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Chemical Composition And Biological Activities Of *Citrus Nobilis* Essential Oil Collected In Dak Lak, Vietnam

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Abstract
Citrus Nobilis, belonging to the family Rutaceae, includes about 17 species worldwide, with citrus fruits such as oranges, tangerines, lemons, grapefruits, and citrons being of great agricultural importance. In Vietnam, about 20 citrus species are widely grown, including *Citrus nobilis*, which is valued for its flavor, nutritional content, and health benefits. Although often considered an agricultural by-product, the peel of *Citrus Nobilis* contains bioactive compounds, including essential oils, flavonoids, and polyphenols, with reported antibacterial, antifungal, antioxidant, and anti-inflammatory activities. However, the chemical composition and biological properties of *Citrus Nobilis* collected in Dak Lak, Vietnam, have not been investigated. Gas chromatography- mass spectrometry (GC-MS) was used to identify the chemical composition of *Citrus Nobilis* essential oil. The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay and the agar disc diffusion method were used to evaluate the antioxidant and antibacterial activities of the essential oil, respectively. The results of determining the chemical composition of *Citrus Nobilis* in Dak Lak province showed that contained 12 natural compounds, indicating considerable chemical diversity. The major constituents were D-Limonene (91.96%), 2-β-pinene (2.11%), cis-limonene oxide (1.85%), Carveol (0.85%), α-Terpinene (0.75%), p-Mentha-1(7), 8(10)-dien-9-ol (0.63%), α-Thujene (0.58%). These distinctive features provide an important basis for evaluating the bioactive potential and pharmacological applications of *Citrus Nobilis* essential oil from Dak Lak.

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Keywords: Essential oil, Citrus Nobilis, D-Limonene, α-Thujene, Dak Lak.

1. Introduction

The citrus plant consists of 17 species that belong to the family Rutaceae and these are found almost everywhere in tropical, subtropical, and temperate regions. Citrus fruits of the genus Citrus are in the family Rutaceae, and come in a wide variety of shapes and sizes (from round to oblong). This group of trees accounts for a large proportion of world agriculture with oranges, tangerines, lemons, grapefruits and citrons being the most important fruits of this genus [1]. In ancient times, these were grown by the inhabitants of South Asia including Indonesia and China, however, currently, they are grown everywhere across the world [2].

In Vietnam, the genus Citrus comprises approximately 20 species and numerous cultivated varieties that are widely grown across different regions of the country [3]. Due to their pleasant flavor, high nutritional value, and recognized health benefits, Citrus nobilis fruits are not only consumed fresh but have also been utilized in the development of various processed products, including orange wine [4], orange cider [5], and soft-dried orange products [6].

Although often treated as an agricultural by-product, the peel of *Citrus nobilis* contains a diverse array of bioactive compounds beneficial to human health, such as essential oils, flavonoids, and polyphenols. The essential oil obtained from *Citrus nobilis* peel consists of a complex mixture of chemical groups, including monoterpenes, sesquiterpenes, diterpenes, monoterpene alcohols, monoterpene aldehydes, monoterpene esters, monoterpene phenols, sesquiterpene esters, and sesquiterpene oxides [7, 8]. Citrus peels also represent a valuable natural source of polyphenols, and several studies have demonstrated their antihyperglycemic effects [9]. Moreover, the essential oil of Citrus sinensis exhibits multiple biological properties, including therapeutic, antiseptic, analgesic, and anti-inflammatory activities, inhibit cancer cells and prevent cardiovascular disease [10, 11]. In addition, the essential oil extracted from *Citrus nobilis* peel has been reported to possess notable antibacterial, antifungal, and antioxidant activities [12].

