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Essential Oil from Lemon (*Citrus aurantifolia*) Grown in Ben Tre Province, Vietnam: Condition Extraction, Chemical Composition and Antibacterial Properties

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In present study, a steam distillation method has been adopted for extraction of the essential oil from Vietnamese lemon (*Citrus aurantifolia*) peel harvested from Tien Giang Province, Vietnam. Various extraction conditions influencing the oil yield were investigated. The resulting essential oil was evaluated for physico-chemical characteristics and antibacterial activities. The chemical composition of oil was investigated by GC-MS. The results showed that with grinded lemon peel, a ratio of water and lemon peels of 3:1 (mL/g), extraction time of 90 min at 120 ºC, the highest essential oil yield was attained at 2.1 %. Bioactive components found at high content included limonene (64.90 %), γ-terpinene (13.70 %), β-pinene (11.89 %), α-pinene (2.11 %), β-cymene (1.80 %) and sabinen (1.52 %). The lemon essential oil in this study was found to inhibit five bacteria strains including *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Salmonella enterica* and *Pseudomonas aeruginosa*.

Keywords: Vietnamese Lemon, *Citrus aurantifolia*, Essential oil, Antibacterial properties.

INTRODUCTION

Traditional medicinal herbs have been widely used as a source of health treatments in many developed and developing countries [1-5]. Natural herbs act as locally available and inexpensive therapeutic agents with minimal side effects. Essential oil could be obtained from different parts of plant material such as seeds, leaves and peels. Extraction has become an increasingly popular technique in the processing and agro-food industries [6-11]. There are different methods which are used for obtaining essential oil including solvent extraction, microwave extraction and hydrodistillation [12-14]. Distillation is the key method applied to extract essential oils due to reduced energy and material consumption and the ability to safely control the extraction process.

In recent years, converting citrus wastes including oranges, lemon peel into high added-value commercial products is an emerging trend. The previous studies illustrated that the main components in citrus fruits were sugars (23 %), cellulose (22 %), pectin (25 %), hemicellulose (11 %), flavonoids (4.5 %) and upto 4 % of essential oil. These products play an important role in different fields such as cosmetics, flavoring, pharmaceutical, and healthcare [15-18].

Citrus aurantifolia L., is a small citrus fruit that belongs to the family *Rutaceae* which is cultivated mainly in tropical and subtropical countries. Lemon possesses a wide range of useful bioactive components such as ascorbic acid, citric acid, minerals and flavonoids. Moreover, *Citrus aurantifolia* L. plant also has different secondary plant metabolites including terpenoids, flavonoids and coumarins [9,19-23]. The citrus peels are potential sources of variable oils. Moreover, a primary function of essential oils is antibacterial properties, which are of great interest in cosmetic, food and pharmaceutical industries. The present study aims to optimize a steam distillation extraction process of essential oil existing in Vietnamese Lemon (*Citrus aurantifolia*) peel. Chemical composition of lemon oil was analyzed using GC-MS and antibacterial activity assessment of the obtained oil was performed against four bacteria of clinical concern namely *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, *Salmonella enterica* and *Pseudomonas aeruginosa*.

EXPERIMENTAL

Preparation of material samples: The material samples of *Citrus aurantifolia* (Lemon fruit) used for this study were collected in the region of Ben Tre province (10º10′N, 106º30′E), located in the southwest of Vietnam. The lemon fruit was washed and dried at room temperature. The lemon meat was removed and its peels was retained for the extraction of essential oils. A grinder (LC-1416B, Alaska, Vietnam) was used to change the size of materials. The peels were put in a non-hygroscopic bag and stored in a cooler at 10 ºC for further experiments.

Essential oil extraction: The lemon (*Citrus aurantifolia*) essential oil was extracted by the steam distillation method. A instrument has five main parts, including a 1000 mL volume flask containing solvent, which was distilled water in this case, another flask of 500 mL capacity was used to contain material of lemon peels. The system was heated by a VELP heating device (Model: ARE, Italy) placed under the solvent flask (Fig. 1). A condensing equipment was placed on the top of 500 mL flask containing materials, in order to condense the steam to collect the essential oils.

