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Research on chemical compositions and anti-microbial activity of the essential oil of the rhizome of *Kaempferia daklakensis* N.H.Tuan & N.D. Trong – A new record from Vietnam flora

Nguyen Hoang Tuan [a,*], Nguyen Thanh Tung [a], Pham Ngoc Khanh [a,b]

[a] Department of Pharmacognosy, Hanoi University of Pharmacy, 13-15 Le Thanh Tong Str., Hanoi, Viet Nam
[b] Institute of Natural Products Chemistry, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Viet Nam

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ABSTRACT

Objectives: *Kaempferia daklakensis* N.H.Tuan & N.D.Trong (local name Up dat) (Zingiberaceae) was collected at Ea So Nature Reserve, Ea Kar district, Daklak Province, Viet Nam and described as a new record for the country's flora.
Methods: An essential oil with 0.99% yield (w/w) was extracted from the absolute dry rhizomes by steam distillation as a pleasant smelling yellow oil which was characterized by thin-layer chromatography (TLC) and Gas Chromatography- Mass Spectrometry (GC–MS) and evaluated its antibacterial activity against pathogenic bacteria by agar disk diffusion method.
Results: A total of 38 compounds (accounting for 67.3% of the total oil) were identified. The major components of the essential oils are α-pinene (**2**, 3.22%), camphene (**3**, 23.63%), camphor (**10**, 4.42%), borneol (**12**, 4.80%), isoborneol (**17**, 5.77%), ishwarane (**21**, 3.29%) and 1,8-cineole (**8**, 2.89%). *K. daklakensis* rhizome oil possessed inhibitory activity against Gram (+) and Gram (−) microbial strains including *Bacillus subtilis, Bacillus cereus, Bacillus pumilus, Staphylococcus aureus, Shigella flexneri, Proteus mirabilis.*
Conclusions: This results suggest that the essential oil of *K. daklakensis* rhizome could be used for the treatment of some infections by Gram(+) and Gram(−) microorganisms. This paper is considered also as an official announce for *Kaempferia daklakensis* species as a new record from Vietnam flora.

1. Introduction

The genus *Kaempferia* (Linnaeus) is medium-sized genus belonging to the Zingiberaceae family. Worldwide, it consists of 60 scientifically described species, which are distributed mainly from India to South East Asia. Thailand is known to be the most biologically diversity region of this genus with 16 *Kaempferia* species. Many papers were published related to *Kaempferia* species originated in Thailand (Techaprasan et al., 2010).

In Vietnam, 8 *Kaempferia* species were reported (Ho, 2002), which are widely growing in the lowland and midland forests, relatively concentrated in dipterocarp forests in the Central Highlands, Vietnam. The species most widely used as medicinal plants and cultivated are *K. galanga* (Binh, 2017). Research has shown that chemical consituents and extracts from *Kaempferia* species possessed a variety of biological properties. Flavon (5-hydroxy-7- methoxyflavon and 5.7-dimethoxyflavon) in *K. parviflora* inhibited viral protease, flavonoids from *K. galanga* inhibited *Mycobacterium tuberculosis* and *Candida albicans* (Techaprasan et al., 2010). *K. galanga* ethanolic extracts had cytotoxic activity against human Hela cancer cell line (Moi et al., 2002). The rhizomes of *Kaempferia* species prepared as alcohol or water decoction are prescribed for the treatment of headache (Picheansoonthon and Koonterm, 2008), rheumatism, joints pain, disgetive disorders, fever, tooth pain, diarrhea and pertussis (Moi et al., 2002). With such potential, research on these *Kaempferia* species is extremely necessary.

Abbreviations: TLC, thin-layer chromatography; GC–MS, Gas Chromatography–Mass Spectrometry.

* Corresponding author.
E-mail address: tuandl50@yahoo.com (N.H. Tuan).

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During a field trip in Dak Lak province, Central Highland, Vietnam, we found a species belonging to the genus *Kaempferia* (locally name: Up dat, which means "ground digging"). Through literature review (Binh, 2017), (Chi 2003), we found that this species possesses characteristics completely distinguished from those of previously described *Kaempferia* species. In our previous paper, the species was morphologically described and reported (in Vietnamese) as new record for the flora in Vietnam as *Kaempferia daklakensis* NH Tuan & N.D.Trong (Tu n and Trọng, 2017). In this paper, we reported our study results on the chemical compositions and antibacterial properties of the essential oil obtained from the rhizomes of this plant, in detail in English in order to announce the scientific findings of this *Kaempferia daklakensis* species as a new record for the flora of Vietnam.