Currently, a number of research works on the extraction and composition of *Citrus Nobilis* essential oil have been published by a number of authors. However, there has been no research on the chemical composition and biological activity of *Citrus Nobilis* essential oil grown in Dak Lak province. Therefore, "Chemical Composition And Biological Activities Of *Citrus Nobilis* Essential Oil Collected In Dak Lak, Vietnam" was carried out with the objectives of: Extracting, quantifying essential oil, isolating and determining the structure of compounds in essential oil. The research is the first step in providing more information on the antibacterial ability of *Citrus Nobilis* essential oil in Dak Lak, as a basis for future research and applications.

2. Material and methods

2.1. Chemicals

Butylated hydroxytoluene (BHT), C7–C30 straight-chain hydrocarbons, reference chemicals for identification, Tween 80, and DPPH were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Other chemical analytical grades, the culture media, and standard antibiotic discs were procured from Merck (Darmstadt, Germany) and Oxoid Ltd. (Basingstoke, Hampshire, UK), respectively.

2.2. Plant Material

The of *Citrus Nobilis* were gathered from Hoa Thang, Dak Lak Province, Vietnam in 2025. To serve as a reference, a voucher specimen (No C-01) was deposited at the Faculty of Natural Science and Technology, Tay Nguyen University, Buon Ma Thuot City.

2.3. Extraction of Essential Oil

Citrus Nobilis were cleaned, cut into small pieces, and subjected to steam distillation for 3hours by a Clevenger-type apparatus. The essential oil obtained was then dehydrated using anhydrous sodium sulfate and stored in a sealed vial at 10°C in the dark prior to subsequent experiments.

2.4. Analysis of Essential Oil by GC-MS

To analyze the composition of the essential oil from *Citrus Nobilis*, a Trace GC Ultra-ITQ900 system (Thermo Fisher Scientific, MA, USA) was used. Data were interpreted by MassFinder 4.0 software. The separation was performed on a fused silica capillary TG-SQC column (30 m × 0.25 mm i.d., 0.25 µm film thickness). The GC operational parameters included injector temperature 250 °C, detector temperature 260 °C, oven temperature program 60 to 260 °C at a heating rate of 4 °C/min, carrier gas helium at a flow rate of 1.0 mL/min, and sample injection volume 1 µL in split mode with a split ratio of 1:10. The mass spectrometer was operated in electron ionization (EI) mode with the following parameter values: ionization energy 70 eV, interface temperature 280 °C, ion source temperature 230 °C, MS quadrupole temperature 200 °C, and scan range 35–650 amu [13]. The retention indices of the essential oil constituents were determined using an HP-5 MS column and standard C7–C30 straight-chain hydrocarbon reference standards (Sigma-Aldrich Chemical Company, USA). The mass spectra and retention indices of individual compounds were identified by comparing them with those in GC-MS libraries (National Institute of Standards and Technology-NIST 08 and Wiley 09th version) and/or with published data. The relative percentages of the identified compounds were calculated based on GC peak areas without applying correction factors.

2.5. Statistical Analysis

All experiments were carried out in triplicate. Analysis of variance (ANOVA) and Statistica 5.5 software (StatSoft Inc., Tulsa, OK, USA) were utilized to analyze the results. The results are presented as the mean ± standard deviation (SD).

3. Results

3.1. Chemical Composition of *Cymbopogon citratus*

Essential oil from the *Citrus Nobilis* was obtained using steam distillation, yielding 0.42% (w/w, based on fresh weight). The chemical composition of the essential oil was identified using gas chromatography–mass spectrometry (GC-MS), as shown in Figure 1 and Table 1.

