operated at 850 W power. The yield (*y*, %) of the extracted oil is found by eq 1. Each experiment was performed in triplicate.

$$y = \frac{V}{m} 100 \qquad (1)$$

where, *V* is the volume of oil collected in milliliters, and *m* is the mass of the raw material in grams.

Preliminary Studies. On the basis of previous work,[33] three parameters, viz., volume of water, rehydration time, and extraction time, were selected for the present study. Volume of water plays an important role while optimizing the process. Water, being a polar compound, absorbs microwave energy and heats up instantly. However, with an increase in water volume, microwave radiation per molecule decreases, which may lead to a delayed extraction. Considering this, water volume was varied from 200 to 500 mL. With an increase in rehydration time, water uptake by plant matrices increases, which may enhance the extraction rate. Water uptake by leaves of lemongrass at different rehydration times was studied and found almost constant after 3 h. Extraction time is a vital parameter to

have a complete extraction. Lower extraction time may lead to an incomplete extraction, and hence, it was varied up to 45 min. Power was kept constant at 850 W.

Design of Experiment. The planning of experiments was done with the help of the Taguchi method.[34−39] Three factors, viz., volume of water (200−500 mL), rehydration time (30−180 min), and extraction time (15−45 min), were studied at four levels. ANOVA was performed to find out the most significant parameter and percentage contribution of each parameter. The analysis of the data was carried out using Minitab software (version 16) (Minitab, Inc., State College, PA, U.S.A.). Complete details of the Taguchi method and ANOVA can be obtained from various literature.[34−39]

Hydrodistillation. Hydrodistillation was performed in a circulatory Clevenger-type apparatus (2 L capacity) under optimized conditions.[42] The operating conditions were 50 g of lemongrass leaves, 700 mL of water, 500 W power, and 1.5 h extraction time. The experiments were performed in triplicate. The essential oil was stored at 2 °C after dehydration.

Analysis by Gas Chromatography−Mass Spectrometry (GC−MS). Essential oil was analyzed by Clarus 600 GC-MS (PerkinElmer, Boston, MA, U.S.A.) using a Rxi-5Sil-MS column (30 m × 0.25 mm × 0.25 µm film thickness) from Restek (Bellefonte, PA, U.S.A). The conditions maintained were[33,42] carrier gas (He) with a flow rate of 0.7 mL min^{-1}; split ratio, 1:50; injection volume, 0.1 µL; injection temperature, 250 °C; oven temperature, progressing from 60 to 200 °C at a rate of 5 °C·min^{-1} and from 200 to 290 °C at a rate of 20 °C min^{-1}, and then held at 290 °C for 2.5 min; and the ionization mode, electronic impact at 70 eV. Identification of the compounds was carried out by comparing the mass spectral fragmentation patterns with those stored in the MS database (NIST, U.S. National Institute of Standards and Technology).

Antimicrobial Activity. An agar well diffusion method[10,43] was employed for determining the inhibitory effect of essential oils extracted by MAE and HD for concentrations ranging from 5% to 25%. A 20 mL nutrient agar media[44,45] containing 0.5% (v/v) Tween-20 was evenly spread on the individual sterilized petriplates and allowed to solidify. An active cell suspension of organisms (1 mL) was uniformly spread on the agar surface. Four wells of 5 mm diameter each were made in the agar petriplates. The measured quantity of 25 µL of essential oil of different concentrations was filled in each well. The oil was allowed to diffuse in the well for a period of 1 h, and plates were then incubated at 37 ± 1 °C for 24−48 h. The zone of inhibition (mm) was measured with a graduated scale after the period of incubation. The microorganisms used for this study were two Gram-positive bacteria, *Staphylococcus aureus* (ATCC-12598) and *Bacillus megaterium* (MTCC-428), and three Gram-negative bacteria, *Escherichia coli* (ATCC-15223), *Salmonella typhi* (ATCC-6539), and *Shigella dysenteriae* (ATCC-25931).

The minimum inhibitory concentration (MIC) of the lemongrass oil at different concentrations was determined using a broth dilution method.[46] The bacterial culture was prepared by mixing the strain with 5 mL of nutrient broth[44,45] and then incubating overnight at 37 ± 1 °C. The solutions of lemongrass oil for the inhibition test were prepared by mixing 10 mL of sterilized tryptic soy broth (TSB), 0.5% (v/v) Tween-20, and different concentrations of lemongrass oil ranging from 0.5% to 0.015% (v/v). TSB with 0.5% (v/v) Tween-20 without oil was used as a positive growth control. An aliquot of bacterial suspension (25 µL) was added in each test tube containing lemongrass oil solution of different concentration. Each tube was incubated at 37 ± 1 °C for 24 h and then 48 h. The tubes were observed for turbidity after the period of incubation. The lowest concentration, at which no visible growth of culture was observed, was considered as the MIC. Each trial was conducted thrice.

