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CHEMICAL COMPOSITION, ANTIMICROBIAL AND CYTOTOXIC ACTIVITIES OF THE LEAF ESSENTIAL OIL OF *KNEMA GLOBULARIA* (LAMK.) WARB. FROM THANH HOA, VIETNAM

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Summary

This study reports the chemical composition and, for the first time, evaluates the antimicrobial activity and cytotoxic effects of the leaf essential oil of *Knema globularia* (Lamk.) Warb. collected in Thanh Hoa Province, Vietnam. The essential oil was obtained by hydrodistillation and analyzed by GC–MS, leading to the identification of 47 compounds representing 97.82% of the total essential oil. The major constituents were *β*-(*E*)-caryophyllene (26.24%), *α*-pinene (18.44%), *β*-pinene (13.04%), and (3*E*,6*E*)-*α*-farnesene (14.36%). The essential oil showed selective antimicrobial activity against Gram-positive bacteria, with IC_{50} values of 64.00–80.00 *μ*g/mL, while no significant activity was observed against Gram-negative bacteria or *Candida albicans* at the tested concentrations. In cytotoxic assays, the essential oil exhibited moderate effects against four human cancer cell lines (KB, HepG2, A549, and MCF-7), with IC_{50} values ranging from 44.82 to 61.28 *μ*g/mL. These results suggest that the leaf essential oil of *K. globularia* is a promising natural source for further studies on antimicrobial and anticancer agents.

Keywords: *Knema globularia; Essential oil; GC-MS; Antimicrobial activity; Cytotoxicity.*

1. Introduction

Knema globularia (Lamk.) Warb. belongs to the genus *Knema*, the third largest genus within the family Myristicaceae. This family is well known for its medicinal importance, and many species are traditionally used as herbal remedies. Species of the genus *Knema* contain a wide range of bioactive constituents, including essential oils [1]. In Vietnam, *K. globularia* (Lamk.) Warb. is a medicinal plant widely distributed in low mountainous regions of northern and central Vietnam, including Son La, Phu Tho, and Thanh Hoa provinces. The species, locally known as “mau cho la nho”, has been traditionally used as a blood tonic and for the treatment of scabies, skin ulcers, back pain, and musculoskeletal disorders [2],[3]. Previous phytochemical studies on *K. globularia* have identified a variety of bioactive constituents, including flavonoids, proanthocyanidins, and sterols, while analyses of its essential oil have reported compounds such as β-elemen, *α*-copaene, *β*-caryophyllene, and α-humulene as major components [4],[5]. The chemical composition of leaf essential oils from plants collected in several regions of Vietnam, including Phu Tho (formerly Vinh Phuc Province), Ho Chi Minh City (formerly Vung Tau Province), and Hue, has been described [5],[6],[7]. However, the chemical composition of leaf essential oil from *K. globularia* collected in Thanh Hoa Province has not yet been reported, despite the species being widely distributed and abundant in this region. Several biological activities of *K. globularia* essential oil have been reported, including inhibition of α-amylase and tyrosinase [6], suppression of nitric oxide production [3], and antioxidant effects [7]. Moreover, cytotoxic activities have been reported for a number of compounds isolated from species of the genus *Knema*, indicating potential anticancer properties. Considering the traditional use of this plant for the treatment of skin infections and wounds, antimicrobial activity also represents a relevant biological property to be evaluated. However, antimicrobial and cytotoxic activities of the leaf essential oil of *K. globularia* have not yet been investigated. Therefore, the present study aimed to analyze the chemical composition of the leaf essential oil of *K. globularia* collected in Thanh Hoa using GC–MS and to evaluate its antimicrobial and cytotoxic activities *in vitro*.

2. Materials and methods

2.1. Materials

Fresh leaves of *Knema globularia* (Lamk.)Warb. were collected in Thanh Hoa Province, Vietnam (20° 29′ 68.58″ N, 105° 54′ 05.89″ E) in May 2025. The plant was

identified by Assoc. Prof. Dr. Nguyen Hoang Tuan from the Faculty of Pharmacognosy and Traditional Medicine, Hanoi University of Pharmacy. A voucher specimen (HMC-TH-001) was deposited at the Plant Herbarium of Hanoi Medical College (HMC), Hanoi, Vietnam.

