A Comparative Metabolite Analysis of Pandanus amaryllifolius Leaves from Different Growth Stages Using GC-MS and Their Biological Activities

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A Comparative Metabolite Analysis of *Pandanus amaryllifolius* Leaves from Different Growth Stages Using GC-MS and Their Biological Activities

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ABSTRAK

Fragrant pandanus leaves (*Pandanus amaryllifolius*) are known as cooking flavoring leaves because of their aroma and biological activities that are beneficial to health. So far, there is uncertainty in using young and fresh leaves and old leaves. This study aimed to compare the phytochemical composition of *P. amaryllifolius* leaf extract on the development of different leaves. GC-MS analysis successfully revealed 16 compounds in old leaves and 21 in young ones. It categorized them into several classes: phenols, prenol lipids, steroids and steroid derivatives, fatty acyls, and other metabolites. The results showed that the compound 2,6,10,14,18,22-Tetracosahexaene,2,6,10,15,19,23-hexamethyl-,(al-E)- was most dominant in old leaves and young leaves at 45.84% and 19.22%, respectively. International library search results show that the compound has potential as an antiaging and antitumor.

Keywords: *Pandanus amaryllifolius*, GC-MS, metabolite, phytochemical

Introduction

Fragrant Pandanus (*Pandanus amaryllifolius*) is a tropical plant belonging to the family Pandanaceae (Omer et al. 2021) found in India, South China, and Southeast Asia, including Thailand (Saenthaweesuk, Naowaboot, and Somparn 2016). The middle leaves are pale green, slightly flabby, and grayish on the underside. Young leaves are bright green, and the tips of the leaves are pointed with a length of about 25-75 cm and a width of 2-5 cm (Bhuyan and Sonowal 2021). *P. amaryllifolius* leaves in 3-month development are light green-dark green, with a smoother leaf surface, 20 cm long and 2 cm wide. Differs in the leaves of 1-year development of dark green-brownish color, whose surface is rougher, 52.1 cm long and 4 cm wide. This is similar to studies that show 1-year Pandanus in Southeast Asia, predominantly 40-80 cm tall with clustered growing leaves (Amnan et al. 2022). The structure of *P. amaryllifolius* has different characteristics in each development (Fig. 1).

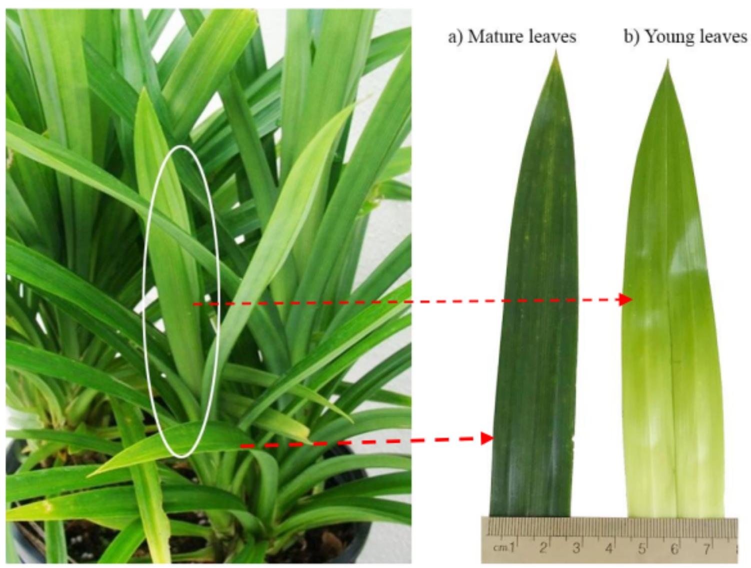


Figure 1. Leaves of *Pandanus amaryllifolius*; a) Age 12 months; (b) Age 3 months.

