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Identification of volatile bioactive compounds from the pericarp and seed extracts of *Alpinia mutica* Roxb. by GC–MS analysis

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Abstract

Alpinia mutica Roxb. is a perennial rhizomatous herbaceous plant of the family Zingiberaceae. The fruits of this plant are commonly used in traditional East Asian medicines. While the phytochemicals of whole *A. mutica* fruits have been previously investigated, the chemical constituents of two parts of the plant, the pericarp and the seed, have not been examined separately. Therefore, the goal of this study was to identify the volatile constituents of *A. mutica* pericarps and seeds. Each part was extracted with dichloromethane, and the extract was further analyzed by gas chromatography–mass spectroscopy (GC–MS). Seventeen compounds were identified in different parts of the *A. mutica* fruit. Slightly different chemical profiles were observed for the pericarp and seed extracts. The main volatile component of the pericarp extract was a diarylheptanoid, specifically 1,7-diphenyl-4,6-heptadien-3-one (45.28%). The most abundant volatile components in the seed extract sample were 5,6-dehydrokawain (64.94%), pinocembrin (22.51%), and farnesol (9.18%). This is the first report of the difference between the chemical components of the *A. mutica* pericarp and seed. Moreover, the phytochemical studies and the compounds identified by GC–MS showed that the *A. mutica* pericarp and seed contain important bioactive compounds, which have been reported as having anti-cancer, anti-inflammatory, and neuroprotective effects. The outcome of this study was the creation of fingerprint analysis based on the GC–MS data and the preliminary identification of the chemical components in *A. mutica* pericarps and seeds, which is related to the reported biological activities and use of the *A. mutica* fruit.

Keywords: *Alpinia mutica; bioactive compounds; GC–MS; orchid ginger; pericarp; seed; volatile component.*

1. Introduction

Alpinia is a large genus of the Zingiberaceae family containing around 230 species. Various plants of this genus have been utilized as ethnomedicines, foods, and spices in many countries, particularly in Asia (Ma, Xie, Miao, Yang, & Yang, 2017). *Alpinia mutica* Roxb. is a perennial rhizomatous herbaceous plant of this genus. It is native to Borneo, Java, India, Myanmar, peninsular Malaysia, Singapore, Sulawesi, Thailand, and Vietnam, where it grows in humid forests on swampy soils close to streams (Puccio, 2000; Dyary, Arifah, Sukari, & Sharma, 2019). It is commonly known as "orchid ginger". The plants have been cultivated as an ornamental, and the rhizomes of this plant are traditionally used to treat stomach problems and flatulence (Sirat, Rahman, Itokawa, & Morita, 1996). The fruits, pericarps, and seeds illustrated in Figures 1(a) and (b) have also been used to treat diarrhea and reduce swelling (Malek, Phang, Ibrahim, Norhanom, & Sim, 2011; Ibrahim et al., 2014; Akram, Ghani, Khamis, & Zulkifly, 2021).

