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Anatomical traits and volatile compounds of acetone extract from *Kaempferia champasakensis*

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Abstract

Kaempferia L. is a genus belonging to Zingiberaceae family, with many species used in indigenous medicines in Asian countries. *Kaempferia champasakensis* Picheans. and Koonterm. are rare species found only in Laos and Vietnam. The aim of this study was to provide the micro-morphological traits and the volatile compounds of the acetone extract isolated from *K. champasakensis* for the first time. Using gas chromatography/mass spectrometry (GC/MS) assay, twenty-three volatile components were identified in the studied extract, among which tyranton (22.41%), 9(E), 11(E)-conjugated linoleic acid (18.58%), palmitic acid (15.32%), phytol (9.95%), 3-hexen-2-one (6.91%), cis-vaccenic acid (6.04%) and β-sitosterol (4.74%) were the major compounds. In addition, using the iodine green-carmine double staining method, the micro-morphological traits of the leaf, root tuber, root and rhizome *of K. champasakensis* were first demonstrated. These results provided the standardization and classification of *K. champasakensis,* which can be applied in the pharmaceutical field and other related areas.

Keywords

acetone extract; anatomical traits; *Kaempferia plant;* volatile compounds

Introduction

Kaempferia L., a genus belonging to Zingiberaceae family, comprises 60 species distributed in the Philippines, Malaysia, Indonesia, Myanmar, Thailand, Cambodia, Laos, Vietnam, Bangladesh, India, China and East Asia (1, 2). So far, eleven *Kaempferia* species have been recorded in Vietnam, including *K. cochichinensis, K. fallax, K. pulchra, K. candida, K. angustifolia, K. galanga, K. fissa, K. harmandiana, K. marginata, K. elegans* and *K. champasakensis* (2-5). The members of this genus have been used in indigenous medicines in Asian countries to treat digestion disorders, cough, infective diseases, pain and wound infections. Additionally, many bioactive compounds such as flavonoids, monoterpenoids, phenolic glycosides, diterpenoids, diarylheptanoids, cyclohexane oxide derivatives and essential oils have also been isolated from *Kaempferia* plants (2, 6-10).

In 2008, Picheansoonthon and Koonterm were the first to discover and describe *K. champasakensis* from Ban Lad Suea, Xanasomboon Town, Champasak Province, Lao PDR (11). This species was later recorded as a new addition to the flora of Vietnam, with specimens collected from Tanh Linh District, Binh Thuan Province and Binh Chau-Phuoc Buu Nature Reserve, Ba Ria-Vung Tau Province (12, 13). The

chemical components and antibacterial properties of the ethanol extracts and the essential oils obtained from *K. champasakensis* have been reported by previous studies (14-16). These results indicate that *K. champasakensis* has the potential to be used as a medicinal plant in the pharmaceutical field, similar to other species in its genus.

So far, the anatomical traits of plant species have been considered a tool that plays an important role in the standardization and classification of medicinal plants (17). The aim of this report is to provide the micro-morphological traits and the volatile compounds of the acetone extract isolated from *K. champasakensis* for the first time.

Materials and Methods

Plant materials

The samples of *K. champasakensis* were collected from Binh Chau-Phuoc Buu Nature Reserve, Ba Ria-Vung Tau Province, Vietnam (Fig. 1). The voucher specimen of this plant was deposited at the Herbarium of this nature reserve. The scientific name of the studied species was identified using the comparative morphological method. Accordingly, the reproductive and vegetative traits of *K. champasakensis* described in prior reports were used to compare with those of the studied plants (11-13).

Anatomical characteristics

The thin slices of the *K. champasakensis* leaf, root tuber, root and rhizome were cut transversally using a razor blade. Javel water was used to bleach these microscopic samples. The thin slices of the different organs of the studied species were stained using the iodine green-carmine double staining method and then washed by distilled water several times before being preserved in 10% glycerol (18, 19). The Olympus BX53 Digital Upright Microscope, equipped with digital microscope cameras and cellSens imaging software, was used to observe and capture images.