2. Material and methods

2.1. Plant material

The whole plant *K. daklakensis* was collected at Ea So Nature Reserve, Ea Kar district, Daklak Province, Viet Nam (at 12°59′17.4″N 108°39′54.2″E, altitude 210 m above sea level), on 16th September 2015. A voucher specimen, HNIP/18153/16, is deposited at Department of Medicinal materials, Hanoi University of Pharmacy.

2.2. Methods

2.2.1. Essential oil extraction

The fresh rhizomes of *K. daklakensis* (Fig. 1) were cleaned, sliced, chopped and subjected to steam-distillation in a Clevenger-type apparatus as described in Vietnamese Pharmacopoeia V (MOH, 2017). The obtained essential oil was dried over anhydrous sodium sulfate and stored in a sealed vial at 10 °C in the dark prior to analysis.

2.2.2. Essential oil analysis by HPTLC and GCMS

The pure essential oil obtained by distillation of water were diluted 100 times with dichloromethane and developed on TLC silica gel 60-F254 (Merck) with an appropriate solvent development system (*n*- hexan: EtOAc (8:2, v/v)) by an automatic sample applicator HPTLC CAMAG LIMONAT 5 (Switzerland). The dried developed TLC was visualized under wavelength 254 nm and by

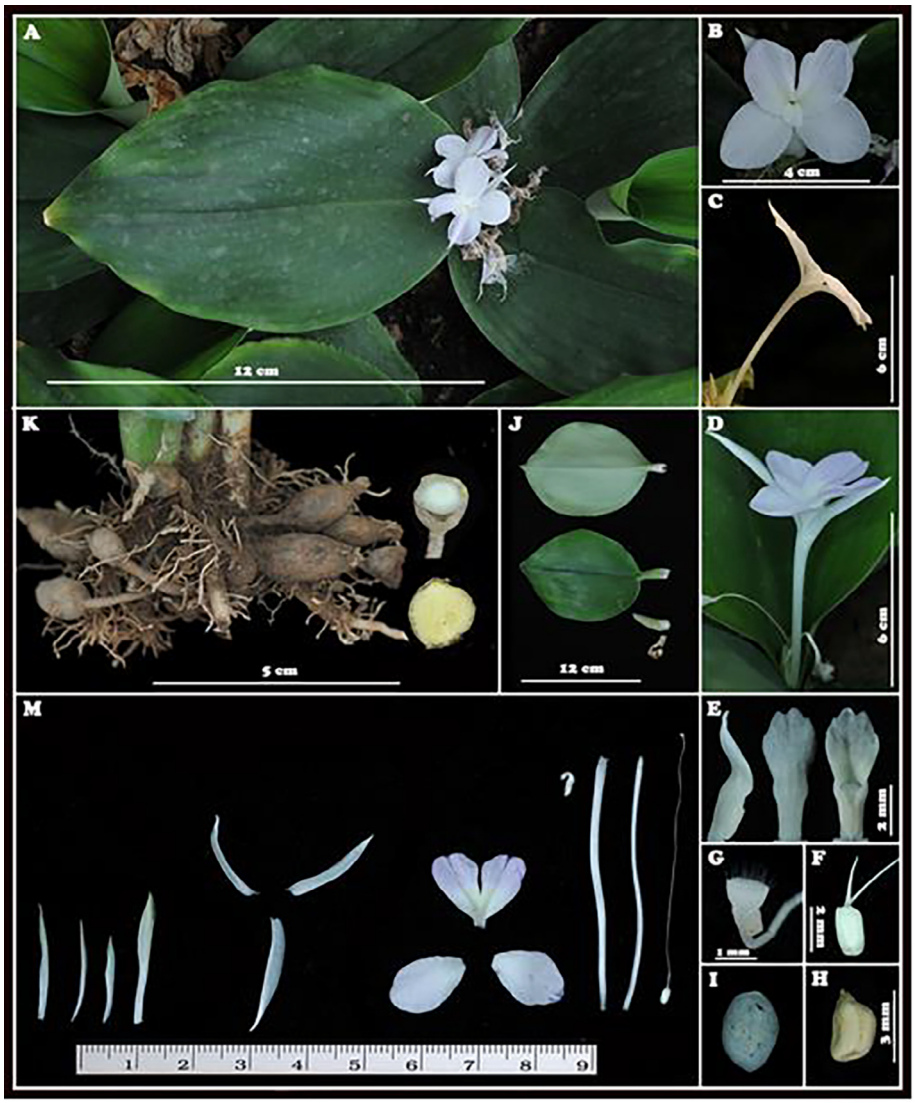

Fig. 1. Photographs of the whole plant and some parts of the *K. daklakensis*. *A. whole plant; B, C, D. Flowers; E. pollen; F. Leaves; G. stigma; F. ovary; I. Cross section of ovary; H. seed; J. Leaf; K. rhizomes and roots, M. Bracts, bracteoles, calyx, carolla, stamens and pistils.*

Table 1
Quantitative analysis of essential oil content in *K. daklakensis* rhizome after 3 times distillation.

No.	Mass of plant materials used for hydro-steam distilation (g)	Material moisture (%)	Essential oil volume obtained (ml)	Essential oil content (%)
1st	40.05	48.25	0.20	1.00
2nd	48.63	48.25	0.25	0.99
3rd	39.40	48.25	0.20	0.98

spraying with vanillin-sulfuric acid reagent followed by heating at 110 °C for 5 min.

Chemical composition of the essential oil from *K. daklakensis* rhizome was analyzed on an Agilent HP mode 7890A gas chromatograph coupled to an Agilent 5975C VL Triple-Axis mass spectrometer, on a fused silica capillary HP5-MS (5% phenyl methyl siloxane) column (30 m × 0.25 mm i.d., 0.25 μm film thickness). Helium was used as carrier gas at a flow rate of 1.0 ml/min. Data acquisition and processing were performed using Agilent MSD productivity Chemstation Rev. E-02.02. Data interpretation was performed using the MassFinder 4.0 software.

2.2.3. GC–MS operation conditions

