

Research article

Comparative Analysis of Chemical Compositions and Biological Activities of Kumquat Leaf Oils from Southern Thailand

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Abstract

The essential oil from fresh and dry kumquat leaves (*Citrus japonica* Thunb.) was extracted and its biological activities were evaluated. The results found that the kumquat leaf oils exhibited the antioxidant activity with dried leaf oil posing higher potency ($IC_{50} = 54.2 \pm 3.3$ mg mL⁻¹) than fresh leaf oil ($IC_{50} = 60 \pm 0.73$ mg mL⁻¹). Moreover, the kumquat leaf oils showed inhibitory effects against *Bacillus subtilis*, and *Staphylococcus aureus* but not against *Escherichia coli*. Greater potency was observed for the dried leaf oil (MIC and MBC values were 3.9 and 3.9 mg mL⁻¹, respectively, against *B. subtilis*, and 7.8 and 7.8 mg mL⁻¹, respectively, against *S. aureus*). The kumquat leaf oils could inhibit MCF7 and NCI-H187 cell proliferation. More than 90% of NCI-H187 cells were eliminated at concentrations of 0.976 mg mL⁻¹ and 1.95 mg mL⁻¹ for fresh and dried leaf oils, respectively. In the case of MCF7 cells, over 90% of cells were obliterated after incubation with the kumquat leaf oil at a concentration of 7.81 mg mL⁻¹. However, their anticancer properties were not significantly different ($P > 0.05$) when IC_{50} values were observed. Thus, the results indicate that kumquat leaf oil possesses remarkable biological activities, highlighting its potential for further in-depth research and development into health-promoting therapeutic agents or products.

Keywords: kumquat leaf oil; chemical profile; antioxidant activity; antibacterial activity; anticancer activity

1. Introduction

Essential oils (EO) are a valuable volatile secondary metabolite synthesized with beneficial function in plants. Plant essential oils possess several compound groups, and the types and numbers of constituents vary among intraspecies and interspecies. The factors affecting this phenomenon are cultivating region, variety, season, weather, age, ripening period and extraction method (Dosoky & Setzer, 2018). Based on the different types and number of constituents resulting in its biological activity variant, plant essential oils contained various biological activities including antibacterial, antifungal, antiviral, anticancer and antioxidant properties (Ali et al., 2015). Thus, to date, plant essential oils

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have gained interest and have been applied in various industries such as food, medical, pharmaceutical and agricultural industries.

Citrus spp., belonging to family Rutaceae, are easy-to-grow plants that are capable of planting in various conditions throughout regions as well as possessing several benefits to health. Thus, the plants have become important economically worldwide. *Citrus* spp. are one of the sources of valuable essential oil. *Citrus* essential oils have popularly been used in various industries, especially in the food industry as a supplement and preservation substance in foods and beverages due to it exhibiting broad antibacterial and antioxidant activities and are also classified in a group generally recognized as safe (GRAS) (Dosoky & Setzer, 2018).

Citrus japonica Thunb. or *Fortunella japonica* (Thunb.) (kumquat) is routinely found in Southeast Asia, where it has been identified as a medicinal plant. To date, the chemical profiles and biological activities of essential oils of kumquat species were previously reported. Earlier studies showed that the fruit peel oil contained various biological activities including antioxidant, antibacterial (Wang et al., 2012; Lin et al., 2021), antifungal (Wang et al., 2012; Ibrahim et al., 2015), anticancer (Jayaprakasha et al., 2012; Fitsiou et al., 2016; Soni et al., 2022; Vrca et al., 2024), and antiviral (Ibrahim et al., 2015) activities. In addition, some reports indicated that kumquat leaf essential oil posed antibacterial, antifungal and antiviral activities against H5N1. In the case of *C. japonica*, there were several studies about its essential oil and activity. The dried leaf oil of *C. japonica* collected in Nepal had linalool as the predominant compound (Satyal et al., 2012). The peel and leaf oils of *C. japonica* cultivated in the central region of Thailand were rich in d-limonene and β -pinene, respectively, and showed antioxidant and anti-gram-positive bacteria (Bunrathep et al., 2015). The peel oils of citrus fruits grown in Cairo, Egypt, were abundant with monoterpenoid limonene (Al-Saman et al., 2019). The dried leaf oil of Palestine-grown samples was rich in γ -muurolene and displayed strong antioxidant, antilipase, antialpha-amylase, and anticancer (Jaradat et al., 2024). The fruit peel and leaf oils of *C. japonica* cultivated in Türkiye were rich in limonene and elemol, respectively (Küçükbay et al., 2024). The peel and flower oils from Greece were predominant with limonene, whereas the leaf oil had sesquiterpenoid germacrene-D as the predominant compound. Moreover, the leaf and flower oils of kumquat grown in Greece displayed inactive or weak antibacterial activity against bacteria and fungi, and only the peel oil exhibited antifungal activity (Ziogas et al., 2024). Likewise, the peel and kernel oils of materials grown in Iran were rich in limonene, and the peel oil gave higher antioxidant activity than kernel oil (Nouri & Shafaghatlonbar, 2015). Thus, *C. japonica* essential oil is interesting as well as the oils of other *Citrus* and kumquat species. Moreover, these demonstrated that several factors could affect the quality of *C. japonica* essential oils as well as other plant essential oils.

Due to different cultivating regions, the plant tissue gave a different quality of *C. japonica* oil. Thus, this study was focused on the constituents and biological activities of essential oil from the leaves of *C. japonica* collected in Southern Thailand. In the present study, we assumed that *C. japonica* leaf essential oil likely contained a different type and number of constituents and therefore biological activities to other *Citrus* and kumquat essential oils derived from plants in another region. In the present study, the antimicrobial and antioxidant activities of *C. japonica* leaf essential oil were determined. In addition, the anticancer activity against breast and lung cancer cells of kumquat leaf oil were studied in this current work.

2. Materials and Methods

2.1 Plant sample

The fresh mature leaves of kumquat (*Citrus japonica* Thunb.) were collected from a single tree in January 2023 at Phang-nga, in the northern part of the southern region of Thailand. The dry leaves were prepared by oven-drying at 60°C for 24 h and ground before essential oil extraction.

2.2 Essential oil extraction

The *C. japonica* leaf oils were extracted with hydrodistillation technique by Clevenger-type device for 4 h. The ratio of leaf to distilled water was 1 g: 10 mL. Subsequently, the fresh and dried leaf oils were kept at 4°C in the dark until the experiments were performed.

2.3 GC-MS analysis

The constituent profile of *C. japonica* leaf oils was determined using gas chromatography coupled with mass spectrometry (GC-MS) following modified conditions from El-Nashar et al. (2021). Briefly, a GC 7890 B, MSD 5977B, Agilent Technologies, USA with a HP-5MS, Ultra Inert (30 m × 250 µm × 0.25 µm) was employed and 1 µL of diluted essential oils (1/2 in hexane) was injected in a mode of split ratio of 100:1, helium carrier gas flow rate of 1.0 mL min⁻¹ and the temperature of the injector of 280°C. The column temperature was set at 80°C for 2 min, rate 5°C min⁻¹ to 120°C (hold time 0 min), rate 2°C min⁻¹ to 200°C (hold time 0 min), rate 10°C min⁻¹ to 300°C (hold time 5 min), and the transfer line temperature at 300°C for 65 min. MS Quadrupole was operated in the electron ionization mode (70 eV), 230°C ion source temperature, and a 35-500 amu scanning range were applied. Finally, the MS spectra patterns of constituents of *C. japonica* leaf oils were compared and identified with those recorded at NIST17 library and Wiley10 & NIST14 libraries (a minimum match factor or similarity score was at least 85%).