Extraction procedures

The whole plant of *K. champasakensis* was harvested and washed under fresh water, then dried at 50ºC until it reached a constant weight. The dried sample was ground into powder using an electric grinder. One hundred mililiters of a 99% acetone solution (Thermo Fisher Scientific, USA) was used to soak 10 g of the powder at room temperature for 72 hr. The extract was filtered using Whatman paper and the process was repeated twice with the residue. The total filtrate was evaporated using a rotary evaporator until a dark brown extract was obtained (14).

Fig. 1. The whole plant of *K. champasakensis*.

Gas chromatography/mass spectrometry assays

The volatile compounds of the acetone extract from *K. champasakensis* were determined using the TRACE 1310 Gas Chromatograph in conjunction with the ISQ 7000 mass spectrometer (Thermo Fisher Scientific, USA). The DB-5MS column (Agilent, USA) was used as the stationary phase. The GC/MS run parameters were set as described in our prior publication (14). The mass spectra of the acquired compounds were compared with those in the NIST 2017 library to identify the volatile components of the studied extract.

Results

Anatomical traits of K. champasakensis

Root (Fig. 2): The cross-section is nearly round and divided into two regions: the cortical area accounts for two-thirds of the cross-section radius, while one-third of the radius is the stele. The piliferous layer consists of a layer of polygonal cells with cellulose walls or impregnated with phellem, which are quite uniform in size, closely arranged and contain many root hairs. The exodermis includes 3-7 layers of polygonal cells, with walls impregnated with phellem, arranged radially. The cortical parenchyma is divided into two regions: the outer region comprises 3-5 layers of polygonal cells wth cellulose walls that vary in size and are arranged haphazardly, leaving small intercellular spaces; the inner region contains 5-7 layers of polygonal or oval cells with cellulose walls that gradually reduce size as they get closer to the center, arranged in concentric rings and radial rows to create small polygonal spaces. There are some secretory cells that are scattered throughout the cortical parenchyma. The endodermis with the Casparian strip includes one layer of rectangular cells that are quite regular. The pericycle consists of a single layer of polygonal cells with cellulose walls,

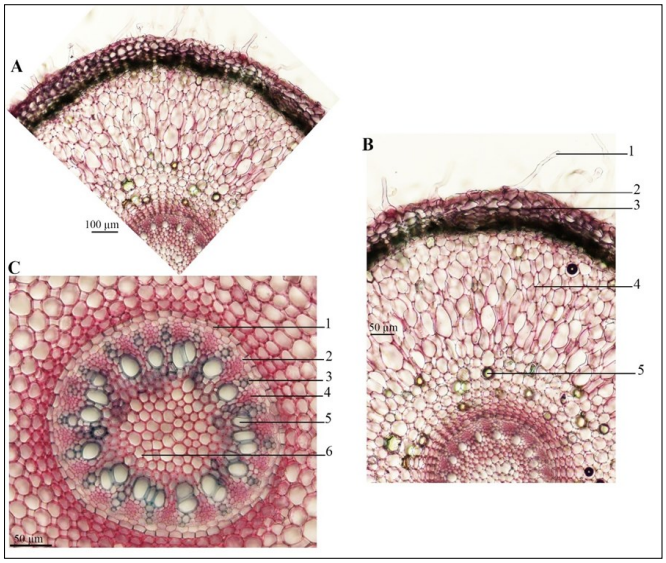

Fig. 2. Cross-section of root. A. The whole view of cross-section. B. Cortical region (1: root hairs, 2: piliferous layer, 3: exodermis, 4: cortical parenchyma, 5: secretory cell). C. Stele (1: endodermis with casparian strip, 2: pericycle, 3: primary xylem, 4: primary phloem, 5: metaxylem, 6: medullary parenchyma).

evenly spaced, interspersed with the endodermis. The vascular system comprises 15-19 primary phloem bundles arranged alternately with 15-19 protoxylem bundles in a ring close to the pericycle. The primary phloem consists of 3-5 layers of polygonal cells with cellulose walls that are irregular and radially differentiated. The protoxylem bundle involves 2-5 vessels that have a polygonal shape and lignin-impregnated walls, also radially differentiated. There are 17-24 metaxylem vessels, which have lignin-impregnated walls and are large, usually arranged in a ring beneath the primary phloem and protoxylem; sometimes there are several metaxylem vessels located in the center of the medullary parenchyma. The medullary parenchyma has 8-10 layers of polygonal cells with cellulose walls that are irregular and tightly packed.