2.2. Methods

2.2.1. Extraction of essential oil:

Fresh mature leaves of *K. globularia* (500 g) were washed and minced into small pieces (≤ 0.5 cm). The plant material was mixed with distilled water at a ratio of approximately 1:3 (w/v), ensuring complete immersion. Hydrodistillation was then carried out using a Clevenger-type apparatus. The process was performed in triplicate and continued until no further essential oil was recovered (approximately 4 h). The obtained essential oil was dried over anhydrous sodium sulfate and stored in amber glass vials at 4°C for subsequent chemical and biological analyses [8].

2.2.2. Gas chromatography–mass spectrometry (GC-MS) analysis:

The volatile composition of *Knema globularia* leaf essential oil was analyzed by gas chromatography–mass spectrometry (GC–MS) using an Agilent 7890B GC system coupled with an Agilent 5977B MSD equipped with an HP-5MS UI capillary column (30 m × 0.25 mm i.d.; 0.25 µm film thickness). The essential oil (1.0 µL injection from a 1:1000 dilution in dichloromethane) was analyzed under the following conditions: carrier gas (helium at 1.0 mL/min), injection mode (split ratio 40:1), MS source temperature (230°C), MS quadrupole temperature (150°C), inlet temperature (300°C), and Aux-2 temperature (250°C). The oven temperature program was initiated at 50°C (3 min hold), increased to 180°C at a gradient of 3°C/min, subsequently increased to 280°C at 5°C/min, and held isothermally for 5 min. Mass spectra were recorded at 70 eV with a scan range of 50–550 *m/z* at a scan rate of 2 scans/s. Identification of the constituents was carried out by comparing their mass spectra and retention indices (RIs), calculated relative to a homologous series of n-alkanes (C_7–C_{30}), with those reported in the NIST 17 and Adams (2007) libraries [9]. The relative percentage of each component was calculated based on the total ion chromatogram (TIC) peak area.

2.2.3. Antimicrobial assay:

The antimicrobial activity of the essential oil from *K. globularia* leaves was evaluated against Gram-positive bacteria (*Staphylococcus aureus* ATCC 13709, *Bacillus subtilis* ATCC 6633, *Lactobacillus fermentum* N4), Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 15442, and *Salmonella enterica*), and yeast (*Candida albicans* ATCC 10231).

The antimicrobial activity was evaluated using the broth microdilution method (serial dilution technique in liquid medium) to assess the antibacterial potency of the tested samples based on the turbidity of the culture medium. The leaf essential oil of *K. globularia* was dissolved in pure DMSO and diluted to obtain concentrations from 1 to 256 μg/mL. Bacterial and yeast strains were prepared at final densities of 5×10^5 CFU/mL and 1×10^3 CFU/mL, respectively. In a 96-well plate, 10 μL of each essential oil concentration was mixed with 190 μL of microbial suspension. The plates were incubated at 37°C for 16–24 hours.

Ampicillin and cefotaxime were used as positive controls for antibacterial activity, while nystatin was used as the positive control for antifungal activity. Dimethyl sulfoxide (DMSO, 100%) was used as the negative control. All experiments were conducted in triplicate [10],[11],[12].

The activity parameters were expressed as IC_{50} (50% inhibitory concentration) and MIC (minimum inhibitory concentration). The MIC value was defined as the lowest concentration of essential oil that completely inhibited visible microbial growth. The IC_{50} value was calculated based on the percentage of growth inhibition using Rawdata software [11].

$$\text{Inhibition (\%)} = \frac{OD_{(+)} - OD_{sample}}{OD_{(+)} - OD_{(-)}} \times 100$$

Where $OD_{(+)}$ is the positive control, $OD_{(-)}$ is the negative control, and OD_{sample} is the absorbance of the treated microbial suspension.

