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Chemical composition, antibacterial, anticancer, and anti-α-glucosidase activities of essential oils from *Alpinia nelumboides*

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ABSTRACT

The chemical composition and biological properties of essential oils extracted from the rhizomes and leaves of *Alpinia nelumboides* were examined in this study. The oils were isolated via hydrodistillation using a Clevenger apparatus and analyzed with GC-FID/MS. Antibacterial activity against antibiotic-resistant bacterial strains was evaluated using the agar microdilution method. Anticancer and α-glucosidase inhibitory activities were assessed through *in vitro* assays. Eucalyptol was identified as the major component, accounting for 71.23% and 32.82% of the rhizome and leaves oils, respectively. Both rhizome and leaf extracts exhibited antibacterial activity against several antibiotic-resistant bacterial strains, with minimum inhibitory concentration values ranging from 8 to 16 mg/ml. The rhizome essential oil demonstrated anticancer properties against NCI-H460 (IC50 = 98.28 ± 5.22 µg/ml) and HepG2 (IC50 = 189.87 ± 3.16 µg/ml) cell lines. Additionally, it showed α-glucosidase inhibitory activity (IC50 = 2.42 mg/ml). The essential oils obtained from *A. nelumboides* possess promising biological activities, including antibacterial, anticancer, and α-glucosidase inhibitory properties. These findings suggest their potential use as healthcare pharmaceuticals in the future.

INTRODUCTION

The Ginger family Zingiberaceae is among the most sizeable plant families, with approximately 50 genera and over 1,300 species thriving in tropical and subtropical forests [1,2]. *Alpinia* is an important and widespread genus, mainly found in South and Southeast Asia as part of the highly diverse Zingiberaceae family which contains approximately 250 species globally and 35 native to Vietnam [3,4]. These *Alpinia* plants are considered folk medicinal plants and spices in some countries including China, Japan, India, and Vietnam. Parts of the plant are commonly used to treat digestive ailments, upset stomachs, vomiting, and intestinal infections. Many species of *Alpinia* have many different medicinal properties and are used as medicine in Vietnamese traditional medicine [5,6]. Most varieties of *Alpinia* contain essential oils and have many potential applications based on their biological activity. Regarding the chemistry of the genus *Alpinia*, studies have centered on analyzing the essential oil components and connected biological functions—antibacterial, anticancer, antioxidant, and so on, across species such as *A. galanga*, *A. zerumbet*, and *A. cancarata* [6–9].

Recently, we have described a new species in *Alpinia* genus, *Alpinia nelumboides* Nob.Tanaka, T.T.K. Van & V. Hoang in Lam Dong, Vietnam, and Laos. This species has the Vietnamese name "Riềng sen" [10] (https://www.ipni.org/n/77315593-1). Thus far, the only studies on this recently documented species have examined the pseudo-stem and rhizome essential oils, analyzing their chemical profile and antioxidant potential [11]. However, other biological activities have not been reported. Therefore, this investigation

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targets elucidating the constituent compounds within the extracted essential oils of *A. nelumboides* rhizome and leaves samples, followed by an assessment of unreported biological properties. We further evaluated their antimicrobial activities against common pathogenic microbes, cytotoxic effects on cancerous and normal cell lines, as well as α-glucosidase inhibitory properties. Results from the phytochemical profiling and bioassays will enhance the current understanding of the chemicals and bioactive potentials of the rhizome, leaves, and pseudo-stem components of *A. nelumboides*. The findings can provide insight into the medicinal values of *A. nelumboides* to support its potential pharmaceutical applications.

MATERIALS AND METHODS

Materials

Alpinia nelumboides (Fig. 1) were collected in Di Linh District and cultivated in Da Lat City, Lam Dong Province (Vietnam). Taxonomic authentication utilized morphological assessment for the plant was conducted [10], https://www.ipni.org/n/77315593-1), and type specimen (PHH1004920, PHH1004921) was deposited at the PHH herbarium of the University of Science, Ho Chi Minh city. Before experimental use, the materials underwent washing with water, cleaning, and storage in clean air.