***Root tuber (Fig. 3)*:** The cross-section is often distorted and rarely round, divided into two regions: the cortex, which is very thick and grows when the tuber enlarges and the stele, which is thin. The piliferous layer consists of rectangular, distorted cells with scattered hairs. The phellem has 4-6 layers of rectangular, flat cells with walls impregnated with cork, arranged radially. The phelloderm includes 2-3 layers of polygonal- shaped cells with cellulose walls that are irregular in size and arranged radially. The endodermis, characterized by a Casparian strip, comprises one layer of fairly regular rectangular cells. The pericycle involves one layer of polygonal-shaped cells with fairly regular cellulose walls. There are 18-22 protoxylem bundles arranged alternately with 18-22 primary phloem bundles per ring, separated by 1-2 rows of medullary rays. The protoxylem bundles consist of 2-3 polygonal xylem vessels, with walls impregnated with lignin and radially differentiated. The primary phloem bundle contains 3-4 layers of polygonal cells, with cellulose walls arranged haphazardly. There are 14-16

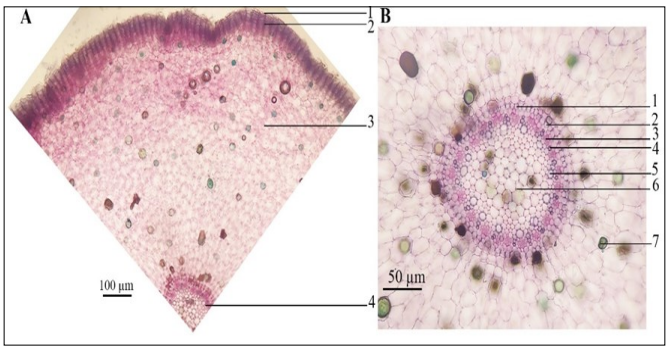

Fig. 3. Cross-section of root tuber. A. The whole view of cross-section (1: piliferous layer, 2: phellem, 3: cortical parenchyma, 4: stele). B. Stele (1: endodermis with casparian strip, 2: pericycle, 3: primary phloem, 4: primary xylem, 5: metaxylem, 6: medullary parenchyma, 7: secretory cell).

metaxylem vessels with polygonal-shaped, lignin-impregnated walls located below the primary phloem and protoxylem bundles; sometimes there are a few metaxylem vessels located in the middle of the medullary ray. The medullary parenchyma comprises polygonal cells and tightly packed cellulose walls. There are many secretory cells scattered in the medullary and cortical parenchyma.

***Rhizome (Fig. 4)*:** The rhizome includes the epidermis, cortex and stele. On the outside of the epidermis, there are leaf scars. The epidermis comprises one layer of rectangular cells with cellulose walls. The phellem consists of 3-5 layers of rectangular cells that are very flat, with walls impregnated with cork and arranged radially. The cortical parenchyma consists of many layers of cellulose wall cells arranged with small intercellular spaces. The endodermis features a Casparian strip. The pericycle consists of a layer of polygonal cells with cellulose walls, arranged alternately below the endodermis. The vascular bundles near the pericycle are closely arranged in a ring formation. The inner vascular bundles are larger and located haphazardly in the medulla, while

Fig. 4. Cross-section of rhizome. A. Cortical region (1: leaf scars, 2: epidermis, 3: phellem, 4: phelloderm, 5: cortical parenchyma, 6: secretory cell, 7: vascular bundle). B. Stele (1: cortical parenchyma, 2: endodermis with casparian strip, 3: pericycle, 4: phloem, 5: xylem, 6: medullary parenchyma).

the vascular bundles are increasingly numerous, contributing to the size of the steel. In the cortical area, the vascular bundles are scattered and arranged in a ring, but their density is less than that of the stele. The vascular bundles are surrounded by sclerenchyma cells that have a polygonal shape and thick walls made of cellulose or lignin. Additionally, there are many cells containing secretions in both the medullary and cortical parenchyma.

