Influence sample sizing of citrus hystrix essential oil from hydrodistillation extraction

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Abstract. Essential oil extracted from kaffir lime leaves through hydrodistillation. The objective of this study is to quantify the oil production rate by identify the significant influence of particle size on kaffir lime leaves. Kaffir lime leaves were ground and separated by using siever into 90, 150, 300 μm and other kaffir lime leaves. The mean essential oil yield of 0.87, 0.52, 0.41 and 0.3% was obtained. 90 μm of ground gives the highest yield compared to other sizes. Thus, it can be concluded that in quantifying oil production rate, the relevance of different size of particle is clearly affects the amount of oil yield. In analysing the composition of kaffir lime essential oil using GC-MS, there were 38 compounds found in the essential oil. Some of the major compounds of the kaffir lime leave oils were detected while some are not, may due to oil experience thermal degradation which consequently losing some significant compounds in controlled temperature.

1. Introduction
Kaffir lime (Citrus hystrix DC) is a sort of plant from Southeast Asian with exceptionally solid scent. It is otherwise called makrut lime, which is a typical tropical herb in the family Rutaceae start all over in Southeast Asia [1]. Kaffir lime has a prickly shrubbery with fragrant leaves and the organic product is dull green with unpredictable uneven surface as appeared in Figure 1. The leaves and organic product peel is the crucial parts of kaffir lime and it is a key fixing in numerous Thai, Cambodian, Indonesian, Laotian, Malaysian, and Philippine cooking styles [2].

Figure 1. Kaffir lime (Ranjit et al., 2013)
By and large, fundamental oil is one of a rich source of biological active compounds. This is on the grounds that key oils have exceptionally complex regular blends which can contain 20 to 60 components. Some of the major component of kaffir lime oil are mostly monoterpene hydrocarbon, with limonene (30.73%) and β-pinene (18.76%). For the minor components are terpinene-4-ol (10.63%), α-terpineol (8.35%), γ-terpinene (6.18%), α-terpinene (5.09%) and terpinolene (4.33%) [3]. In this way, they have been utilized broadly as bactericides, virucides, fungicides, against parasites, and bug sprays in different applications, particularly in the pharmaceutical, sterile, restorative, sustenance and farming commercial enterprises [1].

There are many studies reported on extraction of essential oil from peel of citrus fruits using different methods [4,5,6]. Sud and Badyal [4] extracted peel oil from the rind at various stages of fruit maturity by steam distillation and reported maximum yield in terms of peel oil as 2.05 ml/100 g peels in orange. Brat [5] reported essential oil extraction by flash vacuum expansion of peel of lemon, orange, mandarin and grapefruit with maximum yield of 2.41 kg/tonne of fruits. Bousbia [6] extracted peel essential oil from citrus varieties such as Eureka, lime, Marsh Seedless, Torroco, Washington Naval were extracted by Clevenger apparatus using microwaves hydro-diffusion and gravity reported a significant reduction in extraction time (15 min).

Therefore, the major research efforts have been on extraction of essential oil from various citrus species but some people are still not received attention. If production of essential oil is related to the contact of water with particle size, then exposing to larger surface area of kaffir lime leaves will result more oil yield [7]. The objective of present research work was to evaluate the effect of size on yield of the essential oil from kaffir lime leaves by using hydrodistillation. The size reduction of leaves was aimed to increase the yield of essential oil. The chemical composition of kaffir lime oil produced was also analyzed.

2. Methodology/ Experimental

2.1. Materials
Kaffir lime leaves are obtained from night market at Seksyen 7, Shah Alam, Selangor. n-hexane used in the experimental work were analytical grade.

2.2. Sample preparation
Fresh kaffir lime leaves were washed and dried for two days in a Universal MEMMERT Oven (model 100-800) at 40°C until reach the constant humidity. The final humidity of the leaves is 60.33%. Finally, the air-dried samples were grind by using Waring commercial laboratory blender. The ground kaffir lime leaves were separated according to different sizes by using siever shaker (Octagon Digital Siever) with the range size of 300μm, 150μm and 90μm.

The humidity of kaffir leaves was calculated using Equation 1 as follows:

\[
\text{Humidity (\%)} = \frac{\text{fresh leaves} - \text{dried leaves}}{\text{fresh leaves}} \times 100\%
\]

(1)

2.3. Hydrodistillation extraction
Once the ground kaffir lime leaves were sieve and collected according to the sizes, the experiment was performed by setting up the hydrodistillation equipment. 25g of kaffir lime leaves mix with distilled water was taken into hydrodistillation (Clevenger type) apparatus using ground leaves. The amount water in the ratio of 1:16 for 6 hours. As the boiling of water progressed when reach 100°C, the water and volatile oil in the form of vapor started rising which got condensed in the condenser tube. The condensed water and oil were collected into the graduated cylinder.
2.4. Percentage yield

