CHEMICAL COMPOSITIONS AND ANTIMICROBIAL EFFICACY OF ESSENTIAL OILS FROM *CLAUSENA LANSIUM* (LOUR.) SKEELS PEELS IN THO XUAN DISTRICT, THANH HOA PROVINCE

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Received: 13 May 2024/ Accepted: 15 July 2024/ Published: August 2024

Https://doi.org/10.70117/hdujs.E9.2024.635

Abstract: *The study investigated chemical compositions and antimicrobial efficacy of essential oils from peels of Clausena lansium (Lour.) Skeels. Samples were collected in Tho Xuan district, Thanh Hoa province, in July 2023. Analysis using GC and GC-MS methods revealed that fresh peels of Clausena lansium contained 0.04% (w/w) of the essential oils. Twenty-three compounds were identified, representing 99.57% of the total essential oils. The most abundant constituents were β-phellandrene, α-phellandrene, myrcene, and α-pinene. These compounds comprised 73.73%, 7.19%, 2.6%, and 2.31% of total essential oils, respectively. The results of the antimicrobial activity test showed that essential oils from Clausena lansium (Lour.) Skeels peels inhibited the growth of Aspergillus niger fungus with a minimum inhibitory concentration (MIC) value of 256 µg/mL.*

Keywords: *Clausena, Clausena lansium, chemical compositions, antimicrobial activity*.

1. Introduction

Clausena lansium (Lour.) Skeels (*C. lansium)* a subtropical fruit, belonging to the family Rutaceae, characterized by its unique flavor, high nutrient content, and medicinal value [1]. This species originated from Southern China and is now commercially cultivated in China, Vietnam, Thailand, Malaysia, the Philippines, and India. It has also been introduced to Queensland, Hawaii, and Florida [1, 2]. In Vietnam, this species is primarily grown in the northern and central regions. Notably, Thanh Hoa province boasts a large number of *Clausena lansium* trees cultivated in districts such as Tho Xuan, Trieu Son, Thach Thanh, Bim Son, and Ha Trung.

Clausena lansium fruit is roughly 2.0 cm in diameter, oval-shaped, and typically contains 1-5 seeds. Due to variations in geography and climate, its fruit normally ripens between May and August. The fruit has a yellow peel that's usually discarded before eating. The primary edible part is the pulp, which is rich in valuable compounds. As a good source of vitamins, *Clausena lansium* pulp has vitamin C (548 mg/kg), vitamin E (1.58 mg/kg), vitamin B1 (1.35 mg/kg), vitamin B2 (0.72 mg/kg), niacin (0.33 mg/kg), and β-carotene (0.016 mg/kg) [4]. Additionally, the pulp contains nine essential amino acids (histidine,

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isoleucine, leucine, lysine, methionine, phenylalanine, threonine, valine, and arginine) and free sugars (fructose, glucose, sucrose) [4]. It's important to note that the specific content of these constituents in the fruit can be variable depending on growing conditions, geographic location, and analytical methods used.

Beyond its enjoyment as fresh fruit *C. lansium* offers versatility in food processing. In Southeast Asia, particularly Thailand, *Clausena lansium* fruits are transformed into long-lasting delights like jams, beverages, preserved fruits, and even fermented wine, with dried *C. lansium* being a popular ingredient.

Not only being consumed as a typical food but *C. lansium* also holds medicinal value in traditional Chinese medicine. All parts including leaves, stem, bark, root, peel, and seeds, are believed to have therapeutic properties. This fact is due to different components with great biological activity such as antibacterial, antifungal, and antimalarial activities. The extract of leaves and peel was applied to treat certain dermatological disorders, acute, chronic viral hepatitis, and asthma. In order to treat addressing bronchitis and malarial fever, the root, bark, peel, and stem were used while the pulp and seed were used for alleviating symptoms of cough, dyspepsia, and specific gastrointestinal disorders. The *C. lansium* leave decoction is also used as a hair wash to eliminate dandruff and maintain hair color.

Our study aimed to identify the chemical compositions and to investigate antimicrobial efficacy of essential oils from *Clausena lansium* (Lour.) Skeels peels which were collected in Tho Xuan district, Thanh Hoa province.

2. Materials and methods

2.1. Materials

C. lansium peels were collected in Tho Xuan district, Thanh Hoa province, in July 2023 and were identified by Dr. Trinh Thi Huong (Faculty of Natural Sciences, Hong Duc University with the scientific name as *Clausena lansium* (Lour.) Skeels. A voucher specimen was deposited at the Biological Laboratory, Faculty of Natural Sciences, Hong Duc University.