Leaf (Fig. 5)

Midrib: The cross-section is characterized by a concave upper surface and a convex lower surface. The epidermal cells are rectangular or polygonal and are closely arranged. The angular collenchyma consists of 1-5 layers of thick cells, located in small clusters above the lower epidermis. The spongy parenchyma contains polygonal cells with cellulose walls of irregular sizes. There are vascular bundles with xylem arranged above the phloem, often arranged in two rows within the spongy parenchyma; the lower bundle is larger than the upper bundle. Large intercellular spaces exist among the large vascular bundles. The xylem includes 1-3 metaxylem vessels and 2-4 protoxylem vessels. Sclerenchyma cells, which have thick cellulose walls, are arranged in a ring surrounding the vascular bundles.

Lamina: The upper and lower epidermis consists of one layer of

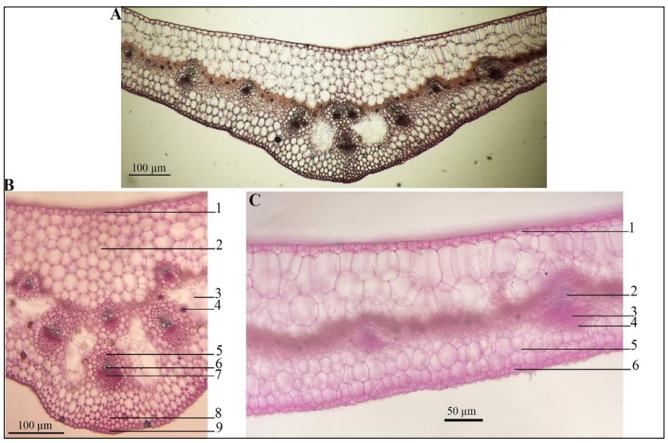

Fig. 5. Cross-section of leaf. A. The whole view of the leaf. B. Midrib (1: upper epidermis, 2: spongy parenchyma, 3: intercellular spaces, 4: secretory cell, 5: sclerenchyma, 6: xylem, 7: phloem, 8: angular collenchyma, 9: lower epidermis). C. Lamina (1: upper epidermis, 2: xylem, 3: phloem, 4: sclerenchyma, 5: spongy parenchyma, 6: lower epidermis).

rectangular cells with cellulose walls and there are scattered stomata in the lower epidermis, that are larger than the epidermal cells in the midrib area. The vascular bundles have a structure similar to that of the midrib, located in a row in the middle of the parenchyma. The spongy parenchyma consists of

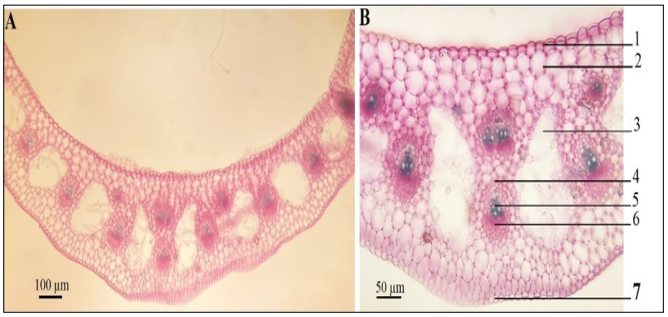

Fig. 6. Cross-section of leaf sheath. A. The whole view of cross-section. B. The central cross-section (1: upper epidermis, 2: parenchyma, 3: intercellular spaces, 4: sclerenchyma, 5: xylem, 6: phloem, 7: lower epidermis)

polygonal cells with cellulose walls, arranged haphazardly to create intercellular spaces.