On completion of extraction process, essential oil that was collected. The oil yield was calculated using Equation 2 as follows:

\[
\text{Yield (\%)} = \frac{\text{weight of essential oil recovered}}{\text{weight of fresh leaves}} \times 100\% \tag{2}
\]

2.5. Gas Chromatography Mass Spectrometer (GC-MS) analysis

When the essential oil samples collected were ready to be analyzed, n-hexane solvent was added into all oil samples. The mixture of the essential oil and solvent was shake and sent to the Gas Chromatography (GC-MS) GC-MS analysis was performed using Varian 450-GC model, mass spectrometry Varian 240-MS inert, while the operation temperature was programmed at a rate of 3°C/min-1 to a maximum temperature of 230°C and held for 10 minutes. The carrier gas for GC was helium with flow rate 1.0 ml/min. The spectrometers were operated in electron-impact (EI) mode, while the scan range was 45-450 amu, and the ionization energy was 70 eV. The inlet, ionization source temperatures were 250°C respectively. The components of the oil were identified by comparing their mass spectra with the recorded spectra in the MS library [8].

3. Results and Discussion

3.1. Effect of size of grind leaves on essential oil yield

The essential oil yield on dry basis varied from 0.1 to 1.0% in kaffir lime leaves. The percentage yields of the essential oil were based on different sizes of kaffir lime leaves. The essential oil was collected as shown in Figure 2. The color of kaffir lime essential oil was colorless. The most oil extracted from hydrodistillation was 90 μm with 0.25 ml, followed by 150 μm and 300 μm with oil extracted of 0.15 and 0.12 ml. The least amount oil extracted to be 0.1 ml from the other size of ground kaffir lime.

Figure 3 shows the relationship between percentage of essential oil yield with the size of kaffir lime leaves was plotted and observed. The percentage of kaffir lime essential oil from 90 μm leaves was 0.81% while for 150 μm and 300 μm leaves, the percentage yields were 0.5226% and 0.418%. However, for other size of grounds kaffir lime leaves, the percentage yield was 0.3484%.

Essential oil yield increased with decrease in particle size (i.e increase in grinding time) of the leaves and reached to almost maximum at 10 min of grinding time. In the mundane grinding process, heat is engendered when energy is utilized to fracture a particle into a more minute size, that engender heat causes temperature elevate in the grinder which is responsible for a loss of volatile oil content[8].

It is evident that oil yield varied widely among particle size. The oil recovery less when extraction was carried out from ground kaffir lime leaves. This is due to small surface area of the leaves and thereby having less contact with water during extraction [9]. The mean yield increased to 0.418% on
the grinding kaffir lime leaves at 300 μm. With further grinding to 150 μm and 90 μm, the oil yield increased further to 0.522% and 0.87%, respectively.

Thus, a very significant increase in oil yield was found on reducing particle size of kaffir lime leaves. This increase in oil yield might be due to increased surface area of kaffir lime leaves and thereby having close contact with water during extraction as well as having reduced resistance to oil water mixture from oil sac to the solution.

3.2. Composition of Volatile Compounds in Essential Oil
There were 38 compounds was found and identified in kaffir lime leaves extracted using hydrodistillation shown in Table 1. There were different numbers of compounds extracted in different sizes of kaffir lime leaves where a total of 31 compounds, 27 compounds, 29 compounds and 30 compounds were identified in size of 90 μm, 150 μm, 300 μm and ground kaffir lime leaves. From here, it can be said that 90 μm size of ground kaffir lime leaves can extract more chemical compounds.

Table 1. Compound of essential oil obtained from the Kaffir lime leaves.