Antioxidant Activity. DPPH Activity. The stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was used to determine the antioxidant activity of lemongrass oil. The ability of the oil as a hydrogen donor or radical scavenger was utilized for this purpose.[47−49] A solution of DPPH (0.1 mM) in ethanol was added to the essential oil solution (essential oil in water containing 2.5% Tween-20 at different concentrations of the oil, 10−300 µg mL^{-1}) in

Table 1. Design Matrix, S/N Ratio, and Total S/N Ratio

exp	factors[a]			yield (v/w, %)			S/N ratio
	A (volume of water, mL)	B (rehydration time, min)	C (extraction time, min)	y1	y2	y3	
1	1 (200)	1 (30)	1 (15)	0.32	0.34	0.30	−9.93
2	1	2 (60)	2 (25)	1.20	1.22	1.22	1.68
3	1	3 (120)	3 (35)	1.40	1.36	1.42	2.88
4	1	4 (180)	4 (45)	1.56	1.58	1.54	3.86
5	2 (300)	1	2	1.66	1.64	1.68	4.40
6	2	2	1	0.24	0.22	0.26	−12.46
7	2	3	4	1.36	1.38	1.32	2.62
8	2	4	3	1.44	1.40	1.46	3.12
9	3 (400)	1	3	1.28	1.30	1.28	2.19
10	3	2	4	1.32	1.34	1.30	2.41
11	3	3	1	0.48	0.50	0.46	−6.39
12	3	4	2	1.04	1.00	0.98	0.05
13	4 (500)	1	4	1.68	1.72	1.72	4.64
14	4	2	3	1.52	1.50	1.48	3.52
15	4	3	2	0.96	0.94	0.92	−0.54
16	4	4	1	0.12	0.08	0.10	−20.36
total S/N ratio (level 1)	−1.51	1.30	−49.13	–	–	–	–
total S/N ratio (level 2)	−2.31	−4.85	5.59	–	–	–	–
total S/N ratio (level 3)	−1.74	−1.43	11.71	–	–	–	–
total S/N ratio (level 4)	−12.74	−13.32	13.54	–	–	–	–
17 (confirmation experiment)	1 (200)	1 (30)	4 (45)	1.86	1.84	1.86	5.36

[a]Values in the bracket of factors A, B, and C show the physical value.

the ratio of 1:3 (v/v). The absorbance of DPPH was measured at 517 nm after 35 min.

H₂O₂ Radical Scavenging Activity. A scavenging activity of lemongrass oil against H_2O_2 was assessed according to the method described Ruch et al.[50] A solution of H_2O_2 was prepared in phosphate buffer (pH 7.4). Essential oil (10−300 μg mL^{-1}) in distilled water containing 2.5% Tween-20 was added to a H_2O_2 solution (0.6 mL, 40 mM). Absorbance of H_2O_2 at 230 nm was determined after 10 min.

Analysis by Scanning Electron Micrographs (SEM). In order to understand the extraction mechanism, the surface morphology of lemongrass leaves was studied using an S-3400 SEM, Hitachi (Tokyo, Japan) with a resolution of 4.0 nm in the high vacuum mode and a high sensitivity semiconductor backscattered electron detector. The examination was carried out with an accelerating voltage of 10.0 kV and a working distance of 20.6 mm.

RESULTS AND DISCUSSION

Optimization Study. *Determination of Optimum Level.* The experiments for three factors at four levels were performed based on an orthogonal array (L$_{16}$ array) of the Taguchi method as shown in Table 1. Because the purpose of the optimization was to maximize the yield of essential oil, the signal-to-noise (S/N) ratio for the "higher is better" case was chosen. The S/N ratio for each run was calculated using the yield of essential oil using eq 2 (Table 1).

$$S/N = -10 \log \left(\frac{1}{n} \sum_{i=1}^{n} \frac{1}{y_i^2} \right) \tag{2}$$

where, n is the number of repetitions performed for a given experiment, and y_i is the yield of the ith experiment.

On the basis of the S/N ratio, the total S/N ratio for each factor at each level was calculated as shown in Table 1 to find out the optimum conditions. With an increase in the volume of water, a decrease in the response was observed indicating a decrease in the yield. A similar trend was observed for the rehydration time. Water, being a polar compound, absorbs a

significant portion of microwaves and hence decreases the penetration depth for the microwave radiation. Moreover, with an increase in water content, power dissipation per volume decreases, leading to a lower temperature rise and finally may lead to an incomplete extraction of essential oil.[33] However, the change in magnitude for both the parameters is not as high as compared to the extraction time. With an increase in extraction time, the yield was observed to be increased. Initially, a rapid increase in the response was observed, followed by a slow rise. It is shown that for up to 25 min of extraction time, the rate of extraction was very rapid and 68% extraction was complete, and then rate decreased.

For selection of the optimal set of parameters, the level corresponding to the maximum total S/N ratio among the four levels for each factor would be chosen. In Table 1, it is shown that the optimum parameters are 200 mL volume of water, 30 min of rehydration time, and 45 min of extraction time.

ANOVA Results. In order to identify the significant effect of each parameter on the yield, the F test was performed (Table 2). A higher value for the calculated F means a greater influence of factor on the experimental results. Extraction time was found to be the most significant factor affecting the process compared to other parameters. The percentage contribution of extraction time was 84.35, and other factors (volume of water and

Table 2. Analysis of Variance (ANOVA)

factors	S	DOF	V	F	%CF
A	22.28	3.00	7.43	0.62	2.80
B	30.25	3.00	10.08	0.84	3.81
C	670.50	3.00	223.50	18.65	84.35
error	71.89	6.00	11.98	1.00	9.04
total	794.92	15.00	252.99		

S = Individual variation; DOF = Degree of freedom; V = Variance; % CF = Percentage contribution of factor.