The Center for Research and Application in Bioscience in Ho Chi Minh City, Vietnam, provided clinically isolated antibiotic-resistant bacterial strains, including *Enterococcus faecium, Staphylococcus aureus, Acinetobacter baumannii, Enterobacter cloacae, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*, and *Candida albicans*. The Department of Genetics at VNUHCM-University of Science provided NCI H460, HepG2, and fibroblast cell lines. A procurement of apramycin was made from GoldBio (USA). The following items were purchased from Sigma (USA): dimethyl sulfoxide, ethanol, trichloroacetic acid, sulforhodamine B, PBS, DPPH, 4-nitrophenyl-α-D-glucopyranoside, trolox, and α-glucosidase.

Extraction of essential oils

The freshly chopped rhizomes and leaves (400 g each) underwent a 6-hour hydrodistillation process using a 2,000 ml capacity Clevenger-esque apparatus loaded with 1,000 ml of water, according to the Vietnamese Pharmacopoeia (The Committee of Vietnamese Pharmacopoeia 2017). Following separation from the hydrodistillation, the hydrophobic essential oil layers were dehydrated using anhydrous sodium sulfate and then kept refrigerated for preservation before impending analysis. The essential oil yields were averaged over three repeated experiments and calculated based on dividing the weight (g) of the essential oil by the weight (g) of the fresh weight of plant materials.

Analysis of the essential oils by GC-FID/MS

Gas chromatography of the essential oils utilized an Agilent 8,860 GC with an Agilent 122-5532 DB-5MS column (30 m × 0.25 mm × 0.25 µm). The GC oven program ramped from 60°C to −240°C at 3°C/minute with the injector and detector maintained at 250°C. The essential oil sample was

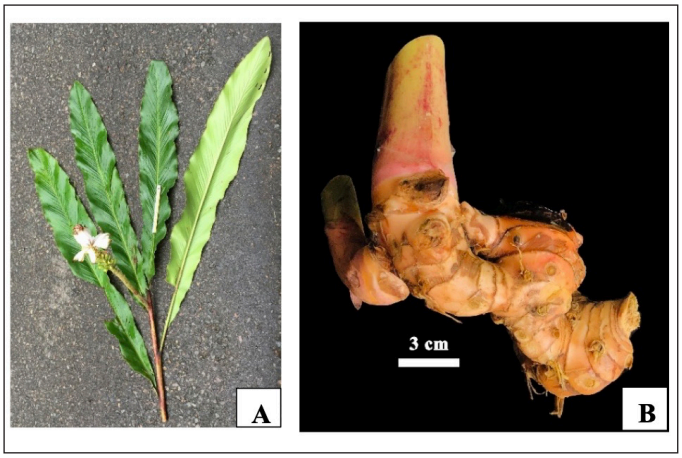

Figure 1. *Alpinia nelumboides* Nob. Tanaka, T.T.K. Van & V. Hoang (A. Leaves, B. Rhizomes).

diluted with hexane solvent at a ratio of 0.1 mg/ml. Samples were injected in splitless mode with 1.00 ml/minute nitrogen carrier gas flow at 11.724 psi. For GC/MS, an Agilent 8,890 GC coupled to a 5977B MSD was equipped with a 19091S-433UI HP-5MS column (30 m × 0.25 mm × 0.25 µm). Helium carrier gas flow was 1.0509 ml/minute at 8.8085 psi. The injection volume was 1.0 µl with a 15:1 split ratio and 70.007 eV ionization voltage [12].

Constituent identification initially utilized comparisons of acquired mass spectra against the NIST 2020 compound library. Supplementary confirmation involved matching retention indices for each constituent with indices reported in the literature, specifically the compilation published by Adams in 2007 [13]. Peak area normalization was used to determine the relative percentage of each component in the oil. Response factors were not computed.

Antimicrobial activity

An agar well diffusion assay assessed essential oil bioactivity against select microbial pathogens [14]. The pathogenic cultures were incubated in nutrient broth at 37°C for 18 hours to reach the exponential growth phase. The cultures were diluted with sterile 0.9% NaCl to produce 1.5×10^8 CFU/ml microbial solutions for each pathogen. On Mueller–Hinton agar (MHA) plates, 100 µl of every microbial solution was distributed. On the MHA plates, 8 mm-diameter wells were created by inserting sterile points. In each well, 25 µl of each essential oil was added. Following 37°C, 16–18 hour incubation of inoculated MHA plates, the potency of test essential oils was assessed by measurements of inhibition zone diameters surrounding wells, compared to apramycin and DMSO as positive and negative controls, respectively.