2.4 DPPH radicle scavenging assay

The antioxidant activity of *C. japonica* leaf oils was evaluated using scavenging capacity against DPPH free radicle following the protocol of Blois (1958). The *C. japonica* leaf oils were gradually dissolved in ethanol to generate the final concentration: 12.5, 25, 50, 100 and 200 mg mL⁻¹, while DPPH was dissolved in methanol to a final concentration of 0.21 mM. Then, 500 µL of *C. japonica* leaf oils were mixed with 1000 µL of DPPH solution and subsequently incubated in the dark for 30 min at room temperature. Positive and negative controls were ascorbic acid and ethanol, respectively. Finally, the absorbance was measured at the wavelength of 517 nm. The experiments were performed in triplicates. The antioxidant activity was calculated using the equation as follows:

$$\text{DPPH radical scavenging (\%)} = [(A_c - A_s)/A_c] \times 100$$

Where A_c is the absorbance of solution without *C. japonica* leaf oils,
 A_s is the absorbance of solution with *C. japonica* leaf oils.

2.5 Determination of antibacterial activity using agar well diffusion assay

The antibacterial activity of *C. japonica* leaf oils was determined against a gram-negative bacterium (*Escherichia coli*) and two gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) using the agar well diffusion assay following the method of Okunowo et al. (2013) with some modifications. Firstly, the fresh bacterial colonies were individually cultured in Muller Hilton broth at 37°C for 24 h. Next, the bacterial suspensions were adjusted to a 0.5 McFarland standard (approximately 10^8 CFU mL⁻¹) and subsequently swabbed on Muller Hilton agar using sterile cotton swab. Then, wells were generated and 40 µL of *C. japonica* leaf oil dissolved in ethanol aliquots (final concentration of 125 mg mL⁻¹) were consequently dropped into the wells. The positive and negative controls were streptomycin (10 µg mL⁻¹) and ethanol, respectively. The agar plates were incubated at 37°C for 24 h. The antibacterial activity was assessed by observing where the clear zone occurs. The experiments were performed in triplicates.

2.6 Determination of minimum inhibitory (MIC) and minimum bactericidal (MBC) concentrations of *C. japonica* leaf oils against pathogenic bacteria

The lowest concentration of *C. japonica* leaf oils inhibiting the growth of test bacteria was investigated using the broth microdilution method following the protocol of Bajpai et al. (2013) with some modifications. First, the stock solutions of *C. japonica* leaf oils were prepared using 7% DMSO as diluent to reach the final concentration as 125 mg mL⁻¹ and diluted to reach the final concentrations of 3.95, 7.9, 15.7, 31.3, and 62.5 mg mL⁻¹. Then, 95 µL of MHB, 5 µL of bacterial suspensions (10^8 CFU mL⁻¹) and 100 µL of each dilution of *C. japonica* leaf oils were dropped into 96 well plates. The positive and negative controls were streptomycin and 7% DMSO, respectively. Next, the 96-well plates were incubated at 37°C for 24 h. After incubation, 30 µL of resazurin solution (0.18%) was added into each well and incubated further for 3 h. The lowest concentration of *C. japonica* leaf oils or streptomycin appeared as a pink color indicated as a MIC value. Subsequently, 10 µL of solutions from a well appearing as a pink color were streaked onto MHA agar and incubated at 37°C for 24 h. The lowest concentration that did not appear with the bacterial growth was indicated as a MBC value.

2.7 Cytotoxicity assay against cancer cells

The cytotoxicity of *C. japonica* leaf oils against cancerous cells including MCF-7 (breast adenocarcinoma cell) and NCI-H187 (small cell lung carcinoma cell) was analyzed using the resazurin microplate assay (REMA) assay following the method previously reported by Brien et al. (2000) with some modifications. Briefly, the cell lines were separately cultured in 96-well plates (3×10^3 cells well⁻¹ for MCF-7 and 6.7×10^4 cells well⁻¹ for NCI-H187) and incubated at 37°C overnight. Then, the cells were incubated with *C. japonica* leaf oil (diluted in 5% DMSO) at a concentration range of 0.244-15.6 mg mL⁻¹. Plates were incubated at 37°C for 3 and 5 days for MCF-7 and for NCI-H187, respectively. Next, 12.5 µL of resazurin (the final concentration was 6.25 µg mL⁻¹) was added to each well and incubated further at 37°C for 4 h. The fluorescence at 530 and 590 nm was read with a bottom-reading mode of fluorometer and calculated using the following equation:

$$\text{Inhibition (\%)} = [1 - (\text{FU}_T/\text{FU}_C)] \times 100$$

Where FU_T is the fluorescence of *C. japonica* leaf oils -treated cells,
 FU_C is the fluorescence of 5% DMSO-treated cells.

Citrus japonica leaf oils concentration causing 50% inhibition (IC_{50}) was calculated from dose-response-curve generated by SOFTMax Pro software (Molecular Devices, USA).

2.8 Statistical analysis

The results were shown as mean $\pm$ S.D and statistically analyzed using one way ANOVA ($P<0.05$).

3. Results and Discussion

3.1 Characteristics of *C. japonica* leaf oils

The physical characteristics of *C. japonica* leaf oil were as follows. The fresh leaf oil was a clear and pale-yellow liquid while dried leaf oil was a clear and pale-green liquid. The extraction yield of dried leaves (1.31%) was higher than the fresh ones with the value of 0.6% (Table 1). After comparison with other reports, the yield of *C. japonica* leaf oil in this study was comparable. The fresh and dried *C. japonica* leaf oils from Pathum Thani (central Thailand)-grown samples were 0.53% and 0.9% (v/w), respectively (Bunrathep et al., 2015). The yield of fresh leaf oil of the Greece-grown sample was 0.082% (v/w) (Ziogas et al., 2024). The dried leaf oil of the Palestine-grown sample gave a yield of 2.22% (v/w) (Jaradat et al., 2024). Moreover, the yield of leaf oil in this study was similar when compared with other kumquat species (ranged: 0.082%-0.27%) (Ibrahim et al., 2015; Ziogas et al., 2024). It is noteworthy that dried leaves yielded more oil than fresh leaves. This might be because drying can leak or rupture the cell membranes of oil-producing cells, potentially allowing more efficient steam penetration and oil release during distillation, compared to fresh material where the cells were intact (Feyzi et al., 2017).

There was evidence identifying that the same plant species from different regions gave a significant difference in oil yield (Ganji et al., 2017; Caprari et al., 2023). Such disparities are attributable to environmental heterogeneity in the cultivation site (e.g., climate, topography, and soil) or to procedural adaptations designed for distinct ecological niches (Tuttolomondo et al., 2015).

Table 1. Physical characteristics and yield percentage of *c. japonica* leaf oils

Essential oils	Physical characteristics	Yield (%)
Fresh leaf	Clear and pale-yellow	0.6
Dried leaf	Clear and pale-green	1.31

3.2 Chemical composition of *C. japonica* leaf oils analyzed by GC-MS

A total of 42 and 41 compounds were identified in the fresh and dried leaf oils, and these contributed to 97.71% and 93.6% of the essential oil compositions, respectively (Table 2).

