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An experimental design approach for the extraction of lemongrass (*Cymbopogon citratus*) oleoresin using pressurised liquid extraction (PLE)

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Abstract

Extraction of lemongrass oleoresin was successfully optimised using Pressurised Liquid Extraction (PLE). Character impact compounds; neral, geranial and geraniol which constituted 72% oleoresin, were monitored during this optimisation study by using GCMSD. Based on maximum extraction of these compounds, the optimised operating conditions for PLE were a temperature of 167°C, a pressure of 1203 psi and a static time of 20.43 min. The quality of PLE extract were compared with conventional extraction methods, hydrodistillation and Soxhlet extraction. The proposed method was found to be better in term of quantity of the targeted character impact compounds.

Introduction

Lemongrass (*Cymbopogon citratus*) is a plant from the grass family and contained about 1–2% essential oil in a dry weight basis. The essential oil is characterised by a high content of citral (constituted by the isomers neral and geranial), which is used as a raw material for the production of ionone, vitamin A and β-carotene. The lemongrass essential oil is a very important in perfumery industry since it blends well with a great variety of essential oil (Carlson *et al*., 2006). The tea made from lemongrass leaves is commonly used as antispasmodic, analgesic, anti-inflammatory, antipyretic, diuretic and sedative (Carlini *et al*., 1986).

The current extraction methods of essential oil from natural products are tedious. These processes consumed a lot of time and amount of solvents. Therefore, the use of Pressurised Liquid Extraction (PLE) can overcome these flaws. Cristina *et al*. (2010) reported that PLE is a better extraction technique in terms of quantification of fat-oil in bread and other derivatives products. PLE combines elevated temperature and pressure with liquid solvents to achieve fast, efficient and reliable extraction. Moreover, PLE has been shown to present a safe and rapid technique for extracting antioxidant compounds from plants (Hossain *et al*., 2011 and Mustafa, 2010).

Recently, Response Surface Methodology (RSM) has been used by many researchers as an effective tool to optimise processes (Wani, 2008; Firatgil-Durmus and Evranuz 2010). It helps in getting relevant information in the shortest time with the minimum number of experiments. A research has been conducted in optimisation of lemongrass essential oil extraction using Supercritical Carbon Dioxide (Huynh *et al*., 2007); but none in PLE extraction. Therefore in this study, extraction of lemongrass marker compounds is optimised with regards to extraction temperature, pressure and static time using PLE.

Materials and Methods

Sample preparation

Fresh lemongrasses were purchased from local supplier in Shah Alam, Selangor. Lemongrass stem was cut 10 cm from root and further cut to 2 mm diameter prior to drying. The initial moisture level of lemongrass was 11%. Samples were then dried in an oven at 40 °C overnight and kept in a sealed bag.

Chemicals

n-hexane used was of Ph. Eur grade (MERCK, Germany). Deionised water used was purified by Milli-Q purification system (Millipore) (Massachusetts, USA). Citral and geraniol standard are of MERCK, Germany.

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Pressurised liquid extraction (PLE)

Extraction of lemongrass essential oil was done using an Accelerated Solvent Extractor ASE 200 (Dionex Ltd. Camberley, Surrey, UK). About 3 g of sliced lemongrass stem were accurately weighed and placed into the 22 mL cells with cellulose filter at the bottom end. The sample cells were closed to finger tightness before being placed into the carousel of the ASE 200 system. Sample was extracted using n-hexane (Ph. Eur).

The other parameters was standardised based on:

1. Time = 5 min
2. Flush volume = 100%
3. Purge time = 60 s
4. Static cycle = 1

The extracted analytes were purged from the sample cell using pressurised nitrogen (861–1034 kPa). Finally the extract was evaporated to dryness to 1 ml using a rotary evaporator to calculate the yield of essential oil (Zaibunnisa *et al*., 2009).

Hydrodistillation

For this method, the Dean Stark apparatus was used. About 900 g sample of fresh lemongrass was weighed in a 500 ml flask and was submitted to hydrodistillation for 12 hours. The distillate was saturated with sodium chloride and added with n-hexane. Then, the ether layer and hydro layer were separated by funnel. After dehydrated by anhydrous sodium sulphate, the n-hexane layer was further dried at 40 °C in a rotary evaporator to concentrate oil to 1 ml and subjected to GC-MS analysis (Guan *et al*., 2007).

Soxhlet extraction

Essential oil of lemongrass was extracted from fresh plant with n-hexane and ethanol as a solvent, for 16 hours using a Soxhlet extractor, following the AACC Method 30-25 (AACC International, 2000). Samples were later dried using rotary evaporator to 1 ml and analysed by using GC-MS.