Each essential oil was added to MHA at a concentration spanning from 0.5 mg/ml to 32 mg/ml at a two-fold dilution to determine the minimum inhibitory concentration (MIC) [15]. On the MHA plates containing a serial dilution of the essential oil, 5×10^5 CFU of each microbial pathogen were deposited. After an 18-hour incubation at 37°C, the MIC was determined as the lowest dilution of oil on MHA that completely inhibited the development of the pathogen. The complete inhibition of microbial growth was

defined as the absence of any visible colonies on the agar plate at the respective essential oil concentration. This conclusion was based on comparing the appearance of colonies to the reference wells containing apramycin and DMSO.

Anticancer activity

The cytotoxicity of essential oils was assessed on three animal cell lines utilizing the Sulforhodamine B (SRB) method [16]. Cells were seeded in 96-well plates at a density of 10^4 cells per well for Hep G2 and fibroblast cell lines and at 7.5×10^3 cells per well for NCI-H460. After 24 hours of culture, the cells were exposed to different concentrations of each essential oil and incubated for an additional 48 hours. The cells were fixed for 1–3 hours with a cold 50% (w/v) trichloroacetic acid solution, rinsed, and stained for 20 minutes with 0.2% (w/v) SRB. Following five treatments with a 1% acetic acid solution, the solubilization of the protein-bound dye was carried out using a 10 mM Tris base solution. At 492 nm and 620 nm, optical density values were determined using a 96-well microtiter plate reader. The growth inhibition percentage (I%) was determined using the formula: I% = (1 - [ODt/ODc]) × 100%, where ODt and ODc denote the optical density values of the test sample and the control sample, respectively. Camptothecin served as the positive control, while DMSO was utilized as the negative control.

Alpha-glucosidase inhibition activity

In a 96-well plate, 120 µl of each essential oil and 20 µl of α-glucosidase at a concentration of 1 unit/ml were dispensed into each well. The mixture was then incubated for 15 minutes at 37°C. Subsequently, 20 µl of a 5 mM p-Nitrophenyl-α-D-glucopyranoside solution was introduced into each well, and the incubation continued for an additional 15 minutes at 37°C. The reaction was terminated by adding 80 µl of a 0.2 M Na2CO3 solution to each well. The quantitative measurement of enzyme activity was performed at an absorbance of 405 nm. All samples were evaluated at five concentrations around the IC50 values. The following equation was used to determine the percentage of inhibition: I (%) = [1—(Asample/Acontrol)] × 100% [17]. The assay used acarbose as the standard control.

Statistical analyses

Statistical analyses were conducted using GraphPad Prism 9. Each experiment was independently replicated at least three times. The data were presented as mean values accompanied by standard deviations and error bars.

RESULTS AND DISCUSSION

Chemical composition of essential oils

Essential oils from the fresh rhizomes and fresh leaves of *A. nelumboides*, growing in Vietnam were yellow and obtained a yield of 0.20% and 0.22% ± 0.02%, respectively. Table 1 and Table 2 (Supplementary Figures 1 and 2) indicate the chemical constituents and their percentages present in the rhizomes and leaves of *A. nelumboides* essential oils. Results revealed 99.75% and 88.19% of detectable substances in rhizomes and leaves essential oils, respectively. There were 14 compounds detected in rhizomes essential oils, while this number was 74 in leaves essential oils.

Notes: The percentage values were determined through FID peak area normalization, employing the response factor exclusively for main components (≥ 10%). LRIs (linear retention indices) on HP-5MS Ultra Inert [Lit: literature [13], Obs: observed].

