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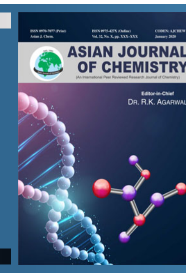

Evaluation of Physical and Chemical Properties of Pomelo (*Citrus grandis* L.) Essential Oil using Steam Distillation Process

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This study attempted the extraction of essential oils from the peels of pomelo (*Citrus grandis* L.) grown in Ben Tre province, Vietnam through hydrodistillation method. In addition, the chemical composition and physio-chemical properties of the essential oils were reported. The results showed that the extraction yield of the extraction process achieved about 1.67%. The physico-chemical index of essential oils is determined by specific gravity (0.8572 g/cm^3), acid index (0.3556 mg KOH/g), ester index (2.4216 mg KOH/g), and refractive index (1.476). The GC-MS analyses of the oil indicated the component with highest content in the oil was α-limonene (96.491%), followed by α-pinene (0.686%), β-pinene (0.248%), β-myrcene (1.644%), α-phellandrene (0.793%) and β-*cis*-ocimene (0.138%). These results suggested that wastes from fruit peeling process can be converted into a new material source with great potential for industrial use.

Keywords: Pomelo, (*Citrus grandis* L.), Essential oils, Vietnam.

INTRODUCTION

Essential oils are a mixture of volatile substances, with a characteristic odor depending on the source from which the essential oils are derived [1-5]. The essential oil does not naturally present in the plant materials, but is rather isolated under mechanical processes or distillation. Essential oils could be extracted from different plant organs including leaves, flowers, fruits, stalks, roots and resins and find a myriad of applications in aromatherapy, perfumes, cosmetics and the food industry [6-11]. Pomelo [*Citrus grandis* (L.) Osbeck], is a fruit that could be classified in both tropical and subtropical categories. The fruit has attracted great scientific interest due to its nutritional and antioxidant properties and the abundance of organic acids, sugar and phenolic compounds, which largely determines taste and organoleptic quality of the fruit [12,13]. The pomelo essential oils, which are often extracted from pomelo peels through hydrodistillation are also a product of interest due to the presence of various valuable components including D-limonene, β-myrcene, α-pinene, β-pinene, γ-terpinene, α-terpinolene, α-caryophyllene, copaene and β-phellandrene [14-16]. The oil is an almost colourless to pale yellow liquid with a characteristically refreshing and pleasant odour.

Pomelo essential oils have been reported to exhibit inhibitory activities against a wide spectrum spoilage-causing microorganism [17]. In addition, the peels of the fruit are also shown to be constituted of important antioxidants including flavonoids, which is characterized by antimicrobial activities [18], and polyphenols. To be specific, while flavonoid in the pomelo primarily consists of hesperidine, narirutin, naringin and eriocitrin, polyphenol composition was found to be made up of caffeic acid, *p*-coumaric acid, ferulic acid and sinapinic acid [19,20]. Other bioactivities of pomelo essential oil may include insecticidal, antiseptic, antimicrobial, anti-bacterial, anti-fungal, astringent and anti-inflammatory properties. These properties have made essential oils extracted from pomelo fruit useful in treatment of cancer, ulcer and arthritis, lowering cholesterol, weight loss therapies, cellular regeneration and skin treatments.

In the large-scale extraction, the oil content in the pomelo peels determines the economic efficiency and often ranges from 1.0 to 2.5%, and the chemical composition of the obtained oil, which varies according to the chemotype and varieties, are the two outcomes of interest. So far, only one study has illustrated yield and compositional differences between pomelo essential oils obtained from materials grown in various locations in Vietnam [21]. However, the extraction technique used in that study was cold pressing, which lacks applicability in real-scale essential oil production and may produce different yield and composition results when compared with other methods. Therefore, the purpose of the present study is two-fold. First, the extraction of essential oils from pomelo fruits was performed with hydro-distillation method at pilot-scale. Second, physico-chemical parameters and chemical composition in the produced oil were determined. The results could open new potentials in diversification of existing cosmetic, pharmaceutical and food products using pomelo essential oil and contribute to the existing knowledge about chemical composition of essential oils extracted from Vietnamese fruits and plants.

EXPERIMENTAL

Plant material: The fresh pomelo fruits were gathered during March 2019 from Ben Tre province (10º14′54′′N 106º22′34′′E) of Vietnam. The fruits were washed with water and peeled to separate the flesh. After peeling 59 kg of materials, 15 kg of peels was obtained.

