

Extraction of essential oil from Patchouli leaves using hydrodistillation: Parametric studies and optimization

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Hydrodistillation has been employed for the extraction of essential oil from the leaves of patchouli plant. The yield of patchouli oil is dependent on solid loading, water volume, size of leaves and extraction time. The optimization of the process parameters has been performed using the Taguchi method while analysis of variance is used to identify the impact of parameters on the yield. The highest yield of patchouli oil (1.53%, w/w) is found at 30 g of solid loading, 900 mL of water volume, 4 mm size of the leaves and 150 min of extraction time. Among the parameters studied, extraction time had a greater impact on the yield of patchouli oil. Amount of patchouliol is maximum (64.9%) in the essential oil obtained under optimized conditions, thus, providing better quality of the essential oil.

Keywords: Analysis of variance, Essential oil, Hydrodistillation, Patchouli, Taguchi method

The bioactive components in volatile oils found from different plant materials are the organic compounds with strong odours and have been extensively used in daily life as flavouring agents, food additives, fragrances and pharmaceuticals ingredients^{1,2}. *Pogostemon cablin* (Blanco) Benth., commonly known as patchouli and belonging to Lamiaceae family, is an aromatic bushy herb known for its high value essential oil derived from its leaves and young twigs. Patchouli is native to the Philippines and grows wild and cultivated in Malaysia, Indonesia, Singapore, China and India³. In India, it is cultivated in coastal areas of South India, West Bengal, Assam, Karnataka, Madhya Pradesh and coastal regions of Gujarat⁴.

Patchouli alcohol is the main component of patchouli oil. This essential oil is also characterized by a large number of other sesquiterpene hydrocarbons such as $\alpha/\beta/\gamma$ -patchoulenes, α -guaiene, seychellene, and α -himachalene. However, the commercial value of patchouli oil depends on the concentration of patchouliol, norpatchoulenol and α -patchoulene⁵⁻⁷. Various therapeutic properties of patchouli oil including antibacterial, antifungal, antiseptic, anti-depressive, anti-inflammatory, astringent, febrifuge, trypanocidal, sedative, diuretic, tonic, antiemetic and insect repellent have been reported⁸⁻¹¹. It is an important essential oil, which is widely used in perfumery, medical, cosmetics and flavourings industries¹²⁻¹⁵. Patchouli oil has been

extracted by steam distillation¹⁶, soxhlet extraction¹⁶, supercritical fluid extraction¹⁷ and pressurized fluid extraction¹⁸. However, long extraction time¹⁹, degradation of thermally labile compounds²⁰, labour-intensive step of sample processing²¹ and high pressure are some limitations existing in these methods. Further, the cost is the major limitation of high pressure extraction systems. Hence, a systematic study for extraction of patchouli oil is needed to explore the benefits of conventional techniques. In case of steam distillation, direct supply of steam to the plant materials may decompose the product while in case of hydrodistillation water acts as a barrier for preventing decomposition. Further, condensation of vapors coming from the water-plant material mixture will result in separation of essential oil and water without any further treatment which is not possible in case of solvent extraction.

Based on the literature survey, the extraction of patchouli oil using hydrodistillation method and its optimization through the Taguchi method has not been reported yet. The optimization using the Taguchi method was studied using four factors; solid loading, water volume, size of leaves and extraction time at three levels. An analysis of variance (ANOVA) was performed to find the most significant parameter affecting the process. Quality of essential oil was assessed by gas chromatography-mass spectrometry (GC-MS).

Experimental Section

Materials

A study on comparative analysis of different cultivars of patchouli demonstrated that cultivar CIM-Shreshtha produced better oil yield than other cultivars of Patchouli²². Hence, patchouli leaves (cultivar CIM-Shreshtha) were collected from the research farm of Central Institute of Medicinal and Aromatic Plants (CIMAP) Research Centre, Bengaluru, India.

The collected leaves were dried under the shade for 7 days for complete removal of moisture. After drying, stems and roots were separated and the dried leaves were stored in tightly packed plastic bags to avoid contamination by water.