Compared with rhizome essential oil, leaves essential oil contains a chemical composition with more abundant components. Eucalyptol is known as one main components in the oil with the highest percentage in both rhizome and leaves essential oil (71.23% in rhizome essential oil and 32.82% in leaves essential oil). *A. nelumboides* was morphologically most similar to *A. kwangsiensis*, but the essential oils of their rhizomes were different which was the main constituent of rhizome *A. kwangsiensis* essential oil was terpinene-4-ol (14.3%) [9]. Differences in the number of compounds detected in the rhizome and leaves essential oils were similar to previous research on *Alpinia vietnamica* with 45 compounds detected from leaves and 36 compounds detected from the rhizome [18]. The essential oil from leaves and rhizomes of *Kaempferia galanga* Linn also showed that 108 and 81 compounds were detected, respectively [19]. Interestingly, the essential oil from this species in the publication of Nguyen Hoang Tuan *et al.* [11] shows that rhizome essential oil contains the main components of 8-cineole, linalool, (E)-citral, (Z)-citral,α-pinene, limonene, and β-pinene which was different from our study. Also in the study of Nguyen Hoang Tuan *et al.* [11], the essential oils of rhizome and pseudo-stem had a large amount of polyphenols, which had high antioxidant capacity, while the essential oils in our study showed weak antioxidant activity (IC50 for DPPH assay was over 35 mg/ml). These differences could be explained by the influence of environmental factors. As reported in previous studies, seasonal variations, geographical locations, and weather conditions can

Table 1. Chemical composition of rhizomes *A. nelumboides* essential oil.

No	Compound name	LRI		%GC	Method of identification
		Lit.(*)	Obs.		
1	(2*Z*)-Hexenol	859	859	0.39	LRI, MS
2	Unknown		868	0.26	
3	α-Pinene	932	933	3.97	LRI, MS
4	β-Pinene	974	977	5.34	LRI, MS
5	p-Cymene	1020	1024	0.33	LRI, MS
6	Limonene	1024	1028	2.68	LRI, MS
7	**Eucalyptol**	1026	1031	**71.23**	LRI, MS
8	Linalool	1095	1101	0.65	LRI, MS
9	Camphor	1141	1145	0.27	LRI, MS
10	Terpinen-4-ol	1174	1177	0.34	LRI, MS
11	α-Terpineol	1186	1191	4.10	LRI, MS
12	*neo*-Fenchyl acetate	1218	1221	0.22	LRI, MS
13	Neral	1235	1241	3.47	LRI, MS
14	Geranial	1264	1271	6.45	LRI, MS
15	Geranyl acetate	1379	1385	0.31	LRI, MS

Table 2. Chemical composition leaves *A. nelumboides* essential oil.