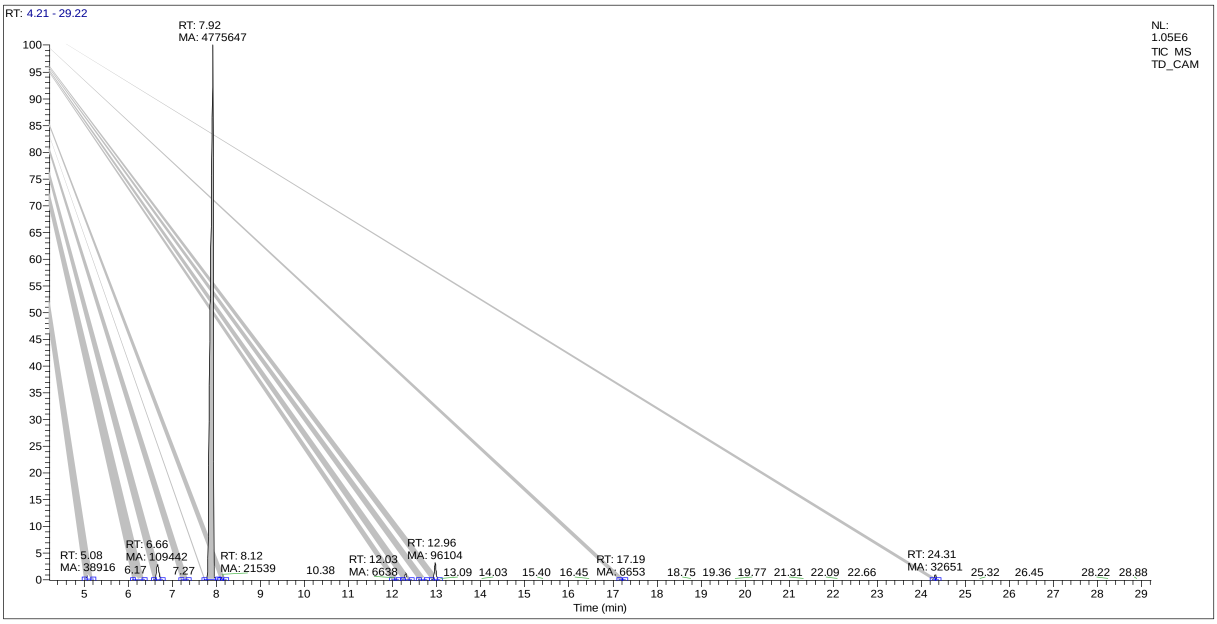

Fig 1: GC-MS total ion chromatogram of *Citrus Nobilis* essential oil.

Table 1: Chemical compositions from the essential oil of *Citrus Nobilis.*

Peak	Retention time (min)	Relative amount (%)	Compounds	Molecular fomular
1	5.08	0.75	α-Terpinene	$C_{10}H_{16}$
2	6.17	0.58	α-Thujene	$C_{10}H_{16}$
3	6.66	2.11	2-β-PINENE	$C_{10}H_{16}$
4	7.27	0.24	Myrtenol	$C_{10}H_{16}O$
5	7.92	91.96	D-Limonene	$C_{10}H_{16}$
6	8.12	0.41	(-)-carvyl acetate	$C_{12}H_{18}O_2$
7	12.03	0.13	trans-2-Caren-4-ol	$C_{10}H_{16}O$
8	12.29	0.85	Carveol	$C_{10}H_{16}O$
9	12.71	0.37	Z-Citral	$C_{10}H_{16}O$
10	12.96	1.85	cis-limonene oxide	$C_{10}H_{16}O$
11	17.19	0.13	L-CARVEOL	$C_{10}H_{16}O$
12	24.31	0.63	p-Mentha-1(7), 8(10)-dien-9-ol	$C_{10}H_{16}O$
Total number of constituents			12	
Total number of constituents identified (%)			12 (100.00%)	
Total number of monoterpenes (%)			4 (33.33%)	
Total number of different compounds (%)			0 (0.00%)	

GC-MS analysis of the *Citrus Nobilis* essential oil sample from Vietnam identified 12 compounds, of which 12 were characterized, accounting for 100.00% of the total composition. The major constituents included: D-Limonene (91.96%), 2-β-pinene (2.11%), cis-limonene oxide (1.85%), Carveol (0.85%), α-Terpinene (0.75%), p-Mentha-1(7), 8(10)-dien-9-ol (0.63%), α-Thujene (0.58%).