| No | Chemical compounds | 90μm (%) | 150μm (%) | 300μm (%) | Ground (%) |
|----|--------------------|----------|-----------|-----------|------------|
| 1  | Arginine           | 0.02     | -         | 0.02      | -          |
| 2  | Ecgonine           | 0.01     | 0.02      | 0.03      | 0.04       |
| 3  | α-Pinene           | 0.01     | 0.06      | 0.01      | 0.00       |
| 4  | 6-Octen-1-ol       | 0.00     | 0.02      | 0.05      | 0.10       |
| 5  | Naphthalene        | 0.06     | 0.09      | 0.08      | 0.04       |
| 6  | 6-octenal          | 1.30     | 0.31      | 16.99     | 0.42       |
| 7  | 1-Penten-3-one     | 0.00     | 0.00      | 0.01      | -          |
| 8  | 3-Carene           | 0.02     | 0.01      | 0.00      | 0.00       |
| 9  | 1H-Imidazole       | 0.00     | 0.00      | 0.05      | 0.03       |
| 10 | Limonene oxide     | 0.20     | 0.12      | 0.02      | 0.08       |
| 11 | Piperidine         | 0.20     | 0.00      | 0.00      | 0.01       |
| 12 | α-Phellandrene     | 0.01     | 0.01      | 0.00      | 0.01       |
| 13 | 7-octen-1-ol       | 1.61     | 0.14      | 0.24      | 0.02       |
|   | Compound                     | 95.54 | 97.64 | 80.08 | 93.53 |
|---|------------------------------|-------|-------|-------|-------|
| 14| Mequinol                     | 0.01  | 0.00  | 0.00  | 0.01  |
| 15| Squalene                     | 0.75  | 0.01  | 0.01  | 4.67  |
| 16| Cis-α-Terpineol              | 0.00  | 0.01  | 0.00  | 0.01  |
| 17| Camphene                     | 0.04  | 0.36  | 1.64  | 0.01  |
| 18| α-Cubebene                   | 0.00  | 0.01  | 0.00  | 0.01  |
| 19| Citronellyl Isobutyrate      | 0.00  | 0.00  | -     | -     |
| 20| 3-Cyclohexen-1-ol            | 0.00  | 0.02  | 0.17  | 0.04  |
| 21| α-Farnesene                  | 0.03  | 0.12  | 0.13  | 0.67  |
| 22| Alloaromadendrene oxide      | 0.00  | -     | 0.18  | 0.04  |
| 23| Spathulenol                  | 0.01  | 0.09  | 0.02  | 0.08  |
| 24| Humulane                     | 0.00  | -     | 0.02  | -     |
| 25| Aristolene                   | 0.00  | 0.03  | -     | 0.02  |
| 26| Azulene                      | 0.15  | 0.25  | 0.18  | 0.12  |
| 27| α-Gualene                    | 0.01  | -     | -     | -     |
| 28| Caryophyllene                | 0.00  | 0.03  | 0.06  | 0.01  |
| 29| α-Vatirrmene                 | 0.00  | -     | -     | -     |
| 30| Phenanthrene                 | 0.01  | 0.00  | 0.00  | 0.00  |
| 31| Eicosene                     | 0.01  | -     | 0.00  | 0.00  |
| 32| ω-Elemene                    | -     | 0.64  | -     | -     |
| 33| ω-Himachalene                | -     | 0.00  | -     | -     |
| 34| α-Panasinsene                | -     | -     | 0.02  | 0.00  |
| 35| Ferrocene                    | -     | -     | 0.00  | 0.01  |
| 36| Camphor                      | -     | -     | -     | 0.00  |
| 37| ω-Gurjuneneoxide             | -     | -     | -     | 0.00  |
| 38| 1-9-Dihydropyrene            | -     | -     | -     | 0.00  |
| 39| others                       | 95.54 | 97.64 | 80.08 | 93.53 |

Some of the major compounds of the kaffir lime leave oils such as α-pinene, limonene and citronellal were detected while some of the major compounds such as sabinene and β-pinene were not detected. This is due to the high temperature while grinding of kaffir lime leaves. This condition may due to oil experience thermal degradation which consequently losing some significant compounds in uncontrolled temperature [7,8,9,10,11]. Throughout the hydrodistillation extraction, the temperature somehow could accidentally exceed 100˚C and cause some of the volatile component like sabinene and β-pinene lost during extraction process. The are many beneficial chemical compounds contains in kaffir lime essential oil. For 90 μm sample, the highest chemical contains is 7-octen-1-ol where is suitable not used for fragrances or flavors while the highest chemical compounds for 150 μm is ω-Elemene. It can be use as flavor and fragrance agents. 300 μm shows 6-octenal is the highest compond and for ground sample indicate that Squalene is the major compounds contains in kaffir lime essential oil. 6-octenal and sequalene can be use as natural substances and extractives [12].

### 4. Conclusion

In conclusion, by the result obtain from the test and analysis, hydrodistillation is still a very good method to extract kaffir lime essential oil although there are many other and modern methods such as automated steam distillation, flash vacuum expansion and many more. The analysis on qualitative and quantitative of Kaffir lime leaves oil extracted using hydrodistillation had been accomplished successfully. In quantifying essential oil production, the relevance of different size of particle is clearly affects the amount of oil yield. Increasing the surface area will cause the contact of water and the kaffir lime leaves particle during the hydrodistillation and reduce resistance to oil water mixture from oil sac in the solution. In analyzing the composition of kaffir lime essential oil using GC-MS,
there were 38 compounds found in the essential oil. Some of the major compounds of the kaffir lime leave oils were detected while some are not, may due to oil experience thermal degradation which consequently losing some significant compounds in uncontrolled temperature.

Acknowledgement

Deepest gratitude to Malaysia Ministry of Education and Universiti Teknologi MARA(UiTM) grant 600-IRMI/MYRA 5/3/LESTARI (0047/2016) for the financial support for this project.

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