The mass spectrometer was operated in electron-impact (EI) mode, the ionization energy was 70 eV, the interface temperature was 250 °C, the ion source temperature was 250 °C, the MS quadrupole temperature was 150 °C, and the scan range was 35–450 amu. A 0.1 μl of the oil sample was injected using split mode with a split ratio of 100:1. Initial temp of GC oven was set 60 °C, temperature increment 4 °C/min to 240 °C.

2.2.4. Identification and quantification of essential oil constituents

Individual compounds in the oil were identified by comparison of their mass spectra and retention indices (RI) with those in GC–MS libraries (MS Wiley 8th and NIST 2008) and with those reported in literatures (Adams, 2007). Retention indices of oil constituents were determined using standard C_8–C_{28} straight chain hydrocarbons (Aldrich Chemical Company, USA) (Adams, 2007).

2.2.5. Quantitative analysis of rhizome essential oil

Essential oil content in percentages (volume/mass of absolute dry plant material) was calculated according to formula $X(\%) = \frac{V.10^4}{A.(100-B)}$, where X(%): Essential oil content (%); V: Essential oil volume obtained (ml), A: Mass of plant materials used for hydro-steam distillation (g) and B: Material moisture (%).

2.2.6. Antimicrobial acitivity assay

Six standardized ATCC strains and 4 known local bacterial strains from laboratory stock cultures were used in the evaluation of the antimicrobial activity of the rhizome oil of *K. daklakensis*. The Gram negative strains were *Escherichia coli* (ATCC 25922), *Proteus mirabilis (BV* 108), *Pseudomonas aeruginosa* (VM 201) and *Salmonella typhi* (DT 220). The Gram positive strains were *Bacillus subtillis* (ATCC 6633), *Bacillus cereus* (*ATCC* 9946), *Bacillus pumilus (ATCC* 10241), *Sarcina lutea (ATCC 9341)* and *Staphylococcus aureus* (ATCC 1128). Fungi and yeast were *Candida albicans, Aspergillus niger, Mycogone* sp.*1 and A*spergillus sp.*1*

The *in vitro* antimicrobial activity assays were carried out by agar disk diffusion assay where the same volume of essential oils (10 μl) in various concentrations are diffused in paper disc (d = 6 mm) and placed into agar medium containing a certain and evenly amount of the test organisms. The essential oil was diluted with DMSO in a concentration range of C_0 as pure essential oils, $C_1 = 10^{-1}C_0$; $C_2 = 10^{-2}C_0$; $C_3 = 10^{-3}C_0$; $C_4 = 10^{-4}C_0$. The susceptibility of bacteria to essential oil were expressed by the diameter of the inhibition zone surrounding the paper disks (in mm) (Tewtrakul et al., 2005) (Cuong et al., 2017).