3.2. Discussion

The distribution of constituents at these proportions indicates that the essential oil sample from Dak Lak exhibits a distinct chemical profile compared with many previous reports from both within and outside Vietnam. The present study identified 12 compounds in *Citrus Nobilis* essential oil from Đak Lak, primarily belonging to the monoterpene hydrocarbon and oxygenated monoterpene groups. Of which, D-Limonene accounts for the majority (91.96%), which is the main characteristic compound of orange peel essential oil. The next important components include 2-β-pinene (2.11%), Cis-Limonene Oxide (1.85%), α-Terpinene (0.75%), p-Mentha-1(7), 8(10)-dien-9-ol (0.63%), α-Thujene (0.58%). Several other monoterpene oxygenated compounds were present at lower concentrations, such as myrtenol (0.24%), Carveol (0.85%), L-Carveol (0.13%), Trans-2-Caren-4-ol (0.13%) and (-)-Carvyl Acetate (0.41%). The Z-citral content in the sample was relatively low, reaching only 0.37%. The above results show that the Dak Lak essential oil sample has a typical chemical structure of orange peel essential oil, in which limonene is absolutely dominant, and at the same time shows the diversity of oxygenated monoterpenes, which contribute to the characteristic aroma of *Citrus nobilis* essential oil.

Compared with previous studies, D-limonene was consistently the major component in the essential oil of orange peel or *Citrus nobilis*, but the specific ratio and diversity of other minor compounds varied according to geographical regions. The results of Phong Xuan Huynh's group showed that the essential oil had a D-Limonene content of 90.42%, followed by β-Myrcene 4.7% and α-Pinene 1.22% [16]. Compared with the Dak Lak sample, the D-Limonene content was similar, however, β-Myrcene in the Dak Lak sample was lower, and monoterpene oxidized compounds such as cis-limonene oxide, Carveol and L-carveol appeared, which reflected a higher chemical diversity in the orange essential oil sample obtained in Dak Lak.

Compared with the research results of the author group

Truong Thi Thu Hien, through GC-MS analysis, 7 components were detected in the orange essential oil of this study. In which, D-Limonene compound is considered the main component with 97.72%, followed by B-Myrcene (1.39%), the remaining compounds such as α-Pinene, β-Pinene, α-Copaene, β-Cadinene and β-Cubebene account for less than 1% [17], the essential oil composition from Dak Lak shows a wider spectrum of compounds (12 components) and a lower D-Limonene ratio (91.96%), showing a more diverse chemical structure. In addition, the study by Yuniantari *et al*. showed that the two main volatile compounds were β-myrcene (3.75%) and dl-limonene (41.08%) [18], with retention times of 2.501 min and 3.113 min, respectively. Compared with the Dak Lak sample, the dl-limonene content was more than twice as high, while β-myrcene was lower. Similarly, the study by Dao Tan Phat *et al*. (2019) identified only three components, with Limonene accounting for 98.343%, followed by β-Myrcene (1.137%) and α-Pinene (0.520%) [19]. In contrast, our results showed a more complex and diverse chemical composition, including several oxygenated monoterpenes such as carveol isomers and limonene oxide which were not present in the study. The significantly lower D-Limonene content in our sample further emphasizes geographical and ecological differences also significantly influence the chemical composition of essential oil samples.

In the study by Tran Thi Ngoc Bich *et al*., five monoterpene compounds accounted for a total of 99.77% in the essential oil of *Citrus nobilis* peel, of which D-limonene accounted for 96.60%, β-myrcene 2.17%, α-thujene 0.14% and α-pinene 0.80% [20]. When compared with the results of our study in Dak Lak, α-thujene was detected at a concentration of 0.58%, about four times higher than that of the published sample. In addition, the essential oil from Dak Lak contained several additional oxidized monoterpene compounds such as carveol, L-carveol, cis-limonene oxide and (-)-carvyl acetate, which were not reported in previous studies. This shows enhanced chemical diversity in *Citrus nobilis* essential oil from Dak Lak, reflecting the influence of the specific ecological conditions of the highlands on the chemical composition of the essential oil.