Methods

Hydrodistillation

Extraction of patchouli oil from dried patchouli leaves has been carried out using hydrodistillation. Hydrodistillation experiments were performed in a circulatory Clevenger-type apparatus (2 L capacity) provided with round bottom flask and water condenser. The experimental set-up is shown in Fig. 1. In order to isolate essential oil by hydrodistillation, the plant material was kept in a round bottom flask and a sufficient quantity of water was added to avoid overheating and charring of the plant material. The mixture was heated and brought to a boiling condition. The essential oil was freed from the oil glands in the plant tissue owing to the influence of hot water. The vapor mixture of water and oil was then condensed using a condenser. Upon condensing, the mixture was directed towards the collector, where essential oil got separated from the water. Moisture was removed from the essential oil by



Fig. 1 – Experimental setup for extraction of patchouli oil

centrifugation and the final product was stored at 2°C. The maximum temperature of the system was the boiling point of water and the system was operated at atmospheric pressure. All the experiments were performed in duplicate. The yield of essential oil was found by the following equation.

$$y = \left(\frac{W_1 \cdot 100}{W_2} \right)$$

where, y is the yield in % (w/w), W_1 is the weight of essential oil collected in g and W_2 is the weight of the plant material in g.

Design of experiment

The aim of this study was to find the optimum values of various factors to have the maximum response and establish the effect of various factors. Using the Taguchi method²²⁻²⁵, four factors viz. solid loading, water volume, size of the plant material and extraction time were investigated with well defined set of experiments and optimum conditions were found accordingly. With the help of ANOVA, the most influential parameter and percentage contribution of each parameter were determined. Details of the Taguchi method and ANOVA can be found in various literatures²²⁻²⁵.

GC-MS analysis

The essential oil samples were analysed for the quality using GC-MS (Perkin-Elmer Clarus 680 GC coupled with Clarus-SQ 8C mass spectrometer). The capillary column used was a bonded phase fused silica capillary column (Supelco (equity-5), 60 m length, 0.32 mm internal diameter, 0.25 μm film thickness). Helium was used as a carrier gas with the flow rate of 1 mL min^{-1} . Column oven conditions were: 70°C for 2 min; 70°C to 250°C at 3°C min^{-1} with hold time of 2 min and then up to 290°C at 6°C min^{-1} with hold time of 5 min. Injector and detector temperatures were maintained at 290°C and 280°C, respectively. Split ratio was maintained at 1:20. The detection was done using the electron ionization (EI) system and mass range was from 40 to 500 amu. Identification of compounds was based on mass spectra library search (NIST/EPA/NIH and Wiley registry of mass spectral data, 7th edition).

Results and Discussion

Study of various parameters

The values of parameters viz. solid loading, water volume, size of the plant material and extraction time, selected for the optimization purpose were based on

the range studied during preliminary experiments. Solid loading was varied from 20 to 60 g. The water volume was studied in the range of 600-1400 mL to avoid charring of the plant material. Size of the plant material was studied in the range of 420 to 4000 micron. Extraction time was varied from 30 min to 180 min to find the time required to complete the extraction. Power should be sufficient enough to reach the boiling point of the water without causing adverse effect on the yield of the extracted oil. Based on initial studies, the power was kept at 90% of power supply of 400 W for all experiments.

Effect of solid loading

The effect of solid loading on the yield of essential oil was studied in the range of 20 – 60 g (Fig. 2). The other parameters were kept constant viz. water volume of 1000 mL, particle size of 1003 microns and 2.5 h extraction time. The results showed that the extraction yield increased significantly with solid loading up to 40 g and thereafter it decreased. A decrease in the yield at a higher solid loading may be attributed to the less amount of heat supplied. It is noteworthy that the heat supplied and time is kept constant for all solid loadings studied. Higher solid content may require longer duration for complete extraction of patchouli oil. Similar observation was made by Moghrani and Maachi while extracting the oil from *Myrtus communis*²⁶.

Effect of water volume

In this study, the experiments were conducted for different water volume (600-1400 mL) keeping other parameters constant viz. 40 g solid loading,