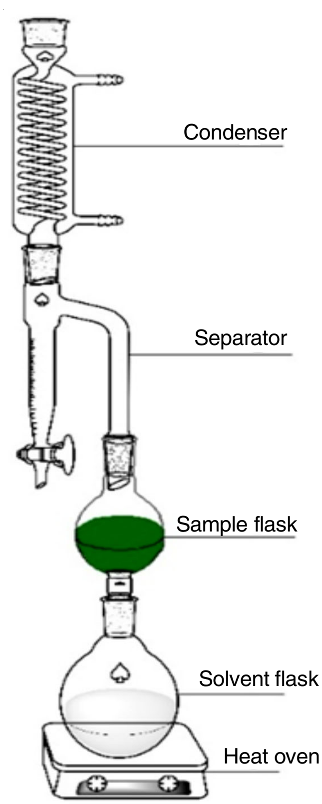

Fig. 1. System of the lemon peels essential oil extraction by the steam distillation method

Single factor investigation for extraction conditions: In this study, four factors influencing lemon peels essential oil extraction were investigated. The material were cutted into quarters, then futher cutted into 8 pieces and finally grinded to a small pieces as shown in Fig. 2. A ratio of water and lemon peels varied from 2:1 to 5:1 mL/g. Time of extraction varied at 15 min intervals from 15 to 120 min. Extraction temperature ranges from 110 to 140 ºC. All the experiments were repeated three times and average values were expressed. To compare the efficiencies of experiments, obtained essential oil, after being unhydrated using sodium sulfate was evaluated for the essential oil yield using the following eqn. 1:

$$\text{Yield (\%)} = \frac{\text{Volume of lemon essential oil obtained (mL)}}{\text{Amount of lemon peels originally used (g)}} \quad (1)$$

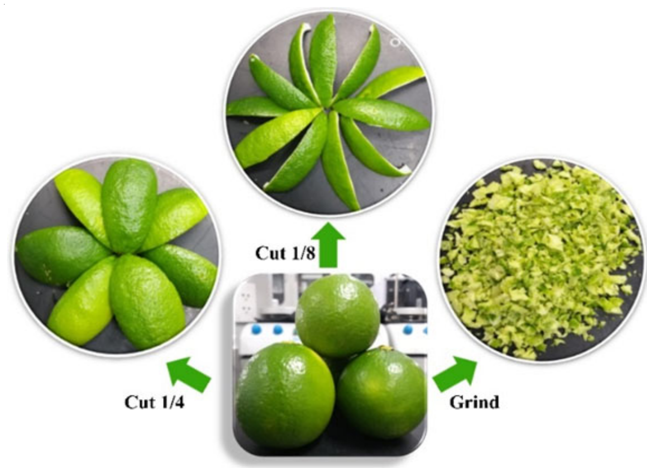

Fig. 2. Material size of the lemon fruit

Chemical composition of essential oils: A GC-MS was used to investigate the composition of the essential oils of all samples. Sample of essential oil (25 µL) was mixed in 1 mL *n*-hexane. The used instrument was GC Agilent 6890N, coupled with MS 5973 inert with HP5-MS column. The head column pressure was set at 9.3 psi. GC-MS system operates under following conditions. A carrier gas was helium at the flow rate of 1.0 mL/min; split ratio: 1:100; injection volume: 1.0 µL; injection temperature: 250 ºC; oven temperature progress included an initial hold at 50 ºC for 2 min, then increased by 2 ºC/min to 80 ºC and increased by 5 ºC/min to 150 ºC, continue rising to 200 ºC at 10 ºC/min and finally rise to 300 ºC at 20 ºC/min for 5 min.

Antimicrobial activity: Five bacteria species *viz. Bacillus cereus, Staphylococcus aureus*, *Escherichia coli*, *Salmonella enterica* and *Pseudomonas aeruginosa* were used in this study. Antibacterial activity of the obtained essential oil was evaluated by using agar-well diffusion method. First, agar plates were prepared with 10 mL of lysogeny broth (LB) solution. Then, 3 mL of liquid cultures were incubated at 37 ºC with aeration (150 rpm) overnight on LB. On the surface of LB agar, 5 mm wells were made and loaded with 20 µL of essential oils and again incubated at 37 °C overnight. Finally, electronic calipers were used to measure the diameters clear zones formed around the wells. In this work, amoxycillin (100 µg/mL) and sterile water were acted as positive and negative control, respectively.