Table 2. Chemical profile of *C. japonica* leaf oils

Compounds	Formula	Fresh Leaf		Dried leaf	
		RT	Ratio (%)	RT	Ratio (%)
(+)- α -pinene	C ₁₀ H ₁₆	5.985	0.94	5.985	1.19
Sabinene	C ₁₀ H ₁₆	6.663	2.45	6.665	2.34
β -pinene	C ₁₀ H ₁₆	6.788	5.70	6.788	5.71
β -myrcene	C ₁₀ H ₁₆	6.895	0.35	-	-
Geraniol	C ₁₀ H ₁₈ O	-	-	6.895	0.25
δ -3-carene	C ₁₀ H ₁₆	7.392	0.24	7.396	0.22
α -terpinene	C ₁₀ H ₁₆	7.515	0.12	-	-
D-limonene	C ₁₀ H ₁₆	7.777	1.01	7.777	1.55
1,8-cineol	C ₁₀ H ₁₈ O	7.836	1.27	-	-
3-carene	C ₁₀ H ₁₆	8.098	2.42	8.098	1.98
γ -terpinene	C ₁₀ H ₁₆	8.424	0.27	8.425	0.22
Linalool	C ₁₀ H ₁₈ O	9.339	6.11	9.312	3.57
Terpinen-4-ol	C ₁₀ H ₁₈ O	11.414	0.52	11.414	0.34
(-)- α -terpineol	C ₁₀ H ₁₈ O	11.766	0.55	11.774	0.26
δ -elemene	C ₁₅ H ₂₄	16.677	3.07	16.683	3.82
β -bourbonene	C ₁₅ H ₂₄	-	-	18.592	0.17
β -elemene	C ₁₅ H ₂₄	18.806	1.20	18.806	1.55
Caryophyllene	C ₁₅ H ₂₄	20.031	3.10	20.047	4.26
Bicyclosquiphellandrene	C ₁₅ H ₂₄	-	-	20.406	0.16
γ -elemene	C ₁₅ H ₂₄	20.502	0.35	20.501	0.52
1,4,7,-cycloundecatriene, 1,5,9,9 tetramethyl-,Z,Z,Z-	C ₁₅ H ₂₄	21.450	0.60	21.454	0.8
γ -muurolene	C ₁₅ H ₂₄	22.454	0.25	22.465	0.36
cis- β -copaene	C ₁₅ H ₂₄	22.770	10.4	22.796	12.85
β -selinene	C ₁₅ H ₂₄	22.951	2.96	22.973	4.13
Bicyclogermacrene	C ₁₅ H ₂₄	23.353	2.48	23.363	3.47
(+)-calarene	C ₁₅ H ₂₄	23.759	0.24	-	-
β -cadinene	C ₁₅ H ₂₄	-	-	23.765	0.26
γ -cadinene	C ₁₅ H ₂₄	-	-	24.102	0.13
Hedycaryol	C ₁₅ H ₂₆ O	24.369	0.5	24.369	0.26
δ -amorphene	C ₁₅ H ₂₄	24.481	0.48	24.486	0.65
β -guaiene	C ₁₅ H ₂₄	25.155	0.11	-	-
Elemol	C ₁₅ H ₂₆ O	25.872	16.2	25.835	13.0
(1Z,4Z)-germacrene B	C ₁₅ H ₂₄	26.123	0.57	26.118	0.88
Nerolidol	C ₁₅ H ₂₆ O	26.300	1.67	26.289	1.52
Spathulenol	C ₁₅ H ₂₄ O	27.038	0.35	27.038	0.29
(-)-globulol	C ₁₅ H ₂₆ O	27.739	0.15	27.739	0.29
Epiglobulol	C ₁₅ H ₂₆ O	27.327	0.12	-	-
3-eudesmen-11-ol	C ₁₅ H ₂₆ O	28.343	0.21	28.332	0.22
τ -cadinol	C ₁₅ H ₂₆ O	29.300	0.25	29.290	0.22
γ -eudesmol	C ₁₅ H ₂₆ O	29.643	11.2	29.627	9.78
Hinesol	C ₁₅ H ₂₆ O	29.948	0.56	29.948	0.48
Aristolene	C ₁₅ H ₂₄	30.178	0.86	30.156	0.76

Table 2. Chemical profile of *C. japonica* leaf oils (continued)

Compounds	Formula	Fresh Leaf		Dried leaf	
		RT	Ratio (%)	RT	Ratio (%)
β -eudesmol	C ₁₅ H ₂₆ O	30.611	11.3	30.579	9.13
α -eudesmol	C ₁₅ H ₂₆ O	30.745	5.55	30.713	5.34
Bulnesol	C ₁₅ H ₂₆ O	31.338	0.39	31.312	0.15
ent-germacra -4(15), 5,10 (14)-trien-1 β -ol	C ₁₅ H ₂₄ O	32.152	0.5	32.146	0.33
Phytol	C ₂₀ H ₄₀ O	51.578	0.17	51.578	0.19
Identification components (%)		97.71		93.6	
1. Monoterpenes hydrocarbon		13.5		13.21	
2. Oxygenated monoterpenes		8.45		4.42	
3. Sesquiterpenes hydrocarbon		26.67		34.77	
4. Oxygenated sesquiterpenes		48.92		41.01	
5. Others		0.17		0.19	

Note: RT is retention time

The prominent compound groups of both *C. japonica* leaf oils were oxygenated sesquiterpenes followed by sesquiterpene hydrocarbons with the amounts of 48.92% and 41.01%, and 26.67% and 34.77% for the fresh and dried leaf oils, respectively. Notable compounds presenting in the fresh leaf oil were elemol (16.17%), β -eudesmol (11.28%), γ -eudesmol (11.22%), cis- β -copaene (10.40%) and linalool (6.11%), while elemol (13.00%), cis- β -copaene (12.85%), γ -eudesmol (9.78%), β -eudesmol (9.13%) and β -pinene (5.71%) were the major substances in the dried leaf oil. The variation in components of *C. japonica* oil derived from fresh and dried leaves were consistent with previous reports which indicated that drying method could affect the constituents of essential oil (Okoh et al., 2008; Usman et al., 2022; Khaiper et al., 2024).

In the case of *C. japonica* leaf oils, our results were distinct from other reports. Satyal et al. (2012) reported that dried leaf oil of *C. japonica* collected in Nepal and extracted by hydrodistillation contained 42 substances that made up 99.6% of all compositions, and linalool (35.1%), eugenol (14.8%), geraniol (12.7%), geranial (7.9%), nerol (5.3%), and (Z)-asarone (5%) were the major compounds. The fresh leaf and peel oils derived from Thailand-grown materials and extracted with hydrodistillation were monoterpene-rich oils by β -pinene (47.44%), d-limonene (10.24%), linalool (9.79%), *trans*-ocimene (7.56%), and α -pinene (7.41%) being the major compounds in leaf oil, in contrast to peel oil, which had d-limonene (87.07%), linalool (1.44%), myrcene (1.32%), and geranyl acetate (1.12%) as the major substances (Bunrathep et al., 2015). Moreover, 45 compounds were found in the dried leaf oil of *C. japonica* grown in Palestine and extracted by hydrodistillation. Its major compounds were γ -muurolene (28.12%), β -eudesmol (10.93%), γ -eudesmol (8.44%), germacrene B (7.39%), and elemol (7.27%) (Jaradat et al., 2024). Ziogas et al (2024) identified 38 compounds in leaf oil derived from *F. japonica* by hydrodistillation. The main substances of *F. japonica* were germacrene-D (12.03%), γ -eudesmol (9.75%), elemol (8.65%), β -elemene (5.26%), and *trans*-caryophyllene (4.97%). The dried leaf oil of Türkiye-cultivated *C. japonica* extracted by hydrodistillation was rich in elemol (13.2-14.8%), β -eudesmol (9.3-11.0%), α -guaial (8.5-10.8%), spathulenol (8.1-10.5%), and alismol (6.5-7.9%) (Küçükbay et al., 2025).

Comparison with other kumquat species revealed that the chemical profiles of leaf oils were different as well. Thirty-eight compounds were identified in leaf oil derived from *F. margarita* by hydrodistillation. The main substances of *F. margarita* oils included germacrene-D (16.4%), elemol (13.17%), α -eudesmol (13.01%), germacrene-B (9.88%), viridiflorol (8.9%), In addition, in the case of *F. crassifolia*, elemol (9.59%), germacrene-D (9.04%), viridiflorol (8.39%), cubenol (7.27%), γ -elemene (6.03%) were the predominant components in the leaf oil (Ziogas et al., 2024).

Moreover, the chemical profile of kumquat oil also differed between plant organs (Ashmawy, et al., 2023). The prominent constituents of kumquat peel oils were D-limonene, germacrene D, β -myrcene and α -terpineol, a profile that was unlike kumquat leaf oil (Wang et al., 2012; Ibrahim et al., 2015; Nouri & Shafaghatlonbar, 2015). Yangshuo kumquats peel oil was limonene and β -myrcene -rich oil (Lin et al., 2021)

Overall, the variation in types and amounts of compounds found in the essential oils in the present and other works is probably attributed to variation in the plant organs, cultivating area, season, environment and extraction method (Nouri & Shafaghatlonbar, 2015; Moghaddam & Mehdizadeh, 2017; Ashmawy, et al., 2023; Khaiper et al., 2024). In case of different regions, climate and soil conditions could lead to variation in the compound production of essential oils in each region (Markabi et al., 2025).