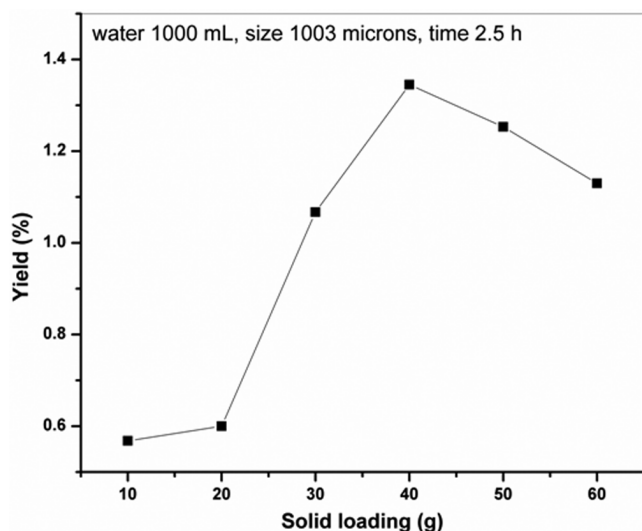


Fig. 2 – Effect of solid loading on patchouli oil extraction

1003 microns size of the leaves and 2.5 h extraction time. The results are plotted in Fig. 3, which reveal that the oil content increased with water volume. However, a drop in the yield was observed for the larger volume of water. When amount of water is less, the plant material might have experienced the overheating or charring, resulting in the decreased yield. For higher water content, the heat could be wasted in heating up the water which might have reduced the efficiency of the process. Also, hydrolytic effect might have contributed to the lower yield²⁷.

Effect of extraction time

The effect of extraction time was studied in the range of 30-180 min and the results are presented in Fig. 4. It was observed that the extraction efficiency increased with increase in time and remained constant

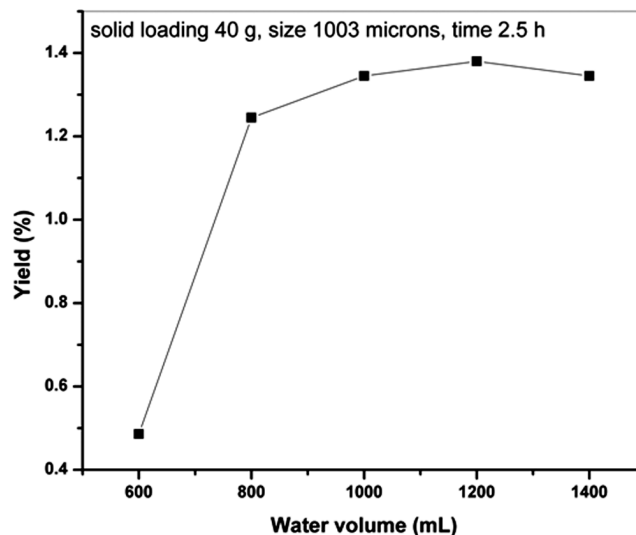


Fig. 3 – Effect of water volume on patchouli oil yield

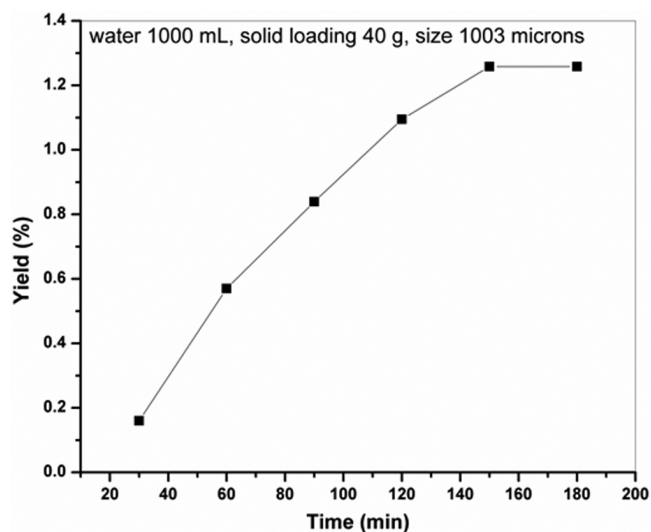


Fig. 4 – Effect of extraction time on patchouli oil extraction

after 150 min. Thermal energy supplied by the boiling water to the leaves could have changed the permeability of cells of the leaves. This might have led to hydrodiffusion of oil from the cell to outer surface^{28,29}. Since the movement of oil from cell to the bulk of water and then vaporization might be time consuming, the extraction time to complete the process was higher.