3. Results and discussion

K. daklakensis N.H.Tuan et. N.D.Trong species found in the Central Highlands, South Vietnam is a perennial grass. *Rhizomes* short, rough surface, brown, cross-sectional, approximately 8–10 mm in diameter, fragrant, light yellow. *Root* tuberous bulb ovale-shaped, the outer shell is brown, the cross-section is nearly circular, about 6–7 mm in diameter, divided into two concentric circles, the outside is white in color, the area inside is milky white, fragrant. *Leaf* single, usually 2 (rarely 3), grows near the ground, elliptic-shaped, size 11–12 cm × 8–9 cm. *Flower* are not stalked, irregular, hermaphroditic, pattern 3. *Corolla* white, stick together at the bottom into a tube 6–6.2 cm long (Fig. 1).

3.1. Quantitative analysis of the rhizome's essential oil

The essential oil content of *K. daklakensis* determined as percent volume per dry medicinal material X(%) was shown in Table 1.

The essential oil of *K. daklakensis* rhizomes obtained in average was 0.99% yield (w/w) to the absolute dry mass of plant materials by steam distillation as a pleasant smelling yellow oil, soluble in organic solvents, and insoluble in water with a specific gravity of 0.875–0.975 g/mL (25 °C) and a refractive index of 1.466 – 1.566 (25 °C).

3.1.1. Identification of components of the rhizome's essential oil by TLC and GC–MS

The essential oil was firstly analyzed with silica gel TLC with different solvent mixtures as mobile phase. The results showed that the solvent mixture of *n*-hexane: EtOAc (8:2, v/v) had the best availability to separate the components of the rhizome oil (Fig. 2).

Fig. 2 and Table 2 showed that the TLC visualized by vanilin/H_2SO_4 solution at normal light had at least 9 spots of non-polar and polar components, where the amount of non-polar compounds (R_f 0.95–0.63) were larger than those of polar compounds ($R_f < 0.50$).

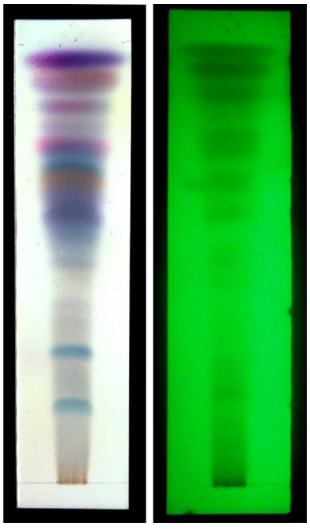

Fig. 2. Thin layer chromatography spectrum of *K. daklakensis* rhizome oil.

Table 2
Qualitative analysis of rhizome oil by TLC.

Spots	R_f	Colour	Classifying
1	0.95	Purple	Non-polar compounds
2	0.92	Orange violet	–
3	0.85	Magnenta	–
4	0.76	Pink	–
5	0.71	Blue	–
6	0.77	Orange	–
7	0.63	Violett	–
8	0.32	Blue	Polar compounds
9	0.16	Blue	–

3.2. Composition of the essential oil of K. daklakensis rhizomes analyzed by GC–MS

The composition of the essential oil is presented in Table 3, whereby all peaks with less than 0.1% area and unknown peaks with less than 0.5% area were not considered for analysis. With these criteria, a total of 45 compounds (accounting for 93.27% of the total oil) were identified. The identification of 45 compounds was obtained through comparison of their mass spectra and retention indices (RI) with those in GC–MS libraries.

