Comparative evaluation of the antibacterial activities of the essential oils of *Citrus grandis* (L.) *Osbeck* obtained by hydrodistillation and microwave assisted extraction methods

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Abstract: The essential oils (EOs) from peels of *Citrus grandis* (L.) *Osbeck* was investigated for their antimicrobial activities against food-related microorganisms by hydrodistillation method (HD), and microwave-assisted extraction (MAHD). The total yield of the volatile fractions obtained through HD and MAHD was 4.4 mL/g and 4.75 mL/g, respectively. The gas chromatography–mass spectrometry (GC–MS) analyses of the oils revealed the presence of different compounds in *Citrus grandis* EOs obtained from MAHD and HD methods, including α-pinene, sabinene, β-myrcene, α-phellandrene and d-limonene. The essential oils obtained from the two extraction methods were found to inhibit six bacteria strains including *Bacillus cereus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *E. coli*, *Clostridium perfringens* and *Salmonella typhi*. The results of antibacterial properties of *Citrus grandis* (L.) *Osbeck* EOs against six bacterial strains showed that essential oil extracted from the HD method were more bacteriostatic than the oil extracted from MAHD method. With this result, the essential oil obtained by the HD method was more active than that of the MADH method against the bacteria.

1. Introduction

In recent decade, using herbs and medicine plants have been receiving a great deal of public attention due to various functions [1-5]. Essential oils are commonly used in daily life, especially in the cosmetics industry such as bathing lotions, cologne waters, shampoos, hair lotions, disinfectant and insecticide ingredient [6-8]. In essential oils, phenolic compounds have antimicrobial activity and protective substances, which is why they are used to deter bacterial development [9].

The pomelo (namely *Citrus grandis* (L.) *Osbeck*), which belongs to the *Rutaceae* family, is a tropical and subtropical fruit. In certain areas, pomelo is not only a fruit but is often used against exhaustion, strength depletion, lack of stamina, swelling, burns, acne, or minor skin disorders in traditional medicine. The previous studies shows the pomelo was used as appetizer, antitoxic, cardiac stimulant, and stomach tonic.
Moreover, over thousands of years plant essential oils have been used in food processing, pharmaceuticals, herbal medical goods and natural therapies. There are various methods of extraction EOs, including hydrodistillation (HD), Steam distillation (SD), microwave hydro diffusion and gravity (MHG) [11], ultrasonic extraction (UE), and so on [11–18]. The varying methods of extraction can alter the properties of essential oils [19-20]. According to Presti et al. (2005), EOs chemical compositions and biological activities were influenced by the essential oil extraction process [21].

In recent years, an alternative method has been created for the extraction of natural products using microwave energy [22-25]. Microwave assisted hydrodistillation (MAHD) is based on microwave heating combination with hydrodistillation at atmospheric pressure. There are some advantages of MAHD comparing with HD such as (1) rapidly of the first essential oil droplet to hit an extraction temperature of 100 °C, (2) high essential oil yield at a reduced energy consumption and (3) high purity of the oil extraction.

This research aims to extract EOs from fresh *Citrus grandis* (L.) Osbeck peels through two methods, including HD and MAHD. Then, phytochemical profile of essential oil was analyzed by the gas chromatography–mass spectrometry (GC-MS), and it was assessed antibacterial ability on Gram negative and Gram positive bacteria.

2. Materials and methods

2.1. Plant material

200 g of *Citrus grandis* (L.) Osbeck was collected between January to February, 2019 from Tien Giang, Viet Nam. Then, pomelo peels are separated and washed several times with water before the extraction process. It was cut into small pieces with size of 0.5-1 cm. Finally, it was put into a 1000 mL volumetric flask with a water-based solvent and placed in different extraction systems.

2.2 Microwave-Assisted Hydrodistillation (MAHD)

MAHD was carried out by a domestic microwave oven MW71E (SAMSUNG) attached to a Clevenger apparatus (Figure 1). The operation power of this MW71E is 100-800W with 250v-50Hz power source. The following conditions were applied: 3:1 of water to raw material ratio; 500W of microwave power and 60 min of extraction time. The flask containing 100 g of *Citrus grandis* (L.) Osbeck, with its deionized water, was put inside the microwave and the obtained essential oils were collected by the Clevenger apparatus.

2.3. Hydrodistillation (HD)

The 1000 mL volum flask containing 100 g of partially dried lemongrass leaves and deionized water were heated using Heating Mantle heater (Glassco Laboratory Equipment Pvt. Ltd., India). Both methods were carried out at 120 minutes.
2.4 Analysis of essential oil sample

The yield of pomelo oil extracted from MAHD and HD methods were removed water by Na$_2$SO$_4$, and calculated by using equation (1):

$$Y (mL/g) = \frac{V (mL)}{m (g)} \times 100$$

where $y$: essential oil yield, $V$: essential oil volume, and $m$: weight of pomelo peels.

The essential oil samples were dissolved in $n$-hexane before subjecting to GC-MS analysis. Name equipments: GC Agilent 6890N, MS 5973 inert. HP5-MS column, head column pressure 9.3 psi. GC-MS were obtained under the following conditions: carrier gas He; split 1:100; flow rate 1.0 mL/min; injection volume 1.0 μL; injection temperature 250°C; oven temperature progress included an initial hold at 50°C for 2 min, then increased to 80°C at 2°C/min, 150°C at 5°C/min, 200°C at 10°C/min and a rise to 300°C at 20°C/min for 5 min. The oil components were determined by comparing retention indices with published mass spectra and Wiley library.

