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RESEARCH ARTICLE

Essential oil compositions of *Fortunella polyandra* from Malaysia

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Abstract
The essential oils obtained from the fresh leaves, peels and twigs of *Fortunella polyandra* growing in Shah Alam, Malaysia were hydrodistilled and analyzed using gas chromatography (GC) equipped with flame ionization detector (FID) and gas chromatography-mass spectrometry (GC-MS). α-Eudesmol (31.0%), hedycrayol (20.1%) and γ-eudesmol (19.7%) were the major compounds in the leaves oil. The peels oil was characterized by high proportion of limonene (71.4%) and γ-terpinene (15.2%), while α-gurjunene (26.4%), hedycrayol (23.5%) and γ-eudesmol (22.1%) were the main compounds in the twigs oil. The study indicates that essential oil of *F. polyandra* parts was rich in monoterpene hydrocarbons, sesquiterpenoids and sesquiterpene hydrocarbons.

***Keywords*:** Essential oils, *Fortunella polyandra*, Rutaceae, kumquat, limonene, GC-MS

INTRODUCTION

The genus *Fortunella* is taxonomically close to the *Citrus* genus. There are six major *Fortunella* species identified either grow wild or cultivated for its fruits such as *F. polyandra*, *F. margarita*, *F. japonica*, *F. hindsii*, *F. crassifolia* and *F. obovata* (Seidemann, 2005). Malayan Kumquat or 'limau pagar' (*F. polyandra*) is a member of Rutaceae family, which is native to Peninsular Malaysia. This plant grows well in a tropical and subtropical areas and mostly cultivated as an ornamental plant. *F. polyandra* is an evergreen shrub or a small tree with 3-5 m height. The leaves is 3-10 cm long, while the fruits are globose in 2-3 cm wide with a bright yellow color (Figure 1). The flesh is sour, and the fruit is eaten together with the peels excluding the seeds. Kumquats exists as an excellent source of nutrients and phytochemicals, including ascorbic acid, carotenoids, terpenoids, flavonoids and essential oils. (Çakmakçi *et al*., 2016; Peng *et al*., 2013). Similar to other *citrus* fruits, *F. polyandra* fruits is also consumed to make various products such as candy, ice cream, wine, marmalade or beverages (Çakmakçi *et al*., 2016; Peng *et al*., 2013).

Previous study on the chemical composition of *F. crassifolia* peel essential oil revealed that this oil contained terpenoid hydrocarbons (85.4%), alcohols (3.3%), ketones (1.8%), esters (1.7%) and aldehydes (0.2%). Characterization of compounds of this oil by their retention indices and mass spectra using GC equipped with flame ionization detector (FID) and GCMS has identified 96.4% of monoterpenes with limonene (93.7%) as the most abundant compound (Choi, 2005). The essential oil also showed a wide range of antimicrobial activity (Wang *et al*., 2012). Extraction of the essential oil of *F. margarita* leaves using three different extraction methods (supercritical carbon dioxide extraction, hydrodistillation and extraction with solvent) disclosed the identification of twenty four compounds each. Eight monoterpene hydrocarbons, six terpene alcohols, one terpene aldehyde, one terpene ketones, four terpene esters and four sesquiterpene hydrocarbons were detected in each oil, in which monoterpenes possessed the highest amount with the percentage value around 98%. This previous research has concluded that the methods to extract essential oil of *F. margarita* did not affect the extracted compound (Sicari & Poiacomna, 2017). The effect of the heat treatment in the extraction of *F. margarita* oil showed that hot water extraction followed by steam distillation produced more yields compared to intact cold pressing method (Peng *et al*., 2013).

Phytochemical study on genus *Fortunella* has started since 1958 (Arch, 1958). Profiling analysis and isolation of phytochemicals had discovered that kumquat was rich in flavonoids. Dihydrochalcones, flavones and flavanones were the major flavonoids, with phloretin, acacetin and apigenin being the main aglycones. Apart from that, diosmetin, luteolin, and vitexin were also reported as flavonoids

Figure 1 Fruits and Leaves of *Fortunella polyandra*

aglycone in kumquat. Bioactivity studies on *Fortunella* plants had discovered that its phytochemicals showed various biological activity such as antioxidant, antimicrobial, tyrosinase inhibitory, antimetabolic disorder, anticancer and antitumor (Tan *et al*., 2016). To the best of our knowledge, there is no report has been conducted concerning the essential oils of *F. polyandra* mainly in Malaysia. Thus, this study report for the first time the chemical compositions of the peels, leaves and twigs of *F. polyandra*.