Apart from the kumquat leaf oils in this study, oxygenated sesquiterpenes- rich oils were reported in other works, and the oils in those studies contained various biological activities. Oils from aerial parts of *Lactuca serriola* and *Pulicaria somalensis* showed antioxidant and allelopathic activities (Abd-ElGawad et al., 2019; Assaeed et al., 2020). *Cistus ladanifer* oils expressed antibacterial and antifungal activity (El Karkouri et al., 2021). The leaf oil of *Rhododendron albiflorum* displayed immunomodulatory activity (Schepetkin et al., 2021). Indian sandalwood (*Santalum album*) oil displayed *in vivo* antioxidant and stress modulatory activity (Mohankumar et al., 2019). Brazilian *Chrysopogon zizanioides* root oil exhibited antibacterial, antiparasitic, and cytotoxic activities (Oliveira et al., 2022). The fresh leaf and twig oils of *Knema angustifolia* were predicted using molecular docking techniques as ligands for *tau* protein involved in neurodegenerative diseases (Barman et al., 2023). Finally, it was interesting that kumquat leaf oils in this study might contain other biological activities, and this hypothesis needs to be further investigated.

3.3 Antioxidant activity of *C. japonica* leaf oils

To investigate whether the kumquat leaf oils could have potential beneficial uses in the food, cosmetic, and pharmaceutical and medical industries, their antioxidant activities were important. The antioxidant activity of the kumquat leaf oils was evaluated using DPPH free radical scavenging assay. The result showed that the kumquat leaf oils were able to scavenge DPPH free radicals in a concentration-dependent manner. At the concentration of 200 mg mL⁻¹, the antioxidant activities of kumquat leaf oils were more than 80% (86.5±0.3% for fresh leaf oil and 82.8±2.1% for dried leaf oil) (Figure 1).

To compare the antioxidant activities, IC₅₀ value was calculated using a dose response function curve. The IC₅₀ values of fresh leaf oil and dried leaf oil were 60±0.73 and 54.2±3.3 mg mL⁻¹, respectively (Table 3). This indicated that the dried leaf oil posed higher activity than the fresh one. Furthermore, the antioxidant activity of the fresh and dried leaf oils was significantly different although their IC₅₀ values were quite similar. This might be because the amount of antioxidant compounds in each oil was of similar quantity

but sufficient enough to produce a significant difference. The result was consistent with other previous reports (Oladimeji et al., 2016; Usman et al., 2022; Yangui et al., 2021).

The antioxidant activity of *C. japonica* leaf oils was reported previously. Bunrathep et al. (2015) showed that *F. japonica* leaf oil collected in May-June 2014 at Pathum Thani, Thailand, possessed the DPPH scavenging activity with IC_{50} of $53.11 \mu\text{g mL}^{-1}$. Moreover, *C. japonica* leaf oils of Palestine displayed antioxidant activity in a depending manner and IC_{50} was $39.8 \pm 1.01 \mu\text{g mL}^{-1}$. These reports revealed higher activity than our result. It might be due to the different chemical compositions of each sample.

In addition to *C. japonica* leaf oils, the potent antioxidant activity of other plant organs was reported in several previous studies, especially fruit peel and kernel oils (Nouri and Shafaghatlonbar, 2015; Lin et al., 2021). The antioxidant activity of kumquat peel oil was involved in a number of compounds in monoterpenes hydrocarbon and oxygenated monoterpenes groups (Warsito et al., 2018), and especially D-limonene and β -myrcene which were its main active constituents (Yu et al., 2021). However, the antioxidant activity of *C. japonica* leaf oils in this study was lower than the peel and kernel oil. Nouri and Shafaghatlonbar (2015) showed that IC_{50} values for DPPH radical scavenging of peel and kernel oils of *C. japonica* were 112 and $165 \mu\text{g mL}^{-1}$. Likewise, the essential oil of Yangshuo kumquats fresh fruit peels extracted by hydrodistillation had antioxidant activity with IC_{50} of 30.01 mg mL^{-1} for the DPPH scavenging assay (Lin et al., 2021). This difference might be dependent on the ratio of antioxidant substances in each oil.

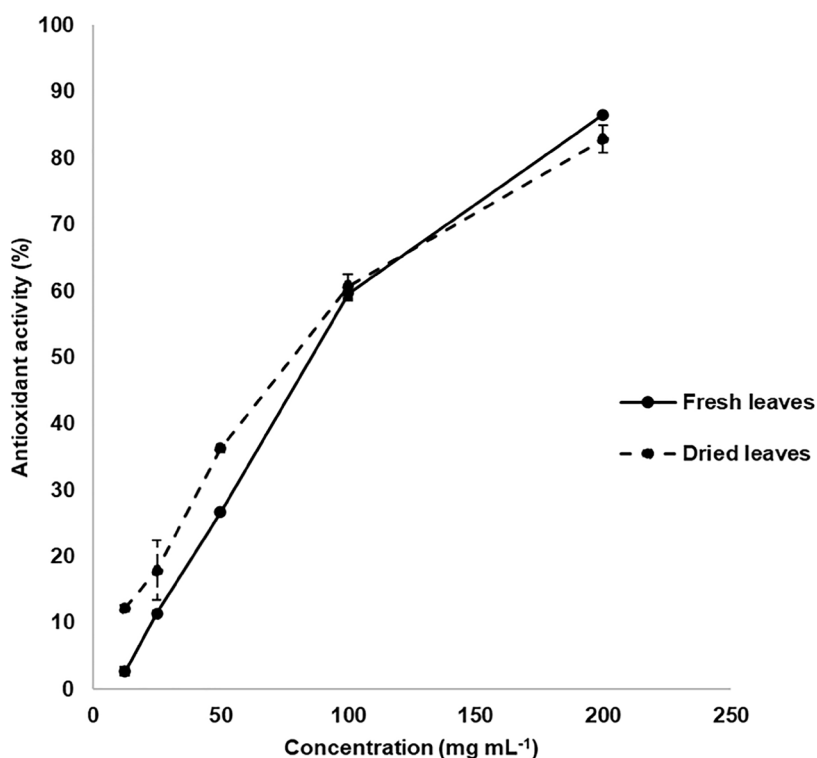


Figure 1. Antioxidant activity of *C. japonica* leaf oils

Table 3. IC₅₀ analysis of *C. japonica* leaf oils using DPPH scavenging assay

Essential Oils	IC ₅₀ (mg mL ⁻¹)
Fresh leaf	60±0.73 ^b
Dried leaf	54.2±3.3 ^c
Ascorbic acid (µg mL ⁻¹)	89.2±1.67 ^a

Note: Different superscript letters indicate significant differences at $P < 0.05$.

3.4 Antibacterial activity of *C. japonica* leaf oils

The antibacterial activity of *C. japonica* leaf oils against *B. subtilis*, *E. coli* and *S. aureus* was assayed using the well diffusion assay. The results indicated that *C. japonica* leaf oils presented antibacterial action against *B. subtilis* and *S. aureus* with inhibition zones ranging from 0.9±0.0 to 1.07±0.1 cm. Conversely, this oil showed no inhibitory activity against *E. coli* (Table 4).

After investigation of MIC and MBC values, it was found that the dried leaf oil showed more bacteriostatic and bactericidal activities than the fresh leaf oil. In detail, the lowest MIC and MBC were presented in the dried leaf oil-treated *B. subtilis* with the values of 3.9 and 3.9 mg mL⁻¹, respectively, followed by the dried leaf oil-treated *S. aureus* with the values of 7.8 and 7.8 mg mL⁻¹, respectively. For the fresh leaf oil, the lowest MIC and MBC were found in treated *B. subtilis* with the values of 7.8 and 7.8 mg mL⁻¹, respectively, followed by treated *S. aureus* with the values of 15.6 and 31.2 mg mL⁻¹, respectively (Table 5).

A few studies have examined the antibacterial activities of *C. japonica* leaf oils. However, the present results were consistent with Bunrathep et al. (2015) revealing that *F. japonica* leaf oil antagonized against *B. subtilis* and *S. aureus* with MIC/MBC values of 1.25/>5 mg mL⁻¹ but was not against *E. coli*. Similarly, Yangshuo kumquat's fresh fruit peel oil possessed antibacterial activity against both gram-positive and gram-negative bacteria with MIC values of 1.56-100 µL mL⁻¹ (Lin et al., 2021).