As shown in Table 1, almost all components of the oil are terpenes including monoterpenes (hydrocarbon and oxygenated monoterpens, 17 compounds, 54.6% of the total oil) and sesquiterpenes (hydrocarbon and oxygenated sesquiterpenes, 24 compounds, 33.9% of the total oil), most of them are oxygenated compounds (14 compounds, 21.3% of the total oil). Compounds were neither monoterpenes nor sesquiterpenes were bornyl acetate (**18**, $C_{12}H_{20}O_2$, 1.37%), pimara-8(14),15-diene (**38**, $C_{20}H_{32}$, 0,42%), (Z)-β -santalol acetate (**43**, $C_{17}H_{26}O_2$, 2.32%) and laurenan-2-one (**45**, $C_{20}H_{32}O$, 6.56%) (accounting for 11.4% of the total oil). The major components of the essential oils are camphene (**3**, 23.63%), apritone (**38**, 6.29%), isoborneol (**17**, 5.77%), borneol

Table 3
Chemical compositions of rhizome oil of *K. daklakensis*.

Peak no.	Compounds	Retention time (min)	RI	Molecular formula	Relative amount (%)
1	α-Thujene	10.17	928	$C_{10}H_{16}$	1.64
2	**α-Pinene**	**10.50**	939	$C_{10}H_{16}$	**3.22**
3	**Camphene**	**11.02**	956	$C_{10}H_{16}$	**23.63**
4	Myrcene	12.13	992	$C_{10}H_{16}$	0.25
5	delta-3-Carene	12.94	1016	$C_{10}H_{16}$	1.01
6	o-Cymene	13.41	1030	$C_{10}H_{14}$	0.35
7	Limonene	13.56	1034	$C_{10}H_{16}$	1.48
8	**1,8-Cineole**	**13.68**	**1038**	$C_{10}H_{16}O$	**2.89**
9	Linalool	15.93	1103	$C_{10}H_{18}O$	0.31
10	**Camphor**	**17.79**	**1156**	$C_{10}H_{16}O$	**4.42**
11	Camphene hydrate	17.99	1161	$C_{10}H_{18}O$	0.43
12	**Borneol**	**18.57**	1177	$C_{10}H_{18}O$	**4.80**
13	Terpinen-4-ol	18.91	1187	$C_{10}H_{18}O$	0.15
14	m-Cymen-8-ol	18.99	1189	$C_{10}H_{14}O$	0.19
15	p-Cymen-8-ol	19.15	1194	$C_{10}H_{14}O$	0.16
16	α-Terpineol	19.37	1200	$C_{10}H_{18}O$	0.27
17	**Isoborneol**	**20.70**	1239	$C_{10}H_{18}O$	**5.77**
18	Bornyl acetate	22.61	1294	$C_{12}H_{20}O_2$	**1.37**
19	Cis-β-Elemene	26.25	1404	$C_{15}H_{24}$	1.63
20	Cyperene	26.71	1418	$C_{15}H_{24}$	1.30
21	β -Acoradiene	28.39	1472	$C_{15}H_{24}$	1.63
22	**Ishwarane**	**28.81**	**1485**	$C_{15}H_{24}$	**3.29**
23	Valencene	28.96	1490	$C_{15}H_{24}$	0.89
24	Aristolochene	29.33	1502	$C_{15}H_{24}$	0.30
25	β-Selinene	29.44	1505	$C_{15}H_{24}$	0.77
26	α-Selinene	29.67	1510	$C_{15}H_{24}$	1.10
27	γ-Cadinene	30.19	1513	$C_{15}H_{24}$	0.64
28	δ-Cadinene	30.38	1531	$C_{15}H_{24}$	0.25
29	Elemol	31.22	1565	$C_{15}H_{26}O$	0.54
30	(E)-Nerolidol	31.38	1570	$C_{15}H_{26}O$	0.32
31	Spathulenol	32.22	1599	$C_{15}H_{24}O$	0.18
32	Caryophyllene oxide	32.42	1605	$C_{15}H_{24}O$	0.20
33	1,10-di-epi-Cubenol	33.26	1635	$C_{15}H_{26}O$	0.63
34	Alismol	33.63	1648	$C_{15}H_{24}O$	0.17
35	epi-α-Cadinol	33.97	1660	$C_{15}H_{26}O$	0.22
36	Neo-intermedeol	34.48	1678	$C_{15}H_{26}O$	1.51
37	Eudesm-7(11)-en-4-ol	35.03	1698	$C_{15}H_{26}O$	1.58
38	Apritone	35.28	1707	$C_{15}H_{24}O$	6.29
39	Eudesma-4(15),7-dien-1β-ol	35.46	1714	$C_{15}H_{24}O$	0.37
40	Cyperotundone	35.71	1723	$C_{15}H_{22}O$	0.23
41	Zerumbone	35.90	1730	$C_{15}H_{22}O$	1.82
42	Unknown oxygenated sesquiterpene (MW = 248)	37.29	1782	$C_{15}H_{22}O$	5.77
43	(Z)- β -Santalol acetate	38.20	1816	$C_{17}H_{26}O_2$	2.32
44	Pimara-8(14),15-diene	42.72	1997	$C_{20}H_{32}$	0.42
45	Laurenan-2-one	46.34	2154	$C_{20}H_{32}O$	6.56
Total number of constituents				45	
Number (%) of constituents identified				93.27	
Number (%) of monoterpene hydrocarbons				31.6 (33.8%)	
Number (%) of oxygenated monoterpenes				19.4 (20.8%)	
Number (%) of sesquiterpene hydrocarbons				11.6 (12.6%)	
Number (%) of oxygenated Sesquiterpenes				19.8 (21.3%)	
Number (%) of other compounds				10.7 (11.4%)	