Pandan leaves are often used for medicine and seasoning in cooking because they have a unique and pleasant taste and aroma (Omer et al., 2021). The presence of 2-acetyl-1-pyrroline is thought to be an aromatic aroma-forming compound (Suryani et al. 2018; Wakte et al. 2010). Traditionally, Pandanus is considered a medicinal plant for the treatment of gout, hyperglycemia, hypertension, and rheumatism (Cheng et al. 2017), antimicrobial, antioxidant, antiviral (Nor et al. 2008), hypoglycemic (Ooi, Sun, and Ooi 2004). Pandanus lowers fever and relieves indigestion and flatulence (Cheeptham and Towers, 2002). In addition, the inhibitory effect of tumor growth from Pandan was proven through a series of pharmacological studies (Peungvicha, Thirawarapan, and Watanabe 1998). *P. amaryllifolius* leaves contain essential oils, carotenoids, tocopherol and tocotrienol (Lee, Su, and Ong 2004), alkaloids (Busqué et al. 2002), fatty acids and esters, and non-specific lipid transfer proteins (Ooi, Sun, and Ooi 2004). Pandanus contains several important phytochemical constituents, such as saponins, tannins, alkaloids, flavonoids, terpenoids, and phenolics, that are relatively high (Thanebal, Vun-Sang, and Iqbal 2021). Flavonoids are one of the secondary metabolites (MS) that act as antioxidants (Anggraito et al. 2018). Pandan leaf extract can be used as a natural antioxidant, so synthetic antioxidants can be reduced by using pandan leaves (Magaretta et al. 2011).

Ingredients such as saponins, tannins, alkaloids, flavonoids, terpenoids, and phenolics are found in most plants, one of which is Maja or bael fruit (*Aegle marmelos*) (Wangkahart et al. 2022). *Aegle marmelos* can also be an excellent source for phenolic and flavonoid content, which ultimately adds antioxidant as well as anti-inflammatory activity (Sonar and Rathod 2020). Flavonoids are found more in leaves than Maja fruits' skin (Atika 2021). The phytochemical content of each plant part varies because secondary metabolite compounds are closely related to the protective function of the plant itself (Syafitri, Bintang, and Falah 2014). The results of the *Helianthus annuus* research study on the roots, stems, leaves, and seeds showed differences in bioactive compounds because each organ had different metabolic processes (Maslakhah et al. 2019).

So far, the part of the pandanus plant used is the leaf part. Although studies of secondary metabolites in pandanus leaves are widely reported, phytochemical variations in pandanus leaves of young development and old development are still rarely investigated. Using the GC-MS analysis technique, this study will provide comprehensive information on variations in the content of secondary metabolite compounds in two types of *P. amaryllifolius* leaves. Therefore, the results of this study can be used as a reference in optimizing utilization in the next *P. amaryllifolius*.

Materials and Methods

Preparation and Identification of Plant Material

Pandanus amaryllifolius leaves development of 3 months and 12 months were taken from farmers in Bogor Regency, Indonesia. Samples of *P. amaryllifolius* were identified by ELSA Botanical Identification Services, the National Research and Innovation Agency (BRIN) Indonesia, and specimens were deposited with voucher number 3079-46085-3. Leaf picking is done by harvesting each part of the leaf directly and then putting it in a Coolerbox for extraction.

Extract preparation

A total of 50 gr of fresh leaves were each washed thoroughly using running aquadest water. Each sample was oven-dried for 72 hours at 40ºC. Sample leaves are blended until smooth for further maceration. Ethanol 99.8% analytical was used as a solvent at maceration for 92 hours. 10 ml of each sample extract was placed in a separate tube and dried at 60°C using a Rotary Evaporator Caliper. The solid residue is re-dissolved with the remaining extract of 200µL (Özbek et al. 2022).

GC-MS Analysis

GC-MS analysis was performed using Agilent Technologies 7890 Gas Chromatograph with Auto Sampler and 5975 Mass Selective Detector and Chemstation data system, following procedures performed by John Bwire Ochola (Ochola et al. 2022) and modified by the BALITRO (Spice and Medicinal Plants Research Center) library. Ethanol plant extract is filtered through a 5 µL syringe filter in split mode (8:1). Helium gas is used as a carrier at a rate of 1.2 mL/min. The injector temperature is 250°C, then the analytes are separated on the silica capillary column (30 m × 0.20 mm I.D × 0.11 mm film thickness). The initial oven temperature is set at 80°C, which is raised to 150°C at a rate of 3°C/min. 1 minute later, the oven temperature is raised to 280°C at a rate of 20°C/min. Once the oven temperature reaches 280°C, it is maintained for 26 minutes. Determination of the mass spectrum using an ionization energy of 70 eV.

Data Analysis

Agilent MassHunter Qualitative Analysis Software performed data analysis, and all components were identified by comparing their mass fragments with the standard mass spectrum. Biological activity data were analyzed using data from the chemistry libraries of NCBI (National Center for Biotechnology Information), NIST (National Institute of Standards and Technology), ChemSpider, WILEY Spectrabase branch, and TMIC (The Metabolomics Innovation Centre) FOOD3 branch (Tang et al. 2022).

