International Food Research Journal 22(6): 2426-2431 (2015)

Journal homepage: *http://www.ifrj.upm.edu.my*

# Chemical composition, antioxidant and antibacterial activities of peels' essential oils of different pomelo varieties in the south of Vietnam

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**Article history**

*Received: 8 January 2015*
*Received in revised form: 7 May 2015*
*Accepted: 20 May 2015*

**Keywords**

*Essential oil*
*Citrus*
*Pomelo*
*Antioxidant*
*Antimicrobial*

**Abstract**

Volatile compounds of citrus essential oil are responsible for its flavor and functional properties. In this study, chemical compositions, antioxidant and antibacterial activities of peels' essential oils extracted from different pomelo varieties grown in the south of Vietnam were investigated. The compositions of volatile compounds of pomelo essential oils varied with different pomelo varieties. The essential oils of DaXanh in DongThap (DX-DT) and DuongCam in DongNai (DC-DN) were mostly limonene (90.1% and 95.7% of total relative concentrations, respectively), whereas NamRoi in VinhLong (NR-VL) and BuoiLong in DongThap (BL-DT) had high amounts of γ-terpinene, α-pinene, α- and β-phellandrene. The results also indicate that the essential oils of NR-VL and BL-DT exhibited the highest antioxidant and antibacterial activities against *S. iniae* and *P. aeruginosa* as compared to the essential oils of other pomelo varieties. Thus, the high amounts of γ-terpinene, α-pinene, α- and β-phellandrene in the essential oils of NR-VL and BL-DT were responsible for the high antibacterial activities of these essential oils, whereas the high amounts of limonene and myrcene had less effect on the tested bacteria. As a result, the different pomelo varieties and growing locations significantly affect the chemical compositions, antioxidant and antimicrobial activities of the peels' essential oils.



## Introduction

*Citrus*, that belongs to the rue family, Rutaceae, is one of the important horticultural crops growing extensively in tropical and subtropical southern regions of Asia. In Vietnam, *citrus* fruits are grown in seven ecological regions in the north, middle and south areas of Vietnam. Vietnamese *citrus* fruits are diversified and each citrus crop varies with the agroecological region (Lan-Phi, 2010). Pomelo (*Citrus grandis* (L.) Osbeck) is one of the most common *citrus* fruits and is the largest fruit among the *citrus* fruit varieties. Pomelos are grown mostly in the South of Vietnam including Vinh Long, Ben Tre, Dong Thap, Dong Nai and Binh Duong provinces although some cultivars are found in the Central and the North of Vietnam such as Hue City, Ha Tinh province, Phu Tho province and Ha Noi City. The annual pomelo production in Vietnam is about 50,000 tons with 5,000 ha produce fruit (FAO, 2004). Like other *citrus* fruits, pomelos are rich in vitamin C (Morton, 1987). They are generally eaten as fresh fruits, and they can be stored well. Moreover, the essential oils extracted from leaves or peels of pomelos are considered to have a variety of health benefits and commonly used in aromatherapy, pharmaceutical and food industries. Ninety-one volatile components are separated and quantified in the peel's essential oils of the Phuc Trach, Van Giang and Nam Roi pomelos in Vietnam (Lan-Phi, 2010). The main compounds in the pomelo essential oils are α-pinene, sabinene, β-pinene, myrcene, α-terpinene, limonene, terpinolene, γ-terpinene and linalool with total relative concentrations of 85.9 - 96.5% (Lan-Phi *et al.,* 2015). The pomelo essential oils have been reported to possess the high antioxidant and antimicrobial activities (Viuda-Martos *et al*., 2008; Choi, 2010; Hung *et al*., 2013; Lan-Phi *et al*., 2015). Viuda-Martos *et al*. (2008) reported that grapefruit essential oil was the best inhibitor of the moulds, *P. chrysogenum* and *P. verrucosum*. The pomelo essential oil was also found to be the most effective against *P. expansum* (Hung *et al*., 2013) and show high effect against the growth of *B. cereus, A. flavus* and *F. solani* with the MIC values of 1.31 - 5.25 mg/ml (Lan-Phi *et al*., 2015). Therefore, the antimicrobial abilities of *citrus* EOs are also shown to be a particularly interesting field for applications

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within the food and cosmetic industries (Caccioni *et al.*, 1998).