RESULTS AND DISCUSSION

Single factor investigation method for extraction conditions: The factors affecting the extraction of lemon essential oil include the lemon peels size, water and peel ratio (mL/g), time extraction (min) and temperature extraction (°C). Their corresponding effects on oil yield are shown in Fig. 3. In Fig. 3a, yield (%) of essential oil of lemon increased when the size of lemon peels decreased, especially when being grinded. The yield attained at grinded materials peaked at 1.9 %. This was because as the size of material decreased, interfacial area between the material and solvent was enlarged, enhancing the ability of a solvent to attract compounds present in the materials, especially polar solvents such as water. However, as the peel material was reduced into small pieces, a characteristic aroma of the essential oil could be lost, suggested that large lemon peel (quarterly cutted pieces) could preserve the flavour of aromatic oils better than the grinded peels.

When the ratio of water and lemon peel increases steadily from 2:1 to 5:1 (mL/g), a overall yield of essential oils decreases from 1.85 to 1.75 % (Fig. 3b). A ratio of 3:1 and 4:1 (mL/g) gave identical yield contents (1.9 %). Therefore, ratio of 3:1 is considered as an optimal factor for further studies. Fig. 3c displayed a yield of essential oils obtained through different time points from 15 to 120 min and observed a yield increased from 0.15 to 1.85 %. However, in this study, the yield of lemon essential oil when passed the optimal point of time of 90 min saw a decrease from 2.0 to 1.85 % because of denaturation of some substances in the oil caused by prolonged exposure with high temperature. Therefore, an extraction time of 90 min corresponds to the yield of 2.0 % was selected.

The temperature was surveyed at four levels above 100 ºC since they were higher than the boiling temperature of water. Fig. 3d indicated that at 120 ºC, the highest yield was achieved at 2.1 %. At further elevated temperatures, some components in the essential oil which were sensitive to temperature might decompose and thus adversely affected the extraction yield, oil quality and the production cost due to increased energy consumption. Therefore, an optimum temperature selected for this process was 120 ºC.

Chemical composition of essential oils: Evaluation of the chemical composition of the compounds in essential oils was conducted with gas chromatography-mass spectrometry (GC-MS). The results are displayed in Table-1 and Fig. 4. There were 15 components accounted nearly 100 % of the total volume of essential oils. The compositions analyzed by GC-MS were similar to previous studies. The main compound in lemon essential oil was limonene (64.90 %). Moreover, in present lemon oil, other components *viz.* γ-terpinene (13.70 %), β-pinene (11.89 %), α-pinene (2.11 %), β-cymene (1.80 %) and