Result

GC-MS analysis proves that *P. amaryllifolius* has different compound content at each development. Found 16 compounds in *P. amaryllifolius* of one-year development (Table 1), with the most compounds named 2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-,(al-E)- as much as 45.84% in leaves of 1-year development. In contrast to the 21 compounds in the leaves of 3-month development (Table 2), which only have 2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-,(al-E)-, as much as 19,22% as the most dominant compounds.

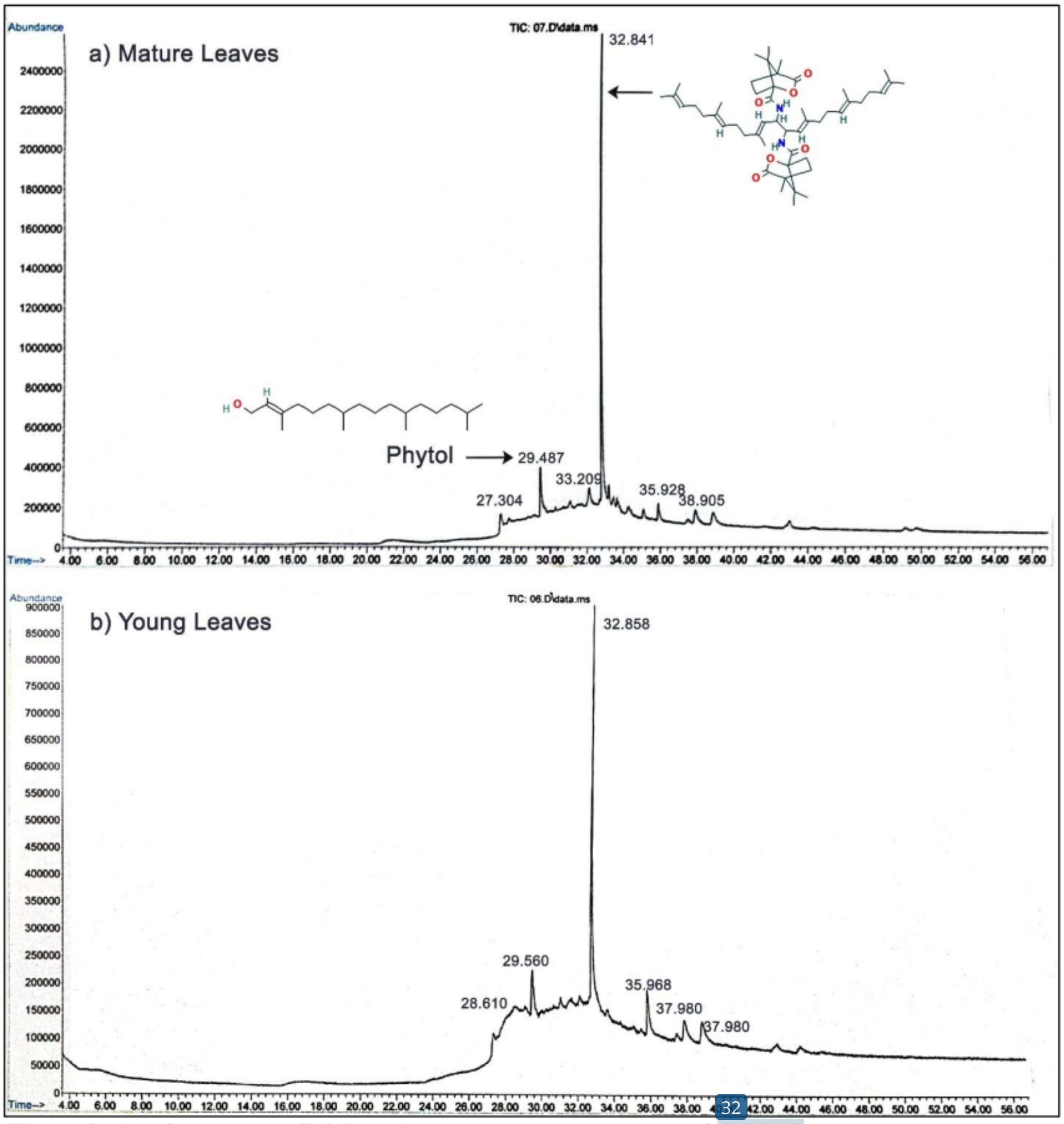
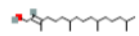
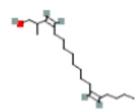
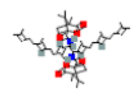
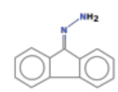
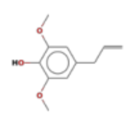
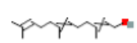
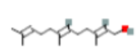
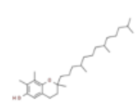
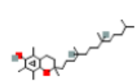


Figure 3. *Pandanus amaryllifolius* chromatogram as an output of GC-MS

The GC-MS analysis found the name of the metabolite compound based on Retention Time (RT) which describes the seconds of discovery of the compound during the GC-MS analysis. GC-MS also found the level of quality of compounds and the amount (% of the area) of each compound. The International Library analyzes Molecules Formula (M.F.), Molecules Weight (M.W.), Chemical Structure, and group division.