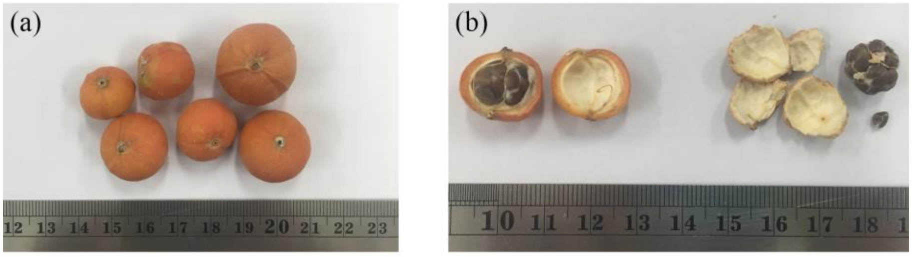

Figure 1 *A. mutica* (a) fruit and (b) pericarps and seeds

In previous reports, the phytochemical investigation of whole *A. mutica* fruits yielded styrylpyrones, flavanones, and chalcones, namely 5,6-dehydrokawain, pinocembrin, alpinetin, flavokawain B, cardamomin, and 2',3',4',6'-tetrahydroxychalcone (Jantan et al., 2004). These compounds have been reported to possess some biological activities, including anti-cancer (Malami et al., 2018; Zahra et al., 2019; Hseu et al., 2020; Zhang et al., 2020), anti-allergy (Hanieh et al., 2017), anti-inflammatory (Wang et al., 2018), and osteogenic (Natsume, Yonezawa, Woo, & Teruya, 2020) effects. Moreover, several phytochemicals from *A. mutica* fruits have been reported to show inhibitory effects on platelet-activating factor receptor binding (Jantan et al., 2004) and human platelet aggregation (Jantan et al., 2008) and cytotoxicity against carcinoma cell lines (Malek et al., 2011). In addition, chemical studies of the rhizome and leaf of *A. mutica* have led to the isolation of flavanones, chalcones, styrylpyrones, and diarylheptanoids (Sirat et al.1996; Sirat & Jani, 2013). Most phytochemical studies of *A. mutica* have focused on the chemical constituents of the rhizomes, leaves, and fruit. A fruit consists of two parts: the pericarp and the seed. The chemical constituents of these two separate parts of *A. mutica* have not been well investigated.

The present study was undertaken to fill this gap by identifying volatile components in the crude dichloromethane extract of the two separate parts, the pericarps and seeds of *A. mutica*, using gas chromatography–mass spectrometry (GC–MS). The presence of specific chemical constituents and their localization in *A. mutica* pericarp and seeds will be useful as supporting data and for further medicinal applications.

2. Material and methods

2.1 Plant materials

Mature fresh fruits of *A. mutica* were collected in May 2018 from the Department of Chemistry, Faculty of Science, Naresuan University, Phitsanulok, Thailand. The voucher specimen (004360) was identified by Assistant Professor Dr. Pranee Nangngam and kept at the Department of Biology, Faculty of Science, Naresuan University, Phitsanulok, Thailand. The pericarps and seeds were separated and dried in air at room temperature.

2.2 Preparation of crude extracts

The samples of mature fresh pericarps and seeds of *A. mutica* were separated for use in the experiments. The dried pericarps (43.22 g) and seeds (44.03 g) were individually macerated in dichloromethane at a ratio of 1:3 (dried sample:solvent) for a week at room temperature. The maceration procedure was repeated twice. The solvent was then filtered and evaporated under reduced pressure using a rotatory evaporator (BUCHI Ltd., Flawil, Switzerland). Two crude dichloromethane extracts of the pericarps (3.10 g) and seeds (3.43 g) were obtained.

2.3 Gas chromatography–mass spectrometry (GC–MS) analysis

Each sample extract was prepared at a concentration of 50 mg/mL in dichloromethane and filtered. The two filtered samples were then injected into a Hewlett-Packard 6870N gas chromatograph equipped with a mass selective detector (MSD5973N; Agilent Technologies, Palo Alto, CA, USA). The condition parameters were as described by Suphrom et al. (Suphrom, Insumrong, Ingkaninan, & Boonphong, 2019). A fused silica capillary HP-5 (5% phenyl methyl siloxane) column (30 m × 0.25 mm i.d., 0.25 μm film thickness) was used for the GC separation. The carrier gas was high purity helium with a constant flow rate of 1.0 mL/min. The injection temperature was set to 250 °C, and the measurements were performed in split mode with a split ratio of 10:1 (volume per volume) in 1 μL. The GC oven temperature program started at 70 °C and was held for 3 min, then raised at 5 °C/min to 280 °C and held for 10 min. The transfer line temperature was set at 280 °C. Measurements were performed in electron impact ionization mode at 70 eV. The mass was scanned from 50 to 550 amu. The identification of the volatile components was conducted by matching the mass spectra with a standard library: Wiley 7n, and the National Institute of Standards and Technology (NIST) Chemistry WebBook. The retention indices (RIs) were determined by analyzing a solution containing the homologous series of *n*-alkanes (C_8–C_{20}) under the same chromatographic conditions and then calculating them as described by Van den Dool and Kratz (Van den Dool & Kratz, 1963). The relative intensity of each volatile compound was calculated as the ratio between the area of the specific molecule and the sum of the areas of all identified peaks (peak area normalization method) in the chromatogram (Kafkas et al., 2006).

3. Results

3.1 Percentage yields and physical properties of extracts

The extraction of the dried material with dichloromethane provided a percentage yield of 7.17% for pericarps and 7.78% for seeds. The crude extracts had different physical appearances. The pericarp extract was brown, and the seed extract was an orange-yellow color.