The standard strains included Gram-negative bacteria (*Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027); gram-positive bacteria (*Bacillus subtillis* ATCC 6633, *Staphylococcus aureus* ATCC 6538); filamentous fungi (mold) (*Aspergillus niger* ATCC 9763, *Fusarium oxysporum* ATCC 48112); yeast (*Candida albicans* ATCC 10231, *Saccharomyces cerevisiae* ATCC 16404). The standard strains (ATCC, Manassas, USA) were provided by the National Institute of Drug Quality Control and stored at the Laboratory of Experimental Biology (Institute of Natural Products Chemistry).

2.2. Chemicals

Tryptic Soy Agar (TSB-Merck); media used for yeast and mold: Saboraud 4% Dextrose Agar (SDA; Merck, Damstadt, Germany).

Standard antibiotics: Gentamycin for Gram-negative bacteria, doxycycline for Gram-positive bacteria and nystatin for filamentous fungi and yeast; antibiotics were provided by Institute of the Drug Quality Control Ho Chi Minh City.

2.3. Methods

2.3.1. The essential oil extraction

One kg (1kg) of *Clausena lansium* peels was cut into small pieces and distilled using the method of steam distillation for 2 hours at normal pressure according to Vietnam Pharmacopoeia V (2017) [11].

2.3.2. The essential oil analysis

The essential oil was analyzed by both GC and GC-MS at the Institute of Natural Products Chemistry, Vietnam Academy of Science and Technology. The details of the GC and GC-MS methods, including column specifications, temperature programs, and identification procedures, are provided.

Gas chromatography (GC): Performed on an Agilent Technologies HP 6890N Plus attached to an FID detector from Agilent Technologies, USA. The HP-5MS chromatography column with a length of 30 m, internal diameter (ID) of 0.25 mm, thin film layer of 0.25 µm was used. The hydrogen was as a carrier gas. The temperature was programmed from 60^{o}C (2 minutes) to 220^{0}C at rate of 40C/minute and then stopped at this temperature for 10 minutes. The used injector and detector temperature were 250^{o}C and 260^{o}C, respectively.

Gas chromatography-mass spectrometry (GC-MS): Qualitative analysis was performed on a gas chromatography and combined spectrometry GC-MS system (Agilent Technologies HP 6890N). The HP-5MS column was 0.25 µm x 30 m x 0.25 mm while the HP1 was 0.25 µm x 30 m x 0.32 mm. The temperature was programmed from 60^{o}C (2 minutes) to 220^{o}C at a rate of 4^{o}C /minute and then increased to 260^{o}C at a rate of 20^{o}C /minute. Heli was a carrier gas. The confirmation of the components is performed by comparing their MS spectrum data with published standard spectra available in the Willey/Chemstation HP library.

2.3.3. Investigation of antimicrobial activity

The essential oils were obtained by steam distillation, and used to investigate the antimicrobial activity.

Diluted the sample with 10% DMSO on a 96-well plate in decreasing concentrations (log2 for 5 concentration ranges). Drop the sample from the template plate onto a 96-well plate (the test plate) and add microbial solution to obtain a sample concentration range of 256-128-64-32-16 µg/mL (repeat 3 times at each concentration). Stored in the incubator at 37^{o}C /24h for bacteria and 30^{o}C/48h for fungi.

0.9% NaCl solution is used as negative control (-) and standard antibiotics are used as positive control (+). A sample is considered to have antifungal and/or antibacterial activity when there is no growth of microorganisms at least one concentration of the test sample compared to the control (-) (*in case of culturing again at this concentration, check on the agar plate that the CFU value is less than 5*). Samples showing antimicrobial activity are tested at a range of different sample concentrations to determine the minimum inhibitory concentration (MIC (µg/mL) which is the lowest test concentration at which microorganisms are inhibited. The sample is determined to be antifungal and/or antibacterial activity when MIC is less than 56µg/mL.

3. Results and discussion

3.1. The essential oils from Clausena lansium peels

Plant - derived natural compounds have attracted much attention from the scientific community. The essential oils are natural bioactive compounds that could be detected in fruits, seeds, flowers and leaves of many plants. *Clausena lansium*, a China - originated small tree, has a great economic and nutrient value. Several previous reports indicated that the essential oil of *Clausena lansium* possess many beneficial biological activities such as anti-inflammatory, antibacterial, antifungal and anticancer activities.

In our study, the *essential oils* were isolated from *C. lansium* peels which were collected in Tho Xuan district, Thanh Hoa province, in July 2023. The *essential oils* were isolated and identified from *C. lansium* peels using both gas chromatography (GC) and gas chromatography and mass spectrometry (GC-MS). The analysis revealed that the **fresh peels yielded 0.04%** of their weight in essential oils. The essential oil is yellow, lighter than water (relative density at 20^0C is 0.8884), and has a fragrant scent. The results of chemical compositions of essential oils from *C. lansium* peels of are presented in Table 1.