The antibacterial activity of other kumquat species was demonstrated in various works. Ibrahim et al (2015) reported that the hydrodistilled oil of *F. margarita* leaf possessed greater antibacterial activity against *B. subtilis*, *S. aureus*, *Sarcina lutea* and *Streptococcus faecalis* than the peel. Moreover, *F. margarita* leaf oil contained higher antibacterial activity than peel oil.

Kumquat oils contained broad spectrum antimicrobial activity against bacteria (gram-negative and gram-positive) (Wang et al., 2012; Huang et al., 2017; Lin et al., 2021), fungi and yeast (Fitsiou et al., 2016; Huang et al., 2017), and virus (Ibrahim et al., 2015). Of these, the antimicrobial activity of kumquat oil was correlated with those of known antimicrobial substances including d-limonene (Hou et al., 2022), linalool (Parasuraman et al., 2022), carvone (Moro et al., 2017), and carvacrol (Souza et al., 2022). Likewise, *C. japonica* leaf oils in this present work were shown to contain d-limonene and linalool as well, and this was the reason why the kumquat leaf oils exhibited antibacterial activity, and the different amounts of these compounds may result in the variations in the antibacterial activity observed.

Table 4. Antibacterial activity of *C. japonica* leaf oils

Essential oils	Diameter of inhibition zone (cm)		
	<i>S. aureus</i>	<i>E. coli</i>	<i>B. subtilis</i>
Fresh leaf	1.03±0.05 ^b	NI	1.03±0.1 ^b
Dried leaf	0.9±0.0 ^b	NI	1.07±0.1 ^b
Streptomycin (µg mL ⁻¹)	1.83±0.05 ^a	2.07±0.05	2.47±0.1 ^a

Note: NI means no inhibition. Different letters in each column indicate significant differences at $P < 0.05$.

Table 5. MIC and MBC of *C. japonica* leaf oils against pathogenic bacteria

Essential oils	MIC/MBC (mg mL ⁻¹)	
	<i>S. aureus</i>	<i>B. subtilis</i>
Fresh leaf	15.6/31.2	7.8/7.8
Dried leaf	7.8/7.8	3.9/3.9
Streptomycin (µg mL ⁻¹)	12.5/12.5	6.25/6.25

Furthermore, *C. japonica* leaf oils inhibited only *B. subtilis*, and *S. aureus* but not *E. coli*, the results which were differed from kumquat peel oils (Wang et al., 2012; Huang et al., 2017; Lin et al., 2021). This might be due to the different antimicrobial content in each oil. Moreover, previous reports indicated that gram-negative bacteria were more resistant to essential oil than gram-positive bacteria (Trombetta et al., 2005). This may be because gram-negative bacteria contain an outer membrane that possesses lipopolysaccharides, a hydrophilic molecule, distributed on them, resulting in hydrophilic barrier formation leading to essential oils being unable to penetrate the cells. Conversely, hydrophobic molecules were easily able to penetrate the cell wall of gram-positive bacteria and thus leading to the ability to act on the cell wall and cytoplasm (Nazzaro et al., 2013).

Interestingly, although the known antimicrobial substances (d-limonene and linalool) were found in the fresh leaf oil at higher levels than the dried leaf oil, the dried leaf oil possessed higher antibacterial activity. This might be possible that other constituents present in each kumquat leaf oil affected known antimicrobial substances in synergistic or antagonistic modes.

3.5 Cytotoxicity activity of *C. japonica* leaf oils against cancer cells

To determine whether *C. japonica* leaf oils possessed anticancer activity, the MCF7 breast cancer cells and NCI-H187 small lung cancer cells were treated with *C. japonica* leaf oils and cell viability was investigated using the REMA method. The results found that *C. japonica* leaf oils had anticancer activity and its activity increased when the dose increased. MCF7 cell death was more than 90% after treatment with fresh and dried leaf oils at the concentration of 7.81 mg mL⁻¹. In case of NCI-H187, cell death was more than 90 % when cells were incubated with fresh and dried leaf oils at the concentration of 0.976 and 1.95 mg mL⁻¹, respectively (Figure 2).

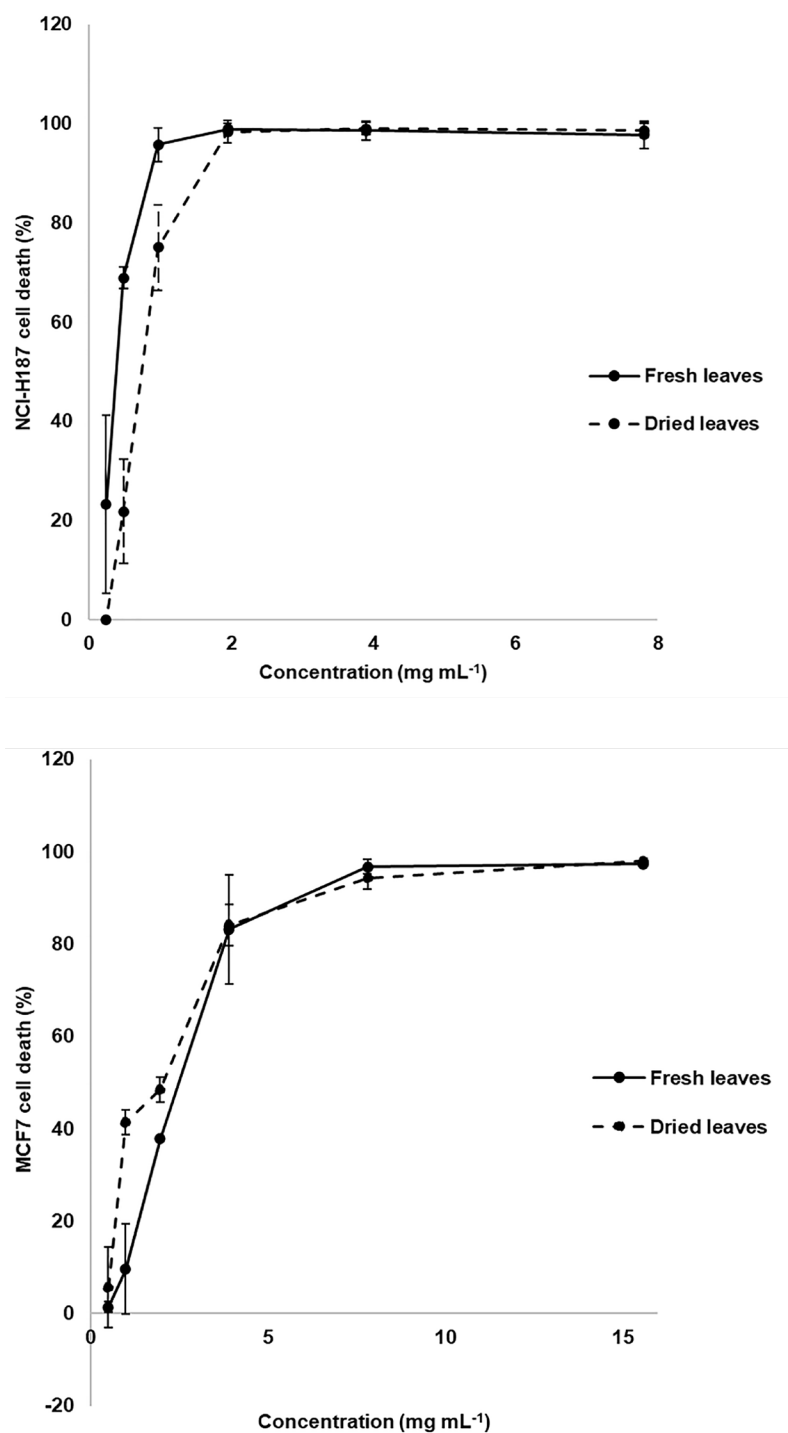


Figure 2. Anticancer activity of *C. japonica* leaf oils against NCI-H187 (upper) and MCF-7 (lower) cells

Subsequently, IC₅₀ of *C. japonica* leaf oils against cancer cells was calculated. The results revealed that the anticancer activity between fresh and dried leaf oils against the same cell line was not significantly different. The IC₅₀ values of the fresh and dried leaf oils for MCF7 cells were 2.7±0.45 and 2.4±1.82 mg mL⁻¹, respectively, and for NCI-H-187 cells were 0.7±0.34 and 0.7±0.05 mg mL⁻¹ (Table 6), respectively. However, interestingly, *C. japonica* leaf oils exhibited different potencies across the two cells tested, when NCI-H187 cells being more sensitive to *C. japonica* leaf oils than MCF7 cells. This result corresponded with Buriani et al (2017), suggesting the different sensitivity of different cancer cells to the same *Pistacia* oils. This might be due to the bioactive compounds of *Pistacia* oils affecting the network of molecular targets, which were specific and different in each cancer cell, affecting the different sensitivity of each cancer cell to the same *Pistacia* oils. Likewise, the anticancer results of the present work showed the same trend. Thus, the above-mentioned factor might be the reason for the different responses of NCI-H187 and MCF7 cells to the same *C. japonica* leaf oils.