3.2 Volatile compositions of the extracts

As shown in Figure 2, slightly different profiles of the volatile compositions were observed in the two extracts. Ten compounds were identified in the pericarp extract, and 11 compounds were identified in the seed extract. The relative amounts (%) of the components were calculated by peak area normalization. As listed in Table 1, the relative amount of total identified compounds in the pericarp was calculated as 94.96%, and in the seed extract, 99.24%. In *A. mutica* pericarps, the major volatile compound (RT 39.50 min) was diarylheptanoid, specifically 1,7-diphenyl-4,6-heptadien-3-one, which was about 45.28% of the detected compounds. In addition, this diarylheptanoid was only found in the pericarp extract. Various groups of compounds, including styrylpyrones, fatty acids, and phenyl derivatives, were present as minor components. These minor compounds also included 5,6-dehydrokawain, palmitic acid, linoleic acid, phenyl butanone, and cinnamaldehyde.

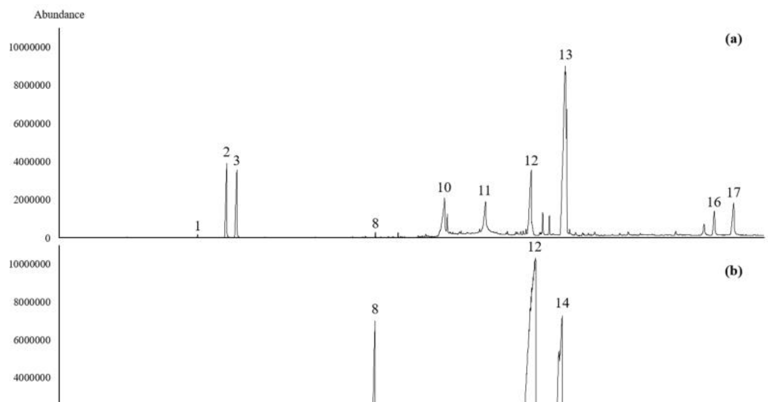

Figure 2 Total ion chromatograms of crude extracts of (a) pericarps and (b) seeds of *A. mutica*. The compounds assigned to the numbers are listed in Table 1.

As seen in Figures 2(b) and Table 1, the seeds were rich in styrylpyrones, flavonoids, and sesquiterpenes, with the most abundant being 5,6-dehydrokawain (64.94%), pinocembrin (22.51%), and farnesol (9.18%). Two of the flavonoids (RT 39.63 min and 42.48 min) were only present in the seed extract, whereas the sesquiterpenes, phenyl derivatives, and styrylpyrone compounds were found in both the pericarp and the seed extracts. Notably, the relative contents of these three groups of compounds were different in the pericarp and seed extracts.

Table 1 Volatile compositions of pericarps and seeds from *A. mutica*.

No.*	**RT[1]**	**RI_{exp}[2]**	**RI_{lit}[3]**	**Identified compounds**	**Molecular formula**	**Classification**	**Relative amount (%)[4]**	
							Pericarp	**Seed**
1	10.79	1164	1170[5]	Borneol	$C_{10}H_{18}O$	Monoterpene	0.21	nd
2	12.95	1240	1243[6]	Phenyl butanone	$C_{10}H_{12}O$	Phenyl derivative	7.77	0.37
3	13.72	1268	1270[7]	Cinnamaldehyde	C_9H_8O	Phenyl derivative	7.64	0.32
4	17.62	1415	1420[8]	Caryophyllene	$C_{15}H_{24}$	Sesquiterpene	nd	0.54
5	18.48	1449	1464[7]	Humulene	$C_{15}H_{24}$	Sesquiterpene	nd	0.10
6	21.65	1579	1589[5]	Caryophyllene oxide	$C_{15}H_{24}O$	Sesquiterpene	nd	0.13
7	24.00	1683	1684[9]	2,3-Dihydrofarnesol	$C_{15}H_{28}O$	Sesquiterpene	nd	0.10
8	24.87	1722	1720[5]	Farnesol	$C_{15}H_{26}O$	Sesquiterpene	0.28	9.18
9	27.16	1830	1812[10]	Farnesyl acetate	$C_{17}H_{28}O_2$	Sesquiterpene	nd	0.10
10	30.08	1976	1969[11]	Palmitic acid	$C_{16}H_{32}O_2$	Fatty acid	7.87	nd
11	33.28	2149	2138[11]	Linoleic acid	$C_{18}H_{32}O_2$	Fatty acid	6.77	nd
12	36.84	2456	2352[12]	5,6-Dehydrokawain	$C_{14}H_{12}O_3$	Styrylpyrone	10.37	64.94
13	39.50	2542	-	1,7-Diphenyl-4,6-heptadien-3-one	$C_{19}H_{18}O$	Diarylheptanoid	45.28	nd
14	39.63	2545	2513[13]	Pinocembrin	$C_{15}H_{12}O_4$	Flavonoid	nd	22.51
15	42.48	2704	-	5,7-Dihydroxy-6-methyl flavanone	$C_{16}H_{13}O_4$	Flavonoid	nd	0.95
16	51.14	-	-	Stigmasterol	$C_{29}H_{48}O$	Triterpene	3.40	nd
17	52.65	-	-	β-Sitosterol	$C_{29}H_{50}O$	Triterpene	5.37	nd
				Total monoterpenes			0.21	-
				Total sesquiterpenes			0.28	10.15
				Total triterpenes			8.77	-
				Total diarylheptanoid			45.28	-
				Total styrylpyrones			10.37	64.94
				Total flavonoids			-	23.46
				Total fatty acids			14.64	-
				Total aromatic ketone			7.77	0.37
				Total aromatic aldehyde			7.64	0.32