2.2.4. Cytotoxic assay:

The cytotoxic effects of the leaf essential oil of *K. globularia* were evaluated against four human cancer cell lines: KB (human oral epidermoid carcinoma, CCL-17™), HepG2 (human hepatocellular carcinoma, HB-8065™), A549 (human lung carcinoma, CCL-185™), and MCF-7 (human breast adenocarcinoma, HTB-22™). The assay was performed using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] method, originally described by Mosmann (1983) [13],[14],[15].

Briefly, cells were cultured in 96-well plates and treated with two-fold serial dilutions of the essential oil prepared in DMSO (initial concentration 20 mg/mL) to obtain final concentrations of 256, 64, 16, and 4 µg/mL. After 72h of incubation under standard conditions, 10 μL of MTT solution (5 mg/mL) was added to each well and incubated for 4 h. Viable cells reduced MTT (yellow) to insoluble formazan crystals (purple) by mitochondrial dehydrogenase activity. The crystals were dissolved in 100 μL DMSO, and the optical density (OD) was measured at 540 nm using a Biotek microplate reader. The percentage of growth inhibition was calculated as follows:

$$\text{Inhibition (\%)} = \frac{OD_{(+)} - OD_{sample}}{OD_{(+)} - OD_{(-)}} \times 100$$

Where $OD_{(+)}$ is the positive control, $OD_{(-)}$ is the negative control, and OD_{sample} is the absorbance of treated cells.

IC_{50} values were determined from dose–response curves derived from the percentage of cell growth inhibition using Rawdata software [13]. Ellipticine was used as the positive control, while 1% DMSO served as the negative control. All experiments were performed in triplicate.

3. Results and discussion

3.1. Essential oil composition

The essential oil obtained from the leaves of *K. globularia* was pale yellow in color and lighter than water. The yield of the essential oil was 0.16 ± 0.01% (calculated on a fresh weight basis). The chemical composition of the essential oil was analyzed by GC–MS, and the results are presented in **Table 1**.