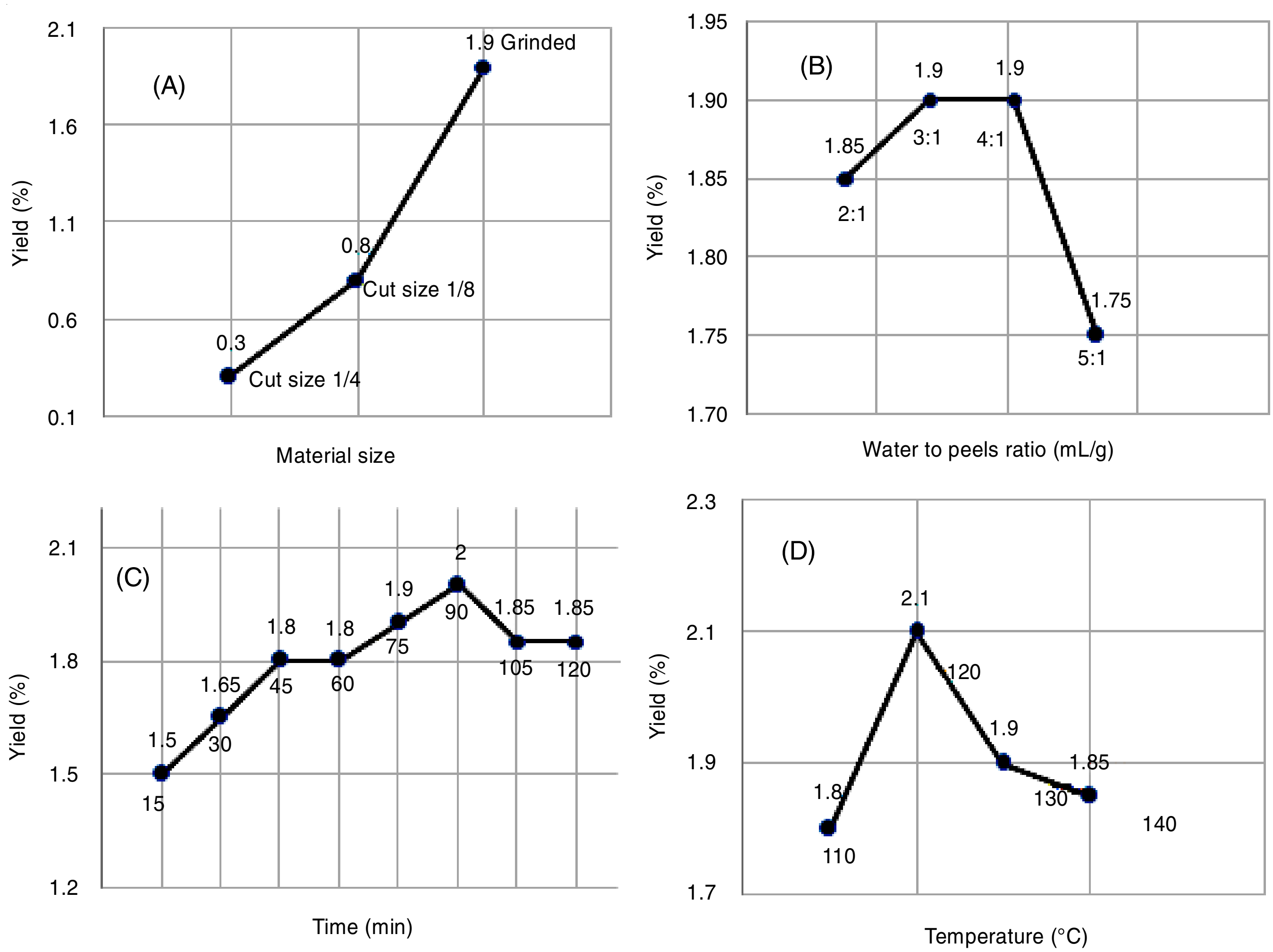

Fig. 3. Factors affecting the yield of lemon essential oil extraction

TABLE-1
COMPONENT OF ESSENTIAL OIL FROM LEMON PEELS AND COMPARE WITH OTHER STUDIES

Name component	Peak	RT (min)	This study (%)	Compare with other studies (%)				
				Italy [23]	Peru [24]	Iran [25]	India [26]	Thailand [27]
α-Thujene	1	7.157	0.491	0.6	1.14	–	–	–
α-Pinene	2	7.387	2.112	2.0	0.87	1.5	0.1	3.05
Sabinen	3	9.144	1.517	1.7	0.28	1.4	0.1	22.75
β-Pinene	4	9.238	11.889	12.3	0.87	–	–	30.48
β-Myrcene	5	10.095	0.789	1.3	1.27	1.8	0.3	–
α-Terpinen	6	11.298	0.256	0.3	–	–	–	–
β-Cymene	7	11.747	1.796	0.1	–	–	–	0.85
Limonene	8	12.061	64.906	52.7	49.66	85.5	46.7	8.13
γ-Terpinene	9	13.703	12.823	16.2	–	2.4	–	–
α-Terpinolen	10	15.449	0.42	0.6	–	–	–	–
α-Terpineol	11	21.085	0.422	0.5	–	–	–	6.61
β-Citral	12	23.281	0.668	1.4	–	–	–	–
β-Elemene	13	28.343	0.202	–	–	–	–	–
β-Caryophyllen	14	29.137	0.669	–	0.48	–	2.6	–
α-Bergamotene	15	29.629	1.04	–	0.80	–	0.1	–