Although there have been several publications reported on the chemical compositions, antioxidant and antimicrobial activities of essential oils of several pomelo varieties grown in Vietnam (Hung *et al.*, 2013; Lan-Phi *et al.*, 2015), the differences in chemical compositions and functional properties of essential oils have been found depending on pomelo varieties and growing locations. Therefore, the objectives of this study are to investigate chemical composition, antioxidant and antimicrobial activities of essential oils extracted from peels of the pomelos grown in the south regions of Vietnam including Ben Tre, Vinh Long, Dong Thap and Dong Nai provinces.

## Materials and Methods

### *Materials*

All pomelo varieties selected in this study were the popular varieties in the southern areas of Vietnam including Da Xanh pomelo in Ben Tre province (DX-BT), Nam Roi pomelo in Vinh Long province (NR-VL), Da Xanh and Buoi Long pomelo grown in Dong Thap province (DX-DT and BL-DT, respectively), Duong Cam pomelo in Dong Nai province (DC-DN). The fruits were collected at mature stage from September, 2013 to December, 2013. Whole and cross-section appearances of the pomelo fruits are shown in Figure 1.

After collecting, the samples were transferred to the laboratory in same day to keep the freshness of the fruits. Then the fruits were washed with tap water and cut into eight equal portions. For each portion, flesh and albedo layers were removed and flavedo layer was collected to extract essential oil.

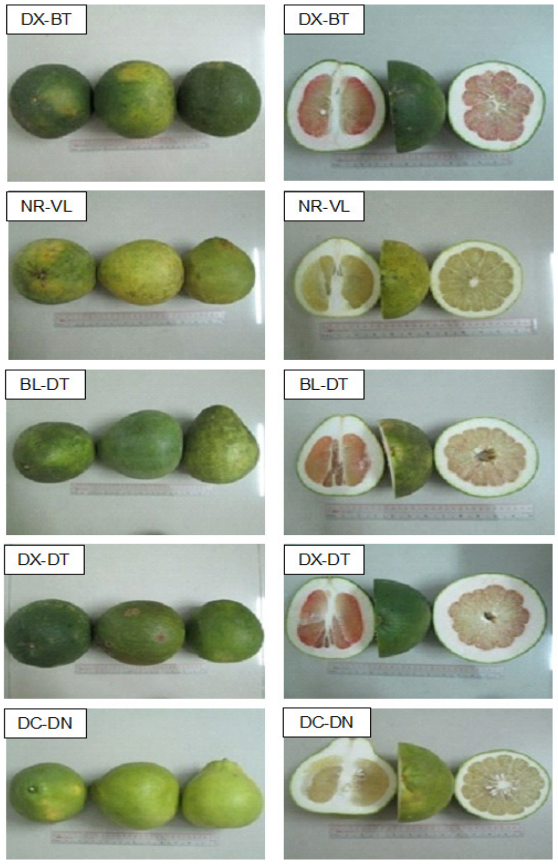


Figure 1. Whole and cross-section views of pomelo fruits in the South of Vietnam. DX-BT, Da Xanh in Ben Tre province; NR-VL, Nam Roi in Vinh Long province; BL-DT, Buoi Long in Dong Thap province; DX-DT, Da Xanh in Dong Thap province and DC-DN, Duong Cam in Dong Nai province.

### *Cold-pressing extraction method*

The essential oils were extracted from peels of the fruits by cold-pressing methods as previously described by Lan-Phi *et al.* (2006). The essential oils were expressed from the flavedo by hand and collected in brine solution on ice. The extract was centrifuged at 4,000 × g for 15 min. The resulting supernatant was dried in anhydrous $Na_2SO_4$ at 5°C for 24 h and then filtered. The oils were stored at -25°C until use.