Gas Chromatography/Mass Spectrometry (GC/MS)

Sample extracted from various method of extraction mentioned were concentrated using a rotary evaporator to 1 mL in GC vial before subjected to GC-MS analysis. Analyses of the samples were performed on a Agilent Technologies Gas Chromatograph-Mass Spectrometer (Model HP 5971 MSD, Hewlett-Packard, USA) using a fused silica capillary column DB-5 (20 m×0.188 mm internal diameter, 0.4 µm film thickness). The program started at 100°C for 1 min. Then, the temperature was increased to 102°C at rate of 1°C/min. Injector and detector temperatures were kept constant at 250 and 300 °C, respectively. The sample volume injected was 2 µL using auto-sampler. Injections were done in triplicates. Finally, compounds detected by GC-MS were referred to Flavour 2L and NIST library from GC-MS software.

Experimental design approach

The optimised extraction condition was applied on PLE extraction. The effect of three independent variables: temperature (°C), pressure (psi) and static time (min) on response variables neral, geranial, geraniol and yield of essential oil were evaluated using Response Surface Methodology (RSM). The RSM experimental design was generated using Design-Expert version 6.0.4 (Stat Ease Software). Each of the variables had levels set at five coded levels: −α, −1, 0, +1 and +α. The results from central composite design (CCD) were assessed using multi-linear regression, using an equation of the form:

$$Y=b_0+b_1A+b_2B+b_3C+b_4A^2+b_5B^2+b_6C^2+b_7AB+b_8AC+b_9BC$$

b_0, b_1 and b_2 = linear coefficients,
b_4, b_5 and b_6 = quadratic coefficients,
b_7, b_8 and b_9 = cross-product coefficients.

The data obtained was analysed using the SPSS 15.0 for Windows Evaluation for analysis of variance and Duncan's Multiple Range test.

Sensory evaluation

The acceptability test conducted was hedonic scale test (9 point scale). About 30 panellists were involved in this test. Samples analysed were lemongrass oleoresins extracted from different methods; the optimised and standard parameters (100°C, 1000 psi, 30 minutes) of Pressurised Liquid Extraction (PLE) and Soxhlet. The parameters tested were lemongrass aroma, chemical aroma, colour and overall attributes.

Results and Discussions

Comparison of PLE with other methods of extraction

Table 3 presents the amount of lemongrass marker compounds (mg/100g) detected and yield of total volatile oil (%) from various extraction method. Each method was tested in triplicate to ensure reproducibility. Detection and quantification of neral and geranial was then determined by GC-MS as shown in Figure 1.

For both PLE and Soxhlet extraction, n-hexane was used as solvent. A preliminary study had been done to select the best solvent for extraction of

Table 1. Central composite design used for the PLE extraction of volatile compounds from lemongrass

	Temperature (°C)	Pressure (psi)	Extraction time (min)
$-\alpha$	40	1000	5.0
−1	80	1250	10.0
0	120	1500	17.5
1	160	1750	25.0
α	200	2000	30.0

Table 2. Comparison of volatile marker compounds (mg/100g) extracted from PLE standard method (100°C, 1000 psi, 30 minutes) using different type of solvents

Solvents	*Marker compounds (mg/100g)*		
	Neral	Geranial	Geraniol
n-hexane	87.36^{a}±0.5	220.32^{a} ±0.3	15.64^{a}±1.6
Water	17.03^{b}±0.4	23.73^{b}±0.5	4.89^{b}±6.3
Ethanol	6.23^{c}±0.7	17.06^{c}±0.7	*n.d*

a-c Same letters within each column indicate no significant (p>0.05) difference according to Duncan's multiple range test.
n.d = not detected

Table 3. Comparison of volatile marker compounds (mg/100g) and yield (%) using PLE, hydrodistillation and Soxhlet extraction from *Cymbopogon citratus*

Extraction Methods	*Marker compounds (mg/100g)*		*Yield of Total*
	Neral	Geranial	*Volatile Oil(%)*
PLE[1]	78.61^{a}±0.1	248.24^{a} ±0.5	2.90^{a} ±0.44
Soxhlet extraction[2]	53.33^{b} ±0.4	82.83^{b} ±0.3	3.81^{a} ±1.12
Hydrodistillation[3]	7.09^{c} ±0.6	12.32^{c} ±0.3	0.01^{b} ±0.00

$^{a-c}$ Same letters within each column indicate no significant (p>0.05) difference according to Duncan's multiple range test.
[1] Pressurised liquid extraction conditions: sample, 3g; solvent, *n*-hexane; temperature, 100°C; pressure,1000 psi; static time, 30 min.
[2] Soxhlet extraction conditions: sample, 2g (air-dried); solvent, *n*-hexane; time,16 h.
[3] Hydrodistillation conditions: sample, 900g (fresh); time, 12 h.

lemongrass marker compounds (neral and geranial). From table 2, it was shown that n-hexane gives significantly ($p<0.05$) highest amount of both neral and geranial (mg/100g) as compared to water and ethanol. This is agreeable with Schaneberg and Khan (2002), where in their study reveals that n-hexane gives significantly ($p<0.05$) highest concentration of lemongrass marker compound as compared to other solvents. Therefore, n-hexane was selected as the extraction solvent in this study.