Table 1. Chemical composition of the essential oil from *K. globularia* leaves

No.	RT (min)	RI (exp.)	RI (lit.)	Compounds	MF	%
1	7.061	929	929	*α*-Thujene	$C_{10}H_{16}$	0.19
2	**7.317**	**937**	**937**	***α*-Pinene**	$\mathbf{C_{10}H_{16}}$	**18.44**
3	7.800	951	952	Camphene	$C_{10}H_{16}$	0.36
4	8.754	975	974	Sabinene	$C_{10}H_{16}$	0.05
5	**8.874**	**978**	**979**	***β*-Pinene**	$\mathbf{C_{10}H_{16}}$	**13.04**
6	9.456	992	991	*β*-Myrcene	$C_{10}H_{16}$	1.02
7	10.793	1026	1025	*p*-Cymene	$C_{10}H_{14}$	0.05
8	10.966	1030	1030	*α*-Limonene	$C_{10}H_{16}$	1.68
9	11.422	1041	1038	*β*-(*Z*)-Ocimene	$C_{10}H_{16}$	0.32
10	11.653	1047	1045	1-Methylhexyl acetate	$C_9H_{18}O_2$	0.37
11	11.873	1052	1049	*β*-(*E*)-Ocimene	$C_{10}H_{16}$	3.89
12	12.282	1061	1060	*γ*-Terpinene	$C_{10}H_{16}$	0.16
13	13.576	1088	1088	*α*-Terpinolene	$C_{10}H_{16}$	0.26
14	14.148	1099	1099	*β*-Linalool	$C_{10}H_{18}O$	0.06
15	14.913	1118	1116	(*E*)-4,8-Dimethylnona-1,3,7-triene	$C_{11}H_{18}$	2.11
16	15.453	1131	1131	(4*E*,6*Z*)-allo-Ocimene	$C_{10}H_{16}$	0.07
17	17.592	1178	1177	Terpinen-4-ol	$C_{10}H_{18}O$	0.07
18	18.132	1188	1187	(*Z*)-Hex-3-enyl butanoate	$C_{10}H_{18}O_2$	0.06
19	18.205	1190	1189	*α*-Terpineol	$C_{10}H_{18}O$	0.26
20	18.347	1193	1192	Methyl salicylate	$C_8H_8O_3$	0.42
21	21.775	1270	1270	Ethyl salicylate	$C_9H_{10}O_3$	0.12
22	24.721	1337	1338	*δ*-Elemene	$C_{15}H_{24}$	0.26
23	26.362	1375	1376	*α*-Copaene	$C_{15}H_{24}$	0.28
24	27.08	1391	1391	*β*-Elemene	$C_{15}H_{24}$	0.31
25	27.672	1405	1406	*β*-(*Z*)-Caryophyllene	$C_{15}H_{24}$	0.82
26	27.793	1408	1409	*α*-Gurjunene	$C_{15}H_{24}$	0.08
27	**28.259**	**1420**	**1419**	***β*-(*E*)-Caryophyllene**	$\mathbf{C_{15}H_{24}}$	**26.24**
28	28.799	1433	1433	*γ*-Elemene	$C_{15}H_{24}$	0.05
29	28.978	1438	1440	Aromadendrene	$C_{15}H_{24}$	0.54
30	29.586	1453	1454	*α*-Caryophyllene	$C_{15}H_{24}$	4.54
31	29.869	1460	1461	Alloaromadendrene	$C_{15}H_{24}$	0.25
32	30.555	1476	1473	*γ*-Gurjunene	$C_{15}H_{24}$	0.16
33	30.891	1484	1486	*β*-Eudesmene	$C_{15}H_{24}$	0.21
34	31.378	1495	1491	(3*Z*,6*E*)-*α*-Farnesene	$C_{15}H_{24}$	1.56
35	31.724	1504	1505	*α*-Bulnesene	$C_{15}H_{24}$	0.2
36	**31.96**	**1511**	**1508**	**(3*E*,6*E*)-*α*-Farnesene**	$\mathbf{C_{15}H_{24}}$	**14.36**
37	32.464	1525	1524	*β*-Sesquiphellandrene	$C_{15}H_{24}$	0.71
38	33.171	1544	1542	*α*-Calacorene	$C_{15}H_{20}$	0.07
39	34.015	1567	1564	(6*E*)-Nerolidol	$C_{15}H_{26}O$	2.17
40	34.136	1570	1568	Palustrol	$C_{15}H_{26}O$	0.09
41	34.424	1578	1576	Spathulenol	$C_{15}H_{24}O$	0.22

No.	RT (min)	RI (exp.)	RI (lit.)	Compounds	MF	%
42	34.618	1583	1581	*β*-Caryophyllene epoxide	$C_{15}H_{24}O$	0.99
43	34.917	1591	1591	Viridiflorol	$C_{15}H_{26}O$	0.14
44	36.227	1633	1631	*γ*-Eudesmole	$C_{15}H_{26}O$	0.08
45	36.547	1643	1640	tau-Cadinol	$C_{15}H_{26}O$	0.05
46	36.898	1655	1655	Pogostole	$C_{15}H_{26}O$	0.23
47	39.886	1765	1762	Benzyl benzoate	$C_{14}H_{12}O_2$	0.21
				Total		**97.82**
	Monoterpene hydrocarbons (Sr. No. 1–9, 11–13, 16)					39.53
	Oxygenated monoterpenes (Sr. No. 14, 17, 19-21)					0.93
	Sesquiterpene hydrocarbons (Sr. No. 22–38),					50.34
	Oxygenated sesquiterpenes (Sr. No. 39–46)					3.97
	Others (Sr. No. 10, 15, 18, 47)					2.75

(RT: Retention time (min); RI (exp.) and RI (lit.): Retention indices obtained from the experiment and the literature, respectively; MF: Molecular formula).