There were a few studies that investigated the anticancer effects of *C. japonica* leaf oils. The γ -muurolene-rich dried leaf oil of Palestine-grown materials displayed anticancer activity against the CaCo-2, HeLa, MCF-7, HepG2, Hep3B, B16F1, and LX-2 cells with IC₅₀ values ranging between 69.7±2.55 to 224.95±2.03 μ g mL⁻¹. This oil showed the best activity against Hep3B (Jaradat et al., 2024). Some results of this research were consistent with our work, as the growth of MCF-7 cells was inhibited by both *C. japonica* leaf oils. However, *C. japonica* leaf oils of the present study exhibited lower anticancer activity after IC₅₀ values were compared. This might be because the different amounts of anticancer compounds present in both oils are differently synergized or antagonized by other compounds in each oil.

Table 6. IC₅₀ analysis of *C. japonica* leaf oils against cancer cells

Essential oils	IC ₅₀ (mg mL ⁻¹)	
	MCF7 Cell	NCI-H187 Cell
Fresh leaf	2.7±0.45 ^c	0.7±0.34 ^b
Dried leaf	2.4±1.82 ^c	0.7±0.05 ^b
Doxorubicin (μ g mL ⁻¹)	15.48±2.53 ^b	0.09±0.04 ^a
Ellipticine (μ g mL ⁻¹)	8.13±2.47 ^a	2.15±1.55 ^a

Note: Different superscript letters in each column indicate significant differences at P<0.05.

The anticancer activity of kumquat oil, especially peel oil, was reported. The peel oil of *F. margarita* could inhibit several types of cancer cells. At a concentration of 200 μ g mL⁻¹, *F. margarita* oil inhibited the proliferation of androgen-dependent prostate cancer cells up to 63% within 72 h by induction of apoptosis and programmed cell death (Jayaprakasha et al., 2012). In addition, Fitsiou et al (2016) showed that *F. margarita* peel oil at a concentration of 100 μ g mL⁻¹ could inhibit Caco2 colon adenocarcinoma and THP-1 leukemic monocytic cells with EC₅₀ values of 0.1±0.027 and 0.1±0.0023 mg mL⁻¹, respectively. The proliferation of HeLa cervical adenocarcinoma and HT-1080 fibrosarcoma was inhibited after incubation with peel oil of *F. japonica* at a concentration of 500 μ g mL⁻¹ with percentage inhibitions of 58% and 96%, respectively (Soni et al., 2022). Moreover, *C. japonica* var. *margarita* peel oil extracted with microwave-assisted distillation (MAD) and Clevenger hydrodistillation revealed cytotoxicity against HeLa, HCT116, and U2OS cells (Vrca et al., 2024).

Several reports identified the bioactive compounds involved in the anticancer activity of kumquat peel and included were limonene, myrcene and ethyl acetate (Choi et al., 2005; Jia et al., 2013; Soni et al., 2022). Limonene and myrcene induced JNK signaling pathway resulted in apoptosis (Soni et al., 2022). Similarly, *C. japonica* leaf oils in the present work also contain limonene and myrcene. This was a reason why the oil could inhibit the proliferation of MCF-7 breast and NCI-H187 small lung cancer cells. Finally, it can be concluded that *C. japonica* leaf oil contained anticancer activity as well as kumquat peel oil.

4. Conclusions

The present work concluded that 42 and 41 compounds were identified in the fresh and dried leaf kumquat oils, respectively, and the major compound groups of essential oil were oxygenated sesquiterpenes followed by sesquiterpene hydrocarbons. The predominant compounds presented in the fresh leaf oil were elemol, β -eudesmol, γ -eudesmol, cis- β -copaene and linalool while elemol, cis- β -copaene, γ -eudesmol, β -eudesmol and β -pinene were the major substances in the dried leaf oil. The kumquat leaf oils were able to scavenge DPPH free radicals in a concentration-dependent manner, and the dried leaf oil showed greater activity than the fresh leaf oil. In addition, the kumquat leaf oils demonstrated antibacterial action against *B. subtilis* and *S. aureus* but showed no inhibitory activity against *E. coli*, with the dried leaf oil possessing greater activity than the fresh leaf oil. Finally, kumquat leaf oils could inhibit the proliferation of MCF-7 breast cancer cells and NCI-H187 small lung cancer cells.

Overall, it was concluded that kumquat leaf oils have potential beneficial uses and applications in the food and pharmaceutical industries, with demonstrated antioxidant, antibacterial, and anticancer properties.

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6. Authors' Contributions

Jaturon Thipwong designed research; performed research; analyzed data; and wrote the paper.

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7. Conflicts of Interest

The author declares no conflict of interest.

References

- Abd-ElGawad, A. M., Elshamy, A. I., El-Nasser El Gendy, A., Al-Rowaily, S. L., & Assaeed, A. M. (2019). Preponderance of oxygenated sesquiterpenes and diterpenes in the volatile oil constituents of *Lactuca serriola* L. revealed antioxidant and allelopathic activity. *Chemistry and Biodiversity*, 16(8), Article e1900278. <https://doi.org/10.1002/cbdv.201900278>
- Ali, B., Al-Wabel, N. A., Shams, S., Ahamad, A., Khan, S. A., & Anwar, F. (2015). Essential oils used in aromatherapy: A systemic review. *Asian Pacific Journal of Tropical Biomedicine*, 5(8), 601-611. <https://doi.org/10.1016/j.apjtb.2015.05.007>
- Al-Saman, M. A., Abdella, A., Mazrou, K. E., Tayel, A. A., & Irmak, S. (2019). Antimicrobial and antioxidant activities of different extracts of the peel of kumquat (*Citrus japonica* Thunb). *Journal of Food Measurement and Characterization*, 13, 3221-3229. <https://doi.org/10.1007/s11694-019-00244-y>
- Ashmawy, N. S., Gad, H. A., & El-Nashar, H. A. S. (2023). Comparative study of essential oils from different organs of *Syzygium cumini* (Pamposia) based on GC/MS chemical profiling and in vitro antiaging activity. *Molecules*, 28(23), Article 7861. <https://doi.org/10.3390/molecules28237861>
- Assaeed, A., Elshamy, A., El Gendy, A. E. -N., Dar, B., Al-Rowaily, S., & Abd-ElGawad, A. (2020). Sesquiterpenes-rich essential oil from above ground parts of *Pulicaria somalensis* exhibited antioxidant activity and allelopathic effect on weeds. *Agronomy*, 10(3), Article 399. <https://doi.org/10.3390/agronomy10030399>
- Bajpai, V. K., Sharma, A., & Baek, K. H. (2013). Antibacterial mode of action of *Cudrania tricuspidata* fruit essential oil, affecting membrane permeability and surface characteristics of food-borne pathogens. *Food Control*, 32(2), 582-590. <https://doi.org/10.1016/j.foodcont.2013.01.032>
- Barman, R., Saikia, J., Sarmah, P., Konwar, P., Kumar, M., Bora, P. K., Bhattacharyya, P., Saikia, S. P., Haldar, S., & Banik, D. (2023). Oxygenated sesquiterpenes, molecular docking, and the trait-linked occurrence of essential oil in *Knema angustifolia* (Roxb.) Warb (Myristicaceae). *South African Journal of Botany*, 159, 617-626. <https://doi.org/10.1016/j.sajb.2023.06.030>
- Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature*, 181, 1199-1200. <https://doi.org/10.1038/1811199a0>
- Brien, J. O., Wilson, I., Orton, T., & Pognan, F. (2000). Investigation of the alamar blue (resazurin) fluorescent dye for the assessment of mammalian cell cytotoxicity. *European Journal of Biochemistry*, 267(17), 5421-5426. <https://doi.org/10.1046/j.1432-1327.2000.01606x>
- Bunrathap, S., Soodvilai, S., Settharaksa, S., & Palanuvej, C. (2015). Chemical constituents and biological activities of *Fortunella japonica* essential oils. *Interprofessional Journal of Health Sciences*, 13(1), 43-49.
- Buriani, A., Fortinguerra, S., Sorrenti, V., Dall'Acqua, S., Innocenti, G., Montopoli, M., Gabbia, D., & Carrara, M. (2017). Human adenocarcinoma cell line sensitivity to essential Oil phytocomplexes from *Pistacia* species: a multivariate approach. *Molecules*, 22(8), Article 1336. <https://doi.org/10.3390/molecules22081336>
- Caprari, C., Fantasma, F., Monaco, P., Divino, F., Iorizzi, M., Ranalli, G., & Saviano, G. (2023). Chemical profiles, in vitro antioxidant and antifungal activity of four different *Lavandula angustifolia* L. EOs. *Molecules*, 28(1), Article 392. <https://doi.org/10.3390/molecules28010392>
- Choi, H. S. (2005). Characteristic odor components of kumquat (*Fortunella japonica* swingle) peel oil. *Journal of Agricultural and Food Chemistry*, 53(5), 1642-1647. <https://doi.org/10.1021/jf040324x>
- Dosoky, N. S., & Setzer, W. N. (2018). Biological activities and safety of *Citrus* spp. essential oils. *International Journal of Molecular Sciences*, 19(7), Article 1966. <https://doi.org/10.3390/ijms19071966>