As shown in Table 3, percentage of total volatile oil detected from Soxhlet extraction (3.81%) and PLE (2.90%) were significantly ($p<0.05$) higher than hydrodistillation (0.01%). This was due to the ability of solvent n-hexane to extract almost all non-volatile and volatile compound, as compared to hydrodistillation which can only extract volatile compounds.

PLE gave the significantly ($p<0.05$) highest amount of neral and geranial (78.61 ±0.1 mg/100g and 248.2 ±0.5 mg/100g, respectively) followed by Soxhlet (53.33 ±0.4 mg/100g and 82.83 ±0.3 mg/100g, respectively) and hydrodistillation (7.09 ±0.6 mg/100g and 12.32 ±0.3 mg/100g, respectively). This shows that not only PLE gives a rapid and low cost extraction, but it is also the best alternative for solvent solid/liquid extraction in terms of quantification of desired compounds from a solid sample. Thus, extraction of lemongrass marker compounds was further optimised using PLE. The extraction parameters optimised were temperature (°C), pressure (psi) and static time (min).

Table 4. Central composite design arrangement for independent variables temperature (°C), pressure (psi) and static time (min) and their responses; neral, geranial, geraniol and yield (%, dry weight basis)

Run	Temperature (°C)	Pressure (psi)	Static time (min)	Neral (mg/100g)	Geranial (mg/100g)	Geraniol (mg/100g)	Yield (%)	Colour of extract
1	119.95	1500.20	17.55	73.68	248.59	21.47	3.94	Yellow
2	119.95	1500.20	17.55	137.37	463.09	35.96	1.98	Yellow
3	167.50	1203.00	10.10	184.77	560.71	37.98	3.28	Brown
4	167.50	1203.00	25.00	192.75	687.51	33.57	3.54	Brown
5	72.40	1203.00	10.10	88.83	486.94	23.36	2.89	Clear
6	167.50	1797.40	25.00	179.88	603.00	35.18	3.50	Brown
7	72.40	1203.00	25.00	3.16	12.16	9.67	2.14	Clear
8	72.40	1797.40	25.00	3.27	6.47	9.99	1.83	Clear
9	72.40	1797.40	10.10	3.07	4.41	10.55	1.77	Clear
10	119.95	1500.20	17.55	189.65	651.92	38.18	1.87	Yellow
11	167.50	1797.40	10.10	143.58	505.24	35.54	2.06	Brown
12	119.95	1500.20	17.55	87.91	330.88	27.86	2.45	Yellow
13	119.95	1500.20	17.55	129.41	420.97	32.75	2.91	Yellow
14	39.98	1500.20	17.55	6.00	9.01	6.36	2.82	Clear
15	119.95	1500.20	30.08	70.42	233.56	22.36	3.67	Yellow
16	119.95	1500.20	17.55	64.90	224.70	21.38	2.55	Yellow
17	119.95	2000.03	17.55	54.16	196.35	18.10	1.75	Yellow
18	119.95	1500.20	5.02	22.62	106.54	13.74	2.00	Yellow
19	119.95	1000.37	17.55	191.4	686.84	40.89	2.65	Yellow
20	199.92	1500.20	17.55	161.89	546.87	99.31	1.17	Brown

Experimental design

Effect of 3 independent variables on response variables neral, geranial, geraniol and yield of total volatile oil were evaluated using Response Surface Methodology. The Central Composite Design (CCD) used in this study was shown in Table 1. 20 experiments were conducted based on CCD. The parameters and responses obtained were shown in Table 3.