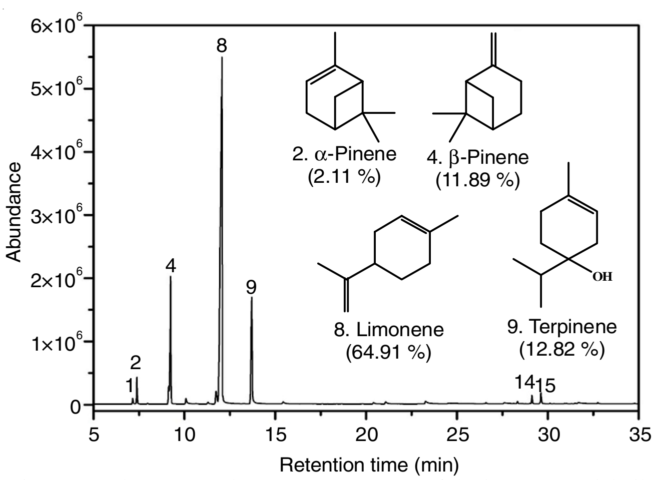

Fig. 4. Gas chromatography-mass spectrometry of the lemon essential oil

sabinen (1.52 %) were also found. In comparison with lemon materials from other regions, lemon essential oil extracted from fresh lemon peels in Vietnam is comparably high in limonene content except from Iran lemon peels (Table-1).

Antimicrobial activity: Table-2 shows the antibacterial activity data of Vietnamese lemon (*Citrus aurantifolia*) oil harvested from Tien Giang Province, Vietnam extracted by steam distillation method on different bacteria strains *viz*. *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Salmonella enterica* and *Pseudomonas aeruginosa*. In general, the antimicrobial properties exhibited against Gram-positive antimicrobial properties were stronger than those against the Gram-negative bacteria. This is because while Gram-negative bacteria had double membranes, the Gram-positive bacteria lacked the outer layer. Among the five bacterial strains, the antibacterial activity of lemon essential oil against *Bacillus cereus* showed to be comparable to that of tetracycline. In addition, lemon essential oil in this study did not inhibit the bacterium *Pseudomonas aeruginosa*. Since, these strains are common in meat, canned products and cosmetics (personal and family care products), it is suggested that lemon essential oil should be used as an ingredient to create flavour or as preservatives in the cosmetic and food sectors.

TABLE-2
COMPARISON OF INHIBITORY ZONE OF OILS BY WELL-DIFFUSION METHOD

Name bacteria	Lemon essential oil (mm)	Tetracyline (mm)
Bacillus cereus	17.67 ± 2.08	18.00 ± 0.00
Staphylococcus aureus	16.33 ± 0.58	23.00 ± 0.00
Escherichia coli	11.00 ± 1.00	23.00 ± 0.00
Salmonella enterica	10.33 ± 0.58	23.00 ± 0.00
Pseudomonas aeruginosa	0.00 ± 0.00	26.00 ± 0.00

*Data presented as mean of triplicate experiments ± SD

Conclusion

In this study, Vietnamese lemon (*Citrus aurantifolia*) were used for extraction of essential oil using hydrodistillation method. In addition, evaluation of chemical compositions and the antibacterial of the essential oil was also performed. The highest yield of the distillation process was 2.1 % with optimal conditions (ratio of water and lemon peels to 3:1 (mL/g), extraction time of 90 min and at 120 ºC). The GC-MS analysis indicated that major constituents in the obtained oil were imonene (64.90 %), γ-terpinene (13.70 %), β-pinene (11.89 %), α-pinene (2.11 %), β-cymene (1.80 %) and sabinen (1.52 %). The obtained oil did not exhibit antibacterial activity against *Pseudomonas aeruginosa*. The steam distillation method of fresh wastes of peel of Vietnamese lemon (*Citrus aurantifolia*) acted as an effective alternative process for the extraction of essential oils from the byproducts of the fruit processing industries.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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