Table 3. Comparison of Different Extraction Techniques

extraction techniques	operating conditions						yield, % (v/w)	power consumption, kWh	CO$_2$ emission, g (100 g plant material)$^{-1}$
	solid loading, g	volume of water, mL	size of the leaves, mm	rehydration time, min	extraction time, min	power, W			
HD	50	700	25	–	90	500	1.80	0.75	1200
MAE	100	200	25	30	45	850	1.85	0.6375	510

rehydration time) had a negligible effect. These results signify that a minor variation in extraction time can affect the yield to a greater extent.

Confirmation Experiment. The confirmation experiment was performed thrice under optimized conditions (Table 1). The yield and S/N ratio for the confirmation experiment were found to be higher compared to the maximum yield and the highest S/N ratio achieved in the L$_{16}$ array. In addition, the predicted (5.62) and actual (5.36) values of S/N ratios were also in good agreement (less than 5% variation). The outcome of this study authenticates the use of the Taguchi method for optimizing the process parameters for extraction of essential oil from the leaves of lemongrass using microwave radiation.

A comparison of MAE with HD was carried out as shown in Table 3. The extraction using microwave radiation was able to treat a higher amount of plant material (100 vs 50 g) with reduced water consumption (200 against 700 mL) in lesser time (45 vs 90 min) with higher yield (1.85 against 1.80%, v/w). Thus, a reduction in energy consumption was possible using MAE with a comparable yield. In addition, the impact of these processes on the environment can be quantified on the basis of carbon dioxide emissions. It is reported that 800 g of carbon dioxide will be released for 1 kWh if obtained by coal combustion.[51] In the case of MAE, lesser carbon dioxide rejection to the environment was observed indicating the environmentally benign nature of the technique.

The above studies clearly show the numerous benefits of MAE from an industrial perspective in comparison to HD. This method can have a variety of applications in processing plant materials at industrial scale and can provide a low break-even point and good rate of return on the investment. In addition, high throughput and reduced operating cost can be achieved.[52,53]

Composition of Essential Oil. To ensure the quality of the oil isolated by MAE under different operating conditions, it was analyzed by GC−MS. The compounds found to be present in essential oil are reported in Table 4. The operating conditions of various experiments (exp 1, exp 2, etc.) can be obtained from Table 1. The oxygenated compounds dominated the composition contributing above 87% of the total composition. Oxygenated compounds are odoriferous and responsible for the characteristic aroma of essential oil and, hence, are valuable.[51] Among oxygenated compounds, citral was the major compound present, ranging from 80 to 89%. Citral is the mixture of acyclic monoterpene aldehydes, neral, and geranial. A fraction of geranial was found to be higher compared to the fraction of neral. A higher percentage of citral (89.33) was found in the oil obtained under optimum conditions. It was not possible to extract caryophyllene oxide, a sesquiterpene, at a lower extraction time (15 and 25 min) irrespective to the changes in other operating conditions, which might be because of higher molecular weight. For different operating conditions, the yield, and the composition were found to be different. Although the trend in the variation was difficult to analyze, the

probable reasons for such variations compared to the optimized conditions are provided in Table 4.

Essential oil obtained in the confirmation experiment (exp 17, Table 1) is composed of oxygenated compounds (95.87%) and hydrocarbons (3.16%). The majority of the compounds present belong to monoterpenes. Caryophyllene oxide (0.05%) was the only sesquiterpene present in the oil. The most representative components were geranial (49.67%), neral (39.66%), α-myrcene (3.07%), and geraniol (1.91%). Other significant compounds were verbenol (1.07%), neryl acetate (1.04%), trans-4,5-epoxy-carane (0.68%), and linalool (0.53%).

The chemical composition of the essential oil extracted by HD was also reported in Table 4 for the qualitative comparison of the oil extracted by the different techniques. The numbers of compounds identified were same though with variation in percentage. The variation in the proportions was probably because of the difference in the heating mechanism. In the case of MAE, attenuation of thermal and hydrolytic effects was observed in comparison with HD, which required a large quantity of water and higher extraction time for the completion of the extraction.[54]

Kinetic Study. A kinetic behavior of the essential oil extraction was investigated with respect to the yield as well as the composition. The kinetic desorption models were applied to study the rate of extraction, and GC−MS analysis was used to examine the variation in composition along with time. The experiments were performed in triplicate under optimized conditions.

Kinetic Desorption Model. In the case of hydrodistillation, modeling was performed by considering it to be a rate process.[55] Similar behavior can be expected for microwave-assisted extraction because in MAE only the mode of the energy supply is changed. It was observed that the microwave-assisted extraction could be controlled by intraparticle diffusion.[33] The kinetic models, which describe the rates of the desorption step as the diffusion-based hot-ball model (one-site) and two-site model, were therefore used to describe these processes. The one-site kinetic desorption model and two-site kinetic desorption model can be presented by eqs 3 and 4, respectively.[56,57]

$$u_t = 1 - e^{-kt} \tag{3}$$

where, u_t is the fraction of the essential oil extracted at time t, and k is the first-order rate constant describing the extraction efficiency in min^{-1}.

$$u_t = 1 - [Fe^{-k_1 t}] - [(1 - F)e^{-k_2 t}] \tag{4}$$

where, u_t is the fraction of the essential oil extracted at time t, k_1 is the first-order rate constant in min^{-1} describing the quickly released fraction, F, and k_2 is the first-order rate constant in min^{-1} describing the slowly released fraction, $1 - F$.