As shown in Table 4, effects of temperature (°C), pressure (psi) and static time (min) on all dependent responses (neral, geranial, geraniol and yield of total volatile oil) were of second order (quadratic). The R^2 values of all interactions are above 0.80, which was suggested by Joglekar and May (1987) as a good fit of model. This shows that interactions between independent and dependent variables were very much reliable. According to the significant model terms, for all equations, temperature (A) and static time (B) has

Table 4. ANOVA for response surface for compounds and yield

Compounds	Model	Lack of Fit (F value)	R^2	Equation	Significant Model Terms
Neral (ppm)	Quadratic Significant	0.49 Not significant	0.8201	Neral = +1107.49 +633.35 A -271.47 B +28.60 C -66.15 A_2 +71.20 B_2 -198.48 C_2 +39.63 AB +162.37 AC+142.59 BC	A, B
Geranial (ppm)	Quadratic Significant	0.58 Not significant	0.8227	Geranial = +3776.58 +2014.63A -1061.48B -24.67C -241.21 A_2 +337.43 B_2 -622.48 C_2 +436.28 AB +872.66 AC +557.97 BC	A, B
Geraniol (ppm)	Quadratic Significant	4.07 Not significant	0.8024	Geraniol = +303.22 +180.50A -36.42B -3.73 C +63.91A_2 -19.65 B_2 -61.70 C_2 +17.58 AB +14.06AC +23.52 BC	A
Yield (%)	Quadratic Significant	1.02 Not significant	0.8184	Yield = +2.74 +0.30 A +0.044B +0.074 C +0.21 A_2 +0.033 B_2 -0.095 C_2 -0.090 AB +0.16 AC +0.11 BC	A, A^2

A= Temperature, °C; B= Pressure, psi; C= Static time, min

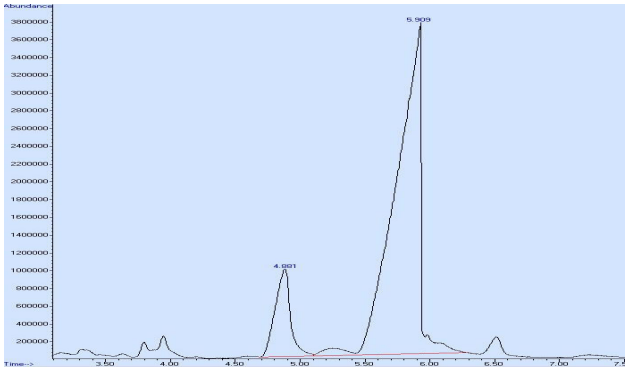

Figure 1. GC chromatogram obtained using optimised PLE condition temperature: 167° C, pressure:1203 psi and static time:20.43 mins

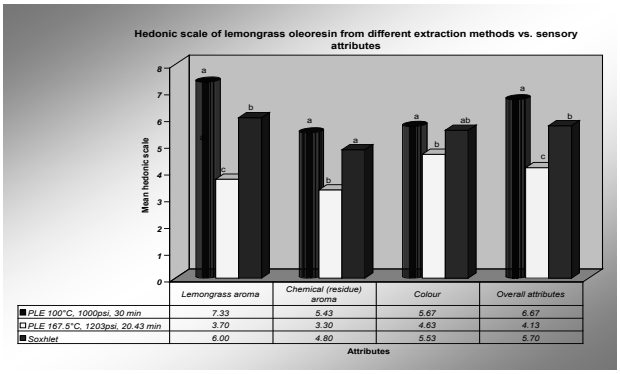

Figure 2. Bar graph showing level of acceptance of lemongrass oleoresin from different method of extractions

the profound effect on the amount of neral (mg/100g) and geranial (mg/100g) obtained. Temperature is the only parameter that affects amount of geraniol (mg/100g) and yield of total volatile oil (%). In this study, static time is the minor variable in extraction efficiency.

The optimised parameters for lemongrass oleoresin PLE extraction were a temperature of 167°C, pressure of 1203 psi and a static time of 20.43 minutes. However, the appearance of lemongrass oleorsin from this optimised condition was undesirable, as it has a burnt smell and dark brown in colour. Sensory evaluation was conducted to measure the acceptance of this oleoresin as compared to extract from standard method (100°C, 1000psi, 30min) and Soxhlet extraction method. According to Figure 2, oleoresin from optimised method was unacceptable for all parameters tested. The oleoresin extracted from this optimised parameter was significantly the lowest ($p<0.5$) as compared to standard method and Soxhlet. The hedonic scale ranges from 3-4 which indicate 'dislike moderately'. However, most panellist prefer lemongrass oleoresin extracted from standard method as it has a stronger lemongrass aroma (7.33) and acceptable colour (5.67).

Conclusion

The optimised operating conditions obtained for PLE extraction of lemongrass oleoresin were, heating at 167°C, a pressure of 1203 psi and a static time of 20.43 min. However, based on sensory evaluation, a standard method of PLE parameters (100°C, 1000psi, 30min) was selected for further lemongrass oleoresin extraction.

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