***Table 1**. The chemical compositions of essential oils from C. lansium peels*

Number	RI	Chemical name	%FID
1	**939**	***α* - Pinene**	**2.31**
2	978	Sabinene	0.75
3	**992**	**Myrcene**	**2.60**
4	**1010**	***α* - Phellandrene**	**7.19**
5	1022	*α* - Terpinene	0.94
6	1030	*o* - Cymene	0.95
7	**1038**	**β- Phellandrene**	**73.73**
8	1049	(E) -*β*-Ocimene	0.14
9	1063	*γ* - Terpinene	0.64
10	1094	Terpinolene	0.37
11	1102	Linalool	0.46

Number	RI	Chemical name	%FID
12	1129	Dehydro - Sabina ketone	0.30
13	1186	Terpinen-4-ol	0.86
14	1196	Cryptone	0.62
15	1431	α - Santalene	0.17
16	1436	β - Caryophyllene	0.26
17	1518	β - Bisabolene	0.16
18	1521	β - Curcumene	0.20
19	1542	(E) - γ Bisabolene	0.16
20	1570	(E) - Nerolidol	0.48
21	1692	(Z)- α - Santalol	0.33
22	**1698**	**unknown (93,218, RI 1698)**	**3.75**
23	1764	α - Sinensal	0.54
		Total	**97.92**
		Monoterpene hydrocarbons	89.62
		Oxygenated monoterpenes	1.62
		Sesquiterpene hydrocarbons	0.95
		Oxygenated hydrocarbons	1.35
		Others	4.37

Note: FDI - Flame ionization detection

According to the GC and GC-MS analysis, twenty-three compositions of essential oils from *C. lansium* peels were detected, accounting for 97.92% of the total components. The composition of essential oil included monoterpenes (91.24%), sesquiterpenes (0.95%), oxygenated hydrocarbons (1.35%) and other compounds (4.37%). In which, four most abundant components were β-phellandrene (73.73%), α-phellandrene (7,19%), myrcene (2.60%) and α-pinene (2,31%) (Table 1).

Our numbers differed slightly from the findings by Xin Huang et al. (2023) who reported that the main essential oils obtained from the *C. lansium* could be 4-terpineol (26.94%), followed by γ-terpinene (14.39%), β-phellandrene (8.24%), sabinene (5.58%), and cymene (5.01%) [4]. The results could be explained by the variations in factors like temperature, and pressure of various extraction methods. However, the presence of β-phellandrene as a major component was consistent with Xin Huang et al (2023) [4, 5].

β-phellandrene was a cyclic monoterpene and a potential antifungal compound, that has been found in various plants. Examining the treatment of five *Candida* species with β-phellandrene, Ma et al. (2021) found out the inhibition zone diameters ranged from 15.6 mm to 22.1 mm [5]. In addition, using the essential oil extracted from *C. lansium*, *Candida. glabrata* was almost inhibited with the inhibition zone diameter of 23.1mm

[12]. These findings could demonstrate the potential antifungal effects of *β*-phellandrene isolated from *C. lansium*.

α-phellandrene was the second most abundant essential oils found in *C. lansium* peels with 7.19%. P. Chokeprasert et al (2005) indicated that the percentage of *α*-phellandrene in the skin of Thailand *C. lansium* fruits was 10.6%) which was much higher than that in our finding.

The third most common compound in *C. lansium* peels selected in Tho Xuan district, Thanh Hoa province was myrcene. Myrcene is an abundant monoterpene that occurs as a major constituent in many plant species. The biological properties of this compound could be anxiolytic, antioxidant, anti-aging, anti-inflammatory, and analgesic properties [4]. Therefore, the compound is usually a main ingredient in products that help relieve pain, relaxation, and promote good sleep. P. Chokeprasert et al. (2006) report the main components obtained from the *C. lansium* seed were monoterpenes (98%), sabinene (84%), *α*-pinene (4.3%), aphellandrene (3.1%) and myrcene (2.9%) [2]. The rate of myrcene in their study was quite the same as in our study (2,6%). X. Huang et al (2023) reported that the rate of myrcene in *Clausena lansium* peel was 3.15% while in other parts, it was 1.1% in leaves, 1.7% in pulp, 2.94% in seed [4].