* These numbers correspond to the numbers in Figure 2, nd: Not detected, [1]RT: Retention time (min); [2]RI_{exp}: Experimental retention index obtained from the relative calculation of to C_8–C_{20} *n*-alkane series; [3]RI_{lit}: Retention index found in reported literature; [4]Relative amount obtained by peak area normalization; [5]Wesołowska, Jadczak, Kulpa, Przewodowski, 2019; [6]Shapi & Hesso, 1990; [7]Hao et al., 2018; [8]Tananaki, Liolios, Kanelis, Rodopoulou, 2022; [9]Milos & Radonic, 2000; [10]Yáñez, Pinzón, Solano, & Sánchez, 2002; [11]Zhang et al., 2017; [12]Stojanovic, Palic, Alagic, Zekovic, 2000; [13]Andriamaharavo, 2014

4. Discussion

Previously, styrylpyrones, flavanones, chalcones, and diarylheptanoids have been isolated from various parts of *A. mutica* (Jantan et al., 2004; Malek et al., 2011; Sirat et al.1996; Sirat & Jani, 2013). Styrylpyrone has been found in the rhizome, leaves, and fruit, while flavanones and chalcones were reported in the rhizome and whole fruit. Interestingly, diarylheptanoid has only been found in the rhizome. However, no scientific studies have been published regarding the chemical composition of pericarp and seeds of the *A. mutica*. In our present study, the volatile component profiles of each were studied using GC–MS. Some identical chemical components were observed in the pericarp and seed. In the pericarp, diarylheptanoid, namely 1,7-diphenyl-4,6-heptadien-3-one, was the major component, and the styrylpyrones, fatty acids, and phenyl derivatives were minor components; conversely, styrylpyrones, flavonoids, and sesquiterpenes were found to be the major constituents in the seed. The three main groups of compounds found in each part corresponded to the study of Jantan and coworkers that isolated these compounds from the whole fruit in a methanol extract of *A. mutica* (Jantan et al., 2004). Moreover, a diarylheptanoid (1,7-diphenyl-5-hydroxy-6-hepten-3-one) has been isolated from the chloroform extract of *A. mutica* rhizome (Sirat et al.1996). The presence of a diarylheptanoid (1,7-diphenyl-4,6-heptadien-3-one) in the pericarp is reported for the first time in this study.

In addition, the farnesol identified in our crude dichloromethane extracts also agreed with the findings of Sirat et al. (2009) They found that the young and mature fruit oils of *A. mutica* obtained by hydrodistillation and characterized by GC–MS contained farnesol (44.3%–51.2%) as the major individual constituent (Sirat, Mohd, Jani, & Basar, 2009). Previously, the chemical components of the essential oils from *A. mutica* fruit obtained by the hydrodistillation method have been reported. For example, the compounds found in the essential oils extracted from many plants: sesquiterpenes, including β-caryophyllene and α-cadinol, were reported as the major components in the essential oil of *A.mutica* fruit collected from Vietnam (Huong, Dai, Thang, Bach, Ogunwande, 2016). Similarly, the group of compounds consisting of farnesol and α-farnesene was also detected in the extracts of fruit from Malaysia (Sirat et al., 2009). Additionally, other sesquiterpenes, such as β-sesquiphellandrene and β-bisabolene, have been identified as the main components in the other *Alpinia* sp. (Sirat & Nordin, 1995; Sirat, Basar, & Jani, 2011). Thus, the area where the plant was collected can cause wide compositional variety. The study of Ibrahim et al. has revealed that the monoterpenes, such as camphor, camphene, β-pinene, 1,8-cineole, and α-pinene, were observed as the major components in the fruit extract of *A. mutica* collected from another region of Malaysia (Ibrahim et al., 2014).