No	Compounds	LRI		%GC	Identification method
		Lit.(*)	Obs.		
1	α-Thujene	924	928	0.22	LRI, MS
2	α-Pinene	932	938	4.24	LRI, MS
3	Camphene	946	950	0.35	LRI, MS
4	trans-m-Mentha-2,8-diene	979	985	5.4	LRI, MS
5	β-Myrcene	988	994	1.35	LRI, MS
6	(E)-β-Ocimene	1044	1056	0.18	LRI, MS
7	γ-Terpinene	1054	1065	0.34	LRI, MS
8	p-Cymene	1089	1093	0.35	LRI, MS
9	Linalool	1095	1108	4.15	LRI, MS
10	endo-Fenchol	1114	1118	0.13	LRI, MS
11	cis-p-Menth-2-en-1-ol	1118	1125	0.18	LRI, MS
12	Pinocarveol	1135	1141	0.4	LRI, MS
13	δ-Terpineol	1162	1170	1.64	LRI, MS
14	Terpinen-4-ol	1174	1182	1.84	LRI, MS
15	α-Terpineol	1186	1201	8.19	LRI, MS
16	Myrtenol	1194	1203	0.24	LRI, MS
17	cis-Isopiperitenol		1206	0.11	MS
18	(Z)-Piperitol	1207	1211	0.11	LRI, MS
19	Carveol,	1215	1222	0.13	LRI, MS
20	Citronellol	1223	1233	0.43	LRI, MS
21	Geraniol	1249	1259	0.61	LRI, MS
22	p-Menthen-9-ol	1294	1301	0.13	LRI, MS
23	Hydroxycitronellol	1359	1370	0.27	LRI, MS
24	α-Copaen-11-ol	1539	1543	0.47	LRI, MS
25	Nerolidol	1561	1567	0.63	LRI, MS
26	1-epi-Cubenol	1627	1632	0.48	LRI, MS
27	epi-α-Cadinol	1638	1645	0.56	LRI, MS
28	α-Cadinol	1652	1658	0.37	LRI, MS
29	Abienol	2149	2156	0.45	LRI, MS
30	Incensole	2158	2166	0.28	LRI, MS
31	Larixol	2265	2267	0.11	LRI, MS
32	Isogeranial	1177	1185	0.16	LRI, MS
33	Neral	1235	1247	2.63	LRI, MS
34	Geranial	1264	1278	3.25	LRI, MS
35	Camphor	1141	1147	0.21	LRI, MS
36	trans-β-Ionone	1487	1488	0.28	LRI, MS
37	Eucalyptol	1035	1052	32.82	LRI, MS
38	cis-Linalool oxide (furanoid)	1067	1077	0.14	LRI, MS
39	cis-Limonene oxide	1132	1136	0.17	LRI, MS
40	Humulene epoxide II	1608	1612	0.61	LRI, MS
41	Disparlure		2227	0.32	LRI, MS
42	trans-Dihydro-α-terpinyl acetate	1300	1309	0.23	LRI, MS
43	cis-Dihydro-α-terpinyl acetate	1316	1322	0.11	LRI, MS
44	Dihydrocitronellol acetate	1319	1325	0.38	LRI, MS
45	Anisyl formate	1330	1337	0.44	LRI, MS
46	Neryl acetate	1359	1365	0.25	LRI, MS
47	Geranyl acetate	1379	1388	0.98	LRI, MS
48	p-Anisyl acetate	1412	1418	0.2	LRI, MS
49	Isobornyl isobutanoate	1431	1434	0.63	LRI, MS
50	Neryl isovalerate	1582	1586	0.38	LRI, MS
51	Silphenene	1345	1350	0.35	LRI, MS
52	α-Cubebene	1345	1352	0.26	LRI, MS
53	α-Copaene	1374	1378	0.48	LRI, MS
54	β-Cubebene	1387	1392	0.7	LRI, MS
55	Caryophyllene	1417	1422	0.16	LRI, MS
56	Citronellyl propanoate	1444	1448	0.26	LRI, MS
57	α-Humulene	1452	1456	0.43	LRI, MS
58	allo-Aronradendrene	1458	1463	0.19	LRI, MS
59	γ-Muurolene	1478	1479	0.2	LRI, MS
60	β-Selinene	1489	1497	0.13	LRI, MS
61	α-Muurolene	1500	1503	0.12	LRI, MS
62	α-Farnesene	1505	1511	0.44	LRI, MS
63	δ-Cadinene	1522	1526	1.06	LRI, MS
64	Grandiflorene	2174	2174	0.38	LRI, MS
65	(2E)-Hexenal	846	850	0.61	LRI, MS
66	6-Methyl-5-hepten-2-one	981	989	0.12	LRI, MS
67	Methyl citronellate	1257	1269	0.15	LRI, MS
68	Ethyl carvacrol ether	1297	1306	0.27	LRI, MS
69	n-Hexadecanoic acid	1959	1970	1.44	LRI, MS
70	Oleic acid	2141	2149	0.15	LRI, MS
71	5-Indanol	1338	1346	0.17	LRI, MS
72	Osthole	2140	2143	1.17	LRI, MS
73	Docosane	2200	2222	0.18	LRI, MS
74	3-Methyloctadecane		2331	0.24	MS

affect the chemical compositions and biological activities of essential oils extracted from medicinal plants. Variations in soil properties, sunlight exposure, rainfall patterns, and so on, contribute to changes in the biosynthesis and accumulation of secondary metabolites in plants grown under different environmental conditions [20]. However, it should be noted that the quantitative data reported in this study were obtained through peak area normalization, which provides only semi-quantitative results without considering potential response factor variations.

Antimicrobial activity

The antimicrobial activity of essential oil from *A. nelumboides* was performed by agar well diffusion method, as shown in Table 3. The essential oil extracted from the rhizomes and leaves of *A. nelumboides* were effective against Gram-negative bacteria, Gram-positive bacteria, and fungi. Rhizome

Table 3. Antimicrobial activity of the rhizomes and leaves essential oil from *A. nelumboides.*

	Microorganisms	Inhibition zone (mm)		
		Rhizomes essential oil	Leaves essential oil	Apramycin
Gram-positive	*Enterococcus faecium*	38	19	18
	Staphylococcus aureus	26	25	21
	Acinetobacter baumannii	16	-	20
	Enterobacter cloacae	15	-	21
Gram-negative	*Escherichia coli*	20	23	24
	Klebsiella pneumoniae	17	12	24
	Pseudomonas aeruginosa	25	-	21
Fungi	*Candida albicans*	18	16	19

essential oil was more active than leaves essential oil which was 6/8 test microorganisms sensitive to the leaves essential oil, 8/8 test microorganisms sensitive to the rhizomes essential oil. On *E. faecium, S. aureus*, and *P. aeruginosa*, the inhibition zone diameters of the rhizomes essential oil (38, 26, 25 mm) were greater than those of apramycin at a concentration of 1 mg/ml (18, 21, 21 mm).