Using the MATLAB program (Mathworks, Natick, MA, U.S.A.), rate constants were determined by minimizing the error between the experimental value and the predicted value as

Table 4. Chemical Composition of Essential Oil[a]

compound name	peak area (%)								
	exp 1	exp 2	exp 3	exp 4	exp 5	exp 6	exp 7	exp 8	exp 9
Major Hydrocarbons									
α-myrcene	3.59	4.56	7.26	7.76	6.29	5.24	7.95	5.37	10.83
E-ocimene	–	–	0.12	0.10	0.08	–	0.11	–	0.20
Major Oxygenated Compounds									
6-methyl-5-hept-en-2-one	–	0.24	0.22	0.19	0.18	0.32	0.15	0.16	0.26
linalool	1.02	0.59	0.57	0.55	0.62	0.72	0.54	0.45	0.64
10-undecyn-1-ol	0.20	0.16	0.21	0.18	0.21	0.24	0.21	0.16	0.31
trans-4,5-epoxy carane	0.75	0.63	1.09	1.00	0.95	0.82	0.93	0.78	1.08
verbenol	1.01	0.76	1.48	1.38	1.33	1.16	1.35	1.21	1.60
citronellol	0.10	–	0.14	0.13	0.12	0.12	0.12	0.11	0.15
neral	40.67	37.71	37.72	37.34	38.55	40.58	38.32	39.14	38.94
geraniol	1.60	1.56	2.50	2.11	1.83	1.89	1.84	2.04	1.87
geranial	45.69	43.10	44.72	45.24	46.64	45.51	44.94	47.10	41.16
neryl acetate	1.12	1.15	1.37	1.43	1.01	0.73	1.30	1.21	1.03
caryophyllene oxide	–	–	0.13	0.14	–	–	0.14	0.10	0.09
total	98.47	97.33	98.58	98.70	98.88	98.79	98.80	99.00	98.93
hydrocarbons	3.76	4.93	7.64	8.12	6.52	5.42	8.33	5.49	11.41
oxygenated compounds	94.71	92.40	90.94	90.58	92.36	93.37	90.47	93.50	87.53
citral	86.36	80.81	82.44	82.58	85.19	86.09	83.26	86.24	80.10
yield (v/w, %)	0.32	1.21	1.39	1.56	1.66	0.24	1.35	1.43	1.29
remarks	less extraction time	less extraction time, higher rehydration time	less extraction time, higher rehydration time	higher rehydration time	higher water volume, less extraction time	higher water volume, less extraction time, higher rehydration time	higher water volume, higher rehydration time	higher water volume, less extraction time, higher rehydration time	higher water volume, less extraction time

compound name	peak area (%)							conf	
	exp 10	exp 11	exp 12	exp 13	exp 14	exp 15	exp 16	exp-17	HD
Major Hydrocarbons									
α-myrcene	8.63	3.68	4.13	7.44	4.49	4.03	7.96	3.07	4.01
E-ocimene	0.13	–	0.11	0.11	0.07	–	0.10	–	–
Major Oxygenated Compounds									
6-methyl-5-hept-en-2-one	0.24	0.26	0.32	0.19	0.12	0.16	0.19	0.12	0.12
linalool	0.59	0.68	0.88	0.53	0.51	0.55	0.50	0.53	0.58
10-undecyn-1-ol	0.23	0.25	0.33	0.26	0.26	0.19	0.22	0.18	0.16
trans 4,5-epoxy carane	1.17	0.84	0.76	0.92	0.78	0.71	0.91	0.68	0.68
verbenol	1.70	1.12	1.10	1.26	1.15	1.06	1.37	1.07	0.98
citronellol	0.18	0.09	0.14	0.11	0.12	0.13	0.15	0.10	0.10
neral	37.70	40.59	40.86	38.81	40.14	40.47	38.53	39.66	39.86
geraniol	2.10	1.96	1.50	1.91	1.86	1.87	2.15	1.91	1.51

Table 4. continued

compound name	exp 10	exp 11	exp 12	exp 13	exp 14	exp 15	exp 16	conf exp-17	HD
					peak area (%)				
geranial	43.50	47.46	46.27	45.64	47.58	48.11	44.87	49.67	49.00
neryl acetate	1.32	0.80	0.73	1.18	1.07	0.93	1.20	1.04	0.92
caryophyllene oxide	0.16	—	—	0.09	0.07	—	0.12	0.05	0.06
total	98.60	99.16	98.59	99.11	99.18	99.29	99.05	99.03	99.18
hydrocarbons	9.02	3.82	4.43	7.67	4.68	4.14	8.28	3.16	4.12
oxygenated compounds	89.57	95.33	94.16	91.44	94.50	95.16	90.77	95.87	95.06
citral	81.20	88.05	87.13	84.45	87.72	88.58	83.40	89.33	88.86
yield (v/w, %)	1.32	0.48	1.01	1.71	1.50	0.94	0.10	1.85	1.80
remarks	higher water volume, higher rehydration time	higher water volume, less extraction time, higher rehydration time	higher water volume, less extraction time, higher rehydration time	higher water volume	higher water volume, less extraction time, higher rehydration time	higher water volume, less extraction time, higher rehydration time	higher water volume, less extraction time, higher rehydration time	—	—