The extraction method is another factor in the identification of compounds of interest. Our study used a conventional extraction method, maceration with dichloromethane, to prepare both extracts. A difference has been observed in the chemical compositions of the crude extracts of macerated *A. mutica* in our study and the hydrodistillation extracts in the publications mentioned above. Monoterpenes and sesquiterpenes were less abundant in our extracts than in the previous studies. Moreover, the main components: styrylpyrones, diarylheptanoids, flavonoids, fatty acids, and triterpene, were only found in our pericarp and seed extracts. These differences could be attributed to several factors, including sources, cultivation, vegetative stage, solvent polarity, and extraction method.

From our results, styrylpyrone (5,6-dehydrokawain), flavanone (pinocembrin), and sesquiterpene (farnesol) were abundant in the *A. mutica* seed. 5,6-Dehydrokawain was found in both the pericarp and the seed. Based on the reported data, this bioactive compound was found in rhizomes, leaves and fruits of *A. mutica* and its dimer has also been found in the leaves (Sirat & Jani, 2013). 5,6-Dehydrokawain, also known as desmethoxyyangonin, is one of the six main kavalactones in the *Piper methysticum* (kava) plant (Pluskal et al., 2019). This compound has been identified as a monoamine oxidase B inhibitor, which can be used to treat neurodegenerative disorders, such as Parkinson's disease and Alzheimer's disease (Chaurasiya et al., 2017), as well as an anti-inflammatory (Nishidono et al., 2020). In addition, it has demonstrated anti-cancer activities (Roman et al., 2017). Some reports have shown that the flavanones pinocembrin, pinostrobin, and alpinetin can be isolated from the rhizomes and fruits of *A. mutica* (Malek et al., 2011; Sirat et al.1996; Jantan et al., 2004). Based on the biosynthesis suggested by Tan and coworkers, pinocembrin is a precursor of pinostrobin and alpinetin formation (Tan et al., 2015). Other studies have shown that pinocembrin has anti-inflammatory (Gan et al., 2021), anti-cancer (Jiang, Yang, Feng, Zhou, & Liu, 2020), and neuroprotective activities (Wang, Zheng, Xu, Tu, & Gu, 2020). Sesquiterpene, particularly farnesol, was the main terpenoid compound in the seed. Farnesol is a natural acyclic sesquiterpene found in the essential oils of various species of flora (Lopes et al., 2021). This sesquiterpene has antibacterial (Lopes et al., 2021), anti-cancer (Jung et al., 2018), and anti-inflammatory biological activities (Jung et al., 2018)

The bioactive compound, diarylheptanoid, has been analyzed in the pericarp for the first time. Diarylheptanoids are natural compounds that possess numerous physiological properties, including anti-cancer (Motiur Rahman et al., 2018) and anti-inflammatory activities (Yao, Huang, Wang, & He, 2018). These compounds have mainly been isolated in the genera *Zingiber*, *Curcuma*, *Alpinia*, *Alnus*, and *Myrica* (Sun et al., 2020). The present study revealed preliminary information on the diarylheptanoid present in *A. mutica* pericarp extract and identified a new opportunity for future researchers and pharmacological companies to formulate various drugs from *A. mutica*.

Likewise, our results revealed that different secondary metabolites could be found in the pericarps and seeds of *A. mutica*, which were also related to the reported pharmacological activities and the uses of *A. mutica* fruits. According to our results, most of the bioactive compounds of *A. mutica* fruit were identified in the seed. Furthermore, the diarylheptanoid in the *A. mutica* pericarp was reported for the first time. However, overlapping peaks and co-elution were observed in our analysis. The comparison of RI values on columns with different polarities should be conducted and applied in a further study to improve the peak resolution and verify this result. Moreover, the identification of chemical constituents by GC–MS in this work was limited to the volatile compounds. Therefore, isolation and identification of other compounds from the pericarps and seeds are still needed.

5. Conclusions

The different volatile compositions of the dichloromethane extracts of the pericarps and seeds of *A. mutica* were analyzed by GC–MS. The predominant component, 1,7-diphenyl-4,6-heptadien-3-one, was reported in the pericarp for the first time. Three bioactive compounds, 5,6-dehydrokawain, pinocembrin, and farnesol, were detected mainly in the seeds. This finding indicated that the pericarps and seeds of *A. mutica* could be a source of these compounds. The information gained in our work can be used to further evaluate the pharmacological roles of these compounds. The isolation of these compounds in both pericarp and seed extracts remains of interest.

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