In general, the essential oil extracted from the rhizomes and leaves exhibited a range of antibacterial and antifungal properties against a variety of bacterial and fungal species. A similar result was found in the study of *E. fimbriobracteata* essential oil [21] and *A. fragrans* essential oil from Iran [22]. According to a study on the antibacterial activity of *Z. spectabile* Griff, the essential oil from the leaves had no antibacterial activity, but the essential oil from the rhizomes did, which was due to a large variation in the chemical composition of essential oil [23]. The results of our analysis could be explained by the difference in concentrations of antibacterial compounds, such as eucalyptol [24], that accumulate in the rhizomes (71.23%) versus the leaves (32.82%). Moreover, eucalyptol is the primary antibacterial component of many antimicrobial-active plants [24,25]. Therefore, the essential oil of *A. nelumboides* rhizomes, whose primary constituent was eucalyptol, is a potential alternative for the control of multiple microorganisms. Following this, we determined the antibacterial activity of essential oil from *A. nelumboides* by determining the MIC of essential oil against susceptible microorganism strains. The MIC values of essential oil against susceptible bacteria strains were obtained in Table 4.

The rhizomes essential oil exhibited antibacterial activity against all 8 microbiological strains, with MIC values ranging from 8 to 16 mg/ml. The antibacterial activity of leaves essential oil ranged from 4 mg/ml to 16 mg/ml against *E. faecium, S. aureus, E. coli, K. pneumoniae*, and *C. albicans*. The anti-*C. albicans* activity of the leaves essential oil was determined to be more impressive than that of the microorganisms examined in this study. The MIC values of rhizomes essential oil from *A. nelumboides* were stronger than *S. abrotanoides* essential oil on *E. coli* (MIC = 64 mg/ml), *P. aeruginosa* (MIC = 64 mg/ml), but its value was identical on *S. aureus* (MIC = 8 mg/ml), and *C. albicans* (MIC = 8 mg/ml) [26].

ANTICANCER ACTIVITY

The anticancer activity of the essential oil of *A. nelumboides* was determined using the SRB method, and their results are shown in Table 5.

Table 4. MIC results of the rhizomes and leaves essential oils from *A. nelumboides.*

	Microorganisms	MIC (mg/ml)	
		Rhizomes essential oil	Leaves essential oil
Gram-positive	*Enterococcus faecium*	≤ 8	≤ 8
	Staphylococcus aureus	≤ 8	≤ 16
	Acinetobacter baumannii	≤ 8	-
	Enterobacter cloacae	≤ 16	-
Gram-negative	*Escherichia coli*	≤ 8	≤ 16
	Klebsiella pneumoniae	≤ 16	≤ 16
	Pseudomonas aeruginosa	≤ 16	-
Fungi	*Candida albicans*	≤ 8	≤ 4

Table 5. Cytotoxic activity of the rhizomes and leaves essential oils from *A. nelumboides.*

Cell line	IC50 (µg/ml)		
	Rhizomes essential oil	Leaves essential oil	Camptothecin
NCI-H460	98.28 ± 5.22	> 300	0.007 ± 0.10
HepG2	189.87 ± 3.16	> 300	3.55 ± 0.12
Fibroblast	> 300	> 300	1.57 ± 0.23

The IC50 value of essential oil greater than 300 µg/ml was considered non-toxic, whereas the IC50 value was between 10 and 50 µg/ml; 50–100 µg/ml and 100–200 µg/ml were evaluated for strong, moderate, and weak toxic, respectively [27]. The IC50 values indicated that the rhizomes essential oil had a moderate cytotoxic effect on the NCI-H460 cell line and a weak cytotoxic effect on the HepG2 cell line. This essential oil was not fibroblast-toxic. The IC50 values of the leaves essential oil for all three human cell lines were greater than 300 µg/ml, indicating no cytotoxicity for these human cell lines.