*a*Exp 1, exp 2, etc. refer to the experiments performed using Taguchi method as described in Table 1.

shown in Figure 1. Table 5 shows the model fit for the extraction of essential oil using HD and MAE under optimized
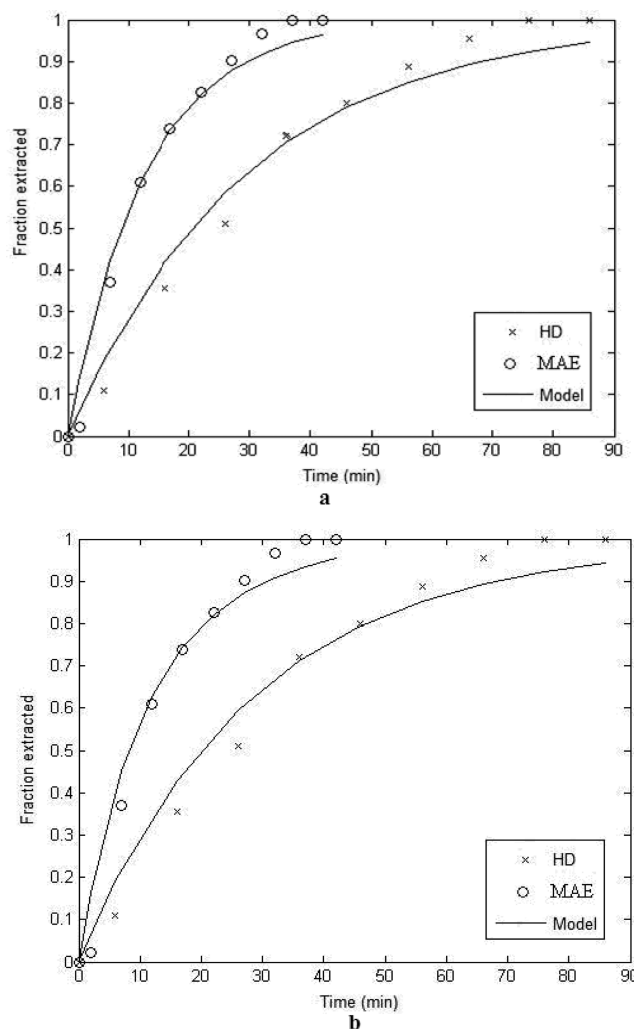

Figure 1. Kinetic desorption model fit for HD and MAE: (a) one-site model and (b) two-site model.

Table 5. Kinetic Model Fit for Different Extraction Techniques

extraction technique	one-site kinetic desorption model		two-site kinetic desorption model		
	k, min^{-1}	error, %	k_1, min^{-1}	k_2, min^{-1}	error, %
HD	0.0339	4.88	0.0300	0.0469	5.23
MAE	0.0776	4.74	0.0644	0.1563	5.78

conditions. Higher rate constants (0.0776 min^{-1} for the one-site model and 0.0644 and 0.1563 min^{-1} for the two-site model) were achieved using MAE compared to the rate constants (0.0339 min^{-1} for the one-site model and 0.0300 and 0.0469 min^{-1} for the two-site model) obtained in HD. The reason might be the mode of energy supplied to the plant material. In the case of HD, the extraction was performed using conventional heating, which was a time-consuming process; hence, a lower rate constant was obtained. In the case of MAE, volumetric heating led to rapid extraction of the essential oil, providing a higher rate constant.

Chemical Composition at Different Time Interval. Each point on the curve presented in Figure 1 in the case of MAE

Table 6. Chemical Composition of Essential Oil Obtained after 45 min and for Different Times of Microwave Radiation (exp 17)

compounds	continuous mode (45 min)	samples collected at different time							
		t_1 = 2 min	t_2 = 7 min	t_3 =12 min	t_4 = 17 min	t_5 = 22 min	t_6 = 27 min	t_7 = 32 min	t_8 = 37 min
Major Hydrocarbons									
α-myrcene	3.07	4.83	3.05	5.00	0.97	2.05	4.41	5.92	1.90
E-ocimene	–	0.07	–	0.08	–	–	–	–	–
Major Oxygenated Compounds									
6-methyl-5-hepten-2-one	0.12	0.21	0.21	0.25	0.16	0.16	0.20	0.17	–
linalool	0.53	0.65	0.61	0.65	0.59	0.65	0.59	0.57	0.52
10-undecyn-1-ol	0.18	0.24	0.22	0.25	0.14	0.20	0.20	0.15	0.16
trans-4,5-epoxy-carane	0.68	0.83	0.72	0.86	0.37	0.55	0.73	0.62	0.55
verbenol	1.07	1.20	0.94	1.22	0.57	0.69	1.10	0.90	0.74
citronellol	0.10	0.11	0.10	0.18	0.05	0.09	0.15	0.09	–
neral	39.66	37.85	37.92	37.93	39.06	36.36	37.98	35.97	35.64
geraniol	1.91	2.08	2.02	2.36	1.36	1.74	2.27	1.60	2.16
geranial	49.67	48.74	50.42	48.00	52.86	52.59	49.11	49.38	51.11
neryl acetate	1.04	–	–	–	1.16	1.09	1.02	–	1.84
caryophyllene oxide	0.05	–	–	–	–	–	–	0.09	0.10
total	99.03	99.10	98.97	98.97	99.17	99.14	99.04	98.36	98.53
hydrocarbons	3.16	5.04	3.16	5.21	1.02	2.16	4.54	6.13	2.03
oxygenated compounds	95.87	94.06	95.81	93.75	98.16	96.99	94.50	92.23	96.50
citral	89.33	86.59	88.34	85.93	91.92	88.95	87.09	85.35	86.75