Table 1. *Pandanus amaryllifolius* leaves in the 12-month development

Metabolites Compound	RT	% of Area	M.F.	M.W. (g/mol)	Chemical Structure	Class
Benzyl (Dideuterated) methyl ethe	27,755	1.03	$C_8H_8D_2O$			
(2E)-3,7,11,15-Tetramethyl-2-hexadecen-1-ol	29,486	8.96	$C_{20}H_{40}O$	296.5		Prenol lipi Acylic Diterpenoids
2-methyl-z,z-3,13-octadecadienol	31,016	1.51	$C_{19}H_{36}O$	280.5		Unknown
2,6,10,14,18,22-Tetracosahexaene,2,6,10,15,19,23-hexamethyl-,(al-E)-	32,844	45.84	$C_{50}H_{76}N_2O_6$	410.72		Prenol lipi Triterpenoid
9H-Fluoren-9-one,hydrazone	33,209	4.38	$C_{13}H_{10}N_2$	194.23		Fluorenes
Phenol,2,6-Dimethoxy-4-(2-propenyl)-	33,464	3.31	$C_{11}H_{14}O_3$	194.23		Phenols: Methoxyphe ls
2,6,10-Dodecatrien-1-ol,3,7,11,trimethyl-	33,664	1.38	$C_{15}H_{26}O$	222.37		Prenol lipi Sesquiterper ds
(2E,6E)-3,7,11-Trimethyl-2,6,10-dodecatrien-1-ol	33,726	1.98	$C_{15}H_{26}O$	220,35		Prenol Lipi Acyclic diterpenoids
2,7,8-Trimethyl-2-(4,8,12-trimethyldecyl)-6-chromanol	35,098	1.85	$C_{28}H_{48}O_2$	416,69		Prenol lipi Tocopherols
dl-.α.-Tocopherol	35,926	3.34	$C_{29}H_{50}O_2$	430.7		Prenol lipi Tocopherols

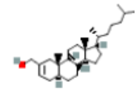
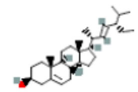
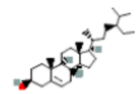
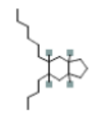
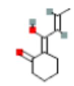
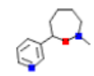
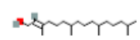
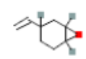
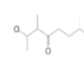
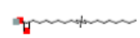
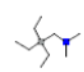
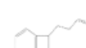
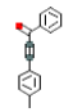
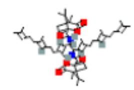
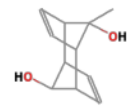
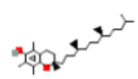
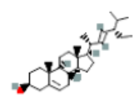
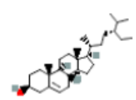
Cholest-2-en-ylmethanol	37,533	1.26	$C_{28}H_{48}O$	400.70		Unknown
Stigmasterol	37,939	5.00	$C_{29}H_{48}O$	412.7		Steroids: Stigmastanes and derivatives
.γ.-sitosterol	38,905	5.22	$C_{29}H_{50}O$	414.7		Steroids: Stigmastanes and derivatives
1H-Indene, 5-butyl-6-hexyloctahydro-	43,049	2.72	$C_{19}H_{36}$	264.5		Benzoxazoles