The fourth most abundant essential oils from *C. lansium* peels in our study was *α*-pinene (2.31%). In comparison with X.Huang et al (2023) report, that rate of *α* - pinene was 9.41%, 1,99%, 2,08%, 4,26% in peel, leaves, pulp and seed, respectively [4]. *α*-pinene, an organic compound of the terpene class, is one of the two isomers of pinene, containing a reactive four-membered ring. This compound can be found in the essential oils of many plants of coniferous trees, especially the Pinus and Picea species. W. Abdelli et al. (2018) showed that the rate of *β*-phellandrene and *α*-pinene isolated from leaves and berries of Algerian *Juniperus phoenicea* were positively correlated with the antibacterial activity [10]. Applying GC-MS method, T.H.Thai et al (2020) identified the chemical composition of essential oils from the needles and branches of the abies *delaveyi Franch* subsp. *Fansipanensis Rushforth* in Lao Cai province, in which the rate of *α*-pinene was 22,28%, *β*-phellandrene was 17.8%, and myrcene was 4.35% [3].

3.2. The antimicrobial activity of the essential oils from C. lansium peels

The essential oils from *C. lansium* peels was evaluated for its antimicrobial activity. It demonstrated positive results against some bacterial strains. Notably, the essential oils from *C. lansium* peels inhibited the growth of the fungus *Aspergillus niger* with a minimum inhibitory concentration (MIC) of 256 µg/mL. However, this antifungal activity was not observed against the filamentous fungus *Fusarium oxysporum.*

Furthermore, the sample did not exhibit any inhibitory effects on the tested Gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa*), Gram-positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus*), or yeast (*Candida albicans*, *Saccharomyces cerevisiae*) (Table 2).

Table 2. *The antimicrobioal activity of the essential oils from C. lansium peels*

Minimal inhibition concentration (MIC, μg/mL)							
Gr (-)		**Gr (+)**		**Filamentous fungi**		**Yeast**	
E. coli	*P. aeruginosa*	*B. subtillis*	*S. aureus*	*A. niger*	*F. oxysporum*	*S. cerevisiae*	*C. albicans*
-	-	-	-	256	-	-	-

Note: *(-): Not determined (not showing activity at the tested concentration)*

Results obtained in this research work revealed the inhibitory potential of the essential oils from *C.lansium* peels against *Aspergillus niger*. The antifungal activity is closely related to their components and contents. In this study, 23 chemical compositions were found, in which four main components were *β*-phellandrene, *α*-phellandrene, myrcene and *α*-pinene.

In earlier studies, inhibition of *Aspergillus niger* by the plant extracts were performed. O. Olakunle et al. (2019) examined the antifungal activity of extracts from several fruit peels (orange, pineapple, cashew, and banana) [8]. The obtained findings showed that the mycelia growth of *Aspergillus niger* was effectively inhibited by these fruit peel extracts. The efficacy order of the fruits was follow: banana, pineapple, cashew, orange [8]. V. Bobbarala et al. (2009) applied fort-nine different plants normally used in traditional Indian medicine to examine the resistance to *Aspergillus niger* [9]. The result pointed out that 86% of tested plants showed inhibition of mycelial growth of *Aspergillus niger*. This activity could be explained by the presence of methanolic chemicals in these plant extracts [9]. Moreover, in our work, the antifungal activities varied with *A. niger* and *F. oxysporum*. This may be due to genetic differences between these two species.

Several previous studies indicated that the different isolated compounds from the *Clausena lansium* showed potential antifungal activity. For example, Yan et al. isolated seven amide compounds from the seeds of *C. lansium* and used for investigation the antifungal activity [7]. The results of the antifungal assay revealed that lansiumamide B and C had excellent antifungal activity against *Sclerotinia sclerotiorum* [7]. Moreover, another part of the *Clausena lansium* (twigs and roots) also showed antimicrobial activity [4]. Y. Ma et al (2021) investigated the antifungal activity of essential oils of *C. lansium* seeds via examining three main components (sabinene, β-phellandrene and 4-terpineol) [5]. The results showed the differences in the antifungal activity efficacy among different types of strains. The antifungal activity of *Clausena lansium* extract against *C. glabrata* and *C. krusei* was higher than that of the Candida strains. In addition, the antifungal efficacy order was as follows 4-terpineol, *β*-phellandrene, sabinene. Interestingly, *β*-phellandrene was the main component isolated from the *C. lansium* peels in our study. This may explain the inhibitory effect of essential oils from *C. lansium* peels against *Aspergillus niger* observed in this study.

4. Conclusion

In conclusion, this study successfully isolated and identified twenty-three chemical compounds of the essential oils from fresh *Clausena lansium* peels collected in Tho Xuan district, Thanh Hoa province, Vietnam. These compounds accounted for 97.92% of the total essential oils, with *β*-phellandrene, *α*-phellandrene, myrcene, and *α*-pinene being the most abundant. The essential oils exhibited antifungal activity against *Aspergillus niger*, suggesting their potential applications. Our findings provide a valuable foundation for further research on the metabolic pathways and toxicological properties of bioactive substances isolated from different parts of *C. lansium.*

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