Table 7. Zone of Inhibition and MIC of Lemongrass Oil

organism	method of extraction	zone of inhibition, mm					MIC, %
		5%	10%	15%	20%	25%	
Gram-positive bacteria							
Staphylococcus aureus	HD	13.33	18.66	23.16	26.33	29.16	0.04
	MAE	14.33	19.66	24.5	28.16	30.33	0.04
Bacillus megaterium	HD	11.5	17.33	21.66	25.00	28.16	0.04
	MAE	12.16	18.83	23.16	26.5	29.66	0.04
Gram-negative bacteria							
Escherichia coli	HD	9.16	14.16	17.83	20.66	24.00	0.09
	MAE	10.33	14.83	18.16	20.83	25.00	0.09
Salmonella typhi	HD	7.16	13.66	18.16	20.16	23.16	0.1
	MAE	9.00	16.16	19.16	21.33	24.50	0.1
Shigella dysenteriae	HD	7.83	11.33	14.16	19.00	22.16	0.11
	MAE	9.00	13.16	16.33	21.16	23.33	0.11

corresponds to a sample of the essential oil collected and analyzed by GC-MS. The volatile constituents extracted in continuous mode as well as at different times using microwave radiation are shown in Table 6. A presence of almost all the compounds with varying proportions was observed.

A percentage of oxygenated compounds varied from 92.23% to 98.16%, and for hydrocarbon, it varied from 1.02% to 6.13%. A higher percentage of geranial (52.86% and 52.59%) and neral (39.06% and 37.98%) was obtained respectively at times t_4 and t_5 and t_4 and t_6. The maximum value of α-myrcene (5.92%) was obtained at t_7. Geraniol had a higher percentage (2.36%) at t_3. A higher amount of neryl acetate (1.84%) was found at t_8. The sesquiterpene, caryophyllene oxide, was found to be present at the end of extraction times t_7 and t_8.

Essential oil extracted by MAE was rich in bioactive compounds such as as α-myrcene (monoterpene hydrocarbon) and neral, geranial, and geraniol (monoterpene oxygenated compounds).

Biological Activity. *Antimicrobial Activity.* The oils extracted by both HD as well as MAE were tested against two Gram-positive (*S. aureus* and *B. megaterium*) and three Gram-negative (*E. coli*, *S. typhi*, and *S. dysenteriae*) bacteria strains, and the results are shown in Table 7.

All of the test organisms showed sensitivity toward both of the oils. Citral, α-myrcene, and geraniol were the major compounds present in both of the oils. These compounds have shown antibacterial activities by disrupting the bacteria membrane activity.[18] Gram-positive bacteria were effectively inhibited at lower concentration of the oil in comparison to Gram-negative bacteria, thus showing more activity of oils against Gram-positive bacteria. The higher activity of Gram-positive could be attributed to the lipophilic ends of the lipoteichoic acids of the cell membrane, which might have facilitated the penetration of the hydrophobic compounds.[58] In contrast to this, highly hydrophilic polysaccharide chains of the external membrane of Gram-negative bacteria might have hindered the diffusion.[59] Similar observations were made in the case of rosemary oil toward its antibacterial activities.[60]

The zone of inhibition was found to increase with an increase in the concentration of the oil. In the case of the oil extracted

by MAE, a higher zone of inhibition was reported compared to the oil obtained by HD, which might be due to a higher fraction of oxygenated compounds. However, the MIC values were found to be the same for both of the cases. The minimum bactericidal concentration, the lowest concentration to kill the bacteria, was found to be the same as the MIC values. *S. aureus* and *B. megaterium* were the most susceptive microbes toward the oils, while *S. dysenteriae* was the least affected bacteria. *E. coli* and *S. typhi* have intermediate susceptibility. The difference in activity might be because of the variation in mechanism of action of essential oil against the microorganisms. The mechanisms involved for the inhibition or death of the bacteria are: change in the permeability of the cytoplasmic cell wall membrane by destructing the phospholipid bilayers, depletion in the proton motive force by inhibiting toxin production, leakages of critical molecules from the cells, and destruction or inactivation of genetic material.[13,61] In the case of *S. aureus*, its structural organization of the cytoplasm was affected to a greater extent compared to the cell, which might have led to intracellular material leakage and morphological changes and finally death of the bacterial cell.[62]

Antioxidant Activity. The generation of free radicals and oxidation of molecules for production of energy are necessary reactions occurring in living organisms. However, the proliferations of oxygen-derived free radicals may lead to the development of chronic diseases like carcinogenesis and cardiovascular diseases and rapid aging.[63,64] A use of natural antioxidants may assuage these health issues without any side effects. In this regard, the ability of lemongrass oils as an antioxidant was determined by measuring the hydrogen atoms or electron donation tendency by reacting with the free radicals (DPPH and H_2O_2), and the percentage inhibition is shown in Tables 8 and 9. The oil extracted by both techniques has shown