Table 2. *Pandanus amaryllifolius* leaves at 3 months of development

Metabolites Compound	RT	% of Area	M.F.	M.W. (g/mol)	Chemical Structure	Class
2-(1-Hydroxybut-2-enylidene)cyclohexane	27,390	2.39	$C_{10}H_{14}O_2$	166.22		Unknown
2-Methyl-7-(3-pyridinyl)-1,2-oxazepane	28,610	18.91	$C_{11}H_{16}N_2O$	192.26		Unknown
(9E)-9-Octadecenoic acid	29,169	6.19	$C_{18}H_{34}O_2$	282.46		Fatty Acyls: Long-chain fatty acids
(2E)-3,7,11,15-Tetramethyl-2-hexadecen-1-ol	29,562	7.94	$C_{20}H_{40}O$	296.5		Prenol Lipids: Acyclic diterpenoids
1,2-Epoxy-1-vinylcyclododecene	30,106	3.17	$C_8H_{12}O$	124.18		Unknown
2-Hydroxy-3,7-dimethyloctan-4-one	30,341	2.67	$C_{10}H_{20}O_2$	172.27		Unknown
Octadec-9-enoic acid	30,541	2.25	$C_{18}H_{34}O_2$	282.5		Fatty Acyls: Long-chain fatty acids
E-9-Tetradecanal	30,748	4.87	$C_{14}H_{26}O$	210		Fatty Acyls
Dimethylaminomethyl(triethyl)stannane	31,134	5.41	$C_9H_{23}NSn$	264.00		Unknown
7-(3-Butenyl)bicyclo[4.2.0] octa-1,3,5-triene	31,747	4.00	$C_{12}H_{14}$	158.24		Benzenoids

Phenyl p-tolylethynyl ketone	32,196	3.28	$C_{16}H_{12}O$	220.26		Ketones
Tricyclo [4.2.1.1 (2.5)] deca-3,7-diene-9,10-diol,9-methyl-,stereoisomer	32,320	1.30	$C_{11}H_{12}O_2$	176.21		Unknown
2,6,10,14,18,22-Tetracosahexaene,2,6,10,15,19,23-hexamethyl-,(al-E)-	32,858	19.22	$C_{30}H_{50}$	410.72		Prenol lipids: Triterpenoids
9-Methyltricyclo [4.2.1.1 (2,5)] deca-3,7-diene-9,10-diol	33,692	1.60	$C_{11}H_{14}O_2$	178.22		Unknown
2,5,7,8-Tetramethyl-2-(4,8,12-trimethyltridecyl)-6-chromanol	35,967	2.61	$C_{29}H_{50}O_2$	430.7		Prenol lipids: Tocopherols
Stigmasterol	37,974	2.29	$C_{29}H_{48}O$	412.7		Steroids: Stigmastanes and derivatives
.β.-Sitosterol	38,981	1.85	$C_{29}H_{50}O$	414.7		Steroids: Stigmastanes and derivatives

The class of Prenol lipids, as the most class found in leaves aged 12 months, is known to have different benefits from the most classes found in leaves aged three months, namely the class of Prenol lipids and Fatty Acyls with the same amount. Whereas, 2,6,10,14,18,22-Tetracosahexaene,2,6,10,15,19,23-hexamethyl-,(al-E)- as most compounds are from the class of prenol lipids, a subclass of triterpenoids. Other lipid prenol groups were also found in as many as 6 in leaves aged 12 months and 3 in three months. Benzyl (Dideuterated) methyl ethe compound as a minor compound found in leaves aged 12 months and leaves aged three months that have Tricyclo [4.2.1.1 (2.5)] deca-3,7-diene-9,10-diol,9-methyl-,stereoisomer as the most minor compound. These compounds have never been reported in the literature and require further research.

Table 3. Comparison of chemical constituents of Pandanus in different stages

Metabolite of Compound	3 months		12 months	
	RT	% of Area	RT	% of Area
(2E)-3,7,11,15-Tetramethyl-2-hexadecen-1-ol	29,562	7.94	29,486	8.96
6- [.Alpha.- (P-tolyl) methylidene] hydrazino-5- (dimethylamino methylene)	32,451 32,609	1.20 1.45		
2,6,10,14,18,22-Tetracosahexaene,2,6,10,15,19,23-hexamethyl-,(al-E)-	32,858	19.22	32,844	45.84
Stigmasterol	37,974	2.29	37,939	5.00

Pandanus amaryllifolius has a different ratio of compound content at each age. Through GCMS analysis, it is known that there are four compounds found at the age of 3 months and 12 months (Table 3). Compounds (2E)-3,7,11,15-Tetramethyl-2-hexadecen-1-ol,2,6,10,14,18,22-Tetracosahexaene,2,6,10,15,19, 23-hexamethyl-,(al-E)-, Stigmasterol, and 6-[.α.- (P-tolyl)methylidene] hydrazino-5- (dimethylamino methylene) have an increasing amount with age. It is known that the four compounds are from the class of prenol lipids, namely (2E)-3,7,11,15-Tetramethyl-2-hexadecen-1-ol, and 2,6,10,14,18,22-Tetracosahexaene,2,6,10,15,19, 23-hexamethyl-,(al-E)-.stigmasterol with steroid grade and steroid derivates. At the same time, the class of 6- [.A.- (P-tolyl) methylidene] hydrazino-5- (dimethylamino methylene) is not yet known.