Similar to many previous investigations, the rhizomes and leaves of *A. nelumboides* essential oil displayed varying degrees of cytotoxicity [26, 28,29]. Eucalyptol is an anti-cancer and metastasis-preventing agent [27]. Variations in the quantities of compounds with cytotoxic activity may

account for differences in the toxicity of various essential oils [26]. Therefore, the increased concentration of eucalyptol in the essential oil of the rhizome may account for its enhanced anticancer activity. According to a previous study, selective index (SI) = IC50 for normal cell line/IC50 for respective cancerous cell line, and a selective index (SI) > 1 denotes a drug with greater efficacy against tumor cells than toxicity against normal cells [30]. Because the IC50 of the rhizomes essential oil was greater than 300 µg/ml on fibroblasts compared with the IC50 on cancer cell lines (NCI-H460 and HepG2), this essential oil could have a selective effect on these cancer cell lines. Thus, the rhizomes essential oil of *A. nelumboides* had a potential alternative for further research on treatment for cancer diseases.

While the results demonstrate promising anticancer effects, testing on additional cancer cell lines from various tissue origins would provide a broader understanding of the essential oil of anticancer potential. Moreover, *in vivo* studies are warranted to validate the anticancer effects observed *in vitro* and investigate any potential toxicity or off-target effects in animal models.

Alpha-glucosidase inhibition activity

Table 6 displayed the α-glucosidase inhibitory activity results of *A. nelumboides* essential oils. *A. nelumboides* essential oil exhibited the α-glucosidase inhibition activity with the IC50 of leaves essential oil was 2.42 ± 0.22 mg/ml while the values of rhizomes essential oil were higher than 3 mg/ml. The IC50 values of *A. nelumboides* essential oil extracted from leaves were lower than those of *Centaurea calcitrapa* essential oil from Iraq (IC50 = 4.38mg/ml) [31]. The hydrolysis of nonreducing α-1,4-linked glucose molecules from disaccharides or oligosaccharides is catalyzed by α-glucosidase. α-glucosidase enzymes complete carbohydrate breakdown into monosaccharides until units are taken into the body [32]. Glucosidase inhibitors have the potential to be utilized to treat diabetes. Therefore, our data preliminary find out the potential of *A. nelumboides* essential oil in diabetes treatment.

The findings of this study demonstrate the potential of *Alpinia nelumboides* essential oils, particularly from the rhizomes, as promising natural sources for pharmaceutical applications. The observed anticancer, antibacterial, and antidiabetic properties present opportunities for developing novel therapeutic agents or functional ingredients. Additionally, this research enhances our understanding of the applicational capabilities of these essential oils compared to the preliminary findings reported earlier since just focus on antioxidation activity [11]. However, further research is required to isolate and characterize the bioactive components, optimize extraction processes, and conduct comprehensive preclinical and clinical evaluations before practical applications can be realized in the pharmaceutical industry.

Table 6. The α-glucosidase inhibition of the rhizomes and leaves essential oil from *A. nelumboides.*

IC50 (mg/ml)	Rhizomes essential oil	Leaves essential oil	Acarbose
	> 3.00	2.42 ± 0.22	0.95 ± 0.72

CONCLUSION

A bearing oil plant, *Alpinia nelumboides* Nob. Tanaka, T.T.K. Van & V. Hoang, named as "Riềng sen" by Vietnamese was found and studied on taxonomy, essential oils, and their bioactivities. The chemical composition of the oils identified by GC-FID/MS and LRI showed that this species was rich in eucalyptol essential oils. By the rich in eucalyptol in particular and rich in oxygenated compounds in general, the essential oils showed high potential for bioactivities. Rhizomes and leaves essential oils exhibited different levels of antibacterial activity, anticancer activity, and α-glucosidase enzyme inhibition to varying degrees.

Authorship

VTTK and TTL carried out experiments, performed statistical analysis, and wrote the manuscript. TTNT conducted experiments chemical analysis experiments. VH and CHN designed experiments, supervised experiments, and reviewed the manuscript. The manuscript underwent review by the full author list before obtaining consent for submission from each party for journal publication.

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AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

DATA AVAILABILITY

All data generated and analyzed have been included in this research article.

SUPPLEMENTARY MATERIAL

The supplementary material can be accessed at the journal's website: Link Here [https://japsonline.com/admin/php/uploadss/4388_pdf.pdf].

PUBLISHER'S NOTE

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USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

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