GC–MS analysis identified 47 constituents, accounting for 97.82% of the total essential oil. The essential oil consisted predominantly of sesquiterpene hydrocarbons (50.34%) and monoterpene hydrocarbons (39.53%), followed by oxygenated sesquiterpenes (3.97%), oxygenated monoterpenes (0.93%), and other compounds (2.75%). The major constituents were *β*-(*E*)-caryophyllene (26.24%), *α*-pinene (18.44%), (3*E*,6*E*)-*α*-farnesene (14.36%), and *β*-pinene (13.04%), which together comprised more than 70% of the total essential oil. Other notable constituents included *α*-caryophyllene (4.54%), *β*-(*E*)-ocimene (3.89%), and (6*E*)-nerolidol (2.17%).

A previous study showed that the leaf essential oil of Vietnamese *K. globularia* was mainly composed of sesquiterpenes, with *β*-elemene (25.48%) and *α*-copaene (17.05%) as the major components, and *β*-caryophyllene at 9.37% [16]. The leaf essential oil of *K. globularia* from Thua Thien Hue also contained high levels of sesquiterpenes, especially *β*-caryophyllene (54.11%) and *α*-humulene (12.67%) [17]. In Ba Ria–Vung Tau, leaf essential oils of *K. globularia* were rich in *β*-caryophyllene (76.06%) and *α*-caryophyllene (10.56%), whereas peel essential oils from the same area were mainly composed of *trans*-*β*-ocimene (45.19%) [18]. These results indicate that the Thanh Hoa essential oil differs not only in the number of identified compounds but also in the proportion of monoterpenes and sesquiterpenes. In most previously reported samples, one or two sesquiterpenes accounted for the majority of the essential oil. In contrast, the Thanh Hoa essential oil contained considerable amounts of both monoterpenes and sesquiterpenes. This composition may be related to its potential biological activities, including antimicrobial, anti-inflammatory, antioxidant, and cytotoxic effects. The differences among samples may be explained by geographical factors (soil, altitude, and climate), harvesting time, plant maturity, plant part used (leaves or peels), and variations in hydrodistillation and GC–MS conditions. Overall, the Thanh Hoa sample shows a distinct chemical composition, supporting the importance of regional studies for the development of standardized essential oils of *K. globularia*.

3.2. Antimicrobial activity of the essential oil

To date, no published studies have specifically reported the antimicrobial activity of essential oil obtained from the leaves of *K. globularia*. Previous research has mainly focused on botanical characteristics, chemical composition, antioxidant activity, and enzyme inhibition, without providing quantitative antimicrobial parameters such as IC_{50} or MIC values [16],[18]. To our knowledge, this study provides the first quantitative evaluation of the antimicrobial activity of *K. globularia* leaf essential oil against Gram-positive bacteria, Gram-negative bacteria, and yeast. The antimicrobial activity is summarized in **Tables 2**, **3**, and **4**.

Table 2. Antimicrobial activity of the essential oil of *K. globularia* leaves (EO) against Gram-positive bacteria

Sample		Conc. (μg/mL)	*Staphylococcus aureus*	*Bacillus subtilis*	*Lactobacillus fermentum*
EO	Inhibition (%)	256	92	52	0
		64	8	49	0
		16	0	46	0
		4	0	0	0

Sample		Conc. (μg/mL)	*Staphylococcus aureus*	*Bacillus subtilis*	*Lactobacillus fermentum*
	Inhibition (%)	1	0	0	0
	IC_{50} (μg/mL)		**80.00 ± 4.05**	**64.00 ± 3.98**	>256
	MIC (μg/mL)		256 ± 0.00	>256	>256
Ampicillin	IC_{50} (μg/mL)		0.020 ± 0.005	3.62 ± 0.15	1.03 ± 0.07
	MIC (μg/mL)		0.125 ± 0.000	32 ± 0.00	32 ± 0.00

Table 3. Antimicrobial activity of the essential oil of *K. globularia* leaves (EO) against Gram-negative bacteria