Discussion

Ethanol is used as an organic solvent with the excess extraction of phenolic compounds studied to be more selective than water (Sánchez-Gomar et al. 2022). Ethanol has an ethane chain whose one of the hydrogens is substituted by a hydroxy group (NCBI). With a molecular weight of 46.07g/mol, it has a safety level that is not harmful to the body. In various studies, ethanol is often used in dissolving extracts (Sánchez-Gomar et al. 2022). Ethanol can identify antioxidant content well in the extraction of mango, olive, and red wine leaves. Ethanol can also identify anti-inflammatories in a solution of *C. sativa* aeroponic root extract (Ferrini et al. 2022). It is even used to identify anticancer substances (Q. Yang et al. 2022). Ethanol can identify 16 compounds in 1-year development and 21 in 3-month development in *P. amaryllifolius* leaf extract. This suggests ethanol is quite good at identifying the chemical substances contained in *P. amaryllifolius* leaf extract.

P. amaryllifolius leaves extracts both dominated by compounds 2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-,(al-E)- with remarkable benefits. This compound is also referred to as squalene which has a linear hydrocarbon structure (Rogowska and Szakiel 2021). Squalene is often found in many plants, such as olive oil, palm oil, and avocado, with levels that depend on plant conditions (Mousavi et al. 2022) and is often used in the pharmaceutical, nutraceutical, and cosmetic industries (Ali et al. 2022). Its primary function is as a precursor to the biosynthesis of sterols (Abuobeid et al. 2022), and triterpenoids (Mus et al. 2022) and beneficial for plants as an antioxidant (Mousavi et al. 2022) and the human body as an antiaging, anti-fatigue, and antitumor (Liu et al. 2021).

The compound 2-Methyl-7-(3-pyridinyl)-1,2-oxazepane is dominant in the extract of *P. amaryllifolius* 3-month development by 18.91%. The compound 2-Methyl-7-(3-pyridinyl)-1,2-oxazepane belongs to the group of 1,2-oxazepines (WILEY). However, no one has yet reported the biological activity of the compound. It is similar to 6- [.α.- (P-tolyl) methylidene] hydrazino-5- (dimethylamino methylene), which has never been reported. Similarly, the compound 7-(3-Butenyl)bicyclo[4.2.0] octa-1,3,5-triene of the benzenoid group (WILEY) has no specific biological activity. However, the benzenoid group and its derivatives predominantly produce a special aroma in plants (A. Gonzalez et al., 2022). Benolenoid-class compounds are also found in *Prunus mume*, which produces a unique aroma in spring (Hao et al. 2022), and in Petunia hybrida flowers that emit a strong aroma at night (Fu et al. 2022). Also found are the Polycyclic hydrocarbons (WILEY) group on the leaves of *P. amaryllifolius* 12 months development, namely, 1H-Indene, 5-butyl-6-hexyloctahydro-. The Polycyclic hydrocarbons group is also found in various studies, but the compound 1H-Indene, 5-butyl-6-hexyloctahydro- still needs more research on its biological activity. There are only ten compounds at 1-year development and eight compounds at 3-month development whose biological activity has been reported (Table 4). Meanwhile, 17 other compounds have not been found and need further research on their biological activity, including 4 compounds on leaves 12 months and 13 compounds on leaves 3 months (Table 5).