Effect of size of plant material

The effect of particle size on the extraction yield was studied in the range of 0.42 – 4.0 mm (Fig. 5). A drastic reduction in the yield (64%) was observed for the plant material of smaller size (0.42 mm). A better extraction was expected in case of a smaller sized plant material because of the increased surface area of the plant material; however, the reverse trend was observed. Here, the process employed for a size reduction i.e. grinding played a major role. During grinding, a rise in the temperature was observed which might have led to the loss of oil

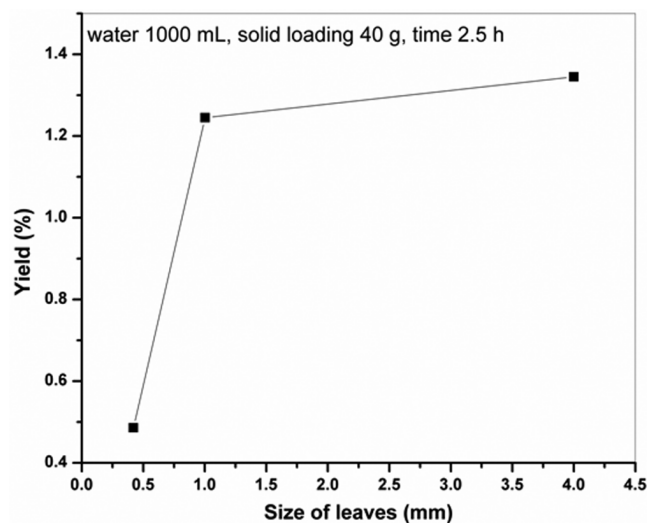


Fig. 5 – Effect of size of plant leaves on extraction of patchouli oil

from the leaves. Similar results were observed by Parikh and Desai³⁰ and Hanci *et al.*³¹ during extraction of volatile oil from lemongrass oil and thyme, respectively.

Design of experiment

Optimization of process parameters

The levels for four factors selected for optimization are given in Table 1. Using the Taguchi method, experimental planning was done (Table 2) and accordingly, experiments were performed. Yield for each run was determined and consequently, the signal-to-noise (*S/N*) ratio was calculated (Table 2). Since the objective of the present study was to maximize the yield, the *S/N* ratio for “higher is better” case was selected and the value was found by the following equation

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

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

Based on the individual *S/N* ratio, total *S/N* ratio was found for each factor at each level as shown in Table 3. For an example, the value of 2.13 in the column of factor A for level 1 is the sum of *S/N* ratios at experiment numbers 1, 2 and 3 in Table 2. These three *S/N* ratios refer to the experiments wherein level of factor A was kept at 1.

Table 1 – Factors and their levels used in optimization study

Factors	Levels		
	1	2	3
A – Solid loading (g)	30	40	50
B – Water volume (mL)	900	1200	1500
C – Size of leaves (mm)	0.42	1.00	4.00
D – Extraction time (min)	90	120	150

Table 2 – L₉ array, yield and S/N ratio for extraction of patchouli oil

Exp. No.	A (g)	B (mL)	C (mm)	D (min)	Yield (%)		S/N Ratio
					Y ₁	Y ₂	
01	1 (30)	1 (900)	1 (0.42)	1 (90)	0.96	0.95	-0.43
02	1	2 (1200)	2 (1.00)	2 (120)	1.03	1.06	0.37
03	1	3 (1500)	3 (4.00)	3 (150)	1.29	1.28	2.19
04	2 (40)	1	2	3	1.40	1.43	3.02
05	2	2	3	1	0.87	0.81	-1.50
06	2	3	1	2	0.98	0.97	-0.18
07	3 (50)	1	3	2	1.15	1.20	1.37
08	3	2	1	3	0.92	0.95	-0.60
09	3	3	2	1	0.74	0.73	-2.66

The maximum value of a level total S/N ratio among each level for a given factor corresponds to the optimum value of that factor. From Table 3, it can be seen that the optimum parameters for the present study are solid loading at level 1 (30 g), water volume at level 1 (900 mL), size of plant leaves at level 3 (4 mm) and extraction time at level 3 (150 min).

Analysis of variance (ANOVA)

In order to identify the significant effect of each parameter on yield, ANOVA was performed (Table 4). In the present study, all the degrees of freedom were consumed and no information was left for the error calculation. In order to facilitate further calculation, factor having the lowest variance can be considered as an error. In this study, variance of factor *C* was found to be the lowest therefore information pertaining to this factor was pooled into error and the *F*-test was carried out.