- El Karkouri, J., Bouhrim, M., Al Kamaly, O. M., Mechchate, H., Kchibale, A., Adadi, I., Amine, S., Ismaili, S. A., & Zair, T. (2021). Chemical composition, antibacterial and antifungal activity of the essential oil from *Cistus ladanifer* L. *Plants*, 10(10), Article 2068. <https://doi.org/10.3390/plants10102068>
- El-Nashar, H. A. S., Eldehna, W. M., Al-Rashood, S. T., Alharbi, A., Eskandrani, R. O., & Aly, S. H. (2021). GC/MS analysis of essential oil and enzyme inhibitory activities of *Syzygium cumini* (Pamposia) grown in Egypt: chemical characterization and molecular docking studies. *Molecules*, 26(22), Article 6984. <https://doi.org/10.3390/molecules26226984>
- Feyzi, E., Eikani, M. H., Golmohammad, F., & Tafaghodinia, B. (2017). Extraction of essential oil from *Bunium Persicum* (Boiss.) by instant controlled pressure drop (DIC). *Journal of Chromatography A*, 1530, 59-67. <https://doi.org/10.1016/j.chroma.2017.11.033>
- Fitsiou, E., Mitropoulou, G., Spyridopoulou, K., Tiptiri-Kourpeti, A., Vamvakias, M., Bardouki, H., Panayiotidis, M., Galanis, A., Kourkoutas, Y., Chlichlia, K., & Pappa, A. (2016). Phytochemical profile and evaluation of the biological activities of essential oils derived from the Greek aromatic plant species *Ocimum basilicum*, *Mentha spicata*, *Pimpinella anisum* and *Fortunella margarita*. *Molecules*, 21(8), Article 1069. <https://doi.org/10.3390/molecules21081069>
- Ganji, S. M. M., Moradi, H., Ghanbari, A., Akbarzadeh, M. (2017). Quantity and quality of secondary metabolites in lavender plant under the influence of ecological factors. *Nova Biologica Reperta*, 4(2), 166-172.
- Hou, C. Y., Hazeena, S. H., Hsieh, S. L., Li, B. H., Chen, M. H., Wang, P. Y., Zheng, B. Q., & Liang, Y. S. (2022). Effect of D-limonene nanoemulsion edible film on banana (*Musa sapientum* Linn.) post-harvest preservation. *Molecules*, 27(19), Article 6157. <https://doi.org/10.3390/molecules27196157>
- Huang, C., Deng, C., Chen, P., Wang, S., Zeng, H., & Zhang, Y. (2017). Optimization of ultrasonic extraction technology of kumquat peel essential oil and its antibacterial, antioxidant activity. *Chinese Journal of Topical Crops*, 38(7), 1345-1352. <https://doi.org/10.3969/j.issn.1000-2561.2017.07.026>
- Ibrahim, N. A., El-Hawary, S. S., Mohammed, M. M. D., Farid, M. A., Abdel-Wahed, N. A. M., Ali, M. A., & El-Abd, E. A. W. (2015). Chemical composition, antiviral against avian influenza (H5N1) virus and antimicrobial activities of the essential oils of the leaves and fruits of *Fortunella margarita* Lour. Swingle, growing in Egypt. *Journal of Applied Pharmaceutical Science*, 5(1), 6-12. <https://doi.org/10.7324/JAPS.2015.50102>
- Jaradat, N., Hawash, M., Al-Maharik, N., Atiani, R., Omariyah, G., Hamodi, L., Mosa, H., Hussein, F., Issa, L., Shakhshir, M. S., Sobuh, S., Makhkamov, T., Sultonov, K., & Abdullayeva, N. (2024). Phytochemical profiling and bioactivity analysis of *Citrus japonica* leaves volatile oil from Palestine. *Chemical and Biological Technologies in Agriculture*, 11, Article 140. <https://doi.org/10.1186/s40538-024-00659-w>
- Jayaprakasha, G. K., Murthy, K. N. C., Demarais, R., & Patil, B. S. (2012). Inhibition of prostate cancer (LNCaP) cell proliferation by volatile components from Nagami kumquats. *Planta Medica*, 78(10), 974-980. <https://doi.org/10.1055/s-0031-1298619>
- Jia, S.-S., Xi, G.-P., Zhang, M., Chen, Y.-B., Lei, B., Dong, X.-S., & Yang, Y.-M. (2013). Induction of apoptosis by D-limonene is mediated by inactivation of Akt in LS174T human colon cancer cells. *Oncology Reports*, 29(1), 349-354. <https://doi.org/10.3892/or.2012.2093>
- Khaiper, M., Poonia, P. K., Redhu, I., Verma, P., Sheokand, R. N., Nasir, M., Tiwari, A., & Kumar, V. (2024). Chemical composition, antifungal and antioxidant properties of seasonal variation in *Eucalyptus tereticornis* leaves of essential oil. *Industrial Crops and Products*, 222(3), Article 119669. <https://doi.org/10.1016/j.indcrop.2024.119669>