Table 4. Biological Activity of the Active Compound on *P. amaryllifolius*

No	Metabolite of Compound	Biological Activity
1	(2E)-3,7,11,15- Tetramethyl-2-hexadecen-1-ol	Antibakteri (Cai et al. 2022)
2	(2E, 6E)-3,7,11-Trimethyl-2,6,10-dodecatrien-1-ol	Anti-inflamasi (Mückter et al. 2022), Antibiotic dan antibakteri (Oliveira et al. 2022).
3	(9E)-9-Octadecenoic acid	Antiproliferative(Ak et al. 2021), anti-inflamasi), (Alzahrani et al. 2021) dan ansiolitik (Fattuoni et al. 2020)
4	.β.- Sitosterol	anti-inflamasi (Tunit et al. 2022) dan antidiabetes (Afifi et al. 2022)
5	.γ.- sitosterol	Anti-penuaan (Younis et al. 2022) dan antikanker (Sánchez-Hernández et al. 2022)
6	2,5,7,8-Tetramethyl-2-(4,8,12-trimethyltridecyl)-6-chromanol	Antidiabetik (H. Yang et al. 2022), Antioksidan, dan antikanker (Civelek and Podszun 2022).
7	2,6,10,14,18,22-Tetracosahexaene,2,6,10,15,19,23-hexamethyl-, (al-E)-	Peningkatan respon imun dan efek anti-penuaan, anti-kelelahan dan anti-tumor (Liu et al. 2021)
8	2,6,10-Dodecatrien-1-ol,3,7,11,trimethyl-	Anti-inflamasi (Mückter et al. 2022) Antibiotic dan antibakteri (Oliveira et al. 2022).
9	2,7,8-Trimethyl-2-(4,8,12-trimethyldecyl)-6-chromanol	Antioxidant (D. F. Gonzalez and Young 2020)
10	2-methyl-z,z-3,13-octadecadienol	Antimikroba (Aguoru 2017)
11	9H-Fluoren-9-one,hydrazone	Antioksidan (Šermukšnytė et al. 2022), antikanker (Naghibi et al. 2022), anti jamur, antivirus, anti-diabetes, antitumor, anti-inflamasi (Alam et al. 2022), dan antibakteri (Angelova et al. 2022)
12	dl-.α.- Tocopherol	antikanker, anti-inflamasi, (Trombino et al. 2022), antioksidan (Daia et al. 2021)
13	E-9- Tetradecanal	Antimikroba (Aguoru 2017)
14	Octadec-9-enoic acid	Antimikroba (Aziz et al. 2021), Antiseptic, Antiparasitic, dan Antibacterial.
15	Phenol,2,6-Dimethoxy-4-(2-propenyl)-	Antimikroba (Oo et al. 2021)
16	Stigmasterol	Penurun Kolesterol, meringankan penyakit hati, anti-osteoartritis, dan antidiabetes (Gładkowski et al. 2022)

Table 5. The active compound of *P. amaryllifolius* leaves that have not been identified as biological activities

No	Metabolite of Compound	Plant Age	Biology Activity
1	1 (2H)-Pentalaneone,hexahydro-3,5,5-trimethyl-,(3.α., 3a.β., 6a.β)-(.+-.)-	3 months	Unknown
2	1,2-Epoxy-1-vinylcyclododecene	3 months	Unknown

3	1-{4'-[Ethyl (methyl) amino] phenyl}morpholine	3 months	Unknown
4	Tricyclo [6.6.0.0 (3,6)] tetradeca-1(8), 4,11-triyene	3 months	Unknown
5	2-(1-Hydroxybut-2-enylidene) cyclohexane	3 months	Unknown
6	2-Hydroxy-3,7-dimethyloctan-4-one	3 months	Unknown
7	2-Methyl-7-(3-pyridinyl)-1,2-oxazepane	3 months	Unknown
8	Tricyclo [4.2.1.1 (2.5)] deca-3,7-diene-9,10-diol,9-methyl-, stereoisomer	3 months	Unknown
9	6- [.Alpha.- (P-tolyl) methylidene] hydrazino-5-(dimethylamino methylene)	3 months	Unknown
10	7-(3-Butenyl)bicyclo [4.2.0] octa-1,3,5-triene	3 months	Unknown
11	9-Methyltricyclo [4.2.1.1 (2,5)] deca-3,7-diene-9,10-diol	3 months	Unknown
12	Phenyl p-totalylethynyl ketone	3 months	Unknown
13	Dimethyl amino methyl (triethyl) stannane	3 months	Unknown
14	Cholest-2-en-ylmethanol	12 months	Unknown
15	Benzyl (Dideuterated) methyl ethe	12 months	Unknown
16	4-Phenyl-1,2,3,4-tetrahydro-8-isoquinolinamine	12 months	Unknown
17	1H-Indene, 5-butyl-6-hexyloctahydro-	12 months	Unknown

In the GC-MS analysis, even phytosterol components were found in *P. amaryllifolius* extracts through ethanol solvents. The phytosterol components (plant sterols) (Wang et al. 2022) have biological functions that are beneficial to the human body, such as antitumor (Karim et al. 2022), anticancer, antioxidant, and antimicrobial (Wang et al. 2022). Stigmasterol was the main phytosterol in both samples, with more in *P. amaryllifolius* extract in 12 months of development. Stigmasterol effectively prevents Alzheimer's disease (Gładkowski et al. 2022) and is supported by research (Hussein et al. 2022), which shows that Stigmasterol can reduce the damaging effects of γ radiation. Other phytosterol components are also found in a 12 months developmental extract of *P. amaryllifolius* named β.-sitosterol as an anti-inflammatory (Tunit et al. 2022), and antidiabetic (Afifi et al. 2022), γ sitosterol as antiaging (Younis et al. 2022) and anticancer (Sánchez-Hernández et al. 2022). This comparison shows that 12 months developmental leaves have higher antioxidant benefits than 3-month development.