Table 4
Antimicrobial activity assay of rhizome oil of *K. daklakensis.*

	Tested microbes	Diameter of inhibition zone (mm)				
	Concentration	10^{o}	10^{-1}	10^{-2}	10^{-3}	10^{-4}
	A. Gram-positive bacteria					
1	*Bacillus subtilis ATCC* 6633	25	20	15	15	15
2	*Bacillus cereus ATCC* 9946	30	25	20	10	6
3	*Bacillus pumilus ATCC* 10,241	20	10	6	6	6
4	*Sarcina lutea ATCC 9341*	–	–	–	–	–
5	*Staphylococcus aureus ATCC* 1128	30	25			
	B. Gram-negative bacteria					
6	*Shigella flexneri DT* 112	20	15	12	10	8
7	*Escherichia coli ATCC* 25,922	–				
8	*Proteus mirabilis BV* 108	30	25	20	10	8
9	*Pseudomonas aeruginosa VM* 201	–	–	–	–	–
10	*Salmonella typhi DT* 220	–	–	–	–	–
	C. Fungi					
11	*Candida albicans*	–	–	–	–	–
12	*Aspergillus niger*	10	8	6	6	6
13	*Mycogone* sp.*1*	–	–	–	–	–
14	*Aspergillus* sp.*1*	12	10	8	8	6

(**12**, 4.80%), camphor (**10**, 4.42%), ishwarane (**21**, 3.29%), α-pinene (**2**, 3.22%), 1,8-cineole (**8**, 2.89%). The contents of the remaining components are below 4%, most of them (35 compounds) even below 2%.

3.2.1. Antimicrobial activity

The results of antimicrobial activity against several bacterial and fungi strains by agar disk diffusion assay were displayed in Table 4.

In the antibacterial assays, the oil at original concentration exhibited significant activity against the Gram positive bacteria *Bacillus subtilis, Bacillus cereus, Bacillus pumilus, Staphylococcus aureus, Shigella flexneri,* and the Gram negative bacteria *Proteus mirabilis.* It had no inhibitory effect on the proliferation of *Sarcina lutea, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Mycogone* sp.*1*