Table 8. Antioxidant Activity of Essential Oil Using the DPPH Method

concentration, $\mu g \ mL^{-1}$	percentage inhibition by oil	
	HD	MAE
25	15.49	18.94
50	24.42	27.61
75	35.88	38.78
100	43.98	46.39
125	54.34	58.41
IC_{50}	114.53	107.51

Table 9. Antioxidant Activity of Essential Oil Using the H_2O_2 Method

concentration, $\mu g \ mL^{-1}$	percentage inhibition by oil	
	HD	MAE
100	27.48	30.44
125	32.59	38.86
150	39.22	43.76
175	44.90	49.81
200	48.83	54.28
225	52.11	–
IC_{50}	213.86	176.11

good antioxidant activities. The presence of citral might have contributed to the free radicals scavenging activity.[65] The results obtained in the study were in accordance with the activity reported by Sacchetti et al.[3] An improved inhibition was

observed by oil isolated by MAE, which might be because of the presence of a bit higher concentration of oxygenated compounds. The half maximal inhibitory concentration (IC_{50}) was measured to know the effectiveness of the oils to reduce the free radicals by half. In the case of MAE oil, IC_{50} values were found to be 107.51 $\mu g \ mL^{-1}$ and 176.11 $\mu g \ mL^{-1}$, for DPPH and H_2O_2, respectively. A comparison with the values obtained for hydrodistilled oil showed there is a minor reduction in IC_{50} values (6.13% and 17.65% for DPPH and H_2O_2, respectively). The difference in percentage inhibition of DPPH and H_2O_2 might be ascribed to the mode of action of the essential oils with these free radicals.

Analysis by SEM. To understand the mechanism of extraction by HD as well as MAE, plant materials before extraction and after extraction were studied using SEM and the respective micrographs are shown in Figure 2.
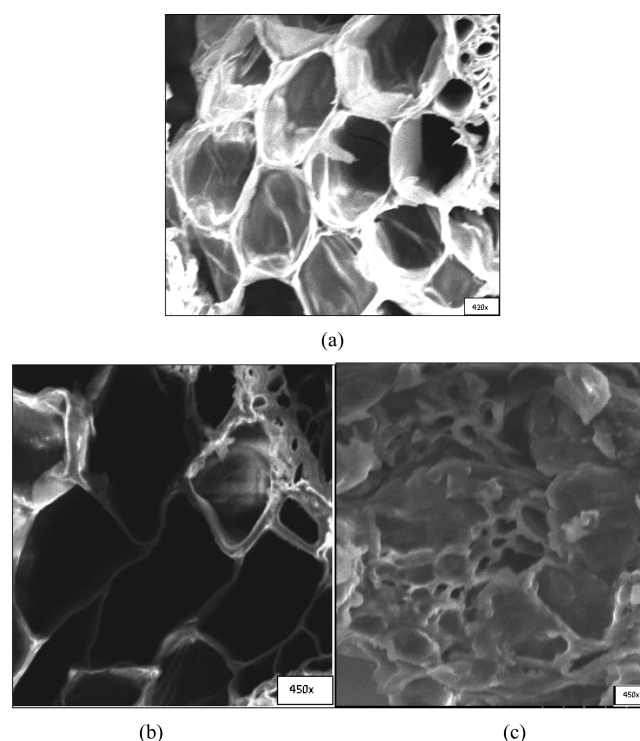

(a)

(b)   (c)

Figure 2. Scanning electron micrographs of lemongrass leaves: (a) prior to extraction, (b) after HD, and (c) after MAE.