The compound (9E)-9-Octadecenoic acid (elaidic acid) in *P. amaryllifolius* extract progressed for three months as much as 6.19%, reported to have anti-inflammatory functions (Alzahrani et al. 2021), and anxiolytics (Fattuoni et al. 2020). The compound (9E)-9-Octadecenoic acid is used in treating inflammation due to COVID-19 infection (Alzahrani et al. 2021). This shows (9E)-9-Octadecenoic acid is very effective in dealing with human inflammation, such as the compound.β.-sitosterol found in leaves of 3-month development. Anti-inflammatory substances were found in as many as four compounds in the leaves of 1-year development, namely (2E,6E)-3,7,11-Trimethyl-2,6,10-dodecatrien-1-ol, 2,6,10-Dodecatrien-1-ol,3,7,11,trimethyl-, 9H-Fluoren-9-one,hydrazone, dl-.α.-Tocopherol. This shows 1-year developmental leaves have higher anti-inflammatory levels. Especially in the compounds 2E,6E)-3,7,11-Trimethyl-2,6,10-dodecatrien-1-ol, and 2,6,10-Dodecatrien-1-ol,3,7,11,trimethyl- which belongs to the farnesol group with natural hydrophobic properties (Mückter et al. 2022), and as aromatic compounds (Sommer et al. 2022). Research on farnesol has proven its effectiveness as an antibiotic, antibacterial (Oliveira et al. 2022), and anti-inflammatory (Mückter et al. 2022).

dl-.α.-Tocopherol has other properties, namely as an antioxidant, as well as the function of compounds classified as tocopherol (Vitamin E) (Sun et al. 2022), such as carotene compounds (pro-vitamin A), coenzyme Q-10, L-carnitine, DL-α-tocopherol acetate (vitamin E), and ascorbic acid reported in the study (Daia et al. 2021). The compound 9H-Fluoren-9-one,hydrazone is often found as a component of anticancer drugs (Huff, Winter, and Dealwis 2022). The four compounds identified as anti-inflammatory substances contain other beneficial benefits.

The compound (2E)-3,7,11,15-Tetramethyl-2-hexadecen-1-ol or phytol has antibiotic properties (Cai et al. 2022) and belongs to the diterpenoid group (Wu et al. 2021). Phytol is found in various plants, namely *Populus sp*. (Wu et al. 2021) and *Vigna unguiculata* (L.) Walp. (Perchuk et al. 2020). Interestingly, phytol is found in higher and low-level plants such as the microalgae *Scenedesmus sp*. (Apandi et al. 2021). Phytol has many benefits, as well as the diterpenoid group in the study (Chen et al. 2022), which has biological activities in the form of analgesics, anti-inflammatories, antiepileptics, and antidepressants. The various biological activities compounds show that *P. amaryllifolius* leaves can be used as cooking ingredients and to maintain a healthy body.

Conclusion

Pandanus amaryllifolius leaves are an essential plant used as a flavoring for dishes and traditional medicinal materials. The differences in components in the leaves of *P. amaryllifolius* have never been studied before. In this study, GC-MS analysis found 16 compounds in the development of *P. amaryllifolius* leaves aged 12 months and 21 in 3 months of development. The most common compound in both samples was named 2,6,10,14,18,22-Tetracosahexaene,2,6,10,15,19,23-hexamethyl-,(al-E)- or squalene which is beneficial for enhancing the immune response, antiaging, anti-fatigue, and antitumor. There are four similar compounds in both leaves, with the increasing age of metabolite content in pandan leaves increasing in age 12 months. Compound 6-[.Alpha.- (P-tolyl)methylidene] hydrazino-5-(dimethylamino methylene) only appeared at the age of 3 months, when the function and class of this compound were not yet known. The international library found a wide range of biological activities beneficial to human health. Our results confirm that *P. amaryllifolius* leaves at 12 months and three months of development can be utilized for human health.

Competing interests

The authors declare that they have no competing interests.

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