Extraction of pomelo essential oil: The peels were then ground with a blender and transferred into the container of the steam distillation apparatus unit with a ratio of 1:3 raw materials-water. The distillation was carried out in 3 h at 105 ºC. The obtained steam and essential oil mixture were then separated into two phases. The upper essential oil layer was collected by allowing the layer to flow into a tank whereas the water underneath was passed back to the distillation apparatus. The essential oil was then dried with anhydrous sodium sulfate to remove the trace amounts of water in the essential oil. The essential oil product was then put into a dark and tightly closed container.

Physico-chemical analyses of pomelo (*Citrus grandis* L.) essential oils: The method of AOAC (2005) was adopted to determine physico-chemical characteristics of the produced essential oils including refractive index, optical rotation, specific gravity, colour, odour and solubility.

Acid index: The acid index is defined as the number of milligrams of KOH needed to neutralize free acids in 1 g of essential oil.

Saponification index: The soap index is defined as the number of milligrams of KOH needed to neutralize all free acids and acid combined as esters in 1 g of essential oil.

Ester index: The ester index is the number of milligrams of KOH needed to neutralize fatty acids in ester form in 1 g of essential oil.

GC-MS analysis of pomelo (*Citrus grandis* L.) essential oil: Chemical composition of pomelo fruit oil was determined by GC-MS analysis using GC Agilent 6890 N instrument coupled with HP5-MS column and MS 5973 inert. The pressure of the head column was 9.3 psi. Essential oil (25 µL) was added with 1.0 mL *n*-hexane and dehydrated with Na_2SO_4. The flow rate of was constant at 1 mL/min. Injector temperature is 250 ºC and the rate of division is 30 ºC. Thermal program for samples was as follows: initiated temperature at 50 ºC, which was kept for 2 min, then increased by 2 ºC/min to 80 ºC, then continued to increase by 5 ºC/min to 150 ºC, then continued to increase by 10 ºC/min to 200 ºC and finally increased by 20 ºC/min to 300 ºC, which was hold for 5 min.

RESULTS AND DISCUSSION

The physico-chemical properties of essential oils from the peels of pomelo (*Citrus grandis* L.) grown in Ben Tre province, Vietnam through hydro-distillation method are illustrated in Table-1. With the conventional distillation method, the distilling of essential oil from the pomelo peels resulted in the yield of 1.67%. The total of 250 mL of essential oil was obtained by distilling 15 kg of materials. This is significantly higher than results of Varkey *et al*. [22] who reported that isolation of grape-fruit essential oil from southern India achieved 0.28-0.78% efficiency. It is worth noted that the performance of essential oil distillation also depends on the source of raw materials and extraction conditions. Pomelo essential oil is a clear liquid, lighter than water and well cleaved in the water (d = 0.872). The oil and has a light yellow colour, spicy taste and a characteristic aroma that is very pleasant. The acid index (IA = 0.3556 mg KOH/g) is relatively low, indicated that no significant thermal oxidation has taken place in the distillation process and the oil is freshly distilled. This is contrary to the high acidity in the long-preserved oil, which is oxidized and contains ester. The refractive index of essential oil is about 1.476, which is moderate due to the presence some double-connected compounds in the essential oil composition.

TABLE-1
SOME PHYSICO-CHEMICAL INDICATORS
OF POMELO ESSENTIAL OIL

Organoleptic characteristics	Vietnamese essential oil
Physical state	Liquid
Colour	Transparent or light yellow
Odour	Specific
Density	0.8572
Acid index	0.3556
Saponification index	2.7772
Ester index	2.4216
Refractive index	1.476

GC-MS analysis: Chemical composition of pomelo essential oil was determined by GC-MS. The analysis results by GC-MS in Table-2 shows that there are six main chemical constitutents in the obtained oil including α-limonene (96.491%), β-myrcene (1.644%), α-pinene (0.686%), β-pinene (0.248%), α-phellandrene (0.793%) and β-*cis*-ocimene (0.138%). The total amount of monoterpene hydrocarbons ranged from 87.90 % to 95.64%. Since, the peak at 12.04 min has the greatest intensity, which suggested that α-limonene is the most important ingredient in pomelo oil (Fig. 1). These reported components are the predominant ingredients in the produced oil, agreeing with results from previous studies of citrus essential oils [23] Additionally, α-phellandrene was found in this study, which is in line with another study where α-phellandrene was also