A periodic arrangement of well-shaped cells was observed in the case of untreated leaves (Figure 2a). Comparison of leaves after HD (Figure 2b) with untreated leaves (Figure 2a) shows that the thermal energy supplied by conventional heating instigated physical changes in the structures of the leaves. Such changes could be attributed to the mechanical strain induced by the boiling water and its vapor. No rupture of the cell wall was observed. The mechanism of extraction could be explained by the hydrodiffusion phenomena. A structure of membranes of plant cells are such that it does not allow the volatile oils to escape. Hence, in the actual process, at the temperature of boiling water, a part of the volatile oil dissolves in the water present within the glands, and this oil−water solution permeates through the swollen membranes by osmosis and finally reaches the outer surface, where the oil is vaporized by the boiling water.[51,66]
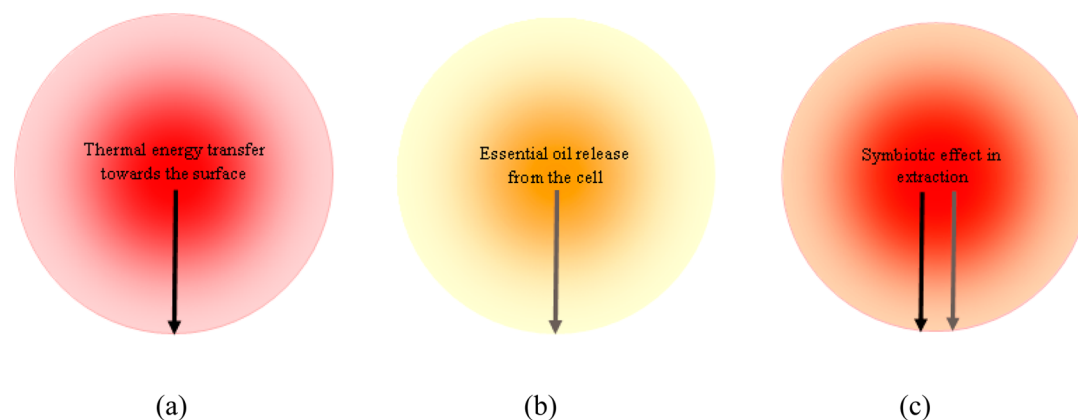
Figure 3. Visualization of transport of mass and heat in MAE: (a) heat transfer, (b) mass transfer, and (c) overall impact on extraction.
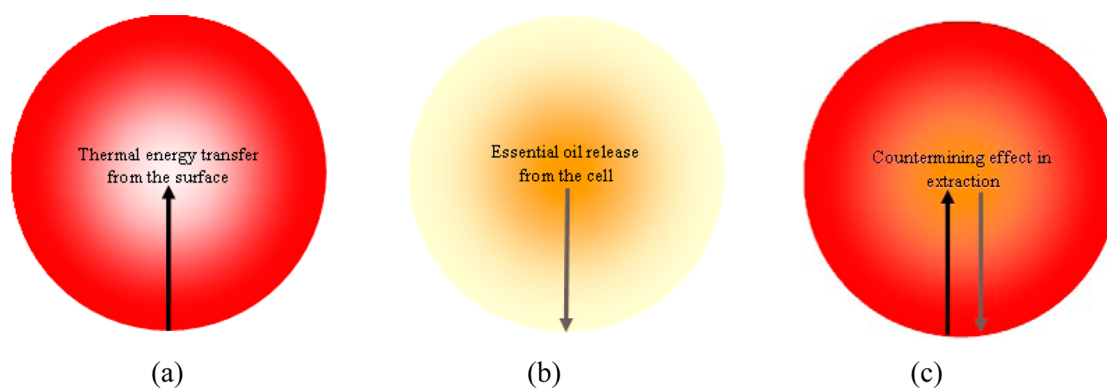

Figure 4. Visualization of transport of mass and heat in HD: (a) heat transfer, (b) mass transfer, and (c) overall impact on extraction.

In the case of MAE, distinguishable physical changes were observed in the leaves after extraction (Figure 2c). Microwave radiation has caused significant damage to the cell structure. After microwave extraction, the cells were empty and ruptured. Because the polar compounds have absorbed the microwave radiation efficiently, instantaneous energy transfer and vaporization of the compounds might have occurred, causing severe thermal stress and localized internal high pressure toward the cell wall. The pressure within the cells could have exceeded their expansion limit, which led to the rupture of the cell walls. This phenomenon caused the release of volatiles from the cell wall, thus emptying the cells. Similar morphological changes were reported in the case of extraction from orange peels[51] and *Calamintha nepeta*,[67] and a rapid cell rupture was observed.

Synergism in Extraction. The processes employed for the isolation of essential oil from the leaves of lemongrass (HD and MAE) were primarily dependent upon the thermal energy. In both of the cases, the mode of energy supplied was different causing a difference in the rate of extraction. In the case of HD, heat is transferred from the surface to the inside of the particle, known as conventional heating. In the case of MAE, heat is generated inside the particles and then moves in an outward direction, known as volumetric heating. The application of thermal energy to the plant materials leads to the release of chemicals residing in the cells. The mass transfer initiates from the cell and then moves toward the surface. In the case of MAE, both of the transport phenomena (heat and mass) progress in a concurrent direction generating synergism in extraction, that is, diffusion of oil toward the surface would have been initiated with an increase in the temperature of the system. Thus, acceleration in extraction rate could be ascribed to this

cooperative phenomenon in MAE, and a visualization of this occurrence is shown in Figure 3.

On the other hand, mass and heat transfers occur in the opposite direction in the case of HD, which might have hindered the extraction process, making it slower (Figure 4). Similar observations were made in the case of essential oil extraction from orange peels.[68]

■ CONCLUSION

The extraction of essential oil from the leaves of lemongrass using microwave radiation was optimized with the help of the Taguchi method. The maximum yield was obtained under optimized conditions, viz., 200 mL volume of water, rehydration time of 30 min, and extraction time of 45 min. The extraction time was the most influential parameter affecting the process. The oil was found to be enriched with oxygenated compounds, mainly citral (80−89%). The good data fit for the one-site and two-site kinetic desorption models suggested that extraction could be governed by intraparticle diffusion. The oil extracted using microwave radiation was found to possess an improved antimicrobial activity and antioxidant activity compared to the oil isolated by hydrodistillation. The microscopic study and symbiotic effect of heat and mass transfer were useful to understand the extraction phenomena of both techniques. A comparison of microwave-assisted extraction with hydrodistillation showed that it was possible to achieve a higher yield with a reduced extraction time and water volume, which was further fortified by higher energy efficiency and reduced environmental impact. Thus, microwave-assisted extraction may prove to be a better alternative to the

conventional method and can be considered for the higher scale.

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Notes
The authors declare no competing financial interest.

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