Higher value for the calculated *F* means a greater influence on the experimental results. As seen in Table 4, the calculated value of *F_D* was substantially higher than *F_A*, *F_B* and *F_C* and factor *C* was found to be statistically insignificant. However, *F* test is useful to provide only qualitative information. Quantitative evaluation can be achieved using the percentage contribution. Percentage contribution of factor *C* (size of leaves) was 7.03 while for factor *D* (extraction time) it was 57.30. The order of factors based on their magnitude of influence is as follows: extraction time > water volume > solid loading > size of the plant material. Hence, a minor change in extraction time can affect the process to a greater extent.

Table 3 – Total S/N ratio for each factor at different levels

Factor levels	A (Solid loading)	B (Water volume)	C (Size of leaves)	D (Extraction time)
1	2.13	3.96	-1.21	-4.59
2	1.34	-1.72	0.73	1.55
3	-1.89	-0.66	2.06	4.61

Confirmation experiment

The confirmation experiment is the crucial and final step in verifying the conclusions drawn based on Taguchi method. The confirmation experiment was performed in duplicate using the optimum conditions obtained by the Taguchi method and the results are reported in Table 5.

Yield and S/N ratio for extraction under optimum conditions are higher than the maximum yield and the highest S/N ratio obtained in the *L₉* array. These results confirmed the validity of the Taguchi method for the extraction of essential oil from the leaves of patchouli using hydrodistillation.

GC-MS analysis of oil

The quality of the extracted patchouli oil sample was estimated by GC-MS and the major compounds thus identified are reported in Table 6. It has been observed that both the samples contained identical components with a little variation. The major constituents identified can be classified as sesquiterpene hydrocarbons (β -caryophyllene, α -guaiene, seychellene, α -patchoulene and γ -patchoulene) and oxygenated sesquiterpenes (pogostol and patchoulol). A higher amount of oxygenated compound was found in both the oils. These compounds are highly odoriferous contributing to the fragrance of the oil³¹. Among these compounds, content of patchoulol was found to be the highest (64-65%). Further, though the amount of

Table 5 – Confirmation experiment

Factors	Confirmation experiment	Exp 4 of <i>L₉</i> array
A – Solid loading (g)	30	40
B – Water volume (mL)	900	900
C – Size of leaves (mm)	4.00	1.00
D – Extraction time (min)	150	150
Yield (w/w,%)	1.55	1.40
	1.50	1.43
S/N ratio	3.70	3.02

Table 4 – ANOVA table

Factors	S	DOF	V	F test	CF
A – Solid loading	3.03	2.00	1.51	1.68	11.84
B – Water volume	6.09	2.00	3.05	3.38	23.83
C – Size of leaves	1.80	2.00	0.90	1.00	7.03
D – Extraction time	14.65	2.00	7.32	8.10	57.30
Error	0.00	0.00	0.00	-	-
Total	25.56	8.00	-	-	100.00
Pooled Error	1.80	2.00	0.90	-	-

S – Individual variance; DOF – Degree of freedom; V – Variance; CF – % Contribution of each factor

Table 6 – Main compounds identified in patchouli oil

S. No.	Compound	Peak area (%)	
		Confirmation experiment	Exp 4 of L ₉ array
Sesquiterpene hydrocarbons			
1	β -Caryophyllene	1.3	1.2
2	α -Guaiene	3.7	3.6
3	Seychellene	2.9	3.1
4	α -Patchoulene	1.7	1.7
5	γ -Patchoulene	5.6	5.5
Oxygenated sesquiterpenes			
6	Pogostol	5.2	5.1
7	Patchoulol	64.9	64.2

α -patchoulene was less (1.7-1.8%), its presence has synergistic effect on performance of the oil. Higher the concentrations of these compounds better will be the quality of the oil and hence the commercial value¹⁷.

Conclusion

The extraction of patchouli oil from the leaves of *Pogostemon cablin* (Blanco) Benth. using hydrodistillation is successfully optimized using the Taguchi method. The optimum conditions are found to be 30 g solid loading, water volume of 900 mL, 4 mm size of the leaves and extraction time of 150 min. Under these conditions, the highest yield of the oil is obtained, i.e., 1.53% (w/w). The most significant parameter affecting the extraction yield is found to be the extraction time and its contribution was 57.30%. Hence, extraction time requires great attention in the present study for extraction of patchouli oil. Patchoulol is the main component (64.9%) of the essential oil, which can increase the commercial value of the oil.

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