Sample		Conc. (μg/mL)	*Salmonella enterica*	*Escherichia coli*	*Pseudomonas aeruginosa*
EO	Inhibition (%)	256	8	0	0
		64	0	0	0
		16	0	0	0
		4	0	0	0
		1	0	0	0
	IC_{50} (μg/mL)		>256	>256	>256
	MIC (μg/mL)		>256	>256	>256
Cefotaxime	IC_{50} (μg/mL)		0.43 ± 0.05	0.007 ± 0.002	4.34 ± 0.15
	MIC (μg/mL)		32 ± 0.00	0.500 ± 0.000	8 ± 0.00

Table 4. Antimicrobial activity of the essential oil of *K. globularia* leaves (EO) against yeast

Sample		Conc. (μg/mL)	*Candida albicans*
EO	Inhibition (%)	256	29
		64	16
		16	0
		4	0
		1	0
	IC_{50} (μg/mL)		>256
	MIC (μg/mL)		>256
Nystatin	IC_{50} (μg/mL)		1.32 ± 0.05
	MIC (μg/mL)		8 ± 0.0

The results indicate that the essential oil exhibited inhibitory activity only against certain Gram-positive bacteria, while no detectable activity was observed against Gram-negative bacteria or yeast at the tested concentrations. As shown in the tables, the Thanh Hoa leaf essential oil exhibited moderate and selective antimicrobial activity. It inhibited Gram-positive bacteria, with IC_{50} values of 80.00 ± 4.05 μg/mL for *S. aureus* and 64.00 ± 3.98 μg/mL for *B. subtilis*. A MIC value of 256 μg/mL was observed only for *S. aureus*. No inhibitory effect was detected against *L. fermentum*, Gram-negative bacteria (*E. coli*, *S. enterica*, and *P. aeruginosa*), or *C. albicans* at concentrations up to 256 μg/mL. These findings indicate that the essential oil mainly acts against certain Gram-positive strains. The lower activity against Gram-negative bacteria may be related to their outer membrane, which limits the penetration of hydrophobic terpenoids. Although *Lactobacillus fermentum* is a Gram-positive bacterium, the essential oil of *Knema globularia* did not inhibit its growth in this study. This may be due to the intrinsic tolerance of lactic acid bacteria to plant-derived essential oils, associated with adaptive mechanisms such as membrane fatty-acid modification, stress-response systems, and detoxification pathways [19]. Moreover, the frequent association of *Lactobacillus* species with plant environments and fermented foods suggests an evolutionary adaptation to phytochemicals [20].

Compared with other *Knema* species, the Thanh Hoa *K. globularia* essential oil shows a narrower antimicrobial spectrum. Essential oils from leaves and twigs of *K. tonkinensis* have been reported to inhibit a broader range of bacteria and fungi [21]. Similarly, *K. squamulosa* leaf essential oil exhibited moderate activity against *S. aureus*, *P. aeruginosa*, and *C. albicans* (MIC 200 μg/mL), although it showed no effect against *E. coli* or *Bacillus cereus*. These differences among species are likely related to variations in chemical composition [22]. The antimicrobial activity of the Thanh Hoa essential oil may be associated with its major constituents, including *β*-(*E*)-caryophyllene, *α*-pinene, *β*-pinene, and *α*-farnesene. Terpenoids are known to affect microbial cell membranes and disrupt cellular processes. In addition, synergistic interactions among essential oil components may contribute to the observed activity [23]. Although the essential oil is less potent than standard antibiotics, its measurable inhibition of Gram-positive bacteria suggests potential for further investigation, particularly in studies on the mechanism of action, fractionation,

and evaluation of individual or combined constituents. Further research is also needed to determine whether the effect is bacteriostatic or bactericidal and to assess its activity *in vivo*.

3.3. Cytotoxic activity of the essential oil

The cytotoxic activity of the leaf essential oil of *K. globularia* against four human cancer cell lines is summarized in **Table 5**.