The study by Nevcihan Gusoy *et al*. identified 14 components accounting for 99.11% of the oil, with Limonene accounting for 76.77% [21]. Compared to this result, the Dak Lak sample showed a much higher D-Limonene content (91.96%), close to the high limonene chemical phenotype commonly found in Southeast Asian citrus species.

Limonene is a monocyclic monoterpene widely known in foods, fruits, vegetables and spices [22]. Previous studies on Citrus sinensis orange peel essential oil from Ben Tre also recorded extremely high Limonene content (98.34%) [23], and a study by Duyen *et al*. showed that Limonene was the main component (90.42%) in C. nobilis orange peel essential oil from Can Tho [24]. The Limonene content of our Dak Lak sample (91.96%) falls within this typical high limonene content range but is still accompanied by a richer chemical composition that sets it apart from these studies.

In contrast, the study by S. Selli, T. Cabaroglu and A. Canbas showed that the volatile composition of Kozan orange juice was significantly more complex, comprising 34 compounds, including esters, aldehydes, alcohols, terpenes, terpenols and ketones, with limonene being one of the main components [25]. Although the matrix (orange juice) was different from the essential oil, this comparison highlighted that Dak Lak essential oil maintained a narrower but characteristic chemical distribution, predominantly monoterpenes, in contrast to the broader volatile spectrum observed in citrus juice.

Furthermore, GC-MS analysis by Nguyen Van Loi, Nguyen Thi Minh Tu and Hoang Dinh Hoa identified 21 components in the essential oil of C. nobilis orange peel from Ham Yen, including L-Limonene, α-Bergamotene, δ-3-Carene, β-Pinene, α-Thujene and others [26]. Compared to their 21-component profile, the Dak Lak sample contained fewer components (12), but the high content of D-Limonene (91.96%) and the presence of oxygenated monoterpenes such as carvyl acetate, limonene oxide and carveol isomers illustrate a unique chemical signature.

The highlight of the *Citrus Nobilis* sample collected in Dak Lak sample lies in the combination of a very high D-limonene content (91.96%) and the chemical diversity of minor monoterpenes, oxygenated monoterpenes, and alcohols, resulting in a distinctive chemical fingerprint that differs markedly from samples from the North and Central regions of Vietnam, as well as from many international studies, where D-limonene sometimes dominates or other minor compounds are present at higher proportions. This difference may be attributed to the unique ecological conditions of the Central Highlands, including strong sunlight, large temperature fluctuations, low humidity, and specific soil characteristics, all of which directly influence the biosynthesis of monoterpenes and oxygenated monoterpenes in the fruit.

Although present at low levels, minor compounds such as cis-limonene oxide, carveol, L-carveol, and p-mentha-1(7), 8(10)-dien-9-ol play a crucial role in shaping the sensory properties and aroma of the essential oil. This indicates that *Citrus nobilis* peel essential oil from Dak Lak is not only distinguished by its high limonene content but also possesses significant potential for applications in food, pharmaceuticals, and the fragrance industry.

4. Conclusion

This study is the first to investigate the essential oil from the *Citrus Nobilis* collected in Dak Lak Province in Vietnam. GC-MS analysis identified a diverse chemical composition comprising 13 natural components. The major constituents were D-Limonene (91.96%), 2-β-pinene (2.11%), cis-limonene oxide (1.85%), Carveol (0.85%), α-Terpinene (0.75%), p-Mentha-1(7), 8(10)-dien-9-ol (0.63%), α-Thujene (0.58%). This result is the first step to provide more information on the antibacterial ability of *Citrus Nobilis* essential oil in Dak Lak, as a basis for future research and applications.

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