- Küçükbay, F., Büyükkormaz, Ç., Özek, G., & Özek, T. (2025). Chemical composition of essential oils from *Citrus fortunella* (kumquat) and its mutants. *Food Processing: Techniques and Technology*, 55(1), 29-44. <https://doi.org/10.21603/2074-9414-2025-1-2553>
- Lin, X., Cao, S., Sun, J., Lu, D., Zhong, B., & Chun, J. (2021). The chemical compositions, and antibacterial and antioxidant activities of four types of *citrus* essential oils. *Molecules*, 26(11), Article 3412. <https://doi.org/10.3390/molecules26113412>
- Markabi, F. S., & Ghavam, M. (2025). The effect of the environmental conditions of the cultivation area on the efficiency, quality and antibacterial activity of lavender essential oil and by-products (residual water & residual herbs) against different wound-causing strains. *Waste and Biomass Valorization*, 16, <https://doi.org/10.1007/s12649-025-03229-9>
- Moghaddam, M., & Mehdizadeh, L. (2017). Chemistry of essential oils and factors influencing their constituents. In A. M. Grumezescu, & A. M. Holban (Eds.). *Handbook of food bioengineering, soft chemistry and food fermentation* (pp. 379-419). Academic Press. <https://doi.org/10.1016/B978-0-12-811412-4.00013-8>
- Mohankumar, A., Kalaiselvi, D., Levenson, C., Shanmugam, G., Thiruppathi, G., Nivitha, S., & Sundararaj, P. (2019). Antioxidant and stress modulatory efficacy of essential oil extracted from plantation-grown *Santalum album* L. *Industrial Crops and Products*, 140, Article 111623. <https://doi.org/10.1016/j.indcrop.2019.111623>
- Moro, I. J., Gondo, G. D. G. A., Pierri, E. G., Pietro, R. C. L. R., Soares, C. P., de Sousa, D. P., & dos Santos, A. G. (2017). Evaluation of antimicrobial, cytotoxic and chemopreventive activities of carvone and its derivatives. *Brazilian Journal of Pharmaceutical Sciences*, 53(4), Article e00076. <https://doi.org/10.1590/s2175-97902017000400076>
- Nazzaro, F., Fratianni, F., De Martino, L., Coppola, R., & De Feo, V. (2013). Effect of essential oils on pathogenic bacteria. *Pharmaceuticals*, 6(12), 1451-1474. <https://doi.org/10.3390/ph6121451>
- Nouri, A., & Shafaghatlonbar, A. (2015). Chemical constituents and antioxidant activity of essential oil and organic extract from the peel and kernel parts of *Citrus japonica* Thunb. (kumquat) from Iran. *Natural Product Research*, 30(9), 1093-1097. <https://doi.org/10.1080/14786419.2015.1101692>
- Okoh, O. O., Sadimenko, A. P., Asekun O. T., & Afolayan, A. J. (2008). The effects of drying on the chemical components of essential oils of *Calendula officinalis* L. *African Journal of Biotechnology*, 7(10), 1500-1502.
- Okunowo, W. O., Oyedeji, O., Afolabi, L. O., & Matanmi, E. (2013). Essential oil of grape fruit (*Citrus paradisi*) peels and its antimicrobial activities. *American Journal of Plant Sciences*, 4(7), 1-9.
- Oladimeji, A. O., Aliyu, M. B., Ogundajo, A. L., Babatunde, O., Adeniran, O. I., & Balogun, O. S. (2016). Identification and comparison of the volatile constituents of fresh and dried leaves of *Spondias mombin* found in North-central Nigeria: in vitro evaluation of their cytotoxic and antioxidant activities. *Pharmaceutical Biology*, 54(11), 2674-2678. <https://doi.org/10.1080/13880209.2016.1178308>
- Oliveira, T. A. S., Vieira, T. M., Esperandim, V. R., Martins, C. H. G., Magalhães, L. G., Miranda, M. L. D., & Crotti, A. E. M. (2022). Antibacterial, antiparasitic, and cytotoxic activities of chemical characterized essential oil of *Chrysopogon zizanioides* roots. *Pharmaceuticals (Basel)*, 15(8), Article 967. <https://doi.org/10.3390/ph15080967>
- Parasuraman, V., Sharmin, A. M., Anand, M. A. V., Sivakumar, A. S., Surendhiran, D., Sharesh, G., & Kim, S. (2022). Fabrication and bacterial inhibitory activity of essential oil linalool loaded biocapsules against *Escherichia coli*. *Journal of Drug Delivery Science and Technology*, 74, Article103495. <https://doi.org/10.1016/j.jddst.2022.103495>

- Satyal, P., Paudel, P., Limbu, K., & Setzer, W. N. (2012). Leaf essential oil composition of *Citrus japonica* from Nepal. *Journal of Essential Oil-Bearing Plants*, 15(3), 357-359. <https://doi.org/10.1080/0972060X.2012.10644059>
- Schepetkin, I. A., Özek, G., Özek, T., Kirpotina, L. N., Khlebnikov, A. I., & Quinn, M. T. (2021). Chemical composition and immunomodulatory activity of essential oils from *Rhododendron albiflorum*. *Molecules*, 26(12), Article 3652. <https://doi.org/10.3390/molecules26123652>
- Soni, S., Parekh, M. Y., Jacob, J. A., Mack, J. P., & Lobo, D. E. (2022). Kumquat essential oil decreases proliferation and activates JNK signaling and apoptosis in HT-1080 fibrosarcoma cells. *Molecular and Cellular Biochemistry*, 477(2), 445-453. <https://doi.org/10.1007/s11010-021-04291-2>
- Souza, V. V. M. A., Almeida, J. M., Barbosa, L. N., & Silva, N. C. C. (2022). Citral, carvacrol, eugenol and thymol: antimicrobial activity and its application in food. *Journal of Essential Oil Research*, 34(3), 181-194. <https://doi.org/10.1080/10412905.2022.2032422>
- Tuttolomondo, T., Dugo, G., Ruberto, G., Leto, C., Napoli, E. M., Potorti, A. G., La Bella, S. (2015). Agronomical evaluation of Sicilian biotypes of *Lavandula stoechas* L. spp. *stoechas* and analysis of the essential oils. *Journal of Essential Oil Research*, 27(2), 115-124. <https://doi.org/10.1080/10412905.2014.1001527>
- Trombetta, D., Castelli, F., Sarpietro, M. G., Venuti, V., Cristani, M., Daniele, C., Saija, A., Mazzanti, G., & Bisignano, G. (2005). Mechanisms of antibacterial action of three monoterpenes. *Antimicrobial Agents and Chemotherapy*, 49(6), 2474-2478. <https://doi.org/10.1128/AAC.49.6.2474-2478.2005>
- Usman, L. A., Oguntoye, O. S., & Ismaeel, R. O. (2022). Phytochemical profile, antioxidant and antidiabetic potential of essential oil from fresh and dried leaves of *Eucalyptus globulus*. *Journal of the Chilean Chemical Society*, 67(1), 5453-5461.
- Vrca, I., Fredotović, Ž., Jug, B., Nazlić, M., Dunkić, V., Jug, D., Radić, J., Možina, S. S., & Restović, I. (2024). Chemical profile of kumquat (*Citrus japonica* var. margarita) essential oil, in vitro digestion, and biological activity. *Foods*, 13(22), Article 3545. <https://doi.org/10.3390/foods13223545>
- Wang, Y. W., Zeng, W. C., Xu, P. Y., Lan, Y. J., Zhu, R. X., Zhong, K., Huang, Y. N., & Gao, H. (2012). Chemical composition and antimicrobial activity of the essential oil of kumquat (*Fortunella crassifolia* Swingle) peel. *International Journal of Molecular Sciences*, 13(3), 3382-3393. <https://doi.org/10.3390/ijms13033382>
- Warsito, W., Noorhamdani, N., Sukardi, S., & Suratmo, S. (2018). Assessment of antioxidant activity of citronellal extract and fractions of essential oils of *Citrus hystrix* DC. *Tropical Journal of Pharmaceutical Research*, 17(6), 1119-1125.
- Yangui, T., Chakroun, H., Dhouib, A., & Bouaziz, M. (2021). Biological properties and chemical composition of essential oils from fresh and shade dried olive leaves of *Olea europaea* L. Chemlali cultivar. *Journal of Essential Oil Bearing Plants*, 24(6), 1389-1401. <https://doi.org/10.1080/0972060X.2022.2026254>
- Yu, F., Wan, N., Zheng, Q., Li, Y. H., Yang, M., & Wu, Z. F. (2021). Effects of ultrasound and microwave pretreatments on hydrodistillation extraction of essential oils from Kumquat peel. *Food Science and Nutrition*, 9(5), 2372-2380. <https://doi.org/10.1002/fsn3.2073>
- Ziogas, V., Ganos, C., Graikou, K., Cheilari, A., & Chinou, I. (2024). Chemical analyses of volatiles from kumquat species grown in Greece - a study of antimicrobial activity. *Horticulturae*, 10(2), Article 131. <https://doi.org/10.3390/horticulturae10020131>