TABLE-2
CHEMICAL COMPOSITION OF POMELO ESSENTIAL OIL DETERMINED BY GC-MS

Peak	Compound	Retention time (min)	Present study	Indian [33]	Malaysian [34]	Korean [31]
1	α-Pinene	7.272	0.686	0.41	0.3	0.75
2	β-Pinene	9.008	0.248	2.25	0.63	0.25
3	β-Myrcene	9.949	1.644	2.06	1.60	22.66
4	α-Phellandrene	10.503	0.793	0.43	0.05	nd
5	α-Limonene	12.040	96.491	89.04	95.10	68.08
6	β-*cis*-Ocimene	13.065	0.138	0.33	nd	0.48

nd: not detected

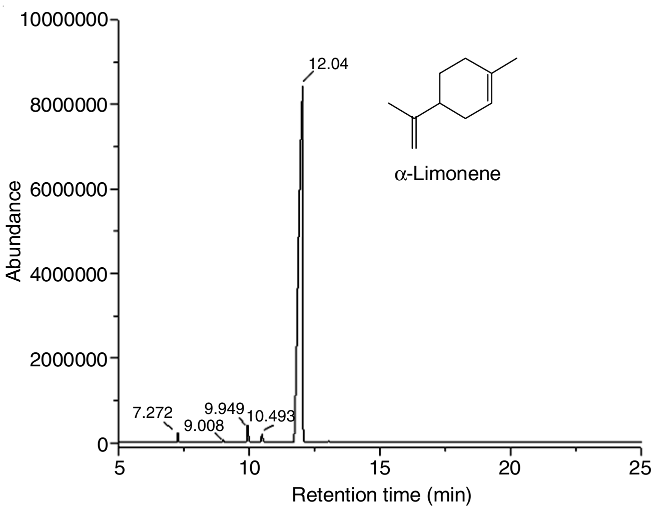

Fig. 1. GC-MS chromatography of components from pomelo peel essential oil

found at the concentration of lower than 1% in various citrus oils [24]. Despite that α-phellandrene had not previously been reported as a pomelo essential oil constituent. Furthermore, individual quantification of aldehydes was also not attempted in this study despite their role as the primary measure of oil quality. The difference in terms of yield and composition of essential oils may stem from biological differences of the plant materials, extraction parameters as well as the habitat conditions in which the plant grows [25].

Generally, compounds detected in the GC-MS analysis were hydrocarbons in nature [26]. According to Ahmad *et al.* [27] who determined the composition of volatile oil of pomelo from Pakistan, monoterpene hydrocarbons constituted 93.98% of the obtained oil and limonene (86.27%), myrcene (6.28%) and α-terpinene (2.11%) were the main compounds. In Korean *C. grandis* essential oil, the predominant components were limonene (68.08%), β-myrcene (22.65%) and γ-terpinene (1.63%) [28]. In another report [29], abundant constituents in pomelo essential oil were limonene (74.45%), β-myrcene (12.85%) and α-pinene (3.74%). These markedly different results indicate that the composition of essential oils of the same plant species might not be identical.

Further examination on the structure of α-limonene (Fig. 2a) revealed that the compound is an aliphatic hydrocarbon and colourless. Limonene is also classified as a cyclic and stable monoterpene that could be isolated without decomposition under high temperature. Since limonene exhibits high volatility and antibacterial activity, strong oxidative property and low solubility in water [30], it is suggested that the essential oil from pomelo could be used as an antibacterial agent, powerful antioxidant and anti-free radical agent [14,31].

For β-myrcene (Fig. 2b) indicated that the compound is an olefinic natural organic hydrocarbon. In other words, the compound is classified as a monoterpene, serving as dimers of isoprenoid precursors. Myrcene is a valuable agent having a pleasant odour and playing the role of an intermediate agent in the manufacture of flavoring chemicals in the perfumery industry [32]. Besides, α-myrcene is effective in preventing inflammation and relieving pain.