EXPERIMENTAL

Plant material

Sample of *F. polyandra* was collected at Taman Botani Shah Alam (GPS coordination: N 3°, 5', 45.1'', E 101°, 30', 42.6'') in February 2018, and identified by Dr. Shamsul Khamis, from Universiti Kebangsaan Malaysia (UKM). The voucher specimen (SK1507/18) was deposited at UKMB Herbarium Universiti Kebangsaan Malaysia.

Extraction of Essential Oils

The fresh peels, leaves and twigs of *F. polyandra* were chopped into small pieces and then separately submitted to a hydrodistillation using Dean-Stark apparatus for 8 hours. The distillates were extracted with diethyl ether, dried over anhydrous magnesium sulfate and kept at 4°C in air tight-capped vials until further analysis.

Gas Chromatography (GC) Analysis

The quantitative GC analysis was performed on a Shimadzu GC-2010 Plus gas chromatograph equipped with a flame ionization detector (FID) and CBP-5 (30 m × 0.25 mm; 0.25 μm film thickness) capillary column. The oven temperature was programmed at initial temperature of 60°C (10 min.) to final temp. 230°C at an increasing temp. rate of 3°C/min. Injector and detector temperatures were set-up at 220°C and 280°C, respectively. Helium was used as the carrier gas and the volume of oil injected was 1.0 μL. The peak areas and retention times were measured by electronic integration.

Gas chromatography-mass spectrometry (GC-MS) analysis

The qualitative GC-MS analyses were performed on an Agilent GC-MS 7890A/5975C Series MSD (70eV direct inlet) equipped with a HP-5MS fused silica capillary column (30 m × 0.25 mm; 0.25 μm film thickness). The column and injector temperatures were the same as those for GC. The mass range was 50-550 in the full scan mode with a rate of 2.91 scans/sec. The total scan time was 67.7 min.

Identification of chemical compounds

The chemical compositions of the essential oils were identified by comparing their Kovats indices (KI) with literature values (Adams, 2007) and also confirmed by matching their mass spectral data with those from the Wiley, HPCH 2205.L and NIST05a.L mass spectral databases. The Kovats indices of the chemical compositions were determined relative to the retention times of a homologous series of *n*-alkanes (C_8-C_{30}).

RESULTS AND DISCUSSION

Hydrodistillation of the fresh peels, leaves and twigs of *F. polyandra* gave pale yellow oils in 0.22%, 0.14%, and 0.06% (w/w), respectively.

F. polyandra Peels Oil

A total of fourteen compounds were characterized from the GC-MS chromatogram which contributed 95.6% of the oil. The identified compounds were monoterpene hydrocarbons (seven compounds; 91.4%), monoterpenoids (three compounds; 2.8%), sesquiterpene hydrocarbon (0.2%), sesquiterpenoids (two compounds; 0.9%) and an alcohol (0.1%). Monoterpene hydrocarbons, limonene (71.4%) being the most abundant compound found in the peels oil followed by *γ*-terpinene (15.2%). Limonene had been reported as the major compound in three varieties of kumquats; *F. crassifolia*, *F. margarita* and *F. japonica* with 50.4%, 55.4% and 51.5%, respectively. (Nouri & Shafaghat, 2015; Sutour *et al*., 2016; Zhonghai *et al*., 2009). The compounds present in the peels oil are tabulated in Table 1.

Table 1 Chemical Composition of *F. polyandra* Peels Oil.

No.	Compound	KI (Calc.)	Percentage (%)
	Monoterpene hydrocarbons		
1	Limonene	1034	71.4
2	*γ*-Terpinene	1073	15.2
3	Myrcene	992	1.7
4	*β*-Pinene	980	1.1
5	*α*-Pinene	939	1.0
6	Terpinolene	1092	0.9
7	*α*-Thujene	931	0.3
	Monoterpenoids		
8	Linalool	1102	0.5
9	Terpinen-4-ol	1183	0.8
10	*α*-Terpineol	1198	1.5
	Sesquiterpenoids		
11	*γ*-Eudesmol	1643	0.4
12	*α*-Eudesmol	1666	0.5
	Sesquiterpene hydrocarbon		
13	Germacrene D	1378	0.2
	Alcohol		
14	(3*Z*)-Hexenol	846	0.1
	Total (%)		**95.6**