***Leaf sheath (Fig. 6)*:** The cross-section is curved with a deeply concave upper surface, thick in the middle and gradually thinner on both sides. The upper and lower epidermis have the same structure, but the upper epidermal cells are larger than those of the lower epidermis, consisting of one layer of rectangular cells. The parenchyma includes polygonal cells. The vascular bundles are arranged in a row in the middle of the parenchyma, with large intercellular spaces among them.

Volatile compounds of acetone extract from K. champasakensis

By using gas chromatography/mass spectrometry assay (Fig. 7 and Table 1), twenty-four volatile components were determined in the studied extract, of which 2-pentanone, 4-hydroxy-4-methyl -(22.41%); 9(E),11(E)-conjugated linoleic acid (18.58%); n-hexadecanoic acid (15.32%); phytol (9.95%); 3-hexen-2-one (6.91%); cis-vaccenic acid (6.04%) and β-sitosterol (4.74%) were the major compounds.

Discussion

In this study, the chemical compositions of the acetone extract of *K. champasakensis* are reported for the first time. However, the chemical components of the ethanol extracts and the essential oils obtained from this species collected in Vietnam

Table 1. Chemical components of acetone are extracted from *K. champasakensis* by using gas chromatography/mass spectrometry assay

RT	Compounds	%
2.05	3-Hexen-2-one	6.91
2.40	Tyranton	22.41
2.59	Diacetonamine	0.34
4.53	2-Acetoxyisobutyryl chloride	1.48
6.37	2-Ethyl-3,5-dimethylpyridine	0.20
6.54	2-Propen-1-amine, N,N-bis(1-methylethyl)-	1.70
7.27	Triacetonamin	1.20
7.94	Piperidin-4-one, 1-ethyl-2,3-dimethyl-	1.43
9.38	Pyrrolidine, 2-butyl-5-heptyl-	0.37
10.36	Caryophyllene	0.38
11.52	Caryophyllene oxide	0.79
12.85	Neophytadiene	0.73
13.53	Palmitic acid	15.32
14.27	Phytol	9.95
14.39	9(E),11(E)-Conjugated linoleic acid	18.58
14.41	cis-Vaccenic acid	6.04
14.49	Octadecanoic acid	3.37
16.46	Ethyl iso-allocholate	0.20
18.35	Squalene	0.60
21.00	Androsta-1,4-dien-3-one, 17-hydroxy-17-methyl-, (17α)-	1.50
22.51	dl-α-Tocopherol	1.13
25.06	Stigmasterol	0.47
26.09	β-Sitosterol	4.74
	Total	99.85

have been documented in several prior studies. For instance, using LC/MS assay, researchers showed that the ethanol extracts from the leaf and rhizome of *K. champasakensis* contained six components: Kaempulchraol A, C, G, I, L and M (20). Furthermore, the essential oil isolated from the rhizosme of *K. champasakensis* was found to be rich in camphene, α-pinene and β-pinene (17), while the leaf essential oil of this plant contained β-caryophyllene, β-elemenone and β-pinene as its main components (18).

In addition, the volatile compositions of other *Kaempferia* plants using gas chromatography/mass spectrometry assays have also been reported. For example, the rhizome essential oil of *K. daklakensis* collected from Vietnam were mainly composed of camphene, camphor, borneol and isoborneol (21). The researchers reported that the chemical constituents of *K. angustifolia* grown in Thailand mainly contained camphene, camphor, α-pinene and borneol (22). Similarly, camphene and α-pinene were the major