### *Chemical composition analysis*

The chemical compositions of essential oils were analyzed using a gas chromatography-mass spectrometry (GC-MS) (Shimadzu GCMS-QP5050A) with a DB-Wax column (60 m x 0.25 mm i.d., 0.25 µm film thickness) and a flame ionization detector (FID) as previously described by Lan-Phi *et al.* (2006). For MS detection, and the electron ionization system with ionization energy of 70 eV and ion source temperature of 250°C was used.

### *Determination of antioxidant capacities of essential oils*

Antioxidant capacity of essential oil was expressed as its ability to scavenging of 2,2′-diphenyl-1-picrylhydrazyl stable radicals (DPPH) based on the method of Choi *et al.* (2010). The essential oil solutions with different concentrations (5, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 100, 120 and 140 mg/ml) were mixed with an equal volume of methanolic DPPH solution (100 µM). The absorbance of resulting solutions was spectrophotometrically recorded at 517 nm using a spectrophotometer (UVD-2960, Labomed, USA) after standing for 15 min at room temperature. The control contained methanol and DPPH solution was used. DPPH inhibition (%) by essential oil was calculated in following equation (Choi *et al.*, 2010):

$$\text{Inhibition (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Where: $A_{control}$ is the absorbance of the negative control and $A_{sample}$ is the absorbance of the tested compound.

The concentration of essential oils expressing 50% inhibition ($IC_{50}$) was calculated from the graph-plotted scavenging percentage against essential oils concentration.

*Determination of antibacterial activities of essential oils*

*Microbial strains*

Two bacteria, a gram-positive strain *(Streptococcus iniae)* and another gram-negative strain *(Pseudomonas aeruginosa)*, were used in this study to test antimicrobial capacity of essential oils of pomelo peels. These bacteria were obtained from Institute of Drug Quality Control in Ho Chi Minh City (IDQC-HCMC). The concentration of bacteria tested in experiment was $10^6$ colony forming unit (CFU)/ml.

*Diffusion method*

The antimicrobial activity of the essential oils was determined by the disc diffusion testing method as recommended by the National Committee for Clinical Laboratory Standards (NCCLS, 1997a). The agar plates were prepared in 90 mm petri dishes with 22 ml of agar medium (Tryptone Soybean Agar, Himedia, India) giving a final depth of 3 mm. A broth culture suspension (100 µL) of bacteria were spread on petri dishes and incubated at 37ºC for 24 h. The essential oil (100 µL) was mixed with absolute ethanol to obtain a concentration of 50% and added into the wells (9 mm in diameter). The control was used by adding the ethanol solution to the well. Antibacterial activity was assessed by measuring the diameter of the growth-inhibition zone in millimeters (including well diameter of 9 mm) for the tested organisms comparing to the controls.

*Dilution method*

A broth dilution susceptibility assay described by the National Committee for Clinical Laboratory Standards (NCCLS, 1997b) was used to determine a minimum inhibitory concentration (MIC) of the essential oil. A serial two-fold dilution of essential oils in absolute ethanol was prepared to obtain concentration of essential oils: 42 mg/ml, 21 mg/ml, 10.5 mg/ml, 5.25 mg/ml, 2.63 mg/ml, 1.31 mg/ml, and 0.66 mg/ml. A volume of diluted essential oils (500 µL) were transferred to the test tubes. Subsequently, a fixed volume (4 ml) of liquid culture medium was distributed into the test tubes and inoculated with 500 µL of bacterial suspension ($10^6$ CFU/ml) and then incubated for 24 h at 37°C. During the incubation period, the tubes were submitted to a manual agitation every hour. After incubation, 100 µL from each tube was spread on agar medium and incubated for 24 h to determine the MIC. The lowest concentration demonstrating no apparent growth was recorded as the MIC. The experiment included three controls: the negative control tube containing culture medium and essential oils only, the positive control tube containing culture medium and microorganisms, and the solvent control tube containing ethanol, medium and microorganism.

*Statistical analysis*

Each parameter was tested in triplicate. Analysis of variance (ANOVA) was applied to the data to determine differences ($p < 0.05$). Significant differences between the means values were determined using Ducan's test. Statistical data analysis was undertaken using the Statistical Package for the Social Sciences (SPSS).