4. Discussion

The chemical compositions of the rhizome oil from *Kaempferia daklakensis* N.H.Tuan & N.D.Trong, distributed in the Central Highlands, South Vietnam, was described in details. The monoterpene profile of the rhizome oil of *K. daklakensis* is characterized by the presence of the most abundant compound camphene (**3**) with 23.63% of total oil, following by apritone (**38**, 6.29%), isoborneol (**17**, 5.77%), borneol (**12**, 4.80%), camphor (**10**, 4.42%), α-pinene (**2**, 3.22%), 1,8-cineole (**8**, 2.89%), α-thujene (**1**, 1.64%), and limonene (**7**, 1.48%). The sesquiterpene profile of the rhizome oil of *K. daklakensis* is characterized by the presence of the most abundant ishwarane (**21)** with 3.29% of total oil, following by *cis*-β-elemene (**19**, 1.63%), neo-intermedeol (**35**, 1.51%), α-selinene (**25**, 1.10%), β-selinene (**24**, 0.77%), γ-cadinene (**26**, 0.64%), 1,10-di-epi-cubenol (**32**, 0.63%), and elemol (**28**, 0.54%). The sesquiterpene profile of the rhizome oil of *K. daklakensis* collected in Vietnam is different from those of the *K. galanga* rhizome oils collected in other countries. The *K. galanga* rhizome oils collected in Malaysia had 54 components with terpenoid constituents amounted to 16.4%, major constituents as ethyl *trans-p*-methoxycinnamate (51.6%), ethyl cinnamate (16.5%). Pentadecane (9.0%), 1,8-cineole (5.7%), 6-car-3- ene (3.3%) and borneol (2.7%) (Wong et al., 1992). The volatile oil of *K. galanga* rhizome collected in Thailand was only 9 compounds identified of which mostly were terpenoid compounds as ethyl-p-methoxycinnamate (31.77%), methyl cinnamate (23.23%), carvone (11.13%), eucalyptol (9.59%) (Tewtrakul et al., 2005). The volatile oil of *K. galanga* rhizome collected in Bangladesh composed of 81 compounds, of which the major components were 2-propenoic acid, 3-(4-methoxyphenyl),-ethyl ester (63.36%), ethyl cinnamate (6.31%), 4-cyclooctene-1-methanol (4.61%), caryophyllene oxide (4.37%), borneol (2.46%) (Bhuiyan et al., 2008). Generally, the major compound of the rhizome oil of K. galanga collected in most contries was identified as ethyl p-methoxy cinnamate, which was absent in our *K. daklakensis* rhizome oil.

Finally, the essential oil of *K. daklakensis* rhizome was aimed to evaluate the antimicrobial activity against several bacterial and fungi strains *in vitro*. In comparison to that of *K. galanga*, at the same amount of 10 μl of pure volatile oil impregnated to assaying paper disc, the activity of *K. daklakensis* essential oil against tested microbacterials was significantly higher. We found that the diameters of inhibition zone induced by *K. daklakensis* against *Staphylococcus aureus, Baccillus subtilis* and *Shigella flexneri* were significantly greater than those induced by *K. galanga* essential oil (25 and 12 mm, 25 and 16 mm, and 20 and 12 mm, respectively). However, the rhizome oil from *K. galanga* inhibited *Caldida albicans* with diameter of inhibition zone of 31 mm (positive sample clotrimazole: 25 mm) (Tewtrakul et al., 2005) while it was negative by *K. daklakensis* rhizome oil. This results suggest that the essential oil of *K. daklakensis* rhizome could be used for treatment of some infections by Gram (+) and Gram (−) microbacterials.

5. Conclusion

Kaempferia daklaknensis N.H.Tuan & N.D.Trong was found and described as a new botanical record in Vietnam. The essential oil from its rhizome was qualtitatively and quantitatively analyzed for the first time. The essential oil of *K. daklakensis* rhizomes was obtained in 0.99% yield (w/w, absolute dry weight of raw material) by hydrodistillation as a pleasant smelling yellow oil insoluble in water but soluble in organic solvents. A total of 45 compounds accounting for 93.27% of the total oil were identified. The major components of the essential oils are camphene (**3**, 23.63%), apritone (**38**, 6.29%), isoborneol (**17**, 5.77%), borneol (**12**, 4.80%), camphor (**10**, 4.42%), ishwarane (**21**, 3.29%), α-pinene (**2**, 3.22%), 1,8-cineole (**8**, 2.89%). *K. daklakensis* rhizome oil possessed inhibitory activity against microbial strains including *Bacillus subtilis, Bacillus cereus, Bacillus pumilus, Staphylococcus aureus, Shigella flexneri, Proteus mirabilis.* This results suggest that the essential oil of *K. daklakensis* rhizome could be used for the treatment of some infections by Gram (+) and Gram (−) microorganisms. This

paper is considered as an official announce for the species *Kaempferia daklakensis* as a new record for the flora of Vietnam.

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Declaration of Competing Interest

We declare that we have no conflict of interest.

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