Fig. 3 showed the difference between the content of α-limonene in essential oil compositions of pomelo in different regions. In combination with data in Table-2 and Fig. 3, it can be seen that α-limonene component of pomelo oil from Vietnam (96.461%) is comparably high to that of pomelo essential oils from materials in other regions. Besides, α-limonene component in pomelo essential oil from Korea (68.08%) is much lower than the rest. However, pomelo essential oil from Korea was offset by the relatively high content of α-myrcene (22.66%) [31,33]. Meanwhile, α-limonene content in pomelo essential

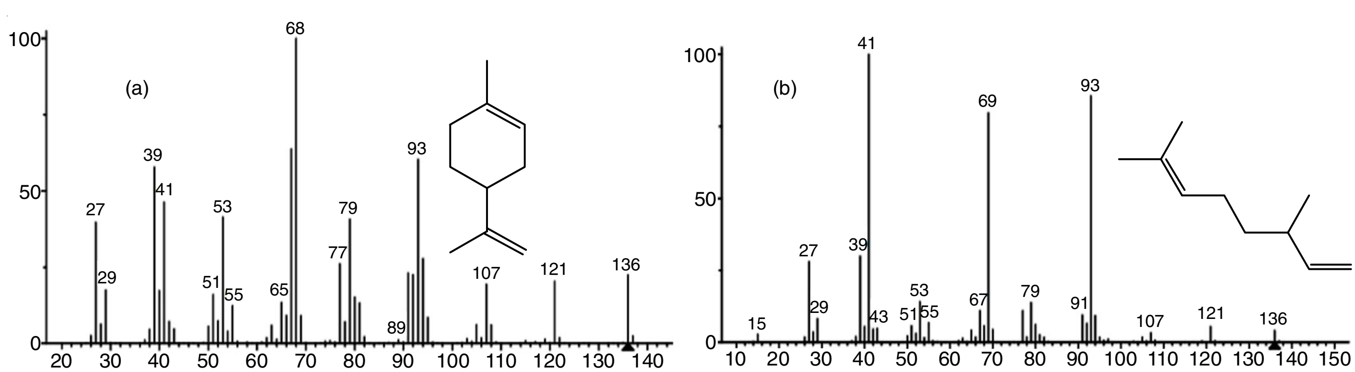

Fig. 2. MS spectrum of α-limonene (a) and β-myrcene (b) from pomelo essential oil

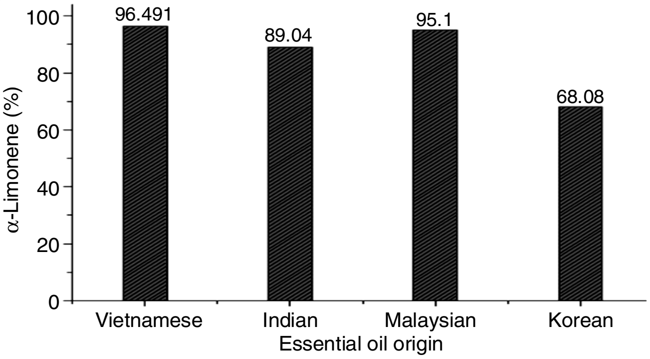

Fig. 3. α-Limonene volatile components of pomelo essential oil in different countries

oil in Malaysia (95.1%) [34] and India (89.04%) [35] was relatively high and approximated pomelo essential oil in Vietnam (96.46%). This difference may be due to species, environmental factors, the climatic conditions and extraction methods [35]. In addition, the use of fertilizers and irrigation can also lead to differences in composition [36].

Conclusion

In present work, the pomelo (*Citrus grandis* L.) peels were used for extraction of essential oil using hydrodistillation method. The obtained oil was evaluated for chemical composition and physico-chemical characteristics. The extraction process achieved the yield of 1.67%. The analyzed physico-chemical parameters were specific gravity (0.8572 g/cm^3), acid index (0.3556 mg KOH/g), ester index (2.4216 mg KOH/g) and refractive index (1.476). Result of GC/MS analysis revealed six predominant components existing in the pomelo essential oils. The most abundant component is α-limonene (96.491%), followed by α-pinene (0.686%), α-pinene (0.248%), α-myrcene (1.644%), α-phellandrene (0.793%) and α-*cis*-ocimene (0.138%). It can be concluded that the chemical composition of pomelo (*Citrus grandis* L.) essential oils correlates with the climatic conditions in which it is grown, as well as a genetic variation and the pomelo essential oil from Vietnam is suitable for use as an antibacterial agent.

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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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