## Results and Discussion

*Extraction yields of cold-pressed pomelo essential oils*

Extraction yields of essential oils extracted from five pomelo varieties grown in the south of Vietnam using the cold-pressing method are shown in Figure 2. BL-DT showed the highest yield of essential oil, followed by DX-BT, DC-DN, DX-DT and NR-VL. DX-BT contained higher amount of essential oil than did DX-DT even though they were the same variety. Likewise, although BL-DT and DX-DT were grown in the same province, BL-DT had higher yield of essential oil than did DX-DT. Thus, the extraction yields varied depending on pomelo varieties and growing location. Lan-Phi *et al.* (2015) also reported that the essential oils accumulated in the *citrus* peels were affected by not only the origin of citrus fruit but also by growing location.

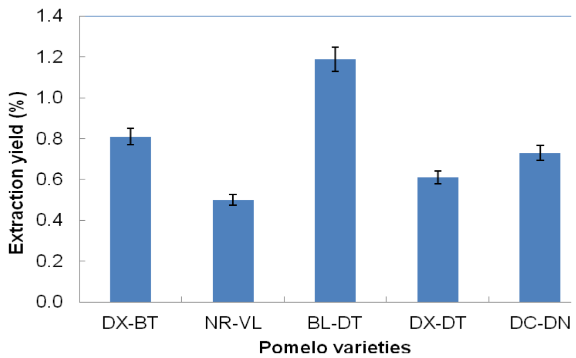


Figure 2. Yield (%w/w) of essential oils extracted from different pomelo varieties in the South of Vietnam. Abbreviations are the same as in Figure 1.

Table 1. Compositions (%w/w) of volatile compounds in peels' essential oils of different pomelo varieties in the South of Vietnam

| | Compounds | RI[a] | Relative concentration (%)[b] | | | | |
|---|---|---|---|---|---|---|---|
| | | | DX-BT | NR-VL | BL-DT | DX-DT | DC-DN |
| 1 | α-pinene | 1035 | 3.28 | 3.12 | 2.22 | 1.08 | 0.572 |
| 2 | β-pinene | 1123 | --- | 0.921 | 0.972 | 0.145 | --- |
| 3 | sabinene | 1132 | 0.833 | 0.606 | --- | 0.219 | 0.106 |
| 4 | myrcene | 1167 | 8.57 | 1.85 | --- | 1.97 | 1.89 |
| 5 | α-phellandrene | 1175 | 1.12 | 1.43 | 2.06 | 0.594 | --- |
| 6 | α-terpinene | 1190 | --- | --- | 0.237 | --- | --- |
| 7 | limonene | 1211 | 69.4 | 67.2 | 77.6 | 90.1 | 95.7 |
| 8 | β-phellandrene | 1220 | 12.8 | 9.21 | 0.421 | 2.95 | 0.283 |
| 9 | *p*-cymene | 1279 | 1.07 | 4.33 | 0.908 | 0.561 | --- |
| 10 | geranial | 1744 | 0.384 | --- | --- | 0.0846 | 0.140 |
| 11 | bicyclogermacrene | 1753 | 0.364 | --- | --- | --- | --- |
| 12 | cis-carveol | 1849 | 0.264 | --- | --- | --- | 0.082 |
| 13 | γ-terpinene | 1255 | --- | 9.85 | 13.5 | 1.92 | --- |
| 14 | terpinolene | 1291 | --- | 0.413 | 0.577 | --- | --- |
| 15 | neral | 1690 | --- | --- | --- | --- | 0.068 |
| 16 | germacrene D | 1725 | --- | --- | 0.921 | --- | 0.558 |
| 17 | carvone | 1753 | --- | --- | --- | --- | 0.106 |
| | Total | | 98.09 | 98.93 | 99.42 | 99.62 | 99.51 |

[a]RI, Identification based on Retention Index.
[b]Abbreviations are the same as in Figure 1.