Table 5. Cytotoxic activity of the essential oils from *K. globularia* leaves (EO)

Sample		Conc. (μg/mL)	KB	HepG2	A549	MCF-7
EO	Inhibition (%)	256	85	88	91	87
		64	52	56	74	53
		16	16.5	28.5	14	18
		4	2	11	3	5
	IC_{50} (μg/mL)		**61.28 ± 1.97**	**53.81 ± 3.10**	**44.82 ± 1.81**	**59.89 ± 1.94**
Ellipticine	IC_{50} (μg/mL)		0.46 ± 0.02	0.47 ± 0.02	0.45 ± 0.02	0.46 ± 0.02

The leaf essential oil of *K. globularia* collected in Thanh Hoa province exhibited moderate and selective cytotoxic activity against human cancer cell lines. The IC_{50} values were 61.28 ± 1.97 μg/mL (KB), 53.81 ± 3.10 μg/mL (HepG2), 44.82 ± 1.81 μg/mL (A549), and 59.89 ± 1.94 μg/mL (MCF-7). The US National Cancer Institute (NCI) classifies the cytotoxic activity (IC_{50}) values as follows: $IC_{50} < 20$ µg/mL in the high category, IC_{50} 20–100 µg/mL in the moderate category, IC_{50} 101–500 µg/mL in the weak category, and $IC_{50} > 500$ µg/mL in the inactive category as an anticancer [24]; therefore, the present essential oil can be classified as moderately active. Slightly stronger inhibition was observed against A549 cells, suggesting some degree of cell-line selectivity. The observed cytotoxicity may be related to the high content of *β*-(*E*)-caryophyllene and other sesquiterpenes, including *α*-farnesene. *β*-Caryophyllene has been reported to show antiproliferative effects in several cancer cell lines and may enhance activity in complex essential oil mixtures [25],[26]. However, the overall potency of the essential oil remains lower than that of the positive control, ellipticine.

Compared with root-derived extracts of *K. globularia*, the leaf essential oil shows a different activity pattern. Most crude root extracts were inactive against KB and MCF-7 at concentrations above 50 μg/mL, while only a few isolated compounds displayed moderate cytotoxicity. In contrast, the leaf essential oil demonstrated measurable activity across all tested cell lines. Although its IC_{50} values are higher than those of the most active isolated compounds, the essential oil exhibited activity against multiple cell lines rather than a limited response [5]. Similarly, studies on fruit-derived constituents reported several isolated compounds, including knecorticosanones A–B, of which knecorticosanone B and malabaricone D showed moderate cytotoxicity (IC_{50} = 8.76–18.74 μM), while others were less active [27]. The stronger activity of these isolated metabolites likely reflects the effect of structurally defined non-volatile compounds. In contrast, the cytotoxicity of the leaf essential oil may result from combined or synergistic effects among volatile terpenoids.

Overall, these findings indicate organ-dependent variation in the cytotoxic profile of *K. globularia*. Root and fruit extracts, rich in non-volatile secondary metabolites, tend to show stronger but more compound-specific activity, whereas the leaf essential oil exhibits moderate activity across several cell lines. Further studies are needed to clarify the mechanisms involved and to evaluate possible synergistic interactions among its major constituents.

4. Conclusions

This study reports the chemical composition of *K. globularia* leaf essential oil from Thanh Hoa province, Vietnam, and presents the first report on its antimicrobial and cytotoxic activities. GC–MS analysis identified 47 volatile constituents, representing 97.82% of the total essential oil, with sesquiterpene hydrocarbons (50.34%) and monoterpene hydrocarbons (39.53%) as the dominant classes. The major components were *β*-(*E*)-caryophyllene, *α*-pinene, *β*-pinene, and (3*E*,6*E*)-*α*-farnesene. The essential oil exhibited selective antimicrobial activity against Gram-positive bacteria (*S. aureus* and *B. subtilis*), while no significant inhibition was observed against Gram-negative bacteria or *C. albicans*. In addition, moderate cytotoxic activity was detected against four human cancer cell lines (KB, HepG2, A549, and MCF-7). Overall, the results demonstrate a distinct chemical profile and organ-dependent bioactivity of *K. globularia* leaf essential oil from Thanh Hoa. These findings emphasize the influence

of geographical origin on essential oil composition and provide a basis for further phytochemical and pharmacological investigations.

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