F. polyandra Leaves Oil

Extraction of the fresh leaves of *F. polyandra* using hydrodistillation gave thirteen compounds, accounting for 99.5% of the total oil compounds. The oil was rich in sesquiterpenoids (75.2%) with *α*-eudesmol (31.1%), hedycrayol (20.1%), and *γ*-eudesmol (19.8%). Apart from that, the leaves oil also comprised of sesquiterpene hydrocarbons (18.0%) with the major compounds were *γ* -muurolene (6.4%) and *β*-selinene (4.8%). Benzeneacetaldehyde (6.3%) was the only aldehyde found in the oil. This study shows that eudesmol is the major compound in *F. polyandra* leaves oil, previous publication on *F. margarita* oil has reported that eudesmol is also the most abundance compound in the leaves (Quijano & Pino, 2009). Table 2 displays the chemical composition of the leaves oil.

Table 2 Chemical Composition of *F. polyandra* Leaves Oil.

No.	Compound	KI (Calc.)	Percentage (%)
	Sesquiterpenoids		
1	*γ*-Eudesmol	1643	19.8
2	*α*-Eudesmol	1666	31.1
3	Hedycrayol	1562	20.1
4	Nerolidol	1568	4.3
	Sesquiterpene hydrocarbons		
5	*γ* -Muurolene	1480	6.4
6	*β*-Selinene	1486	4.8
7	*δ*-Elemene	1330	1.0
8	*β*-Elemene	1386	1.8
9	*γ* -Elemene	1428	0.7
10	*β*-Farnesene	1452	1.4
11	*β*-Gurjunene	1592	1.7
12	*α*-Gurjunene	1660	0.1
	Aldehyde		
13	Benzeneacetaldehyde	1028	6.3
	Total (%)		**99.5**

F. polyandra Twigs Oil

The analysis of the twigs oil have identified twenty one compounds, representing 96.6% of the oil sample. The twigs oil consisted of (number of sesquiterpenoids) sesquiterpenoid (59.9%), (number of sesquiterpene hydrocarbons) sesquiterpene hydrocarbons (34.8%), and a monoterpene hydrocarbon, limonene (1.9%). Hedycrayol (23.5%), *γ*-eudesmol (22.1%), and elemol (7.8%) were predominant in sesquiterpenoid fraction while, *α*-gurjunene (27.0%) was abundance in sesquiterpene hydrocarbons fraction. Elemol has been previously reported in *F. margarita* and *F. japonica* leaves oil with total composition of 18.8% and 9.1% resspectively (Quijano & Pino, 2009;

Sutour *et al*., 2017). The chemical composition of the twigs oil are listed in Table 3.

Table 3 Chemical Composition of *F. polyandra* Twigs Oil.

No.	Compound	KI (Calc.)	Percentage (%)
	Sesquiterpenoids		
1	Hedycrayol	1562	23.5
2	*γ*-Eudesmol	1643	22.1
3	Elemol	1604	7.8
4	*α*-Eudesmol	1666	0.3
5	Eudesmol	1642	2.2
6	Amorpha-4,9-dien-2-ol	1771	0.5
7	Oplopanone	1870	2.2
8	Amorpha-4,7(11)-diene	1944	0.8
9	Acetyoxyelemol	1923	0.5
	Sesquiterpene hydrocarbons		
11	*α*-Gurjunene	1494	0.5
11	*δ*-Elemene	1330	0.3
12	*β*-Elemene	1386	0.5
13	*α*-Gurjunene	1660	26.4
14	Sesquisabinene	1460	0.4
15	*γ* -Amorphene	1488	1.6
16	*δ*-Amorphene	1512	0.3
17	*β*-Curcumene	1517	0.4
18	*δ*-Cadinene	1531	0.3
19	*γ* -Cadinene	1615	3.5
20	Amorpha-4,7(II)-diene	1630	0.6
	Monoterpene hydrocarbon		
21	Limonene	1034	1.9
	Total (%)		**96.6**

CONCLUSION

This study is the first attempt to investigate the chemical composition of essential oils from the peels, leaves, and twigs of *F. polyandra.* The GC and GC-MS analysis of the essential oil showed that the peels oil is rich in monoterpene hydrocarbon (91.4%) with limonene (71.4%) as the most abundance in the oil, whereas sesquiterpenoids comprised as the major content for the leaves oil (75.2%) and twigs oil (59.9%) with *α*-eudesmol (31.1%) and hedycrayol (23.5%) as the major compound respectively.

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