*Composition of volatile compounds of pomelo essential oils*

Volatile compounds in essential oils extracted from pomelo varieties are given in Table 1. Seventeen main volatile components were detected in DX-BT, NR-VL, BL-DT, DX-DT and DC-DN accounted for 98.09, 98.93, 99.51, 99.62 and 99.42% relative concentrations of essential oils, respectively. The compositions of volatile compounds of pomelo essential oils also varied with different pomelo varieties. The essential oils of DX-DT and DC-DN were mostly limonene (90.1% and 95.7% of total relative concentrations, respectively), whereas DX-BT, NR-VL and BL-DT contained much lower amounts of limonene as compared to DX-DT and DC-DN (69.4%, 67.2% and 77.6%). However, DX-BT had high amounts of β-phellandrene, myrcene and α-pinene, while NR-VL had high amounts of γ-terpinene, β-phellandrene, p-cymene and α-pinene, and BL-DT contained high amounts of γ-terpinene, α-pinene and α-phellandrene. Lan-Phi *et al.* (2015) also reported that the pomelo varieties grown in the north and middle regions of Vietnam contained three components having more than 1% of total oils that were limonene (78.44% - 80.55%), myrcene (2.16% - 6.43%) and γ-terpinene (7.63% - 10.90%). These results indicate that the differences in chemical compositions of essential oils depended not only on varieties but also on growing locations with variable soil textures (Hussain *et al*., 2008) and different climatic factors such as temperature and drought (Milos *et al*., 2001). The differences in chemical compositions of essential oils resulted in different physicochemical and biological properties such as flavour, antioxidant and antimicrobial activities of these essential oils.

*Antioxidant capacities of pomelo peels' essential oils*

In this study, antioxidant activity of pomelo peels' essential oils was evaluated by DPPH radical scavenging assay and expressed in terms of 50% inhibition concentration ($IC_{50}$). The essential oils which had higher antioxidant activities exhibited the lower $IC_{50}$ values. The results indicate that the pomelo peels' essential oils possessed high antioxidant activities with the $IC_{50}$ values of 43.8 – 63.1 mg/ml (Figure 3). The essential oils of NR-VL and BL-DT exhibited the highest antioxidant activities, followed by DX-DT, DC-DN and DX-BT. The previous study also reported that the DPPH scavenging capacities of 34 kinds of *citrus* essential oils ranged from 17.7% to 64% (Choi *et al.*, 2000). The difference in antioxidant activities of the essential oils was due to the different chemical compositions of the essential oils. NR-VL and BL-DT contained higher amounts of β-pinene, sabinene and γ-terpinene than other essential oil, whereas the essential oil of DX-BT had high amount of limonene and myrcene and low amounts of β-pinene, sabinene and γ-terpinene. As a result, the β-pinene, sabinene and γ-terpinene might contribute to the antioxidant activities of the essential oils rather than limonene and myrcene. These results

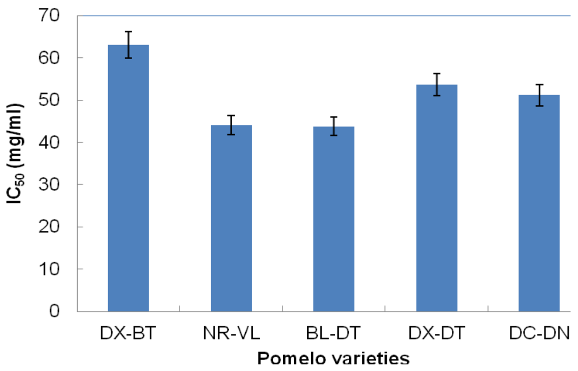


Figure 3. Antioxidant capacity ($IC_{50}$, mg/ml) of peels' essential oils of different pomelo varieties in the South of Vietnam. Abbreviations are the same as in Figure 1

are consistent to the previous studies that reported the terpenes included a higher content of terpinolene play an important role in antioxidant activities of the citrus essential oils (Choi, 2010) and limonene and myrcene would not play the principle role in determining the scavenging activity for the radical (Hung *et al.*, 2013; Lan-Phi *et al.*, 2015).