2.5. Antimicrobial activity testing

*Bacillus subtilis* NRRL B-354, *Staphylococcus aureus* NRRL B-313, *Bacillus cereus*, *Pseudomonas aeruginosa* NRRL B-14781, *E. coli* NRRL B-409, *Clostridium perfringens* and *Salmonella typhimurium* YS1646 were collected from Applied and Environmental Microbiology Research Group at the University of Fort Hare, then subjected to agar diffusion assay to measure the antimicrobial activity. 3 mL of liquid cultures were grown at 37 °C with shaking (150 rpm) overnight on LB agar plates. Plates inoculated with the test organism was loaded with essential oil samples. All plates were incubated (37°C) overnight. The inhibition zone was calculated by using an electronic calipers to divide the total halo diameter (mm) by a disk thickness (5 mm). As positive tests, amoxicillin (100 μg/ml) was used, negative tests were distilled water. Both experiments were done in triplicate.
3. Results and discussion

3.1. Yields and chemical composition of the essential oil

The essential oil yield of the pomelo peels obtained from the microwave-assisted hydrodistillation method was 4.75 mL/g while that obtained from hydrodistillation was 4.4 mL/g. The chemical compositions of \textit{Citrus grandis} (L.) Osbeck essential oil is described along with their respective retention indices determined by comparing with Wiley library and published mass spectra (Figure 2). Five compounds were identified, including sabinene, \textit{α}-pinene, \textit{α}-phellandrene, \textit{β}-myrcene, and \textit{d}-limonene (Table 1).

Table 1. Chemical composition of pomelo peels essential oils extracted from HD and MAHD.

| R.T. | Compound Structure | Compound Name   | Content (%) | MAHD | HD  |
|------|-------------------|----------------|------------|------|-----|
| 7.24 | ![Structure](image1) | \textit{α}-pinene | 0.533      | 0.645|
| 8.966| ![Structure](image2) | Sabinene       | 0.13       | 0.206|
| 9.917| ![Structure](image3) | \textit{β}-myrcene | 1.12       | 1.31 |
| 10.461| ![Structure](image4) | \textit{α}-phellandrene | 0.58       | 0.713|
| 11.883| ![Structure](image5) | \textit{d}-limonene | 97.637     | 97.127|

Yield (mL/g) | 4.75 | 4.4
Figure 2. Chromatogram of pomelo essential oil from hydrodistillation (HD) and microwave-assisted hydrodistillation (MAHD).

3.2. Antibacterial activity

Citrus grandis (L.) Osbeck essential oil were screened against B. cereus, B. subtilis, P. aeruginosa, E. coli, C. perfringens and S. typhi. As shown in Table 2 and Figure 3, the pomelo essential oil was extracted by a microwave-assisted hydrodistillation method has the antibacterial ability increased from S. typhi < B. subtilis < E. coli < P. aeruginosa < C. perfringens < B. cereus. Meanwhile, for the hydrodistillation method, the antibacterial capacity increased from B. subtilis < E. coli < P. aeruginosa < S. typhi < C. perfringens < B. cereus. Throughout this analysis, the degree of the antibacterial capacity of essential oils derived from hydrodistillation was higher than that of the process of microwave-assisted hydrodistillation. It is a fact that the oil collected by the hydrodistillation method has a higher content of α-pinene, sabinene, β-myrcene, α-phellandrene which possess effective antibacterial and antimicrobial function than that of compounds in the hydrodistillation process supported by microwave. Such chemical components impose their antimicrobial action on microorganisms by interrupting the cohesion of the membrane of bacteria. However, the difference in membrane structure of Gram positive and Gram negative bacteria could have caused varied bacterial responses to the antibacterial agent.

Table 2. Diameter of inhibition zone of Citrus grandis (L.) Osbeck essential oil

| Tested bacteria           | MAHD (mm)  | HD (mm)  |
|---------------------------|------------|----------|
| Bacillus cereus           | 24.63 ± 0.11 | 31.66 ± 0.13 |
| Bacillus subtilis         | 11.33 ± 0.33 | 16.11 ± 0.58 |
| Pseudomonas aeruginosa    | 19.36 ± 0.25 | 19.41 ± 0.17 |
| E. coli                  | 16.36 ± 0.17 | 18.41 ± 0.25 |
| Clostridium perfringens   | 20.69 ± 0.15 | 22.40 ± 0.26 |
| Salmonella typhi          | 8.69 ± 0.08  | 19.70 ± 0.12 |

* Data presented as mean of triplicate experiments ± SD

* Values followed by the same letter in the same column are not significantly different (p≤0.05)
Figure 3. Inhibition of pomelo essential oil by well diffusion assay. Wells contained essential oil obtained by Microwave extraction (well B1), 20μl essential oil obtained by hydrodistillation (well B2), add 20μl Amoxicillin (100 μg/ml) (well +) and 20μl sterile water (well H2O). Ba: Bacillus cereus, B. Sub: Bacillus subtilis, PA: Pseudomonas aeruginosa, E. coli: E. coli, Clos: Clostridium perfringens, SAL: Salmonella typhi.

4. Conclusion

Citrus grandis (L.) Osbeck peels essential oils were examined by hydrodistillation and microwave-assisted extraction processes for their antimicrobial activity against food-related microorganisms. The total oil yield obtained from HD and MAHD was 4.75 mL/g and 4.4 mL/g, respectively. GC-MS analyses identified five components in the Citrus grandis (L.) Osbeck essential oils, including sabinene, α-pinene, α-phellandrene, β-myrcene and d-limonene. Moreover, this research showed that the antibacterial activity of hydrodistillation oil higher than microwave solvent-free extraction. Overall, the oils collected from HD were more effective than the oil extracted by MAHD against the examined bacteria.

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