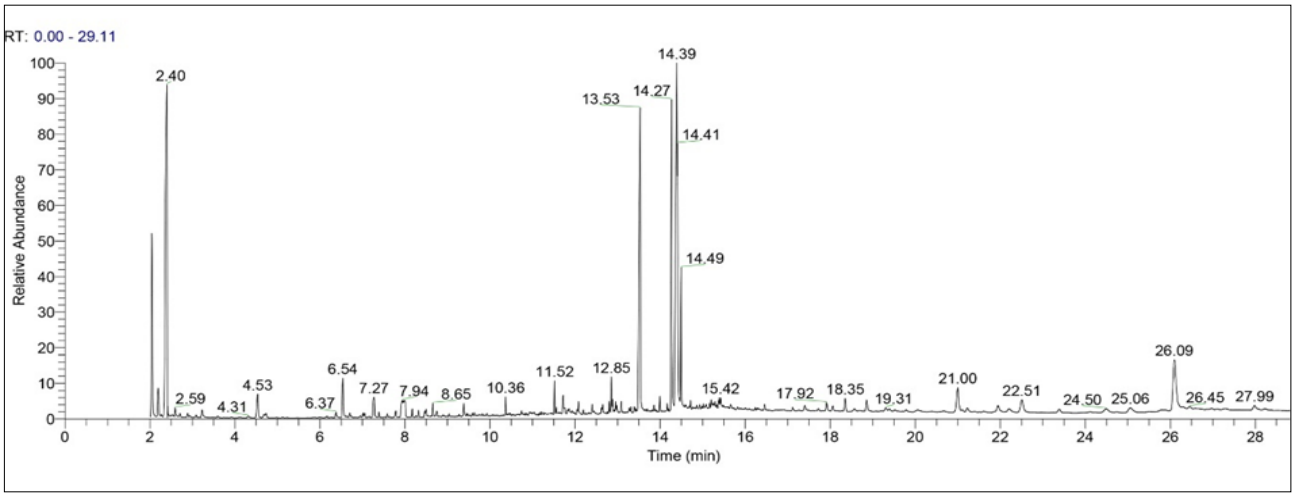

Fig. 7. The GC chromatogram of acetone extract from *K. champasakensis*.

compounds found in the root and rhizome of *K. larsenii* collected from Thailand (23). The major chemical compounds of the *K. rotunda* rhizome from Malaysia were pentadecane, bornyl acetate, benzyl benzoate and camphor (24). The leaf essential oil of *K. galanga* collected from Bangladesh was characterized by a high presence of linoleoyl chloride, caryophyllene oxide, cubenol and caryophyllene, while the rhizome oil contained 2-propenoic acid, 3-(4-methoxyphenyl),-ethyl ester; ethyl cinnamate and 4-cyclooctene -1-methanol as the major compounds (25). Meanwhile, isoamyl *p*-methoxycinnamate, *n*-pentadecane and ethyl cinnamate were the abundant components in the rhizome essential oils isolated from the *K. galanga* rhizome collected from China (26).

The ethanol extract of the *K. galanga* rhizome grown in India comprised ethyl p-methoxycinnamate and 3-methyl-2-(2-oxopropyl) furan as the mainly abundant components (27). The methanol extract of the *K. galanga* leaves collected from India was found to be rich in 2-(3,4-dimethoxyphenyl)-7-hydroxy-3-methoxy-4H-chromen-4-one; 2-(3-hydroxy-4-methoxyphenyl)-3,7-dimethoxy-4H-chromen-4-one and octamethyl cyclotetrasiloxane, whereas the rhizome extract was reported to contain ethyl p-methoxycinnamate, ethyl cinnamate and pentadecane as the main compounds (28). The hexane, ethanol and dicholomethane extracts of *K. rotunda* Linn. rhizome collected from Thailand were mainly composed of pentadecane, benzyl benzoate and crotepoxide (29).

Conclusion

In this study, the anatomical characteristics and the volatile compounds of the acetone extract isolated from *K. champasakensis* were first investigated. Twenty-three volatile compounds were identified in the studied extract, among which tyranton, 9(E),11(E)-conjugated linoleic acid and palmitic acid were the major constituents. Based on the chemical compounds and the details of micro-morphological traits of the leaf, root tuber, root and rhizome provided, the pharmacological potential of *K. champasakensis* is further clarified.

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Authors' Contributions

TTLT and HTDN participated in the design of the study and performed the experiments and statistical analysis. MPN, HNV, HTV, TVP, VSL, QHN and TTL conducted the experiments and managed the research data. TTLT and HTDN drafted the manuscript and addressed all queries from editors and reviewers. All authors read and approved the final manuscript.

Compliance with Ethical Standards

Conflict of interest: The authors have no conflict of interest to declare.

Ethical issues: None

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