*Antimicrobial activities of pomelo peels' essential oils*

Antimicrobial activities of pomelo peels' essential oils against two bacteria, *S. iniae* and *P. aeruginosa,* are shown in Table 2. Both growth inhibition zone diameters and broth dilution assay were used to evaluate the antibacterial activities of the essential oils. The essential oil of BL-DT exhibited the highest antibacterial activities against both *S. iniae* and *P. aeruginosa* with inhibition zone diameters of 26.3 and 25.1 mm, respectively. The essential oils of NR-VL and DX-DT showed the high antibacterial activities against *S. iniae,* whereas those of NR-VL and DC-DN had high antibacterial activities against *P. aeruginosa.* The minimum inhibition concentration (MIC) of the essential oil of BL-DT was 2.63 mg/ml for both *S. iniae* and *P. aeruginosa*, meaning that this essential oil exhibited strong antibacterial activity against these bacteria. The MIC of the essential oil of NR-VL against *S. iniae* was 5.25 mg/ml, whereas MICs of the essential oils of DX-BT, DX-DT and DC-DN against *S. iniae* were 10.5 mg/ml. The MICs of the essential oils of NR-VL and DC-DN against *P. aeruginosa* were 10.5 mg/ml, while the MICs of the essential oils of DX-BT and DX-DT against *P. aeruginosa* were 21.0 mg/ml. As a result, BL-DT and NR-VL had strong antibacterial activities against *S. iniae* and *P. aeruginosa* as compared to the essential oils of other pomelo varieties. Lan-Phi *et al.* (2015) also reported that the essential oils of pomelos grown in the north and middle regions of Vietnam also exhibited strong antibacterial activities against

Table 2. Antibacterial activities of peels' essential oils of different pomelo varieties in the South of Vietnam

| Samples | *S. iniae* | | *P. aeruginosa* | |
|---|---|---|---|---|
| | Inhibition zone (mm) | MIC (mg/ml) | Inhibition zone (mm) | MIC (mg/ml) |
| DX-BT | 21.2 ± 0.1a | 10.5 | 20.3 ± 0.2a | 21.0 |
| NR-VL | 23.2 ± 0.3c | 5.25 | 21.2 ± 0.3b | 10.5 |
| BL-DT | 26.3 ± 0.3d | 2.63 | 25.1 ± 0.1c | 2.63 |
| DX-DT | 22.2 ± 0.3b | 10.5 | 20.1 ± 0.3a | 21.0 |
| DC-DN | 21.5 ± 0.2b | 10.5 | 21.4 ± 0.3b | 10.5 |

[a]Data presented as mean of triplicate experiments ± SD.
[b]Values followed by the same letter in the same column are not significantly different ($p \leq 0.05$).
[c]Abbreviations are the same as in Figure 1

positive- and negative-bacteria (*S. aureus, B. cereus*, *S. typhi* and *P. aeruginosa*) with inhibition zone diameters of 17.17 - 27.17 mm. Thus, the high amounts of γ-terpinene, α-pinene, α- and β-phellandrene in the essential oils of NR-VL and BL-DT were responsible for the high antibacterial activities of these essential oils, whereas the high amounts of limonene and myrcene had less effect on the tested bacteria. Other previous studies also reported that the antibacterial activities of the essential oils were affected by their chemical compositions (Cowan, 1999; Lan-Phi *et al.*, 2015) and the mechanism actions of essential oils against microorganisms were related to chemical composition and amount of essential oil and their possible interaction (Lis-Balchin *et al.*, 1998).

## Conclusion

Chemical compositions, antioxidant and antimicrobial activities of the essential oils extracted from different pomelo varieties grown in the south of Vietnam were investigated in this study. The results indicate that chemical compositions of the essential oils were affected by the pomelo varieties and growing locations and the differences in antioxidant and antimicrobial activities were due to the different chemical compositions of the essential oils. The high amounts of γ-terpinene, α-pinene, α- and β-phellandrene in the essential oils were responsible for the strong antioxidant and antibacterial activities of these essential oils, whereas the high amounts of limonene and myrcene would not play the principle role in determining the antioxidant and antibacterial activities of the citrus essential oils.

## Acknowledgement

The authors thank the National Foundation for Science and Technology Development, Vietnam (NAFOSTED) for the financial support, Research Grant No. 106.99-2012.97.

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